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ORIGINAL RESEARCH

Evaluation of Temporomandibular Joint Dysfunction in Patients with Chronic Neck Pain

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Abstract

Objective: The aim of this study was to compare the presence of temporomandibular joint dysfunction (TMD) in individuals with and without chronic neck pain (CNP).

Materials-Methods: The study included 41 subjects with neck pain and 41 subjects without neck pain. Temporomandibular Disorders were evaluated according to the Research Diagnostic Criteria (TMR). The amount of mouth opening was measured with a ruler (cm). Presence of voice in TMJ movements was evaluated. Pain intensity (Visual Analog Scale (VAS)) was evaluated on palpation of chewing muscles. Active joint motion of the cervical region was evaluated with a goniometer. Pain-related disability was assessed with the Neck Disability Indicator/NDI.

Results: The results of our study showed that the pain intensity of the temporomandibular joint (TMJ) was significantly higher than the control group. The severity of pain felt during rest, activity (active mouth opening) and chewing in the study group was significantly higher than the control group ($p<0.05$). Six-way ROM of the cervical region of all individuals and TMJ active joint movements results were found to be significantly lower in the study group than in the control group ($p<0.05$). Painful response and voice findings on muscle palpation were found to be significantly higher in the study group ($p<0.05$).

Conclusions: This study revealed that the signs and symptoms of TMD are seen at a higher rate in individuals with CNP than in healthy individuals. Therefore, we think that TMJ should be included in the routine evaluation program for people with CNP.

Keywords: Cervical, Chronic Neck Pain, Temporomandibular Joint Dysfunction, Physiotherapy

INTRODUCTION

Neck pain is an important health problem occurring in up to 20% of adults[1]. Chronic neck pain (CNP) is defined as persistent or severe neck pain lasting more than 3 months[2]. It was reported that approximately half of the patients recovered within one year of treatment, whereas approximately 10% of the patients reported that their pain became chronic[3].

Another musculoskeletal problem that is adjacent to the cervical region and negatively affects the quality of life is temporomandibular joint (TMJ) disorders[4]. Temporomandibular joint dysfunction (TMD) often occurs because of musculoskeletal disorders associated with the masticator muscles and jaw joint[5]. It was reported that the prevalence of TMD reaches 16% and the rate of severe TMD requiring treatment varies between 3.6% and 7%. It is seen 4-6 times more frequently in women than in men, especially in the premenopausal period[6].

Some recent studies have shown a significant association between cervical spine disorders and TMD[7-8]. Although it has been reported that symptoms of cervical region dysfunctions are seen in patients with TMD, the mechanism of the relationship between cervical region pathologies and TMD has not been elucidated. It has been considered that changes in one of the two regions may affect the other region, as the cervical spines

are directly connected to the cranium and masticatory structures through muscle, joint, and neurovascular structures[9-10].

Results of a study showed that TMD, whether chronic or not, is associated with neck muscle tenderness and disability[10]. In another study, it was found that patients with myofascial pain in the masticatory muscles associated with CNP had more widespread pain and distal hyperalgesia compared to patients with CNP alone [11]. Bevilaqua-Grossi et al. reported that as the severity of TMD increased, symptoms related to the cervical spine diseases also increased, but it was not vice versa[9]. In contrast, Matheus et al. concluded that TMD was not associated with craniocervical dysfunction[4]. Despite studies showing the contrary, the results of few studies examining the relationship between TMD and neck pain have demonstrated this relationship. It is seen in the literature that the functional status, especially the pain parameter, is also examined. Increasing the number of studies on the subject will provide a multifaceted approach to patients in treatment and reduce unnecessary medical expenses and labor losses.

The primary aim of this study was to compare the presence of temporomandibular joint dysfunction (TMD) in people with and without chronic neck pain (CNP).

MATERIALS AND METHODS

Ethical considerations

Ethics committee approval of this study was obtained from the Non-Interventional Medicine Ethics Committee of Pamukkale University (no:2018-05). All individuals included in the study signed the voluntary consent form.

Participants

This study is a prospective, observational study. It is included individuals with (study group) and without chronic neck pain (control group). This study was conducted at Viranşehir State Hospital between March 2018 and June 2018. Forty-one volunteers who met the inclusion criteria, presented to the Physical Therapy Clinic, and were determined to have chronic neck pain problems by the specialist physician were included in the study.

In both the study and control groups, individuals aged 20-50 years were included. The study group included individuals who had a complaint of neck pain for at least 3 months, marked a value above 0 on the Visual Analogue Scale (0-10 cm), and scored at least 5 on the Neck Disability Index (NDI). Those who met the following criteria were excluded from the study: Those who reported musculoskeletal pain in any region other than the neck region; those who have undergone surgery due to any pathology in the cervical region, temporomandibular joint dysfunction (TMD), or an orthopedic problem; those with cervical and/or other musculoskeletal system problems that may affect the cervical region such as impingement and thoracic outlet, where specific pathological conditions such as malignant condition of the TMJ, fracture, systemic rheumatoid disease were shown; those undergoing facial paralysis; those actively receiving cervical and/or TMD-related therapy; those with a diagnosed psychiatric illness; and, those with communication difficulties.

Evaluation methods

TMJ clinical evaluation

The Temporomandibular Disorders/Investigational Diagnostic Criteria (TMD/IDC), widely used in epidemiological and randomized controlled clinical trials, were used for clinical evaluation. According to this classification, TMD is divided into three groups. Group I: Muscle Disorders - Myofascial Pain Syndrome (MPS) a) MPS without limitation in mouth opening b) MPS with limitation in mouth opening; Group II: Disc displacements a) Disc displacement with reduction b) Disc displacement without reduction - limitation in mouth opening c) Disc displacement without reduction - without limitation in mouth opening; and Group III: TMJ degenerations a) Arthralgia, b) Arthritis, c) Arthrosis. In our study, mouth opening measurements, presence of noise in TMJ movements, palpation parameters of chewing muscles and TMJ, which are among the physical examination findings included in TMD/IDC, were used as outcome measurements [12].

TMJ Movements: While individuals were sitting in an upright position on the chair with their arms close to the body, maximal depression, lateral deviation (right and left), and protrusion (maximum forward movement) movements of TMJ were measured. A 15-cm ruler was used in the evaluation [13]. For measurement reliability, the maximum opening movement and maximum forward movement values were corrected by the amount of overbite (a condition characterized by the upper teeth covering the lower teeth by more than a third) and overjet (a condition characterized by the upper teeth being positioned more than two millimeters ahead of the lower teeth), respectively [14]. All measurements were performed 3 times and the mean value was recorded.

Muscle palpation: Temporal muscle, masseter

muscle, and lateral and medial pterygoid muscles were evaluated bilaterally in the palpation of chewing muscles, and the results were recorded as 'pain' or 'no pain'. Noise in TMJ functions: Noise was evaluated with the aid of a stethoscope at the beginning, middle, or end of the mouth opening and closing movement, and it was recorded as a 'click' or 'crepitation' [14].

Evaluation of pain severity

VAS was used to assess the severity of pain. VAS is an assessment scale with proven validity and reliability in assessing musculoskeletal pain [15]. Participants were asked to mark the intensity of pain they felt at rest and activity in the cervical region and at rest and during chewing in the jaw region on a 10-cm scale (0: no pain, 10: unbearable pain).

Cervical region active normal joint movement

Flexion, extension, lateral flexion (right and left), and rotation (right and left) movements of the cervical region were measured using a universal goniometer. Measurements were taken while the participants were sitting on a chair with the head and torso upright [16]. Before the measurements were taken, the physiotherapist who made the evaluation showed the movements as a model and allowed the participants to try each movement once. All measurements were performed 1 times and the mean value was recorded.

Neck disability assessment

The Neck Disability Index was used to determine eligibility for study criteria in both groups. It is used to determine the severity of disability in patients with neck pain. The questionnaire was developed by Vernon and Mior [17-18] and it was adapted to Turkish by Telci et al. [19]. The questionnaire consists of 10 sections (frequency of pain, personal care, carrying heavy objects, headache, concentration, work, driving, sleep, and recreation). Each section is scored from 0 (No obstacle) to 5 (Full handicap). A score of 0-4 from the questionnaire indicates no disability, while a score of 35 and above indicates complete disability [19].

Statistical analysis

The number of participants planned to be included in the research was decided by the G-Power program [20]. It was calculated that when at least 40 people (20 studies, 20 controls) were recruited, 90% power would be obtained with 95% confidence. The data were analyzed with the SPSS package program. Continuous variables are presented as mean ± standard deviation and categorical variables as numbers and percentages. When the parametric test assumptions were met, the Test of Significance of the Difference Between the Two Means was used to compare independent group differences. When parametric test assumptions were not met, the Mann-Whitney U test was used to compare independent group differences. In addition, the differences between the categorical variables were examined by the Chi-square analysis.

RESULTS

The mean ages of the individuals in the study and control groups were 33.6± 8.8 years and 29.73± 5.8 years, respectively. The two groups were similar in terms of age, height, weight, BMI, years of education, and gender (p>0.05). Majority of the participants were married (61%) and employed (74.39%) (Table 1).

Table 1. Sociodemographic data of the study and control groups

Variables	Study Group n=41 X(SD)	Control Group n=41 X(SD)	P
Age (year)	33.63 (8.8)	29.73 (5.8)	**0.091***
BMI (kg/m^2)	25.08 (4.04)	24.03 (4.12)	**0.695****
Education Duration (year)	2.78 (1.17)	3.39 (1.09)	**0.297****
Gender	**n (%)**		
Sex			
Female	24 (%58)	21 (%51)	**0.376*****
Male	17 (%42)	20 (%49)	
Marital Status			
Married	32 (%78)	18 (%43.9)	
Single	9 (%22)	23 (%56.1)	
Occupation			
Housewife	13 (%31.7)	8 (%19.51)	
Employed	28 (%68.3)	33 (%80.49)	
Retired	0 (%0)	0 (%0)	

*Mann-Whitney U test, **Student's-t test, ***Chi-square test

According to TMD/IDC, there was no individual without TMD in the study group. In 19 patients (46.3%), Group 1 (Muscle disorders) signs and symptoms were recorded. In 17 patients (41.5%), Group 2 (Disc disorders) signs and symptoms were recorded. In 5 patients (12.2%), Group 3 (Joint disorders) signs and symptoms were recorded. In the control group, these values were 22 (53.7%), 8 (19.5%) and 2 (4.9%), respectively. TMD was not detected in 9 patients (22.0%) in the control group. The difference between the study group and the control group was found to be significant in terms of the levels of dysfunction signs and symptoms (χ^2=13.745 p=0.003). While disc- and joint-related TMD was more common in the study group, myofascial TMD was more common in the control group (Table 2).

Table 2. Comparison of the evaluation results of the Individuals in the study and control groups according to the TMD/IDC classification

		Group I n=41 n(%)	**Group II n=41 n(%)**	**Total n=82 n(%)**	**χ^2**	**p**
TMD/IDC Classification	No TMD	0	9 (%22.0)	9 (%11.0)	13.745	**0.003***
	Group1 (Muscular)	19 (%46.3)	22 (%53.7)	41 (%50.0)		
	Group2 (Disc)	17 (41.5)	8 (%19.5)	25 (%30.5)		
	Group3 (Joint)	5 (12.2)	2 (%4.9)	7 (%8.5)		

*Pearson Chi-Square

The mean neck pain duration of the individuals in the study group was 44.48 ± 35.79 months. The mean neck pain according to VAS was 3.82 ± 1.96 cm at rest and 7.73 ± 1.59 cm in activity. Thirty-one people (75.6%) in the study group and 8 people (19.5%) in the control group stated that they had TMJ pain complaints. There was a statistically significant difference between the two groups in terms of the incidence of pain in TMJ (p=0.000). The pain intensity felt at rest and during activity (active mouth opening and closing) and chewing in the study group was significantly higher than that in the control group (p<0.05) (Table 3).

Table 3. Comparison of study and control groups in terms of pain levels in the TMJ

Variables	**Study Group n=41 X(%)**	**Control Group n=41 X(%)**	**P**
TMJ Pain			
Yes	31 (%75.6)	8 (%19.5)	**p <0.001***
No	10 (%24.4)	33 (%80.5)	
	X(SD)		
TMJ Pain at Rest (cm)	0.80 (1.49)	0.07 (0.47)	**0.004****
TMJ Activity Pain (cm)	2.95 (2.63)	0.53 (1.77)	**p <0.001****
TMJ Chewing Pain (cm)	3.61 (2.84)	0.68 (1.59)	**p <0.001****

*Pearson Chi-Square, **Independent Samples Test

Six directions of Active Range of Motion (AROM) (flexion/extension, right and left lateral flexion, right and left rotation) belonging to the cervical region of all individuals and the total values of these movements were recorded. All measurement results were significantly lower in the study group than in the control group (p<0.05). TMJ active joint movements (AJM) (maximum opening, maximum right lateral, maximum left lateral, maximum forward movement amounts) were recorded. It was determined that all measurement results were significantly higher in the control group than in the study group (p<0.05) (Table 4).

Table 4. Comparison of the active range of motion values of the study and control groups for the cervical region and TMJ

Variables	Study Group, n=41, X(SD)	Control Group, n=41, X(SD)	P
Cervical Region AROM Values (°)			
Flexions	36.83 (10.86)	45.08 (8.84)	**p <0.001***
Extension	26.22 (8.76)	33.70 (8.70)	**p <0.001***
Lateral Flexion			
Right	32.35 (7.51)	35.79 (7.31)	**0.039***
Left	33.00 (9.57)	37.89 (7.92)	**0.014***
Rotation			
Right	51.26 (10.08)	63.49 (8.85)	**p <0.001***
Left	51.30 (10.30)	62.41 (7.27)	**p <0.001***
TMJ Lower Jaw AJM Values (mm)			
Maximum opening movement	42.22 (8.93)	45.88 (7.38)	**0.047***
Maximum right lateral movement	6.82 (2.66)	9.48 (2.28)	**p <0.001***
Maximum left lateral movement	7.10 (3.26)	9.72 (2.46)	**0.001***
Maximum forward movement	4.99 (2.70)	6.69 (2.01)	**0.002***

*Independent Samples Test; Active Range of Motion (AROM); TMJ active joint movements (AJM)

Pain response (pain/no pain) in the masticatory muscles was evaluated bilaterally. The greatest pain response occurred in the left lateral pterygoid muscle in the study group. It was determined that the presence of pain in all masticatory muscles except the right masseter muscle was significantly higher in the study group than in the control group ($p<0.05$). Clicking was observed in 31 individuals (75.6%) in the study group and 18 individuals (43.9%) in the control group. TMJ clicking in the study group was 1.72 times that of the control group. While there was no difference between the two groups in crepitation finding ($p>0.05$), it was determined that clicking was significantly higher in the study group than in the control group ($p<0.05$) (Table 5).

Table 5. Comparison of the study and control groups in terms of pain with palpation and noise data

Presence of pain on palpation	Study Group, n=41, X (%)	Control Group, n=41, X (%)	P
Right masseter	14 (%34.15)	7 (%17)	0.064
Left masseter	17 (%41.46)	6 (%14.63)	**0.006***
Right temporal	14 (%34.15)	6 (%14.63)	**0.035***
Left temporal	16 (%39)	6 (%14.63)	**0.012***
Right lateral pterygoid	21 (%51.22)	7 (%17)	**0.001***
Right medial pterygoid	18 (%43.9)	4 (%9.75)	**p <0.001***
Left lateral pterygoid	23 (%56)	7 (%17)	**p <0.001***
Left medial pterygoid	20 (%48.78)	4 (%9.75)	**p <0.001***
TMJ	27 (%65.8)	8 (%19.5)	**p <0.001***
TMJ Sound Finding			
Click sound	31 (%75.6)	18 (%43.9)	**0.003***
Crepitation	6 (%14.63)	4 (%9.75)	0.369

*Pearson Chi-Square

DISCUSSION

It was found in the present study that TMD findings were found in all patients with chronic neck pain. The most important result obtained in this study was that the incidence of TMD-related problems was significantly higher in individuals with CNP than in individuals without CNP.

According to many epidemiological studies, at least one symptom of TMD (such as movement anomalies, joint noise, limitation, and tenderness on palpation) is seen in an average of 75% in the general population without specific complaints[19,21]. In our study, at least one symptom was observed in 78% of individuals in the control group. No symptoms were observed in only 9 individuals in the control group. Kraus reported in a study that the presence of neck pain was associated with TMD at a rate of 70% [22]. In our study, symptoms of TMD were detected in all patients with CNP, which is higher than that belonging to healthy individuals.

In our study, in the study group consisting of patients with chronic neck pain, the severity of TMJ pain (at rest and during activity and chewing) was significantly higher than that in the control group. Furthermore, regarding TMJ pain intensities in the study group, it was seen that the lowest mean pain intensity was found at rest and the highest pain intensity mean value was obtained during chewing. In TMJ pain assessment, the outcome that the TMJ pain level felt during active mouth opening and chewing was significantly higher in the CNP group compared to the control group was explained by Stiesch-Scholz via the anatomical and functional relationships between trigeminal and cervical innervated structures in the craniofacial and cervical region[23]. Lauriti et al. [2014] measured the activity of the masticatory muscles with electromyography and found that the activation of the masticatory muscles was significantly correlated with the severity of TMD, and that TMJ pain intensity was felt at a lower level at rest[24-25].

In this study, we determined that the normal joint movements of the TMJ and cervical region in the study group were significantly lower than those of the control group. Similar to our results, De Laat et al. [1998] found that cervical movements were more restricted in individuals with TMD than in those without TMD[26]. This result shows that both joint movements are related to each other. Micarelli et al. [2020] evaluated a total of 254 patients, including patients with TMD or cervical region pain and healthy individuals. As a result, they found that people with TMD had limited cervical normal range of motion[27]. It is supported by the literature that individuals with TMD have limited range of motion in the cervical region[27-30].

In our study, when we compared the range of motion of the mandible between the two groups, we found that there were reduced movements in the CNP group compared to the control group. This result is similar the results reported by Rodrigues et al. [2015], who showed that TMJ movement capacity in patients with CNP decreased more than TMJ movement capacity in healthy individuals [31]. In addition, we did not find any limitation in our study in the left shifting movements of the chin in patients with CNP and we think that the reason for the limitation we found in the right shift movement may be that the left lateral pterygoid muscle is the muscle that produces the most pain response. In this study, the fact that the lateral pterygoid, which provides the anterior movement of the mandible, was the muscle that produced the most pain response, may explain the most common limitation in the maximum forward movement in the CNP group. In addition, the lack of joint limitations in this way shows us that there may not be internal irregularity in the TMJ, and that the TMJ problem may be caused by muscular structures[27]. In our study, we found that 50% of the individuals evaluated had myofascial TMD, and this rate was 46.3% in the study group, and myofascial TMD was highest in the group with CNP.

Matheus et al. [2009] reported that the relationship between cervical problems and TMD is due to muscular structures rather than joint structures [4]. In our study, in the comparison of pain on palpation of the masticatory muscles between the two groups, painful response on palpation in individuals with CNP was significantly higher compared to that of the control group. The muscle with the highest frequency of pain response on palpation in the CNP group was the left lateral pterygoid muscle. Furthermore, we determined that the frequency of pain response with muscle palpation was significantly higher in the study group than in the control group. When we examined the groups in terms of TMJ palpation, 65.89% of the individuals with CNP had a painful joint response, and it was 19.5% in the control group. The pain response revealed by TMJ palpation was significantly higher in the study group consisting of patients with chronic neck pain compared to the control group.

Our results showed that the muscle palpation and pain response due to myogenic factors seen in the form of excessive, frequent, and long-lasting load on the TMJ articular cartilage in the CNP group was higher than those of the healthy individuals.

The first sign of TMD is joint noise. The presence of noise-clicking in TMJ is mostly related to internal irregularity, but there may also be clicking in myofascial pain due to TMD [21]. In their study of 251 patients with TMD, Dalkız et al. [2001] reported clicking in 75.6%, locking in 7.1%, limitation in mouth opening in 90.4%, dislocation in 54.9%, and varying degrees of occlusal irregularities, temporal pain, facial pain, headache, or joint pain in all individuals. In the present study, the rate of TMJ clicking from both joints or one joint was 75.6% in the CNP group and 43.9% in the control group. Our results showed that the presence of noise-clicking is a common symptom in TMD, in line with the literature, and revealed that these symptoms are seen at a higher rate in individuals with CNP than in healthy individuals[21].

The limited number of studies in the literature comparing the signs and symptoms of TMD in patients with chronic neck pain and healthy individuals is the strength of this study. However, the relatively low number of cases to examine the parameters associated with TMD, especially in the study group consisting of patients with chronic neck pain was one of the limitations. The study population consisting of young individuals was another limitation of the study.

CONCLUSION

Although the incidence of TMD is high in our society, it is seen that there is no consensus in the literature on whether there is a relationship between TMD and CNP, and the number of studies on this subject is insufficient. In conclusion, TMD incidence was found to be significantly higher in patients with CNP than in healthy individuals. For this reason, we think that TMJ should be included in the routine evaluation program in people with CNP complaints, so a more effective improvement can be achieved in terms of pain and disability parameters.

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Ethics statement

In this study, all the rules stated in "the Higher Education Institutions Scientific Research and Publication Ethics Directive" were followed; and, we undertake that none of the actions specified under the title of "Actions Contrary to Scientific Research and Publication Ethics" of the aforementioned directive have been carried out.

Ethics committee approval of this study was obtained from Pamukkale University Non-Interventional Medicine Ethics Committee (no: 2018-05). All individuals included in the study signed the voluntary consent form.

Disclosure statement: The authors have no conflicts of interest to declare.

Author contributions: Conceptualization: [HCG, EAT]; Design: [HCG, EAT]; Writing: [HCG AA]; Investigation/Data collection: [HCG, AA]

Conflict of interest: There is no potential conflict of interest relevant to this article.

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ORIGINAL RESEARCH

Is There a Relation between The Lower Extremity Mechanics and Patellofemoral Pain Syndrome?

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Abstract

Objective: It has been theorized that changes in the lower extremity mechanics may lead to develop Patellofemoral Pain (PFP) in the young population. The present study aims to investigate the effects of lower extremity mechanics on Patellofemoral Pain Syndrome in private university students.

Material-Method: Kujala Patellofemoral Score (KPS) of 400 Yeditepe University students ages of 18-30 years was performed**.** Students having a less or equal point of 85 in KPS (n=30) and healthy groups randomly selected in students with KPS=100 (n=30) were measured in terms of Feiss Line, navicular drop, subtalar angle, tibial torsion, knee valgus angle, Q angle in standing and supine position and hamstring tightness.

Results: The prevalence of PFP among students was found to be 10.5% (n = 42). In the PFP group, 16 (%53.3) students and in the control group, 3 (%10) students had 2nd Pes Planus (PP). A statistically significant difference was found between groups in navicular drop, subtalar angle, tibial rotation, Q angle in supine and hamstring tightness (p-value <0.05).

Conclusion: The results from this study show that students with PFP have higher severity degrees of pes planus, navicular drop, subtalar angle, tibial torsion, and hamstring tightness than nonpainful students. Therefore, these parameters that are related to lower extremity mechanics may be investigated in PFP examination and be considered while preparing a treatment plan.

Keywords: Pes Planus, Patellofemoral Pain, University Students.

INTRODUCTION

Patellofemoral Pain (PFP) is frequently seen in physically active populations, especially in young adults and mostly greater in females compared to males[1]. Squat position, ascending, and descending stairs or hills, sitting a long time with knees in flexion may elicit the onset of pain in PFP[1,2]. Pain at the patellofemoral joint during described positions or activities may lead to restrictions to participation in physical activities among young adults[3].

Lower extremity alignment may have an important role to understand the pathogenesis of PFP[2,4]. It starts with an abnormal form of the feet especially pes planus (PP), which is related to the absence of medial longitudinal arch and excessive pronation (hind foot valgus)[5]. To control this abnormal pronation, the tibia rotates and forces the knee to valgus, which may cause a decrease in the contact surface of patella and femur. The position of patella affects the Q angle, which is also known as "quadriceps angle." As a result, excessive compression to lateral patella facets and abnormal patella tracking may lead to PFP[4,6].

Muscle tightness or shortness is frequently reported as an objective sign in Patellofemoral Pain Syndrome (PFPS) patients and represents a target for treatment. Actually, relieving the tightness of specific muscles is the common clinical target in physiotherapy. Although the effect of hamstring tightness is thought to affect knee pain[7], in the literature, the impact of muscle length on PFPS is investigated for a group of muscles[8], such as hamstrings, tensor fascia lata and quadriceps. There are several studies revealing the individual effect of

hamstring tightness on knee pain[9]. However, the results of those studies are unclear to explain the association between hamstring tightness and knee pain.

There are several factors; such as, muscle weakness, overuse, and lower extremity mal-alignments that may contribute to PFP. However, the consensus on the etiology of PFP is not defined[4]. Moreover, in the literature, there are a limited number of studies investigating the risk factors of this problem in a particular population[9].

Plenty of uncertain contributing factors to PFP may be the main reason why there is no definitive treatment[1,4]. Describing the cause of PFP at the early stages of life, before pain becomes worse at an older age, may be the key to the treatment. The purpose of this study was to investigate the prevalence of PFP among private university students and to investigate and compare the lower extremity mechanics of students with and without PFPS. It was hypothesized that students with PFPS would have more pes planus degree, navicular drop, subtalar angle, tibial rotation, and Q angle than students without PFPS.

MATERIALS AND METHODS

Study design

The present study was a case-control design study and was conducted from 04. 04. 2018 to 10. 08. 2018. The subjects were asked to sign an informed consent form that had been approved by the Human Research Ethics Committee of Yeditepe University in 04.04.2018 (Approval number: 37068608-6100-15-1469)

Participants

Four hundred university students aged between 18 and 30 years were included in this study. Students were asked to fill the Kujala Patellofemoral Score (KPS) to determine the PFP status. 30 patients who had 85 or lower scores were included in the PFP group[10]. Sixty students who had PFP were excluded due to other orthopedic problems (n=48) or unwilling to participate in evaluations (n=12). Also, thirty students who had higher scores from KPS were included in the present study as a control group. Participants excluded from the study if there is a trauma and fracture history of the knee or lower extremity, a musculoskeletal system surgery, a diagnose for any disease of lower extremity, a regular usage of drugs which may influence muscular, skeletal, or neurological systems, or an in-line injection and systemic diseases (Figure 1)

Outcome measures

Kujala Patellofemoral Scoring Questionnaire was developed for the people who have PFP by Kujala et al.[11] in 1993. Also, validity, reliability, and sensitivity of this questionnaire was shown by Crossley et al.[12]. For this research, the Turkish version of the Kujala, which is demonstrated by Kuru et al. was used[1]. This questionnaire has 13 questions asking about how the pain is during a weight-bearing position, walking, jumping, running, squatting, ascending, and descending stairs and sitting with the knee bent in a long time. Also, the questionnaire asks the patients if they have complaints of swelling, limping, abnormal painful kneecap, atrophy of thigh muscles, flexion deficiency, and inquire that whether weight bearing is painful or not. It is determined that the highest point is 100, and the lowest one is 0[1,11,12].

Pes planus was measured by the Feiss line. The navicular tuberosity, the apex of the medial malleolus, and the plantar aspect of the first metatarsophalangeal joint were marked with pencil on patients in sitting position than standing position. For a first-degree flatfoot, the tubercle had to fell one-third of the distance to the floor; for a second-degree flatfoot, it had to fell two-thirds of the distance; if it was very closed to the floor, it meant a third-degree flatfoot. The difference between the height of navicular tuberosity from the ground in sitting and standing positions was recorded as "navicular drop"[13].

Subtalar angle was measured by recording the midline of Achilles tendon and ankle joint, and the midline of the calcaneus with the ruler. Then, the pivot point was the midline of the ankle joint. Next, the angle between Achilles and calcaneus line was measured in both prone and standing positions. The difference between standing and prone position of subtalar angle was recorded with goniometer in degrees for three times. For example, if the subject in prone position had 3 degrees of varus and in standing position had 4 degrees of valgus, the difference between them was recorded as 7 degrees[14].

Tibial torsion was measured by checking the line between apexes of medial and lateral malleolus according to the floor in the supine position. The angle between the lines was calculated with the goniometer as a tibial torsion for three times, and the average value was recorded in degrees[13].

Knee valgus angle measurement was performed with a goniometer in the upright position. After marking the mid popliteal pili, one arm of the goniometer was placed to the tuber ischium popliteal pile, and other arm was placed on the

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popliteal pili and mid-line of Achilles tendon. This value was measured for three times, and the average value was recorded in degrees[14].

The Q angle was measured in two different positions (supine position and standing with the double limb) by using the lines between (Anterior Superior Iliac Spine) ASIS and the mid-point of the patella, and the mid-point of the patella and the tibial tuberosity[13].

Hamstring tightness was measured by 90/90 Passive knee extension test, which has excellent inter-rater and good reliability[15]. While testing the participants in the supine position, knee and hip were taken 90-90-degree flexion, then one arm of the goniometer was placed on the femur and other on fibula and knee moved passively to the extension. Then, the angle between the fibula and the floor was noted. This measurement was applied for three times, and the average results revealed the tightness of hamstring numerically[16].

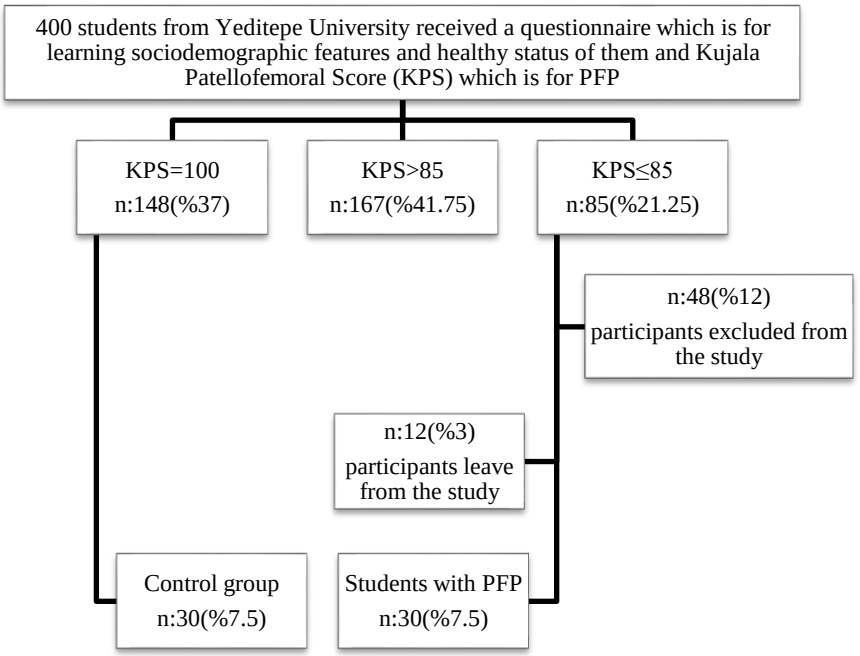

Figure 1. Distribution of subjects in the study. "PFP" and "n" indicates Patellofemoral Pain and number of students respectively.

Statistical analysis

Statistical Package for Social Sciences (SPSS) Version 25.0 program was used for the data analysis. Descriptive statics, mean ± standard deviation (X ± SD) or percentages (%), were gathered. The level of significance was accepted as p-value <0.05. Before statistics analysis, One-Sample Kolmogorov-Smirnov normality test was applied to obtained data. For comparison of groups Mann Whitney U test was used.

RESULTS

The demographic and clinical characteristics of participants showed in Table 1. The mean age was 22 ± 1.41 years in the PFP group, and that for the control group was 22.6 ± 1.32 years.

Data expressed as mean and ± standard deviation. Mean values expressed as are significantly different at $p < 0.05$ and $p < 0.01$.

Table 1. Demographic data in students with PFP and Control Groups [a]

	PFP Group(n=30)	Control Group(n=30)	p value
Female (n[%])	24(%80)	24(%80)	
Age (years)	22.0±1.41	22.6±1.32	0.158(NS)
Height (cm)	166.70±7.52	169.46±7.96	0.172(NS)
Weight (kg)	60.35±12.08	62.16±13.66	0.615(NS)
BMI	21.56±3.05	21.45±3.40	0.779(NS)

BMI, body mass index (kg/m^2);n, number of subjects; NS, nonsignificant difference; PFP, patellofemoral pain.

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This study showed that the prevalence of PFP in a private university was 10.5%. Besides, the frequency of the PFP syndrome among women and men was revealed as 9% and 1.5%, respectively (Figure 2).

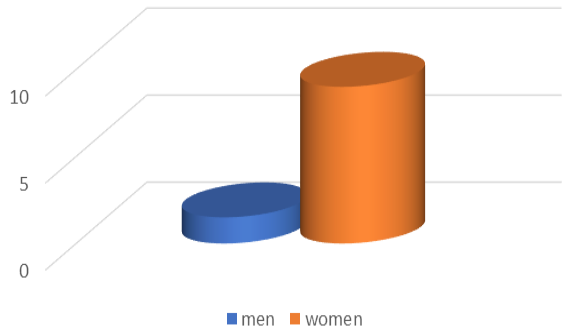

Figure 2. Distribution of percentage of gender in PFP individuals.

While 16 (%53.3) students in the PFP group had 2nd degree PP, 3 (%10) students in the control group had 2nd degree PP. Compared to controls, 14 (%46.7) students had 1st degree PP in the PFP group (Figure 3).

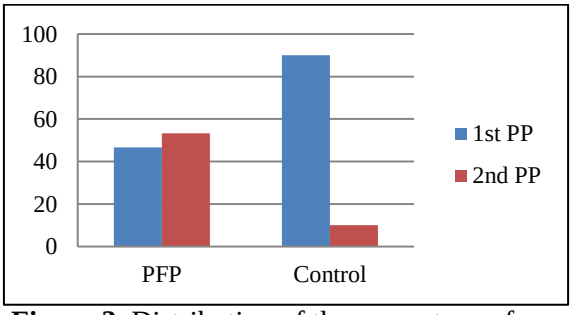

Figure 3. Distribution of the percentage of pes planus degrees between students with PFP and healthy students

The navicular drop, subtalar angle, and tibial torsion were found significantly higher in the PFP group than the control group ($p<0.05$) (Table 2). While the Q angle showed statistically significant results in the PFP group, compared to controls in the supine position ($p<0.05$), in the standing position there was no significant difference of Q angle in between the groups ($p=0.06$). Table 2 also demonstrates that there was no significant difference in genu valgum angle between the groups ($p=0.257$). 90/90 Hamstring tightness test showed statistically significant results in the PFP group compared to controls ($p<0.05$) (Table 2).

Table 2. Lower limb measurements in students with PFP and control groups.

	PFP Group(n=30)	**Control Group(n=30)**	***p value***
	Mean±SD	**Mean±SD**	
Navicular drop(mm)	5.06±2.42	2.50±1.71	***0.000[a]***
Subtalar angle (°)	5.30±3.49	2.7±1.66	***0.004[a]***
Tibial torsion (°)	12.04±3.64	10.33±2.83	***0.040[b]***
Q angle in supine position (°)	19.63±4.28	15.7±3.93	***0.001[a]***
Q angle in standing position (°)	19.56±4.86	17.15±4.62	*0.063*
Genu valgum angle(°)	8.74±3.59	7.79±3.48	*0.257*
90/90 Hamstring tightness test(°)	14.06±6.12	6.55±3.96	***0.000[a]***

**According to results of Mann Whitney U test*
SD, Standard Deviation; n, number of subjects; PFP, Patellofemoral pain
Data expressed as mean and ± standard deviation. Mean values expressed as are significantly different at $p<0.05$ and $p<0.01$.

DISCUSSION

This study showed that the prevalence of PFP in a private university was 10.5%. Besides, the frequency of the PFP syndrome among women and men was revealed as 9% and 1.5%, respectively (Figure 2). Similarly, to the results of Roush JR. and Curtis R[17], in our study women were more likely to have PFP than men, which may be a result of the alterations of lower extremity biomechanics in women, such as, the wider pelvis and larger Q angles.

In the literature, the reason for having PFP has not been adequately clarified yet. One of the risk factors might be pes planus since the foot posture may affect the lower extremity alignment[18]. To the best of our knowledge, even though the relationship between PFP and pes planus has theoretically known, literature has been debatable about this topic.

In the current study, in the PFP group, 16 (%53,3) students and in the control group, 3 (%10) students had 2nd PP. Our results showed the number of students having first- and second-degree PP was higher in the PFP group (Figure 3). As in our study, Kosashvili et al.[18] reported that moderate and severe PP might be associated with PFP among adolescents. Therefore, it may be recommended that patients with PFP may be assessed and treated in terms of the posture of the feet.

The present study demonstrated that subjects with PFP had significantly higher navicular drop results than subjects in the control group (Table 2). Consistent with our study results, Barton et al.[19] reported that subjects with PFP had significantly higher navicular drop results compared to controls. Similarly, Mølgaard et al.[20] have also shown that patients with PFP had a higher navicular drop and navicular drift diverge than their control groups. Therefore, it is possible to conclude that individuals who have a navicular drop may have an increased risk for PFP.

According to our results, a significant difference between the two groups was found in terms of subtalar angle (Table 2) (p=0.00). Our results also showed a greater subtalar angle in the PFP group compared to the control group. Similar results were reported by the research of Dileep et al.[21], in which the patients with PFP within the ages of 20-30 years were evaluated according to their foot posture and PFP syndrome and found an association between these two components. In contrast to our study, Hetsroni et al.[22] concluded that there is no consistent association between the incidence of anterior knee pain and foot pronation by measuring the subtalar joint displacement angle. Different from the study of Hetsroni, we selected a quite narrow age ranges to prevent the interference of age-related problems. With the selection of a population within specific age, we aimed to emphasize the mechanical links to PFP. Therefore, these controversial issues may result from the simultaneous evaluation of many risk factors that can cause PFP in the studies. Since there is more than one risk factor affecting PFP, lower extremity biomechanics may be recommended to be evaluated separately.

In this study, the mean values of tibial torsion in the PFP group were higher than the control group. In the PFP group had significantly greater tibial torsion compared to subjects in the control group (Table 2) (p = 0.04). Powers et al.[23] reported that tibial torsion and foot pronation were not different between subjects with and without PFP. In contrast to Powers et al.[23], Levinger and Gilleard[24] found greater inversion angle of the subtalar joint and eversion of the calcaneal joint in women with PFP compared to controls. Increased pronation of the foot may be considered as a cause of PFP and may mechanically cause tibial torsion in subjects with PFP. Besides, abnormal movement of the subtalar joint leads to the abnormal tibial rotation, which may result in injuries of the lower limb.

In this study, even though the Q angle in the supine position was different between groups (p=0.00), the Q angle in standing position was not significantly different (Table 2) (p=0.06). According to mean values, the Q angle in supine and standing positions were higher in the PFP group than the other group (Table 2). Some studies showed that an increased Q angle (greater than 20°) might lead to increased retro-patellar pressure, which may result in PFPS and degeneration of the articular cartilage. However, this issue has been controversial in the current literature (4). In their study, Kaya et al.[25] showed that individuals with PFP had significantly higher Q angle degrees in standing position than the individuals without PFP. On the contrary, the results of Caylor et al.'s study[26] did not reveal any difference between the Q angle degrees of the subjects with or without PFP in the standing position. In the current literature, the Q angle, being an indicator of PFP, has remained suspicious. However, it has been one of the most frequently used parameters to evaluate the risk of PFP among patients with PFPS[13]. Therefore, the effects of the Q angle are still discussed for subjects with or without PFP as the same in the literature.

Our genu valgum angle results were not statistically significant between PFP and control groups (Table 2). However, the mean of the genu valgum angle was higher in the group with PFP than in the control group (Table 2). Consistent with our study results, a systematic review of 973 study summaries and 20 full-text articles examining the predisposing factors of PFPS reported that static knee valgus was not associated with PFPS[27]. Despite the results of some studies showing a low association of genu valgum

in PFP, it is still considered to be a risk factor in clinical practice as the expectation of the clinicians is in the way that genu valgum may cause lateralization of the patella[28].

Likewise, the study of White et al.[29], which revealed an increased hamstring tightness in the PFP group, in our study, a significant increase in terms of hamstring tightness was apparent in the PFP group compared to control group. Consistent with our study results, a systematic review reported that hamstring tightness might be the contributing factor of PFP since it may also cause a decrease in knee flexion to result in increased quadriceps forces[30]. Therefore, hamstring tightness may induce PFP by overloading the patella-femoral joint.

Limitations of the study

In our study, we used the score of Kujala to determine the PFP, feiss line to measure the navicular drop. However, more objective assessments, such as MR images of patellofemoral joint or kinematic analysis, could be used to increase the reliability.

CONCLUSION

The prevalence of PFP in students of a private university was found % 10,5 (n=42). The results from this study showed that students with PFP have higher severity degrees of pes planus, navicular drop, subtalar angle, tibial torsion, and hamstring tightness than nonpainful students. However, the effects of Q angle and genu valgum angle on patients with PFP were controversial in our study. The foot type of patients with the PFP should be examined. However, future work should be conducted to determine the correlation between PFP and PP.

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Author contributions: Conceptualization: [BK, AA]; Design: [BK, AA]; Supervision [AA]; Materials [BK, AA]; Data collection and processing [BK]; Analysis and Interpretation [BK, AY, DA]; Literature Review [BK, AA, GKA, ED]; Writing [BK, AA, DA, AY, GKA, ED], Clinical review [AA]

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ORIGINAL RESEARCH

Attitudes of Infertile Women towards Complementary and Alternative Medicine Methods

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Abstract

Objective: To determine the complementary and alternative medicine practices used by infertile women and to evaluate their attitudes towards these practices.

Material-Method: This descriptive and correlational study was carried out between February-May 2022 with 94 infertile women reached by snowball sampling on the online platform. In the collection of data, the "Questionnaire on Complementary and Alternative Medicine Methods" and the Complementary, Alternative and Conventional Medicine Attitude Scale has been used.

Results: The mean age of the women participating in the study was 34.48±7.66 years. It was determined that the participants got an average of 109.69±18.81 points from the Complementary, Alternative and Conventional Medicine Attitude Scale. After the diagnosis of infertility, 60.4% of women used any complementary and alternative medicine methods, and among these methods, onion cure (30.2%) as a nutritional supplement, applying honey directly into the vagina (46.4%), going to spas (50.0%), and praying and worshiping (74.7%) were preferred.

Conclusion: It was determined that majority of the infertile women use any complementary and alternative medicine method, and their attitudes towards traditional and complementary medicine are moderate level. It is recommended to carry out experimental and evidence-based studies with larger samples in order to determine the benefit/harm status of the applications.

Keywords: Infertility, Complementary and Alternative Medicine, Traditional Method

INTRODUCTION

Infertility is defined as the inability to conceive or maintain a pregnancy despite having regular unprotected sexual intercourse for a year[1]. Infertility is known as a public health problem affecting many women in the world, and it is estimated that 8-12% of couples are affected by infertility all over the world[2]. The prevalence of infertility in Turkey varies between 10-15%[3], and one out of every six married women has infertility problems[4]. While infertility may occur due to female-related, male-related, and both female and male-related causes, it can also occur for "unexplained" reasons that are not accompanied by any pathological findings. The most common causes in women are ovulation disorders, anovulation, tubal factors, peritoneal factors, uterine factors and cervical factors. In men, testicular problems that affect the production or function of sperm are sperm function problems and sperm conduction problems[5,6]. In addition to these problems, the age of the couple, sexual intercourse habits, previous pelvic operations, alcohol, smoking and substance use, sexually transmitted diseases, drugs and chemical expose, stress are risk factors for infertility[5].

In infertile couples, treatment is planned specific to the underlying problem. With the general evaluation of the couple and the determination of the cause, medical treatment and surgical treatment and assisted reproductive techniques (ART) are used and a long treatment process awaits the couples[5]. In this long treatment process, the couple, who think that they cannot fulfill the duty of having a child and being a companion-mother, which is of great importance to the society, is negatively affected psychosocially. In this process, couples may experience emotional problems, lack of control and self-esteem, marital problems, anxiety, stress,

and depression[7-10].

ART are applications that require high cost and time. The high rate of treatment failure and the increased cost and stress resulting from repeated IVF cycles lead couples to alternative treatments[11]. Traditional and alternative complementary therapies (CAM) are practices based on traditions, beliefs, and experiences in order to maintain the continuity of health in addition to diagnosis and treatment[12]. It is known that CAM applications have been used in infertility for about thirty years, but studies with high evidence value are limited[11,13]. Patients aiming to increase the likelihood of conception tend to use complementary and supportive care interventions that are safer, more effective, and affordable[14]. In Turkey, the rate of CAM use of infertile couples was determined as %51-82[11,15-17], while this rate changed between %41-91 in other countries[18-20]. Although the CAM methods used vary from country to country according to culture, geography, and traditions, it is known that acupuncture, massage, nutritional supplements, herbal methods, mind and body applications (hypnosis, yoga, meditation), homeopathy and psychotherapy applications are mostly used in infertility[21].

This study was aimed to determine the complementary and alternative medicine practices used by infertile women and to evaluate their attitudes towards these methods.

MATERIALS AND METHODS

Type of the study

This study was conducted with a descriptive and correlational type.

Study sample

The study population consisted of infertile women who could be accessed online during the study period, while the sample consisted of 94 infertile women who could be accessed online with the snowball sampling method and met the inclusion criteria of the study.

Inclusion criteria

- Being a woman diagnosed with infertility
- Receiving fertility treatment or preparing for the treatment process (all the treatment types included such as oral medication, injections, intrauterine insemination, oocyte pick-up, or embryo transfer)
- Being able to read and understand Turkish

Exclusion criteria

- Being pregnant after IVF treatment
- Not participating in the study voluntarily.

Data collection

This study was conducted online between February-May 2022. Online survey links were sent to the participants, and it took approximately 15 minutes for each participant to fill out the data collection forms.

Data collection tools

Introductory Information Form, Questionnaire on Complementary and Alternative Medicine (CAM) Methods and Complementary, Alternative and Conventional Medicine Attitude Scale (CACMAS) were used as data collection tools.

Introductory information form

It was created by researchers and consists of 13 questions questioning sociodemographic characteristics and infertility history.

Questionnaire on complementary and alternative medicine (CAM) methods

It was created by researchers in line with the literature[11] and consists of 13 questions regarding the applied CAM methods.

Complementary, alternative and conventional medicine attitude scale

This scale is a 27-question scale consisting of three subscales. 22 of the scale items consist of positive statements and 5 of them are negative statements. Scoring of the scale is made in a 7-point Likert scale ranging from 1-7 from “strongly disagree” to “strongly agree”. The scale does not have a cutoff value, but as the score obtained from the scale increases, it is interpreted as a positive attitude towards traditional and complementary medicine[22]. In the validity and reliability study of the scale, the Cronbach's alpha coefficient was found to be 0.808. In this study, the Cronbach's alpha coefficient of the scale was found to be 0.751.

Ethical approval

Ethical approval was obtained for the research from the Human Research Ethics Committee of Istinye University (Protocol no: 22-05)

Statistical analysis

The online data of the study were transferred to the SPSS 26.0 program, and descriptive analysis including numbers, percentages, means, and standard deviation values has been done. The Kolmogorov-Smirnov test was used to evaluate the normal distribution. Since the data does not distribute normal, nonparametric tests were used such as Mann-Whitney U, Kruskal Wallis, and Spearman correlation for intergroup comparisons, and Bonferroni correction was used for further analysis and considering a significance level alpha as $p<0.05$.

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RESULTS

The mean scores of the participants from the CACMAS are given in Table 1 and total scale mean score was found 109.69±18.81 points (min:71; max:160) .

Table 1. CACMAS Scores

Features	Min-max	Mean± S.D
Philosophical congruence with complementary and alternative medicine subscale	10-56	31.81±10.91
Dissatisfaction with conventional medicine subscale	10-56	26.05±11.33
Holistic balance subscale	15-63	51.83±9.29
CACMAS Total Score	71-160	109.69±18.81

SD: standard deviation

The age of 94 infertile women participating in this study was 34.48±7.66 years, and the findings regarding the socio-demographic characteristics of the participants are given in Table 2.

Table 2. Characteristics of the Participants and Comparison with CACMAS Score

Features		Min-max	Mean± S.D	Test/p
Age		22-60	34.48±7.66	r_s: 0.043 p: 0.67
Length of marriage (year)		1-35	10.50±7.98	r_s: 0.163 p: 0.11
		n	**%**	**Test/p**
Education level	Primary or lower level	42	43.8	KW: 0.677 p: 0.71
	High school	33	34.4	
	University and above	21	21.9	
Income level	Less than expenses	24	25.0	KW: 0.186 p: 0.91
	Equal to expenses	59	61.5	
	More than expenses	13	13.5	
Working status	Yes	33	34.4	Z: -0.660 p: 0.50
	No	63	65.6	
Have you been pregnant before?	Yes	34	35.4	Z: -0.671 p: 0.50
	No	62	64.6	
Do you have any living children?	Yes	30	31.3	Z: -0.075 p: 0.94
	No	66	68.7	
Cause of infertility	Female	35	36.5	KW: 2.129 p: 0.54
	Male	16	16.7	
	Both female and male	10	10.4	
	Unexplained	35	36.5	
How long have you been receiving fertility treatment?	Less than 1 year	29	30.2	KW: 13.497 **p: 0.00***
	1-3 year	26	27.1	
	3-5 year	14	14.6	
	More than 5 year	27	28.1	
At what stage are you currently in treatment?	Ovulation follow up	18	18.8	KW: 6.306 p: 0.17
	IUI	13	13.5	
	OPU	8	8.3	
	ET	11	11.5	
	Not started to medical treatment / waiting for IVF list	46	47.9	
If treatment has been started, what type of treatment are you receiving?	Oral medication	22	22.9	KW: 0.170 p: 0.91
	Injections	38	39.6	
	Not started	36	37.5	

Z: Mann-Whitney U test; KW: Kruskal Wallis test, *: significance level as p<0.05 S.D: standard deviation

When the use of CAM methods by women is examined; the rate of participants using any CAM method after the diagnosis of infertility was determined as 60.4%, and it was determined that 53.1% of the participants using CAM methods learned about this method from family members and friends. It was determined that 62.5% of the participants used nutritional supplements as a CAM method, and onion cure (30.2%) was the most used nutritional supplement. The rate of participants who applied directly into the vagina was 20.8%, and it was determined that the most (46.4%) application was rubbing honey to the vagina. In this study, 50% of the participants had been to people with religious qualifications. The most common recommendations of these people were to make a vow and visit the shrine (25.5%), to wear amulets (23.4%), to drink prayed water or to eat food (23.4%). The rate of participants who made hot application as a CAM method was 40.6%, and it was determined that these participants mostly performed the practice of going to spas (50%). Among the other methods, it was determined that the method of praying and worshiping (74.7%) was preferred most frequently. While 70.8% of the participants thought that CAM methods were not effective, 16.7% stated that they would recommend CAM methods to others (Table-3).

Table 3. Findings on Traditional, Complementary and Alternative Medicine

Features		**n**	**%**
Have you used any complementary and alternative medicine (CAM) methods after being diagnosed with infertility?	Yes	58	60.4
	No	38	39.6
If you have used a TAT method, from whom did you hear about it? *	Social media/internet	49	51.0
	Family and friends	51	53.1
	Doctor/Nurse/Midwives	22	22.9
	I created myself	11	11.5
Have you used nutritional supplements as a CAM method?	Yes	60	62.5
	No	36	37.5
If so, which ones did you use?	Virgin Mary grass	11	11.5
	Onion cure	29	30.2
	Walnut cure	13	13.5
	Stinging nettle	8	8.3
	Other	5	5.2
Have you applied directly into the vagina?	Yes	20	20.8
	No	76	79.2
If you have made an application, what are these?	Placing a trout in the vagina	3	3.1
	Rubbing sheep tail oil	3	3.1
	Rubbing honey	13	46.4
	Placing wild daffodils and pansies	3	10.7
	Other	6	21.4
Have you been to people you think have religious qualifications? (Imam, saint, hodja, etc.)	Yes	48	50.0
	No	48	50.0
If so, what practices did these people recommend?	Making / putting on amulets	11	23.4
	Make a vow	12	25.5
	Prayed water/food meal	11	23.4
	Visiting the shrine	12	25.5
	Other	1	2.1
Did you apply hot?	Yes	39	40.6
	No	57	59.4
Which of the hot applications have you done?	Go to hot springs	19	50.0
	Getting into the hot sand	6	15.8
	Sitting on hot ash or brick	9	23.7
	Other	3	7.9
	Nothing	1	2.6
Which of these methods did you use?	Massage	9	10.3
	Exercise	9	10.3
	Acupuncture	3	3.4
	Praying/Worship	65	74.7
	Other	1	1.1
If you have used CAM methods, do you think it works for your fertility treatment?	Yes	28	29.2
	No	68	70.8
Would you recommend CAM methods during fertility treatment?	Yes	16	16.7
	Partially	55	57.3
	No	25	26.0

DISCUSSION

In this study, which was conducted to determine the CAM applications used by infertile women and to evaluate their attitudes towards these applications, it was determined that 60.4% of the participants used any CAM method after the diagnosis of infertility, and their attitudes towards traditional and complementary medicine methods were moderate level. However, it was found that the characteristics of the participants did not significantly affect the CACMAS Scores, except for the duration of treatment. In the literature, the rate of women undergoing fertility treatment to use CAM methods varies between 29-82%[14,15,23-25] and usage rates vary according to culture, geography and sociodemographic characteristics. Most of the infertile individuals state that they see CAM applications as a hope for them in order to do their best to have a child[6]. In addition, the fact that infertility treatment is a costly and difficult process may cause couples to resort to inexpensive, easily accessible, and easily applied CAM methods[26]. The fact that most of the participants used a CAM method in this study can be associated with this situation and it can be said that as the duration of treatment increases, women turn to different alternatives to cope with this process, and they look at CAM positively with the hope of doing everything to have a child and achieving a successful pregnancy.

In this study, it was determined that 62.5% of the participants used nutritional supplements as a CAM method, and onion cure (30.2%) was the most used nutritional supplement. When we look at the studies in the literature, it is seen that infertile women frequently use honey and onions from nutritional supplements, similar to this study[6,11].

In this study, it was determined that half of the participants applied to people with religious qualifications, and the majority of them did practices such as pray and worship. It is known that praying, especially in case of illness, increases well-being and calmness, reduces anxiety and increases well-being. For this reason, people can resort to methods such as worship and prayer through their relatives and people with religious qualifications[27].

Although most of the participants were using any CAM application, it was determined that most of them thought that these methods were not effective. Women prefer these methods because they believe that they contribute to the usefulness of the treatment, they do the application themselves, they have autonomy in their own treatment, and they provide psychological relief[23]. Therefore, as in this study, it can be thought that women apply these methods not only because they are effective in treatment, but also because of their positive effects on well-being.

CONCLUSION

Most of the infertile women use any CAM method and it has been determined that the attitudes of women towards traditional and complementary practices are moderate level. The most preferred CAM methods were determined as onion cure, applying honey to the vagina, praying/worshiping, and going to the spa. It is essential to determine the benefits and harms of CAM methods used in infertility. In this context, it can be recommended to conduct experimental studies with larger samples and examining CAM methods on evidence-based basis.

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ORIGINAL RESEARCH

Impact of *Capsella bursa-pastoris* (Shepherd's Purse) Herbal Tea Preparations on Symptoms and Severity of Hemorrhoidal Disease: A Prospective Randomized Study

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Abstract

Objectives: Medicinal herbs have been used to treat hemorrhoidal disease for centuries. Given its anti-oxidant and anti-inflammatory mechanisms, *Capsella bursa-pastoris* (Shepherd's purse) may potentially lead to regression of the hemorrhoidal plexuses. In this context, the objective of this study is to investigate the effect of *Capsella bursa-pastoris*, an affordable and easy-to-use herbal medicine, on the severity and physical examination findings of hemorrhoidal disease in patients, who receive conventional treatment for hemorrhoids.

Material-Method: This study was designed as a prospective, randomized study. The population of the study comprised symptomatic patients who presented with second and third-degree hemorrhoids to the Internal Medicine and General Surgery departments of Istanbul Medipol University Hospital, İstanbul, Turkey, between October 2021 and February 2022. The patients were randomized into two groups: the control group, that is, the group of patients, who were to receive conservative treatment only for three months, and the study group, that is, the group of patients who were to receive conservative treatment and use herbal tea preparations of *Capsella bursa-pastoris* for three months. Demographic (age and gender) and baseline and 3-month clinical characteristics were recorded. The study's primary outcome was the changes observed in the symptoms and physical examination findings of hemorrhoidal disease in the study group relative to those in the control group.

Results: The study sample consisted of a total of 218 patients, who were randomized into the study and control groups, with 109 patients in each group. Significantly older patients were seen in Group CT (39.5 ± 14.6 and 35.5 ± 11.7 years, p=0.030). There was no significant difference between the groups in terms of other characteristics (p>0.05). At the 3rd-month follow-up examination, it was determined that there were significantly fewer patients with bleeding and difficulty during defecation in the study group compared to the control group (p=0.001 for both cases). Patients in the study group had significantly lower VAS scores for pain and the number of affected quadrants compared to those in the control group (p<0.001 for both cases). The proportion of patients with Grade II and III hemorrhoidal disease was significantly lower in the study group.

Conclusion: The findings of this study, which revealed significant improvements in the symptoms and physical examination findings of hemorrhoidal disease, suggest that the use of *Capsella bursa-pastoris* herbal tea may benefit patients with symptomatic hemorrhoids.

Keywords: Hemorrhoids, Phytotherapy, Medicinal Herbs, *Capsella bursa-pastoris*

INTRODUCTION

Herbal products have enjoyed widespread medicinal use globally throughout history[6,13]. Turkey's noted use of plants for medicinal purposes owes much to the relatively rich diversity of its flora, and its extensive history and culture of such usage[4]. Although there are different indications for each medicinal plant in different regions of the World, documentation of the medicinal utility of the less known plants has gained importance for several decades[4,6]. Different herbal plants have been investigated for their potentially beneficial effects in patients with hemorrhoidal diseases[2,10,11,19,24]. It has been suggested that plants with anti-inflammatory, analgesic and venotonic properties may prevent and cure hemorrhoidal diseases[19].

Capsella bursa-pastoris (Shepherd's purse), a member of the Cruciferae family, is an edible plant. Its leaves and roots can be eaten both raw and cooked.[3] The tea formulations made from the whole plant or the dried herb have been used for different purposes.[2] In addition to its hemostatic and oxytocic properties, *Capsella bursa-pastoris* is also known

for its anti-oxidant, anti-inflammatory, anti-ulcer, and in-vivo anti-cancer activities.[2,3,6,21,24,27] As a result, its use has been investigated in several experimental and clinical treatments, including for hemorrhoids,[3] hepatic steatosis and hypercholesterolemia,[4] urinary tract infections,[13] cataracts,[26] nose bleeding,[4] and heavy menstrual bleeding.[6,20]

Capsella bursa-pastoris has been used as a traditional medicine to treat hemorrhoidal disease in Anatolia and the Middle East.[2,3,25] Furthermore; it is also used as a foodstuff in many regions of Turkey.[15] Apaydin Yildirim et al.[3] reported the anti-oxidant activity of *Capsella bursa-pastoris* in an experimental rat model of hemorrhoids. However, there is no clinical study to date, in which the use of *Capsella bursa-pastoris* was evaluated in patients with hemorrhoids.

In view of the foregoing, the objective of this study is to evaluate the effect of *Capsella bursa-pastoris*, an affordable and easy-to-use herbal medicine, on the severity and physical examination findings of hemorrhoidal disease in patients, who receive conventional treatment for hemorrhoids.

MATERIALS AND METHODS

Research design

This study was designed as a prospective, randomized study. The study protocol was approved by the local ethics committee at Istanbul Medipol University (15.11.2021, E-10840098-772.02-5855). The study was carried out in accordance with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from each patient included in the study.

Study population

Patient older than 18 years of age, who presented with symptomatic second and third degree hemorrhoids to the Internal Medicine and General Surgery departments of Istanbul Medipol University Hospital, Istanbul in Turkey between October 2021 and February 2022 comprised the study population. The diagnosis of hemorrhoids was made based on a detailed history of symptoms and rectal and anoscopic examinations. The disease severity was determined according to the Goligher scale.[7] Patients with 1st and 4th-degree hemorrhoids, perianal pathologies, a previous interventional or surgical hemorrhoid treatment history, coagulation disorders, and those who were pregnant,, or lactating, or using anticoagulants, were excluded from the study. The patients were informed that they were free to withdraw from the study at any time.

Study sample

A pilot study was carried out to determine the sample size. Accordingly, 20 hemorrhoid patients were divided into two groups: patients treated with conservative medical treatment only and those treated with conservative medical treatment plus the herbal tea, *Capsella bursa-pastoris*. The type I error (α value) was 0.05, and the power of the study (1-β) was 80%. The comparative analysis of the VAS scores for pain revealed a 1.4±1.5 point decrease in the VAS scores in the group treated with conservative medical treatment plus *Capsella bursa-pastoris* compared to the score in the group treated with conservative medical treatment alone. Accordingly, it was determined that at least 19 patients must be included in each group. The sample size was increased by 10% to allow for possible dropouts. As a result, it was determined that the sample should include a minimum of 42 patients, with 21 patients in each group. The sample size calculation was conducted using the MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

Interventions

All patients included in the study underwent a complete physical examination and symptom assessment. Patients were divided into study and control groups via sequential randomization. Patients in the control group were administered the conservative treatment only, whereas the patients in the study group were administered the conservative treatment and herbal tea preparations of *Capsella bursa-pastoris* (Temmuz Organic Company, Konya, Turkey). Both the conservative treatment and the treatment using herbal tea preparations were continued for three months. The conservative treatment included diosmin plus hesperidin (Daflon®, diosmin 450 mg, hesperidin 50mg, tablet, twice a day, Servier Turkey, Istanbul, Turkey) and trimebutine plus ruscogenin (Recbutin®, trimebutine base 5.8%, ruscogenin 0.5%, rectal cream, twice a day, Abdi Ibrahim, Istanbul, Turkey). The patients in the study group were instructed to drink one cup (200 ml) of *Capsella bursa-pastoris* tea one hour before sleep. A total of 90 tea bags were provided to each patient in the study group, and their consumption was checked at one-month intervals.

Data collection

Patients' demographic (age and gender) and clinical characteristics (difficulty and bleeding during defecation, visual analogue scale (VAS) score for pain, severity of the hemorrhoidal disease, and the number of affected quadrants of hemorrhoids) were collected during the face-to-face interviews conducted at the beginning of the study. The patients rated the degree of pain they were feeling in relation to the hemorrhoidal disease using a visual analogue scale (VAS) between zero (no pain) and ten (most severe pain). The investigator who assessed the VAS scores of the patients was blinded to the groups. The severity of the hemorrhoidal disease was graded via a rectal examination with anoscopy as grade 2 or 3.[7] Based on the quadratic distribution, the number of the quadrants of the hemorrhoidal plexuses was also recorded. Hemorrhoids present in all four quadrants were defined as circumferential hemorrhoids.[22]

At the 3rd-month follow-up examination, the patients were asked about the difficulty and bleeding they had during defecation and to re-rate the degree of pain they were feeling in relation to the hemorrhoidal disease using VAS. The severity of patients' hemorrhoidal disease was re-graded, and the number of the affected quadrants was recorded once more. Any adverse clinical events that might be caused by the consumption of *Capsella bursa-pastoris* tea preparations were queried in detail.

Statistical analysis

The study's primary outcome was the changes observed in the symptoms and physical examination findings of hemorrhoidal disease in the study group relative to those in the control group. For this reason, statistical analyses of the clinical variables associated with the hemorrhoidal disease and its outcomes in line with the different treatment modalities administered were conducted both within and between the groups.

Descriptive statistics were expressed as mean ± standard deviation values in the case of continuous variables that were determined to conform to the normal distribution and as median values along with minimum-maximum values in the case of continuous variables that were determined not to conform to the normal distribution. Categorical variables were expressed as numbers and percentages. The Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests were used to determine whether the numerical variables conformed to the normal distribution.

The independent samples t-test was used to compare the two independent groups, i.e., the study and the control groups, where numerical variables, including age, conformed to the normal distribution. On the other hand, the Mann-Whitney U test was used to compare two independent groups, i.e., the study and the control groups, where numerical variables including VAS scores and the number of the affected quadrants, did not conform to the normal distribution.

The Pearson's chi-squared test and the Fisher-Freeman Halton test were used to compare the differences between categorical variables (gender, severity of the hemorrhoidal disease, bleeding, and difficulty during defecation).

The changes in bleeding and difficulty the patients had during defecation over time were analyzed separately for each group using McNemar's test.

"Jamovi project (*Jamovi*, version 2.3, 2022, retrieved from https://www.jamovi.org) and JASP software package (Jeffreys's Amazing Statistics Program, version 0.16.1, retrieved from https://jasp-stats.org) were used in the statistical analysis. Probability (p) values of ≤ 0.5 were deemed to indicate statistical significance.

RESULTS

The study sample consisted of 218 patients, who were randomized into the study and control groups, with 109 patients in each group. The patients in Group CT were significantly older than those in Group Caps (39.5 ± 14.6 and 35.5 ± 11.7 years, p=0.030). Female patients comprised the majority in both groups (91.7% in the study group and 89.95% in the control group). There was no significant difference between the groups in terms of gender, bleeding and difficulty during defecation, VAS score for pain, the disease severity, and the number of affected quadrants (p>0.05) (Table 1). The changes observed in the symptoms and physical examination findings of hemorrhoidal disease in the study group relative to those in the control group are given in Table 2.

Accordingly, there were significantly fewer patients with bleeding and difficulty during defecation in the study group compared to the control group at the end of the study period (p=0.001 for both cases). Patients in the study group had significantly lower VAS scores for pain compared to those in the control group and the number of affected quadrants was also comparatively lower (p<0.001 for both cases) (Figure 1). The proportion of patients with Grade I hemorrhoidal disease was significantly higher in the study group compared to that in the control group (71.6% vs.

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20.2%). In parallel, the proportion of patients with Grade II and III hemorrhoidal disease was significantly lower in the study group than in the control group (Table 2).

Table 1.Baseline demographic and clinical characteristics of the groups.

	Group CT[a] (n=109)	Group Caps[b] (n=109)	p-values
Age (year) †	39.5 ± 14.6	35.5 ± 11.7	**0.030***
Sex ‡			
Male	11 (10.1)	9 (8.3)	0.814
Female	98 (89.9)	100 (91.7)	
Bleeding during defecation ‡	75 (68.8)	75 (68.8)	0.999
Difficulty during defecation ‡	80 (73.4)	80 (73.4)	0.999
VAS score for pain §	6.0 [1.0- 9.0]	6.0 [1.0- 9.0]	0.999
Number of affected quadrants §	2.0 [1.0- 4.0]	2.0 [1.0- 4.0]	0.873
Grades for hemorrhoids ‡			
Grade II	52 (47.7)	52 (47.7)	0.999
Grade III	57 (52.3)	57 (52.3)	

a: conservative treatment group, b: herbal tea group, †: mean ± standard deviation, ‡: n (%), §: median [min-max], VAS: Visual analog scale

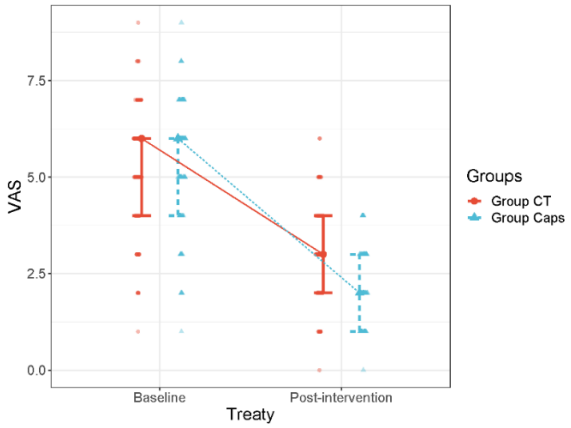

Figure 1. Box violin plot graphs for post-interventional VAS scores for pain

Table 2. Inter- and intra-group analyses of the changes in the clinical symptoms and physical examination findings between the study groups.

	Group CT (n=109)	Group Caps (n=109)	p-values
Bleeding during defecation ‡			
Baseline	75 (68.8)	75 (68.8)	0.999
Post-intervention	59 (54.1)	34 (31.2)	**0.001**
p-value	**<0.001**	**<0.001**	
Difficulty during defecation ‡			
Baseline	80 (73.4)	80 (73.4)	0.999
Post-intervention	79 (72.5)	54 (49.5)	**0.001**
p-value	0.999	**0.005**	
VAS[c] score for pain §			
Baseline	6.0 [1.0- 9.0]	6.0 [1.0- 9.0]	0.999
Post-intervention	3.0 [0.0- 6.0]	2.0 [0.0- 4.0]	**<0.001**
p-value	**<0.001**	**<0.001**	
Number of affected quadrants §			
Baseline	2.0 [1.0- 4.0]	2.0 [1.0- 4.0]	0.873
Post-intervention	2.0 [0.0- 3.0]	1.0 [0.0- 3.0]	**<0.001**
p-value	**<0.001**	**<0.001**	
Grades for hemorrhoids ‡			
Grade I	22 (20.2)	78 (71.6)	**<0.001**
Grade II	66 (60.6)	29 (26.6)	
Grade III	21 (19.3)	2 (1.8)	

a: conservative treatment group, b: herbal tea group, c: Visual analog scale, ‡: n (%), §: median [min-max]

Additionally, the comparison of groups' baseline and post-intervention clinical features revealed that there were significant improvements in the hemorrhoidal disease-related clinical symptoms and physical examination findings of the patients in both groups and that these improvements were significantly more prominent in the study group (Table 2). Accordingly, in the control group, there was a significant post-interventional decrease in the number of patients with bleeding during defecation ($p<0.001$), but not in the number of patients with difficulty during defecation ($p=0.999$), whereas in the study group, there was a significant post-interventional decrease in both the number of patients with bleeding during defecation ($p<0.001$) and the number of patients with difficulty during defecation ($p=0.005$). In addition, there were significant post-interventional decreases in the median VAS score for pain and the number of the affected quadrants in both groups.

DISCUSSION

The findings of this prospective randomized study revealed that the combination of *Capsella bursa-pastoris* herbal tea preparations with the standard conservative treatment significantly affected the hemorrhoidal disease-related symptoms and the physical examination findings in a positive direction when compared with stand-alone standard conservative treatment.

The positive effects of *Capsella bursa-pastoris* on the hemorrhoidal disease-related symptoms and the physical examination findings have been noted in the literature and attributed to its anti-oxidant and anti-inflammatory properties.[12] Additionally, it was reported that *Capsella bursa-pastoris* plays a role in the biosynthesis of several active substances, including tannins, sulforaphanes, tyramines, sterols, flavonoids, choline, acetylcholine, and histamine.[3,20,25] Apaydin Yildirim et al.[3] demonstrated the anti-hemorrhoidal effects of ethanol and water extracts of *Capsella bursa-pastoris* on croton oil-induced hemorrhoids in rats. Accordingly, *Capsella bursa-pastoris* reduced the severity of hemorrhagic necrotic enteritis and the levels of cytokines and lipid peroxidation in serum, as well as myeloperoxidase and anti-oxidants in the recto-anal tissues. Naafe et al.[20] and Ghalandari et al.[6] reported the positive effects of hydroalcoholic extracts of *Capsella bursa-pastoris* on heavy menstrual bleeding and early post-partum hemorrhage creating contractions in the uterine smooth muscle cells and hormonal effects. Although the underlying physiological action mechanisms of *Capsella bursa-pastoris* in creating the positive effects noted, were not considered in previous studies or in this study for that matter, it would not be unreasonable to speculate that the anti-inflammatory properties of *Capsella bursa-pastoris* were primarily responsible.

Gulec et al.[8] investigated the anion and cation contents of the herbal products in Turkish folk remedies used for hemorrhoidal diseases. Sargin et al.[1] reviewed the ethnobotanical survey of medicinal plants in a region in Turkey. In two reviews from Iran, Hashempur et al. (6) and Memariani et al. (21) did not mention *Capsella bursa-pastoris* among 105 medicinal plants belonging to 51 families that were used to treat hemorrhoidal symptoms. However, *Capsella bursa-pastoris* has not been mentioned in any of these studies for its effects on hemorrhoidal diseases. This may be simply due to the lack of clinical studies on the usage of *Capsella bursa-pastoris* in patients with hemorrhoids.[1,8] Hence, one of the objectives of this study is to fill this gap in the literature.

There are several phytotherapeutic substances with various application routes used in the treatment of hemorrhoids. Malekuti et al.[16] reported the effect of Myrtus communis anti-hemorrhoidal ointments on the hemorrhoidal symptoms of post-partum women. In another study conducted in Iran, the authors investigated the use of Allium ampeloprasum subspecies Iranicum (Leek) extract cream in patients with symptomatic hemorrhoids.[19] Such herbs have active substances such as tannins, saponins, flavonoids, phenocolic acids, and alcohol.[7,9,10,16,19] It was reported in Hashempur's review that more than half of the herbs with anti-hemorrhoidal usage in Iran exhibited anti-inflammatory and analgesic effects.[11] Several mechanisms have been put forward to explain the anti-hemorrhoidal properties of these active substances including improvement of the venous tone, increases in lymphatic drainage, protection of capillary bed microcirculation, inhibition of inflammatory reactions and decreases in capillary permeability.[9] Additionally, it has been reported that flavonoid-containing herbs were more potent in controlling hemorrhoidal symptoms, given their edema protective mechanisms.[9] Taken together with the results reported in the literature, the findings of this study suggest that a herbal product's active anti-hemorrhoidal ingredients are more

important than the type and application route of the herbal products in inducing the clinical improvements in the symptoms of hemorrhoidal disease.

The medicinal herbs used in the treatment of hemorrhoids are administered in different forms, including oral capsules or creams/ointments.[9,10,14,17,19,23] Herbal tea represents one of the most common forms of use of medicinal herbs. *Capsella bursa-pastoris* was used in both capsule and drop form in previous human studies.[6,20] Another study investigated the effect of vagitories, which contained shepherd's purse, on vaginitis.[5]

Limitations

Apart from its strengths, such as being the first study to demonstrate the anti-hemorrhoidal activity of *Capsella bursa-pastoris* in humans and to analyze the herbal tea formulations of *Capsella bursa-pastoris* on hemorrhoidal symptoms, there were also some limitations to this study. First, it was designed as a single-center study. Secondly, patients' adherence to herbal tea consumption was not measured in detail. Lastly, the groups differed significantly in age, which might have produced a bias in the outcomes.

CONCLUSION

The findings of this study, which revealed significant improvements in the symptoms and physical examination findings of hemorrhoidal disease, suggest that Capsella-bursa pastoris herbal tea products may be recommended to patients with symptomatic hemorrhoids.

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Authors sontributions: The idea, design, data collection and statistical evaluation: [TM]; Drafting of the article, evaluation and interpretation of the findings, and review of the article for intellectual content: [EY].

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ORIGINAL RESEARCH

The Effects of Kinesiotape on Injury Risk in Young Tennis Players: A Randomized Trial

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Abstract

Objective: Injury prevention is very important factor affecting success in tennis and Kinesio Taping is a newly rising method for decreasing injury risk in athletes. This study was carried out to determine whether Kinesio Taping reduces the risk of injury to tennis players and, if it decreases, which Kinesio Tape material should be used.

Material-Method: Thirty-three young tennis players were included in our study. Assessment was done bare (no tape), after 45 min of Performance Plus Kinesio Taping application and after 45 min of Gold Text Finger Print Kinesio Taping application. Thermal analysis was done for risk of injury including both lower extremities. Quadriceps muscle was chosen from upper leg and gastro soleus muscle was chosen from lower leg region for thermal analysis.

Results: Statistically significant differences were found at lower leg and upper leg assessments for both dominant and non-dominant sides in skin temperatures between bare assessment (no tape), Performance Plus Kinesio Taping application and Gold Text Finger Print Kinesio Taping application ($p<0.05$) in triple comparison. When comparing Performance Plus Kinesio Taping application and gold text Finger Print Kinesio Taping application, there were no statistically significant differences in skin temperature ($p>0.05$).

Conclusion: The results show that Kinesio Taping may be an effective application for decreasing risk of injury but there is no difference between the types of Kinesio Taping material used, as long as it is applied with the same technique.

Keywords: Sports, Athletic Injuries, Thermography, Kinesiotape, Pragmatic Study

INTRODUCTION

Injury prevention is very important factor affecting success in tennis. According to The National Collegiate Athletic Association, tennis has the incidence of injury as much as contact sports[1]. Also, tennis practice involves repetitive stresses that make players more vulnerable and lead to musculoskeletal injuries. Data about the frequency of injury in tennis sports were also reported in several studies. The incidence of acute musculoskeletal injuries at lawn tennis varies from 0.04 to 3.0 injuries/1.000 playing hours. According to the data obtained, the injury prevalence in tennis players is not low and many of the injuries are joint injuries[2–4].

In the researchers conducted to prevent injuries, some methods have been mentioned to take precautions against injuries. Functional Movement Screen, online monitoring systems, infrared thermography are some of the methods for the detection of injury risk in sports practice[5–7]. The use of infrared thermography in preventive rehabilitation is an effective, safe, and relatively cost-effective for detecting changes in the skin surface temperature[8].

Kinesio Taping is a newly rising method for decreasing injury risk in athletes. Kinesio Tape with the elastic, acrylic adhesive structure differs from regular white athletic tape because of the wavelike design on the adhesive surface. The structure and elasticity of the tape provides a tensile force to the skin and is purported to lift the fascia and soft tissue, thus increasing interstitial space. When the skin is

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lifted by this technique, the flow of blood and lymphatic fluid beneath the skin improves and several therapeutic benefits occurs[9]. The effects of Kinesio Taping treatments on injury risk prevention are due to the therapeutic benefits outlined above, as well as influencing range of motion, proprioception, and strength[10]. Yet no studies have been conducted on the effects of Kinesio Taping application on injury risk prevention by thermal injury risk analysis.

As time changes, Kinesio Taping applications and materials used change due to technological developments. Today, 4 types of different Kinesio Tape materials are presented: Kinesio Tex Classic, Kinesio Tex Performance Plus, Kinesio Tex Gold Fingerprint and Kinesio Tex Gold Light Touch Plus. The differences between these tapes are presented as nano technological differences[11]. Classic and Performance Plus tapes are said to be effective in muscle correction techniques. For the usage in field, no studies have been found to present the difference between these two tapes and which one to use would affect performance better.

Therefore, current study had two aims; to determine the effectiveness of Kinesio Taping application on decreasing injury risk for young tennis players and to determine which Kinesio Taping material is more effective.

MATERIALS AND METHODS

Design

Randomized, double-blind trial. Clinical trial number of this study is NCT04059575. The study protocol was approved and required permissions were taken from related tennis club officials, and a written consent was obtained from all the participants' parents about the study. Evaluations were made in the centers of the tennis clubs to which the participants were affiliated.

Thirty-three young tennis players aged between 9 and 12 were included in the study (age 11.16±1.56 years; BMI 18.49±2.57 kg/m^2). Assessments were done bare (no tape), after 45 min of performance plus Kinesio taping application (PP application) and after 45 min of gold text fingerprint Kinesio taping application (GT application). Players were randomly taped with either PP or GT taping applications using an online random allocation software program (GraphPad Software QuickCalcs, GraphPad Software Inc., La Jolla, CA, USA). Thirty minutes of rest was given between 2 taping applications after the removal of the first tape. One participant was not able to complete the second taping procedure. The flowchart of the study is shown at Figure 1. PP and GT taping applications were done by using Kinesio taping muscle facilitation techniques to Quadriceps and Gastrosoleus muscles. I tape with a tension of 10-35% was used for muscle facilitation (Figure 2-3)[12]. All patients were assessed by an experienced physiotherapist, and the tapings were done by another experienced physiotherapist. Participants and physical therapist who performed the evaluation was blind to the study to provide a double-blind structure of the study.

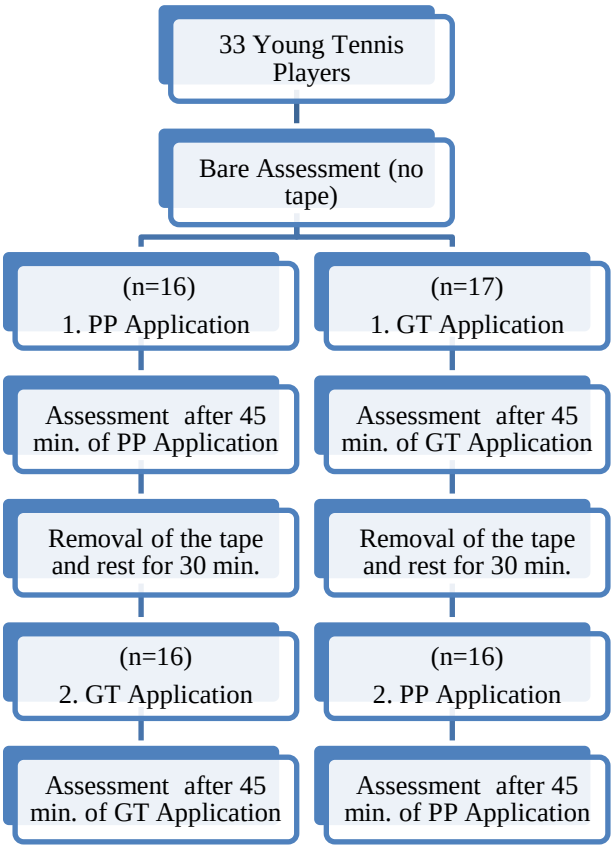

Figure 1. Flow-chart of the study

Subjects

Thirty-three young tennis players aged between 9 and 12 years were recruited in the study. The exclusion criteria included those (a) with soft tissue or bone problems affecting lower extremity, (b) who had acute inflammation affecting lower extremity region, (c) had scoliosis, (d) who had undergone any orthopedic surgery, (e) who had defined any pain or painful area at lower extremities and (f) who were obese (BMI>30 kg/m2).

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Figure 2. PP and GT taping applications for quadriceps muscle

Figure 3. PP and GT taping applications for gastrosoleus muscle

Methodology

Thermal analysis was done for the analysis of risk injury including both lower extremities. Quadriceps muscle was chosen for upper leg and gastro soleus muscle was chosen for lower leg region for thermal analysis. The thermographic assessment was performed by using FLIR E5 (FLIR Systems AB, Sweden) thermal camera to evaluate which muscles had the highest thermal activity while maintaining stable upright posture. The part of muscle with more activation (greater heat) was measured by the thermographic camera and the heat was recorded in centigrade. Each participant was thermographically evaluated in the same room (ambient temperature, 21°C)[13] and participants were left for 10-20 minutes to 'acclimatize' to the thermographic imaging environment [14,15]. FLIR E5 Thermal Camera, with a resolution of 120 x 90 pixels was used for thermal imaging and the Color Palette iron was chosen for displaying the images. It is indicated that IR imaging might be a reliable and valid measure of treatment outcomes with clinical utility and sensitivity[16].

Statistical analysis

The power analysis indicated that 33 participants for total were needed with 80% power and a 5% type 1 error. The power analysis of our study showed a power of 80% with tissue temperature as the primary outcome. The data were analyzed using statistical software (SPSS version 18, Inc., Chicago, IL, USA). All the statistical analyses were set a priori at an alpha level of $p<0.05$. The tests for homogeneity (Levene's test) and normality (Shapiro-Wilk) were used to determine the appropriate statistical methods. According to the test results, nonparametric Friedman test was used for comparisons between baseline, first taping and last taping. Wilcoxon test was used for possible differences which might occur between taping applications in order to identify the application that provided the difference. Parametric test assumptions were not possible due to small sample size and inhomogeneous parameters.

RESULTS

The flowchart of the study is shown at Figure 1. The trial was ended when the number of participants calculated in the power analysis was reached (n=33). One participant in PP application group was not able to complete the second taping procedure.

Statistically significant differences were found at lower leg and upper leg assessments for both dominant and non-dominant sides in skin temperatures between bare assessment (no tape), PP application and GT application ($p<0.05$) in triple comparison (Table 1).

Considering pairwise comparisons, both performance PP application and GT application are found significantly different than bare assessment in terms of skin temperature ($p<0.05$). But when comparing PP application and GT application, there

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were no statistically significant differences in skin temperature (p>0.05) (Table 2).

Table 1. Differences between skin temperatures for different taping applications

	Bare Assessment n=33 (X±SD)	PP Application n=33 (X±SD)	GT Application n=32 (X±SD)	p
Upper Leg-Dominant Side (°C)	28.05±2.2	29.25±2.56	29.15±2.31	≤.01*
Upper Leg-Nondominant Side (°C)	27.63±2.42	29.05±2.49	28.76±2.54	≤.01*
Lower Leg-Dominant Side (°C)	30.00±1.75	30.66±1.93	30.81±1.96	0.024*
Lower Leg-Nondominant Side (°C)	30.13±1.62	30.82±1.61	30.84±1.82	≤.01*

Friedman test, *p<0,05, PP: Performance Plus Tape, GT: Gold Text FP Tape

Table 2. Pairwise skin temperature comparisons for different taping applications.

	Bare Assessment - PP Application	Bare Assessment - GT Application	PP Application - GT Application
Upper Leg-Dominant Side (°C)	≤0.01*	≤0.01*	0.505
Upper Leg-Nondominant Side (°C)	≤0.01*	≤0.01*	0.383
Lower Leg-Dominant Side (°C)	0.013	≤0.01*	0.800
Lower Leg-Nondominant Side (°C)	≤0.01*	≤0.01*	0.902

Wilcoxon test, *p<0,05, PP: Performance Plus Tape, GT: Gold Text FP Tape

DISCUSSION

In current study we found that Kinesio Taping might be an effective application for decreasing risk of injury. Also, the types of Kinesio Tape used would be more effective than other type for decreasing risk of injury and found that both types of Kinesio Tape material (Performance Plus Tape and Gold text FP) have equal effects on decreasing risk of injury.

According to many studies in the literature, tennis has the risk of injury to the lower extremity; the most common (51%) followed by the upper extremity (24%) and the trunk (24%) [17–19]. The principal findings of these studies were that there is a great variation in the reported incidence of tennis injuries and most injuries occur in the lower extremities, followed by the upper extremities and then the trunk. We found that Kinesio Taping application on bilateral lower extremities normalizes thermally assessed risk of injury in tennis players. Also there is a research indicating that Kinesio Taping application to Gastro soleus and Quadriceps muscles might provide injury prevention in triathletes [20]. The reason for us to apply taping on Gastro soleus and Quadriceps muscles were because of common lower extremity injuries.

Studies about tennis state that there are no randomized controlled trials investigating injury prevention measures in tennis [21]. Measurement of injury risk is a very complicated assessment because of multifactorial influence of the player during training or playing. Due to these factors, there might be minor injuries to the muscle, which might not give symptoms to the player, but might increase the temperature of injury area as a symptom of injury inflammation process. These minor injuries might form a major injury of the muscle during an exercise or training. For the evaluation of this type of injuries thermal risk analysis is a commonly used assessment method since 1900's. Thermal risk analysis is indicated as a cheap and non-invasive method used for detecting minor traumas[22,23]. According to our results, Kinesio Taping application has shown a decrease in injury risk depending on decrease in skin temperature. We believe that Kinesio Taping application has regularized the skin temperature on areas that create injury (max 4.9%).

Our limitation was that we investigated the immediate effects of KT, but the long-term effects could have been investigated. Further studies are needed to see long-term effects of KT either PP or GT during sports activities. Also, it would be better to compare with different types of tapes as well as the sham group. However, during the study period, no negative side effects of both GT and PP were observed and none of participants reported any discomfort during assessments. Furthermore, we believe that in future studies, different sample sports can be safely applied.

There are no studies yet comparing the types of Kinesio Tape material used in neither study about performance nor injury risk. But there are some studies about the comparison of original Kinesio tape material and other taping materials. These studies indicate that Kinesio taping enhances dynamic muscle support and functional performance as much as non-elastic tape for ankle region [19,24]. One of the hypotheses of our study was to understand which kind of Kinesio Taping material should be used to decrease injury risk better. According to the results of two different types of Kinesio Taping application, we might say that there is no difference between the types of Kinesio Taping material used if it is applied with the same technique.

Practical applications

Considering the results of our study, we might be said that Kinesio Taping has regularized the skin temperature of both Gastro soleus and Quadriceps muscle region. According to the knowledge we have about thermal risk analysis, we might say that Kinesio Taping decreases risk of injury on most injured area. This finding might be very important for the training programs and prevention from injury of tennis players.

CONCLUSION

Our results had contributed to the knowledge of temperature changes in skin which was associated with risk of injury.

Both types of Kinesio Tape material (Performance Plus Tape and Gold Text Finger Print) had equal effects on decreasing risk of injury.

There was no difference between the types of Kinesio Taping material used, as long as it was applied with the same technique.

For future research, it could be beneficial to see long-term effects of KT either PP or GT during sports activities and add sham taping to applications.

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Author contributions: Conceptualization: [GB, NOP]; Design: [GB, NOP, GH]; Writing: [SCD, BU]; Investigation/Data collection: [GB, NOP, SCD, GH, BU].

Conflict of interest: There is no potential conflict of interest relevant to this article.

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ORIGINAL RESEARCH

The Effect of Kinesio Tape in Chronic Neck Pain: Randomized Controlled Study

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Abstract
Objective: This study aimed to compare the effects of Kinesio tape and conventional physiotherapy in addition to home exercise in patients with chronic neck pain.
Material-Method: A total of 44 individuals were randomly divided into two groups. Conventional physiotherapy methods were applied to all subjects for 15 sessions. In addition to the study group, Kinesio tape application was performed each session. Pain, pressure pain threshold, range of motion, muscle strength, muscle endurance, pectoralis minor muscle length, disability level, quality of life, and depressive symptoms were evaluated. The first evaluation was conducted prior to the Kinesio tape application, the second was conducted 24 hours after the procedure, and the third was after 15 sessions.
Results: A significant improvement was obtained in depression scores, muscle endurance, and patient satisfaction in favor of the study group (respectively p = 0.021, p=0.029, p= 0.009).
Conclusion: Kinesio tape can be used to increase muscle endurance in the short term. Also, it can be said that Kinesio tape application provides additional benefits to the conventional physiotherapy method in terms of treatment satisfaction.
Keywords: Neck Pain, Kinesio Tape, Pain, Quality of Life

INTRODUCTION

The prevalence of chronic neck pain varies between 12.1% and 71.5%. Especially in developing countries, it is considered an essential public health problem as it causes socioeconomic issues such as loss of labor and disability[1].Chronic neck pain is in the fourth rank among diseases causing disability[2].
The main goal of treatment is to increase joint range of motion (ROM), muscle strength, endurance, and coordination, to ensure independence in activities of daily living, and to improve the quality of life in chronic neck pain. Many different methods have been defined in its treatment. Exercise therapy, which requires the patient's active participation, is the most effective treatment method preferred by physiotherapists[3].Electrotherapy, hot pack-cold pack applications, manual therapy techniques, cervical traction, and neck collar use, which are among passive treatment applications, are applied to reduce inflammation, pain, and muscle spasm secondary to nerve root irritation and to improve functions[4]. In addition to all these treatment options, Kinesio taping technique (KT) has become popular, especially in musculoskeletal problems Kenzo Kase developed the KT, but its use has become more prevalent in recent years[5]. The hypothesis proposed for the effects of KT can be listed as pain inhibition, stimulation of blood circulation, reduction of edema by increasing lymph circulation, correction of joint position by providing muscle relaxation, support, and stability to muscles and joints without limiting the ROM. KT application seems to be preferred in the literature for many musculoskeletal problems such as knee pain, chronic low back pain, neck pain, shoulder pain. Different results of studies conducted with individuals with low back and neck pain in order to reduce pain and increase functionality are included in the literature. While some studies discuss the benefits of KT, other studies show the opposite effects.The number and variety of studies in the literature are insufficient to establish clinical evidence for using KT in patients with neck pain.
Therefore,the aim of the study was to compare the effects of KT and conventional physiotherapy in addition to home exercise in patients with chronic neck pain.

MATERIALS AND METHODS

In our study designed with pretest-posttest control groups in randomized parallel groups, the allocation ratio is 1:1.

Fifty-two individuals who applied to the clinic with complaints of chronic neck pain and fulfilled the inclusion criteria participated in the study. The groups were divided into two groups with online computer randomization software[10]. The physical therapist who generated the random allocation sequence was blind to patients' clinical data. Oral and written information was given to each individual about the study, and their written consent was obtained.

Ethical permission was obtained from the Non-Interventional Research Ethics Committee of Pamukkale University. Also, this study was supported by Pamukkale University Scientific Research Projects Coordination Unit (Date: 03/05/2014/No: 60116787-020/14168). Written informed consent was obtained from each patient, and assessments were carried out according to the Declaration of Helsinki.

The study was carried out between April 2014 and December 2015 at Şifa University Göztepe Hospital Physical Therapy and Rehabilitation department in İzmir. Criteria for inclusion in the study; between the ages of 30-55, neck pain lasting at least three months, pain between the superior nuchal line and spina scapula, a score of at least five on the Neck Disability Index, and being in the first two levels of the Neck Pain Task Force[11].Criteria not included in the study; the presence of conditions that will prevent evaluation or communication (such as cognitive problems), being illiterate, patients with discitis and/or spondylitis and/or spondylolisthesis, having undergone neck, back, or waist region physiotherapy within the previous six months; having undergone cervical region and spine surgery; having impingement or thoracic outlet syndrome; malignancy; having a fracture; having systemic autoimmune diseases; having neurological issues, and having been diagnosed with a mental illness that will prevent evaluation and treatment.

Materials

Age, sex, height, weight, Body mass index (BMI), education level, occupation, marital status, and smoking history of the participants were recorded before the treatment. Pain assessment was made with the evaluation of pain history, pain intensity, and pressure pain threshold. In the evaluation of the pain story, the type, duration, and activities that increase and decrease pain were questioned.

Visual Analogue Scale (VAS) was used to evaluate pain intensity. Pressure pain threshold was evaluated with an objective method, a digital algometer. Jtech Medical Commander Algometer device was used in the evaluation. The participants were asked to report the pressure sensation as soon as they returned to the feeling of pain and the compression applied after the patient notification was terminated. Two measurements were made a 30-second interval was taken between both measures.

Measurements were taken bilaterally from three regions in the prone position: the midpoint of the upper edge of the upper trapezius muscle (the lateral edge of the acromion and the midpoint of the region between the midline), the levator scapula muscle (2 cm above the lower medial edge of the scapula where the levator scapula muscle inserts), suboccipital region (2 cm lateral to the cervical line consisting of spinous processes, just below the occipital region). The results are recorded in Newton / cm^2[12]. Cervical range of motion (CROM) was evaluated with the CROM device. The CROM device measures CROM with three inclinometers and a magnetic amplifier placed on the neck[13,14]. The evaluation was made while the participants were sitting in the chair in an upright position with their arms adjacent to the body. The measurement results of actively performed movements were recorded in degrees[15]. Isometric muscle strengths of the cervical region were evaluated by Hand-Held Dynamometer (HHD). In the evaluation, Jtech Medical Commander Powertrack II Hand-Held Dynamometer device was used, and the result measurements were recorded in Newton. The pectoralis minor muscle shortness of the participants was evaluated with a standard bilateral ruler and the endurance of the deep neck flexor muscles with a chronometer; Muscle shortness was recorded in centimeters, and muscle endurance was recorded in seconds[16].Disability levels of the participants were assessed with the NDI[17,18], quality of life with Nottingham Health Profile (NHP)[19], emotional status with Beck Depression Inventory (BDI)[20,21], and treatment satisfaction with theVAS[22]. All evaluations except treatment satisfaction were conducted prior to the Kinesio tape application, the second was conducted 24 hours after the procedure, and the third was after 15

sessions by the same physiotherapist who was not blind to patients' treatment. Treatment satisfaction was evaluated on the second day of treatment and after treatment.

Methods

Conventional rehabilitation treatment

The standardized physical therapy methods and exercise programs were applied to all participants by the same physiotherapist. Unlike the control group, Kinesio tape was applied after each conventional treatment session.

Within the physical therapy methods, 20 minutes of the hot pack, 5 minutes of ultrasound (1.5 watts / cm^2), and 20 minutes of conventional transcutaneous electrical nerve stimulation (TENS) were applied. In the exercise program, active joint movement exercises, stretching exercises, isometric strengthening exercises, and posture exercises were used. All of the exercises were given to the participants in the form of brochures. In addition to the ones applied during the treatment session, the participants were asked to apply two more sets of home exercises on the same day.

On the first day of treatment, all participants were provided with patient education on the principles of pain control, proper posture, and ergonomics that should be considered in daily life to protect neck health.

Kinesio taping procedure

The inhibition technique was applied to the upper trapezius muscle to reduce pain and spasm and to support weak muscles. For this technique, 15-25% tension was achieved with I tape. The facilitation technique was applied to cervical paravertebral muscles. A tension of 15-35% was obtained with the Y band. The correction technique with 15-35% tension X-band was used in the rhomboideus major muscle. "Star application" was used in the area where the most pain and tenderness were felt during palpation. (Figure 1)[5].

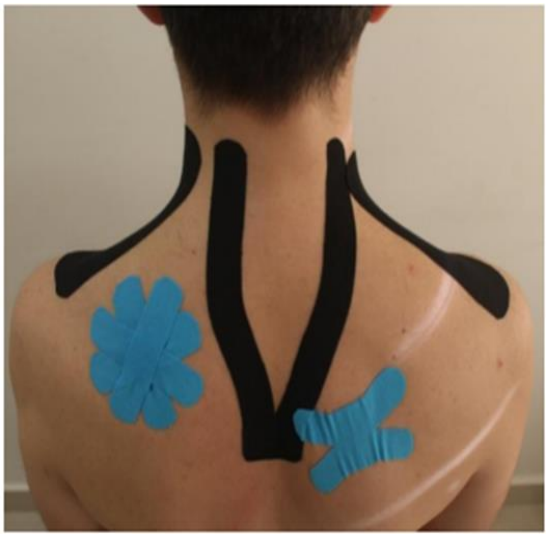

Figure 1. Application of Kinesio tape (KT)

KT was performed every day of conventional treatment immediately after the session ended. In case of complaints such as redness, burning, or itching in the area where the tapes were applied, the participants were told that the tapes should be removed using baby oil, etc. At the same time, individuals were advised that the tapes were water-resistant, but they should not be dried with a dryer. The participants who were treated five days a week were asked not to remove the tapes at the weekend.

Statistical analysis

The data were analyzed with SPSS 20.0 package program. Continuous variables are expressed as mean ± standard deviation, median (minimum and maximum values), and categorical variables as numbers and percentages. When parametric test assumptions are provided, independent groups t-test in comparison of differences; when parametric test assumptions are not provided, the Mann-Whitney U test was used to compare independent group differences. In the dependent group comparisons, Friedman Test and Wilcoxon paired-sample tests were used. The differences between categorical variables were examined by chi-square analysis. In all analyses, $p < 0.05$ was considered statistically significant.

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RESULTS

Fifty-two individuals with chronic neck pain participated in the study. One of the participants was excluded from the study because she was diagnosed with ankylosing spondylitis during the study period, two of them did not come to the last session of the treatment, and five of them did not attend the treatment regularly. When 44 people were included in the study (22 for each group), it was calculated that 80% of power would be obtained with 95% confidence. The study was completed with forty-four participants. Figure 2 shows the flow diagram for patient recruitment, reasons for exclusion, and drop-out cases.

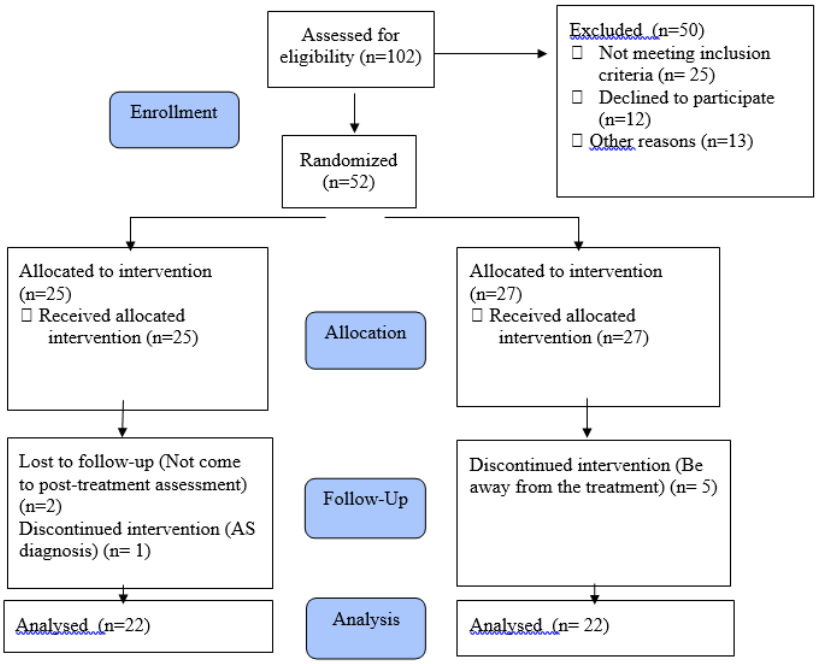

Figure 2. Flow diagram

The two groups were compared in demographic features, education level, properties of the neck pain, total pressure pain threshold, ROM, muscle endurance, muscle shortness, disability level, quality of life, and sex variables. In the pre-treatment evaluation, the muscle strength of the study group was found to be significantly higher than the control group (p=0.012) (Table 1). When the difference in BDI scores (delta values) after treatment and on the second day of treatment was compared, a significant decrease was obtained in favor of the study group (p = 0.021). The comparison was made in terms of muscle endurance differences before and on the second day of treatment, again in favor of the study group (p = 0.029). In the study group, patient satisfaction after treatment was significantly higher than on day 2 (p=0.009), but there was no significant difference in the control group (p=0.085) (Table 2).

DISCUSSION

As a result of our study, in addition to conventional physiotherapy methods in chronic neck pain, KT was found to have positive effects on BDI scores and patient satisfaction. Also, it was observed that muscle endurance increased after 24 hours after KT application in chronic neck pain.Controlling pain in the presence of chronic pain is important for symptomatic treatment. There are fewstudies in the literature evaluating the short-term effect of KT application on pain severity in neck pain. Ay et al.[23] divided the participants into two groups in their study on cervical myofascial pain. The first group received KT and exercise, and the second group received placebo taping and exercise, repeated every three days for a total of 5 sessions over three weeks. Improvement was observed in both groups, while a reduction in pain intensity was reported in the KT and exercise groups. However, there was no significant difference between the three treatment modalities. According to our results, a significant decrease in pain intensity was detected at the end of both treatments applied during 15 sessions. However, there was no significant difference between the two groups.

Another common clinical picture of chronic neck pain is pressure-sensitive painful points[24]. Tender

points in the cervical region are due to excessive muscle activity, poor posture, and psychological factors. Tender points can be palpated by hand or detected by an algometer[25]. Since the low-pressure pain threshold and pain are directly related to each other, treatment methods used to reduce pain can also be used to increase the pressure pain threshold[26,27]. Ay et al.[23] in their study, compared KT application and placebo taping in subjects with cervical myofascial pain and observed an increase in pressure pain threshold in both groups at the end of 15 days of treatment and reported that this increase was higher in the KT-treated group. In our study, the pressure pain threshold after treatment was significantly higher in both groups than before treatment. When the treatment groups were compared, there was no significant difference between the two groups.

Table 1. Demographic characteristics education status and neck pain variables.

Variables	Studygroup (n = 22)		Control group (n = 22)		p
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	
Age (years)	41.9±6.9	41 (30- 55)	39.6±7.4	38.5 (30- 55)	0.154*
Height (cm)	167.9±12	163.5 (150-198)	169.0±10	168.5 (150-190)	0.755**
Weight (kg)	69.8±16.2	67.5 (47-117)	66.5±11.9	66 (50-86)	0.442**
BMI (kg/m^2)	24.6±3.6	24.9 (19.3-32.4)	23.2±2.7	22.9 (18.6-28.7)	0.139**
Education status (years)	13.4±2.9	15 (5 – 20)	14.2±3.2	15 (8 – 22)	0.445*
First pain time (months)	62.4±3.7	30 (3-240)	41.7±44.8	24 (3-168)	0.268**
Last neck pain duration (months)	6.4±3.5	4 (3-12)	9.3±9.3	4 (3-36)	0.144**
Neck pain intensity (0-10)	6.3±2.3	6.6 (1-10)	5.6±2.4	5.9 (2-10)	0.334**
Total of pressure pain threshold (N/cm^2)	213.7±72.0	201 (108.8-350)	172.4±71.0	166.8 (47.7-366.3)	0.062*
ROM (^{0})	312.5±39.3	316 (211-370)	324.6±42.5	326 (220 - 396)	0.335**
Muscle strength (N)	308.1±98.2	283.8 (162.8-491)	230.1±98.8	218.4 (103.4-59.2)	0.012**
Muscle endurance (sn)	18.9±10.6	17.5 (3 - 40)	22.6±16.0	22 (3 - 75)	0.557*
Muscle tightness (cm)	18.9±2.5	20 (14 - 24)	18.2±2.5	18 (12 - 22)	0.423*
Disability level	13.9±6.4	14 (5 - 28)	16.9±6.6	16(6 - 30)	0.125**
Quality of life (0-600)	133.0±82.4	117.5 (0-329.9)	145.6±102.0	111.7 (10.5-55.9)	0.655**
	n (%)		n (%)		
Sex					
Female	14 (63.6)		18 (81.8)		0.176***
Male	8 (34.4)		4 (18.2)		

BMI body massindex; ROM range of motion; Mean±SD mean±standard deviation; *Mann-Whitney U Test; ** Independent T-Test; *** Pearson's Chi-squared test

Gonzalez-Iglesias et al.[8]reported that the increase in ROM was significantly higher in patients with acute whiplash injury than in the placebo taping group immediately after and in a 24-hour follow-up Erdoğanoğlu and Bayraklı[28] reported the measurement results before elastic adhesive tape application and after 24-hour follow-up, and a significant difference was found between ROM values. In our study, ROM evaluation was performed with the CROM device, which is an objective and reliable method[14,29]. Our results showed that ROM in both groups was increased after treatment and the treatment methods were effective on ROM.

Table 2.Comparison of the differences in outcome measures total values between the groups and treatment satisfaction within and between groups.

Variables		Baseline-2nd day Mean±SD	Baseline –After treatment Mean±SD	2nd day –After treatment Mean±SD
Disability level	Study	2.73±3.71	7.18±5.91	4.45±4.24
	Control	2.86±5.79	7±6.44	4.14±5.29
	p^1	0.926*	0.923*	0.827**
Pressure pain threshold (N/cm^2)	Study	-11.59±34.44	-45.06±52.82	-33.48±40.68
	Control	-17.32±42.97	-32.96±46.59	-15.64±39.74
	p^1	0.628*	0.656*	0.149**
ROM (^{0})	Study	-16.82±25.69	-42.27±24.14	-25.45±22.51
	Control	-16.5±32.83	-36.23±49.86	-19.73±24.88
	p^1	0.411*	0.385*	0.428*
Muscle tightness (cm)	Study	0.45±1.06	1.36±1.79	0.91±1.6
	Control	0.82±1.59	1.27±1.58	0.45±1.95
	p^1	0.451**	0.889**	0.807*
Muscle strength (N)	Study	-16.86±42.76	-52.8±62.19	-35.95±43.28
	Control	-12.85±35.46	-39.89±52.33	-27.04±40.04
	p^1	0.467**	0.46*	0.483**
Neck pain intensity (0-10)	Study	1.02±1.73	4.17±2.41	3±2.62
	Control	0.37±0.89	3.08±2.36	2.7±2.2
	p^1	0.126*	0.142*	0.688**
Muscle endurance (sn)	Study	-6.07±8.5	-12.5±11.04	-6.43±7.66
	Control	-0.82±11.55	-10.77±17.45	-9.95±11.55
	p^1	**0.029****	0.323**	0.24**
Quality of life (0-600)	Study	32.88±48.01	81.25±61.21	48.37±44.8
	Control	15.47±54.2	66.93±85.14	51.46±86.34
	p^1	0.324*	0.241**	0.296**
Beck Depression Inventory (0-21)	Study	1.23±3.15	3.95±5.46	6.2±5.5
	Control	1.32±2.83	1.64±3.65	8.3±6.0
	p^1	0.92*	0.105*	**0.021****
		2nd day Mean±SD	Aftertreatment Mean±SD	p^2
Treatment satisfaction	Study	9.3±1.1	9.9±0.2	**0.009******
	Control	9.1±1.4	9.3±1.0	0.085*****
	p^1	0.751***	0.148***	

ROM range of motion; Mean±SD mean±standard deviation; *Independent T-Test; **Mann-Whitney U Test; ***Friedman'stwoway ANOVA; **** Wilcoxonsigned-rank test; p^1intergroup; p^2intragroup.

Exercise is an effective treatment method for muscle strengthening in chronic neck pain[16].The muscle strength values before treatment were compared in both groups, and the total muscle strength was found to be high in favor of the study group. After the treatment, an increase in muscle strength was detected in both groups, but there was no difference between the groups. In other words, KT application is helpful for muscle strength but does not provide any additional benefit compared

to conventional treatment. Similar to our study, Copurgensli et al.[30]applied conventional therapy with exercise, Mulligan mobilization, and KT in cervical spondylosis and found no significant difference in muscle strength between the groups at the end of the treatment.

In individuals with chronic neck pain, cervical flexor muscle activity decreases, causing the head to go to the anterior tilt position. It causes muscle spasms and pain[31]. Researchers have noted that Alvarez-Alvarez et al.[32]recorded a significant increase in the endurance of the back extensor muscles in healthy individuals as a result of the evaluation made immediately after the application of KT to the back extensor muscles. Differently, Stedge et al.[33]evaluated the effects of KT applied to the gastrocnemius muscle on muscle endurance at the 24th and 72nd hours and concluded that it did not affect. In our study, in which we evaluated the cervical flexor muscle endurance, our results show thatmuscle endurance was increased after treatmentin both groups. However, the results of our study showed that KT increased muscle endurance over 24 hours and provided additional benefits to conventional treatment.

Another consequence of postural changes caused by chronic neck pain is the shortness of the pectoralis minor muscle[16]. In our study, it was found that there was a significant decrease in pectoralis minor muscle shortening at the end of treatment in both groups, but there was no difference between the groups. Our results showed that KT and conventional treatment methods effectively reduce muscle shortness, but KT application does not provide any additional benefit.

In our study, disability caused by neck pain was evaluated with the NDI[18]. Saavedra-Hernandez et al. [34] divided individuals with mechanical neck pain into two groups, cervical manipulation in one group and KT in the other group. In our study, the evaluation made at the end of the 24-hour follow-upand after treatment, the decrease in the level of disability was similar in both groups.

Neck pain adversely affects the quality of life with parameters such as ROM, muscle strength, and muscle endurance[35]. Cuesta-Vargas et al.[36]reported that a multimodal physiotherapy program, which includes therapeutic exercise, patient education, and swimming, increases the quality of life in individuals with chronic nonspecific neck pain. Our results showed that there was an improvement in the quality of life after treatment in both groups, but there was no significant difference between the groups. However, no study evaluating the effect of KT application on quality of life in neck pain has been found.

Emotional status, as well as the quality of life, is an essential indicator of health and has an important place in chronic pain[37]. Benlidayi et al.[38]have two groups in their study of temporomandibular joint disorders; the first group received KT, exercise, and patient education, and the second group received only exercise and patient education. After the treatment, it was observed that the emotional status improved in favor of KT group. In our study when the difference in BDI scores after treatment and on the second day of treatment was compared, a significant decrease was obtained in favor of the KT group. However, when the scores obtained from the BDI before treatment, on the second day, and after the treatment are analyzed, it is seen that all the values are below the limit value of 17 points, which indicates severe depressive symptoms that require treatment. This result shows that individuals do not have severe emotional problems during the treatment process.

According to Hills and Kitchen[39], the results obtained from the treatment are related to the expectations and satisfaction of the individuals. In the literature, no study evaluating the satisfaction of individuals with chronic neck pain from KT application has been found. In the KT group, while patient satisfaction showed a significant increase after treatment compared with the second day, there was no significant change in the only conventional treatment group.

Many authors evaluated the effectiveness of KT in chronic neck pain, but changes in the application area, applied tension, and duration of application affected the outcome of the studies[40]. Gonzalez et al. [8], in their study on changes in applied tension, found that the tension between 15-25% was more effective than KT applied without tension. In our study, KT was applied using 15-25% tension for inhibition and 15-35% for facilitation. We think that the way KT is applied is also valuable for the compliance of our results with the literature.

The strength of our study was to examine the effect of KT, which is applied together with conventional physiotherapy methods, on many parameters in chronic neck pain, where there are not enough studies in the literature yet. The limitations in our study's lack of a sham KT group are that the placebo effect could not be investigated, and lack of long-

term follow-up of the patients.

In the literature, studies comparing the effectiveness of KT and conventional physiotherapy in chronic neck pain are controversial and, number of studies is few. Our results show that KT can be used to increase muscle endurance in the short term. Also, it can be said that KT application provides additional benefits to the conventional physiotherapy method in terms of treatment satisfaction. The study's main findings can be attributed to the effects of KT to increase local circulation, reduce local edema fasting the target muscle, provide positional stimulation to the skin-muscle or facial structures, and provide suitable afferent inputs for the central nervous system.

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Authors contributions: Methodology: [HD, EAT]; Formal analysis and investigation: [HD, EAT, MPK]; Writing - original draft preparation: [HD, MPK, EAT]; Writing - review and editing: [MPK, HD, EAT]; Funding acquisition: [HD, EAT]; Supervision: [EAT].

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ORIGINAL RESEARCH

The Effects of Manual Therapy and Inspiratory Muscle Training on Respiratory Parameters in Young Adults with Postural Problems: A Randomized Trial

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Abstract

Objective: It was aimed to compare the effects of manual therapy (MT) and Inspiratory Muscle Training (IMT) on respiratory functions and postural problems of young adults in this study.

Material-Method: Thirty-five volunteers were randomly allocated into IMT and MT groups. All participants received twenty minutes of IMT twice a week for four weeks. Eight sessions of manual therapy were applied to the MT group in addition to IMT. Forced Expiratory Volume in 1 Second (FEV1), Forced Vital Capacity (FVC), FEV1/FVC ratio, Peak Expiratory Flow (PEF), forward head posture (FHP), and thoracic hyperkyphosis were evaluated before and after the treatments.

Results: Although there were significant improvements in the FEV1, FVC, FEV1/FVC ratio, and PEF scores of the MT group ($p<0.05$), no significant difference was found in the IMT group at the post-treatment ($p>0.05$). The comparisons of post-treatment scores of the FEV1, FVC, FEV1/FVC ratio, and PEF between the groups revealed greater improvements in the MT group than IMT group ($p<0.05$). Significant changes were demonstrated in postural problems in the two groups ($p<0.05$). However, no significant differences were analyzed between the group in the comparison of the post-treatment postural changes ($p>0.05$).

Conclusion: We observed in our study that IMT and MT might be effective in correcting FHP and thoracic hyperkyphosis in young adults. It was recommended to add MT to IMT for the young adults with thoracic hyperkyphosis and FHP, due to greater improvements shown in the respiratory functions in our study.

Keywords: Inspiration, Kyphosis, Manual Therapy, Posture, Respiratory Muscles

INTRODUCTION

Young adults are prone to have postural problems such as thoracic hyperkyphosis and forward head posture (FHP) due to prolonged static postures in computers and mobile phones. The prolonged anterior shifting with the FHP may lead the thoracic hyperkyphosis, stiffness in the thorax, and flattening in the diaphragm.[1] Thoracic hyperkyphosis, defined as a thoracic curvature higher than normal ranges, is among the reasons that decrease the mobility of the thorax and the respiratory functions.[2] Previous studies claimed that the increase in thoracic kyphosis and the decrease in the mobility of the thoracic spine are associated with the decrease in respiratory functions such as Forced Expiratory Volume in 1 Second (FEV1) and Forced Vital Capacity (FVC).[2] FHP is suggested to cause impaired respiratory functions due to biomechanical effects on accessory inspiratory muscles such as the scalene muscles, and the sternocleidomastoid muscles.[1] Previous researchers showed that FVC, FEV1, and accessory inspiratory muscle strength were lower in the individuals with FHP, in comparison with the non-FHP individuals.[3] Manual therapy (MT) consisting of different techniques (manipulation, joint mobilization, and soft tissue mobilization) applied to the cervical and thoracic spine have been shown to lead to a significant reduction in thoracic hyperkyphosis and FHP.[4,5] Previous studies revealed that MT includes spinal manipulative therapy, manual diaphragm release, and rib cage mobilization can be an effective approach to improve respiratory function

by increasing the mobility of the thoracic region and diaphragm.[6] Previous researchers have shown beneficial effects of spinal thoracic manipulations on the FEV1, FVC, and Peak Expiratory Flow (PEF) in people with thoracic hyperkyphosis, chronic neck pain, stroke, and chronic obstructive pulmonary disease (COPD).[6-8] The underlying mechanism of the increase of respiratory functions after the MT was claimed as an increase in joint mobility, increase of the inspiratory muscle length, and decrease of muscle tone and pain.[6,8,9] The effectiveness manual diaphragmatic release technique in patients with COPD also has been suggested in previous studies.[10,11]

Inspiratory Muscle Training (IMT) consists of resisted breathing exercises to improve the strength of respiratory muscles and aerobic capacity of healthy adults and patients. IMT is a technique aimed to improve the strength and endurance of the inspiratory muscles with a pressure threshold device.[12,13] Recent research demonstrated that the treatment program that combined the MT and IMT further increases thoracic mobility, inspiratory muscle activity, and respiratory functions in smokers, healthy adults, and individuals with chronic obstructive pulmonary disease (COPD), asthma, and stroke.[14-17] Several researchers revealed that respiratory exercises might have beneficial effects on FHP and thoracic hyperkyphosis. It was stated that the addition of MT and therapeutic exercise protocol to IMT in healthy and asthmatic individuals was more effective than IMT in improving FHP and thoracic hyperkyphosis.[14] In a previous study was suggested that the mechanism of improvements in the FHP with respiratory exercises was associated with the reduction of the activity of upper trapezius, sternocleidomastoid, scalene, and cervical erector spinae muscles.[18] However, the research about the effects of IMT and MT on respiratory functions and postural problems is limited in the literature. In this regard, the aim of our study was to evaluate the effects of MT and IMT on the FEV1, FVC, tiffeneau index (FEV1 / FVC), PEF, FHP, and thoracic hyperkyphosis in young adults.

MATERIAL AND METHODS

Thirty-five university students, between the ages of 18-24 years old, non-smoking, with thoracic hyperkyphosis and FHP, and with low physical activity levels according to International Physical Activity Questionnaire-Short Form (IPAQ)[19] participated in this study. Individuals with moderate or high levels of physical activity, history of traumatic deformity in the thoracic spine, scoliosis of 20° and above, cervical trauma, cervical spine surgery, asthma, cancer history, heart disease, diabetes, hypertension, systemic disorders, smoking history and used oral corticosteroids or antibiotics within one month were excluded from the study. Ethics committee approval was obtained and all participants gave informed consent (Ethic File Number: 69396709-300).

The participants were randomly allocated to IMT (n=16) and MT groups (MT: n=19) by the coin toss. All the interventions were applied twice a week for four weeks and at least two days between sessions. All the measurements were performed pre-intervention and at the end of the four-week of interventions. All measurements and applications in the study were carried out by the same physiotherapist MicroQuark (COSMED, Albano Laziale, Italy) USB spirometer was used for the measurements of respiratory functions. The measurements were performed by the 2019 updated spirometer measurement standardizations of the American Thoracic Society (ATS) and European Respiratory Society (ERS)[20]. After three tests performed by the standards, FEV1 and FVC were determined and recorded by the test device with the highest total, as suggested by ATS. FEV1, FVC, FEV1/FVC ratio, and PEF scores were recorded.

Thoracic hyperkyphosis was determined by Occiput Wall Distance (OWD) measurement. Participants were asked to touch their occiput against the wall with their back and heels resting and touch the wall and head facing forward. The presence of thoracic hyperkyphosis was considered positive if the wall could not be touched with the occiput. During the measurement, two rulers were used. The first ruler was placed parallel to the floor on the occiput, and the second ruler was placed between the first ruler and the wall to measure the vertical distance. Since there were studies that it would be more accurate to use C7 in measurement. The perpendicular distance from the C7 spinous process to the wall was also measured in the same position in this study.[21] Both measurements were repeated three times in a row with a short rest period and the mean values were recorded.

FHP was evaluated with a Cervical Range of Motion (CROM) device. The CROM device is a reliable method for the measurement of FHP.[22] The participants were asked to sit upright on the chair and not to move their heads. The head forward arm was attached to the CROM mainframe and the lower end of the control arm (vertebra locator) was

held by the investigator on the C7 spinous process. The vertebra locator was placed at a 90° angle with the forward arm of the CROM with the help of a bubble indicating that the instrument was straight. The distance between the participant's bridge of nose and C7 was recorded in centimeters. This measurement was repeated three times in total and mean values were calculated. The mean values greater than 17 centimeters are considered as the presence of FHP.[22]

Intervention Protocols

Inspiratory muscle training (IMT)

The participants in the IMT group (n=16) only performed IMT. For all intervention groups, IMT was performed with Powerbreathe Classic-Light Resistance (Powerbreathe, IMT Technologies Ltd., Birmingham, UK) device. To determine the intensity of the training, MIP values were measured with the help of the respiratory pressure meter-RP Check (MD Diagnostics Ltd. RP Check MIP & MEP) device before each training. The pressure corresponding to 50% of the initial MIP value in the Powerbreathe (IMT Technologies Ltd., Birmingham) device was determined as intensity. IMT was applied for 20 minutes, two days of each week, for four weeks. During each IMT session, the participants were asked to maintain diaphragmatic breathing. Each IMT session included five breaths and five sets with 30 seconds between each set. IMT program has previously been used by several studies to improve respiratory muscle strength.[14,17]

Manual therapy (MT)

In addition to IMT, a total of eight sessions of MT (manipulation, joint mobilization, and soft tissue mobilization) approaches, two days a week for four weeks and at least two days between sessions; were applied by an experienced physiotherapist to the participants in the MT group. MT approaches included manual diaphragm release, thoracic mobilization, and thoracic high-velocity low amplitude (HVLA) thrust manipulation and cervical mobilization.

The participants were in a supine position, while the physiotherapist was standing behind the person, in contact (pisiform, hypothenar region, and the last three fingers) with the lower part of the seventh and tenth costal cartilages during the manual diaphragm release. Throughout the breathing, the physiotherapist raised his hand slowly to accompany the rising movement of the ribs and deepened the contact during expiration. The maneuver was carried out in two sets of 10 deep breaths. During the thoracic mobilization, the participants were asked to cross their arms in front of the chest while sitting. The physiotherapist stood behind the participant, wrapped the crossed arms of the participant with his left arm, and performed stretching, extension, lateral flexion, and thoracic rotation with his right hand. For the thoracic HVLA thrust manipulation, the participants were asked to cross their arms in front of the chest, with their hands on the opposite shoulder, in the supine position. The physiotherapist first grasped the participant’s neck and shoulders with his supporting hand and placed his upper chest on the subject's elbows. The physiotherapist positioned his other hand on the transverse processes of the lower vertebrae of the localized hypomobile vertebrae. Then, the physiotherapist placed his supporter hand on the subject's elbows and applied HVLA thrust in the posterior-anterior and inferior-superior directions with the help of her body. For the cervical mobilization; the physiotherapist wrapped the right arm around the participant's face around the back of the neck and placed it on the localized hypomobile vertebrae. The physiotherapist positioned her supporting hand’s index finger and thumb on the lower vertebrae of localized hypomobile vertebrae. Mobilization was performed using the right hand in the directions of flexion, extension, right-left rotation, and lateral flexion.

Statistical analysis

Priori power analysis was performed using the G-Power 3.1 program, and the sample size was calculated as 42, with a large effect size (0.8), a significance level of 0.05, and a power of 0.80. Due to the COVID-19 pandemic, the study was completed with 35 subjects. According to posthoc power analysis for the 34 sample size, with a large effect size (0.8) and significance level of 0.05 the statical power of this study was 0.74.

The statistical analyses were carried out using IBM SPSS Statistics software, version 20 (SPSS, Chicago, IL, USA). The normal distribution of the data was obtained from the Shapiro-Wilk test in the study. Mean, standard deviation, and percentage were calculated in the measurement data. The comparison of outcome measurements between pre-treatment and post-treatment within the groups was examined by Student’s paired t-test. The post-treatment changes were measured with an independent sample t-test. Pearson correlation analysis was used to examine the relationship between the pre-treatment values and post-treatment values of OWD and C7-Wall distance measurements. The level of significance was set as $p<0.05$.

RESULTS

Thirty-five university students (20.94±1.55 years; 17 female and 18 male) were recruited in this study (Table 1).No statistically significant differences were analyzed between the pre and post-treatment FEV1, FVC, FEV1 / FVC, and PEF of the participants in the IMT group (p>0.05). A statistically significant improvement was found in the FEV1, FVC, and PEF of the participants of the MT group in comparison between the pre and post-treatment (p<0.05). However, no statistical change was found in the comparison of FEV1/FVC measurements before and after the intervention in the MT group (p> 0.05). We found a significant improvement in the comparison of the pre and post-treatment values of FHP measurements and OWD and C7-wall distance measurements in both intervention groups (p<0.05; Table 2).

Participants who received MT had greater improvement in the FEV1, FVC, and PEF (p<0.05). However, no statistically significant difference was found in the comparison of the changes in FEV1/FVC, FHP, OWD, and C7-wall distance measurements between the groups (p>0.05; Table 2).

Table 1. Demographic characteristics of participants

		IMT n (%)	MT n (%)	
Gender	**Female**	8 (50)	9 (47.4)	
	Male	8 (50)	10 (52.6)	
		Mean (SD)	**Mean (SD)**	**p***
Age (years)		20.5 (1.41)	21.32 (1.6)	0.543
Height (cm)		171.25 (8.53)	169.73 (1.6)	0.347
Weight (kg)		67.37 (11.55)	70.36 (14.47)	0.308
BMI (kg/m^2)		22.88 (2.91)	24.26 (3.50)	0.295

BMI: Body Mass Index; IMT: Inspiratory Muscle Training; MT: Manual Therapy; SD: Standart Deviation; *Independent sample t test p<0.05.

Table 2. Post-training outcome measures and changes between post and pre-intervention scores

	IMT			**MT**			**Between the groups**
	Baseline Mean (SD)	**Change Mean (SD)**	**p***	**Baseline Mean (SD)**	**Change Mean (SD)**	**p***	**Δ p****
FVC	4.25 (0.77)	0.06 (0.71)	0.726	3.9 (0.68)	0.63 (0.94)	0.009	0.020**
FEV1/FVC	86.76 (4.4)	0.03 (5.39)	0.982	85.53 (5.02)	1.85 (5.87)	0.185	0.332
PEF	7.26 (1.57)	0.38 (2.26)	0.512	6.55 (1.41)	2.13 (1.56)	0.001	0.014**
FHP	18.63 (1.42)	0.87 (1.51)	0.003	18.16 (1.36)	0.84 (1.48)	0.001	0.81
OWD (C7)	7.41 (1.8)	2.00 (1.86)	0.001	7.32 (1.38)	2.93 (1.29)	0.001	0.094
OWD (C0)-Wall	5.92 (2.52)	2.88 (2.30)	0.000	5.76 (1.33)	3.47 (1.77)	0.001	0.398

FEV1: Forced expiratory volume in the first second; FVC: Forced Vital Capacity; PEF: Peal Expiratory Flow; FHP: Forward Head Posture; IMT: Inspiratory Muscle Training; MT: Manual Therapy; OWD: Occiput Wall Distance; SD: Standard Deviation; *Paired Sample t test; ** Independent Sample T test p<0.05.

DISCUSSION

FEV1, FVC, and PEF are suggested as gold measurements for the decision of obstructive pulmonary diseases such as COPD and asthma.[23,24] Previous studies revealed that FEV1 and FVC are decreased in individuals with thoracic hyperkyphosis and FHP due to biomechanical effects on inspiratory muscles.[1-3] MT applied to the diaphragm, cervical and thoracic region have been shown to increase respiratory functions.[6] In our study, an increase was observed in the FEV1, FVC, and PEF at the end of the MT intervention. However non-significant increase was found in the comparison of the FEV1/FVC ratio before and after the intervention in the MT group. In contrast, Wall et al. stated a single session of MT applied to the thoracic spine and thorax is not effective to improve the FEV1, FVC, and FEV1/FVC ratio in healthy adults.[25] However, previous studies showed improvements after the MT among the patients.[6,8,9] In a recent review, Roh et al. revealed that MT

might be beneficial to decrease the FEV1 and FVC; and increasing FEV1/FVC[6]. Park and Chon found that thoracic mobilization might be effective to improve FVC and PEF in patients with chronic low back pain[9]. Joo et al. suggested that a single thoracic spinal manipulation might increase the FVC and FEV1 of individuals with stroke[8]. In our study, it was suggested that the administration of long-term (two days a week for four weeks) MT to healthy non-smokers might be caused a significant change in FEV1, FVC, and PEF.

The effects of IMT on FEV1 and FVC remain inconsistent between healthy individuals and patients, with some research showing improvements in the FEV1 and FVC after the IMT and others not. In a previous study, three weeks of IMT in rugby players did not show significant increases in FEV1 and FVC.[26] Ramos et al. also did not show significant increases in FEV1 and FVC at the end of the twelve sessions of IMT in roller hockey players[27]. In a systematic review, HajGhanbari et al. did not reveal a significant increase in FEV1 after IMT in the athletes[28]. Our results showed similarly no significant effects in the FEV1, FVC, and PEF after the eight weeks of IMT. On the other hand, IMT showed beneficial effects on the FEV1, FVC, and PEF of patients with respiratory muscle weakness, obstructive pulmonary diseases, expiratory flow restrictions, and healthy smokers in the previous studies.[12-15] In a recent review Shei et al. suggested that these heterogeneous results might be related to different training protocols, study populations, and sample size[29].

We observed a greater increase in FEV1, FVC, and PEF in the MT group than in the IMT group. Alvarez et al. similarly observed an increase in the PEF after the IMT combined with cervical and thoracic MT in moderate smokers[17]. However, they did not show a significant increase in the FEV1 and FVC. Villanueva et al. demonstrated no significant effects in the FEV1, FVC, and PEF after the IMT and IMT combined with cervical and thoracic mobilizations in individuals with asthma[14]. The explanation of these results was hypothesized by Villanueva et al. as no significant improvement in the thoracic hyperkyphosis[14]. The mechanism of the observed improvement in the pulmonary functions after the IMT combined with MT was thought as a possible increase in joint mobility and inspiratory muscle length in our study.

Our results showed that there was a significant decrease in the degree of thoracic hyperkyphosis and FHP in both groups after the treatment, although the two treatment groups were not superior to each other. While a few studies aimed to compare the effectiveness of IMT combined with MT and IMT alone, the results of these studies were not consistent with each other. In agreement with our results, Alvarez et al. suggested IMT combined with cervical and thoracic MT is beneficial to decreasing FHP and thoracic kyphosis in healthy individuals with moderate smoking[17]. However, they did not observe an improvement in postural measurements after the IMT alone. Villanueva et al. reported that IMT combined with MT and therapeutic exercise was more effective than the IMT alone for improving FHP in individuals with asthma, while they did not demonstrate significant improvement at thoracic kyphosis after the two interventions[14]. In contrast, in a previous study, a four-week respiratory muscle exercise program significantly reduced the angles of thoracic and lumbar curvatures.[30] Haghighi et al. also reported that the degree of thoracic hyperkyphosis decreased significantly after 12 weeks of IMT[31]. The data obtained from our study reinforced the relationship between the musculoskeletal and respiratory systems. The mechanism of improvement in the FHP is thought to be related to the reduction of the activation of the inspiratory accessory muscles. It was also thought that the pressure in the spine with thoracic expansion might be related to the decrease in the degree of kyphosis.

This study had some limitations. There is no consensus about the most relevant measurement to assess the effectiveness of IMT. MIP, maximal expiratory pressure, transdiaphragmatic pressure, the thickness of diaphragm muscle, and respiratory muscle power output have also been used to assess responses to IMT in previous studies. In our study, we did not measure the MIP to determine respiratory muscle strength. Also, it was impossible to blind the participants and the therapist because of the type of interventions in our study. Due to the COVID-19 pandemic, an equal number of participants in both groups could not be provided in this study.

In conclusion; this study showed that the addition of MT involving the diaphragm, cervical and thoracic region to the IMT might improve respiratory functions in healthy individuals with thoracic hyperkyphosis and FHP. It was also demonstrated that IMT alone and IMT combined with MT might be effective in the reduction of FHP and thoracic hyperkyphosis in healthy individuals.

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Author contributions: Conceptualization: [SY, LAB]; Design: [SY, LAB]; Writing: [SY, LAB, SÖ]; Investigation/Data collection: [SY, LAB, SO]
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ORIGINAL RESEARCH

In Vitro Inhibitory Potential of *Lawsonia inermis* Extracts against Multidrug Resistant Clinically-Relevant Bacteria: a Phytochemical, Quantitative Antimicrobial and Toxicological Assessment

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Abstract

Objective: Majority of the current antibiotics have become less effective due to widespread of multidrug-resistant microorganisms. Medicinal plants are promising candidates that could be used to manage this menace. Therefore, phytochemical, toxicological and antimicrobial potentiality of *Lawsonia inermis* extracts against MDR clinical bacteria were carried out.

Material-Method: Henna leaf and seed were extracted by cold maceration technique using methanol and water and screened phytochemically. Eight MDR isolates, four of which are ESβL-producers were used for this study. *In vitro* antimicrobial efficacy and quantitative antimicrobial potency of extracts were estimated. MIC and MBC were determined using broth macrodilution technique. Cytotoxicity test was conducted using brine shrimp lethality assay and LC_{50} was determined.

Results: The findings of this study revealed that aqueous leaf extract possesses maximum percentage yield of 25.58%. Tannins and phenolic compounds were detected in all extracts, while steroid was absent. Methanol seed extract showed the highest antimicrobial efficacy against all bacteria with 100 percent activity. The highest and lowest zones of inhibition were recorded at 30.0±0.00 and 10.0±0.00 mm, respectively. The zones of inhibition of extracts differed significantly. All extracts displayed highest activity index against the ESβL-producing *Enterobacter aerogenes* 196 that was isolated from wound with highest value at 4.28. *Pseudomonas aeruginosa* U_{109} showed maximum susceptibility index (93.75%); majority of MIC values recorded were within the range of 1.95-62.5 mg/mL. Cytotoxicity test of methanol and aqueous extracts displayed $1000<LC_{50}>1000$, respectively.

Conclusion: Findings from this study elucidate the efficacy of *Lawsonia inermis* as a potential remedy to manage MDR-related infectious bacteria.

Keywords: Antimicrobial Resistance, Multidrug Resistant Microorganisms, ESBL, Antimicrobial Activity, Indigenous Plant, Phytotherapy.

INTRODUCTION

Antibiotics, the 20th-century wonder drugs, have played a major role in the treatment of infectious diseases the world over[1]. However, in part, as a result of irregular, irrational, inappropriate and extensive use of these drugs, antimicrobial resistance (AMR) has surfaced and has led to the development and widespread of multidrug-resistant (MDR) pathogenic bacteria[2]. Antimicrobial resistance is now a leading cause of death globally. Typically of COVID-19 that has swept across the globe; another pandemic (AMR) is spreading silently and rapidly with no regard for border, race or colour[3]. In the Review on Antimicrobial Resistance, 700,000 deaths a year are attributed to AMR and regrettably estimated more 10 million lives each year to be lost in 2050[4]. Not far-fetch, report from first comprehensive global burden associated 4.95 million lives with and attributed 1.27 million deaths to bacterial antibiotic-resistant infections, in 2019 alone[5], hence corroborating earlier report. Yet, the invention of new drugs has reduced drastically in the last three decades[6], and resistance to microorganisms continues at a faster pace. The development of new therapy threatens global efforts to contain drug-resistant infections, where previously treatable illnesses are (becoming) hard-to-treat and even kill[7].

Medicinal plant, however, shows promising effect

in mitigating, if not eradicating the problems of antibiotic resistance. Unlike conventional medicines that microbes find easy to develop resistance to due to a single active ingredient for the same therapeutic target, herbal medicine uses a combination of efficacious natural active ingredients to breakdown the cell wall and cell membrane of microorganisms, which can lead to the release of cellular contents, protein binding domain disruption, enzyme inactivation, and ultimately leading to cell death[8,9]. More specifically, a 1000-year-old antimicrobial remedy was formulated from Bald Leechbook using the ancient's technique and found to be more effective than conventional drug, vancomycin, against methicillin-resistant *S. aureus*[10]. Moreover, medicinal plants continue to enjoy human patronage because they are cheap, readily available and free from side effects often associated with conventional antibiotics[11]. Antimicrobial activities of useful plants vary: the majority act in synergy, reducing the side effect of synthetic drugs while others act as quorum quenchers[9,12].

Lawsonia inermis L. (synonym *Lawsonia alba*), is the sole species of the genus *Lawsonia* belonging to the family *Lythraceae*[13]. It is popularly called 'Mehndi' or 'Henna'; 'Laali' among the Yoruba-speaking people, 'Lalle' among the Hausa-speaking people. Henna is famed for its cosmetic uses worldwide and continuous use for celebrations of women's fertility and marriage in the eastern Mediterranean since the Bronze Age[14,15]. Its seeds have been reported to possess deodorant action and are used in most cases of gynecological disorders[16]. The potency of the plant has been evaluated against an extremely large variety of human pathogenic bacteria. In most cases, aqueous extracts, employing hot or cold water, methanol, ethanol and even acetone, of the leaves or whole plant, and in a few cases, the stems and bark, have been found to have the highest efficacies[14]. This corresponds to the traditional intake of decoctions prepared from the leaves of henna for variety of ailments associated with bacterial infections[17,18]. Studies on *L. inermis* leaf extracts demonstrated antibacterial activity against Gram-positive and Gram-negative bacteria[19,20]. Its extract act against pathogenic organisms from Urinary Tract Infection and found methanol leaf extracts had various degrees of antibacterial activities[21]. Similarly, clinical isolates from wound infection were treated with extracts of the leaves using agar well diffusion methods[22]. Results showed that the henna leaves extracts were able to inhibit the growth pattern of *A. niger* and *F. oxysporum*, *Streptococcus* sp. and *S. aureus*. Decoction of its leaf is used for aseptic cleaning of wounds and healing[23], and this suggests the wound healing management and potential of the plant.

With the current scientific and ethnomedicinal report of *L. inermis*, we therefore proceeded to investigate the phytochemical, antimicrobial and toxicological properties of aqueous and methanol extracts of this plant (leaf and seed), against multidrug resistant and ESβL-producing clinical bacteria that were isolated from urine, wound, sputum and amniotic fluid samples.

MATERIAL AND METHODS

Collection and processing of plant materials

L. inermis plant was collected from a nearby plantation in Akinyele Local Government, Ibadan with the assistance of the Chief Technologist, Herbarium, Department of Botany, UI. The leaves and seeds of the plant were immediately plucked from the stem, washed thoroughly with clean water and then air-dried away from the sun- and fluorescence light. The plant materials were milled twice into a very fine powder using an electrical blender (Model: BL1085BA-CB) disinfected with 70% ethanol. It was then stored in a sealed air-tight container under dark conditions at room temperature for further use.

Extraction of plant materials

The plant materials were weighed using an electric weighing balance (Model: YP-B100002) into sterile bottles for the cold maceration technique, as described by Gull *et al*.[20] and Habbal *et al*[24] with slight modifications. A ratio of 1:6 plant materials to solvents was employed; this is presented in Table 1. Methanol was used as an organic solvent while sterile distilled water served as an aqueous extractant. The extracts were chosen based on the ethnomedicinal preference as reported by Idowu[25]. All bottles were properly covered and left for three days with frequent agitation. After 72 hours of cold maceration with frequent agitation, muslin cloth was first employed to remove particles and ease the passage through filter paper. Accordingly, the contents were filtered through a four-layered muslin cloth, then through a Whatman filter paper No.1 and, where necessary, cotton pluck was employed. All filtrates of the same extract were pooled together and concentrated. While all organic extracts were concentrated *via* a vacuum rotary

evaporator; the aqueous extracts were freeze-dried to avoid the denaturation of the active constituents. These were stored at 20ºC for further use.

The percentage yields of crude extracts were calculated as follows:

$$\textbf{Extraction yield}\ (\%) = \frac{\text{Weight of the concentrated extract (g)}}{\text{Weight of the milled dried plant sample used for the extraction (g)}} \times 100$$

Table 1. Plant to solvent ratio (1:6) of *L. inermis* extraction

Plant Part	Plant material (g)	Methanol (mL)	Aqueous (mL)
Leaf	321	1926	1926
Seed	450	2700	2700
Overall (solvent)	NA	4626	4626

NA: Not applicable

Phytochemical screening of *Lawsonia inermis* (leaf and seed) extracts

The following phytochemical screening of aqueous and methanol extracts of *L. inermis* was performed to determine the phytoconstituents present in the plant materials, using standard methods: terpenoids, tannins[26]; phenolic compounds[27]; quinones, cardioglycosides[20]; saponins, alkaloids[28]; steroids[29]; flavonoids[30]; glycosides[31]; proteins and amino acids[32].

Source and maintenance of isolates

All the isolates used in the present investigation were eight (8) MDR bacterial strains from clinical sources; four (4) of which are ESβL-producers. They were obtained from the Microbiology Department Culture Collection, UI and have been characterized up to molecular level. Table 2 shows their names, assigned codes, resistance phenotype as well as the specific niche they were isolated from. Upon plating, they were preserved on nutrient agar slant. Pure cultures obtained by subculturing on the same medium were used for further studies.

Table 2. Source of isolates used in this study

	Assigned code	Name of Isolate	Source	Resistance Phenotype
	U_9	*Acinetobacter baumannii*	Urine	AMC, CTX, CPD, CIP, GEN, TET
	U_{30}	*Klebsiella oxytoca*	Urine	CTX, CPD, CIP, GEN, TET
	U_{87}	*Salmonella enterica*	Urine	CTX, CPD, CIP, GEN, TET
	U_{109}	*Pseudomonas aeruginosa*	Urine	AMC, CTX, CPD, CIP, GEN, TET
ESβL-PRODUCERS	129	*Klebsiella pneumoniae*	Urine	CRO, CIP, CN, FEP, SAM, AMC, ATM
	190	*Escherichia coli*	Amniotic fluid	AZM, CAZ, CPD, CTX
	195	*Enterobacter cloacae*	Sputum	FOX, CN, FEP, SAM, AMC
	196	*Enterobacter aerogenes*	Wound	CRO, CIP, CAZ, FOX, CN, FEP, SAM, AMC, ATM

AMC: Amoxicillin/Clavulanate
CTX: Cefotaxime
CPD: Cefpodoxime
CIP: Ciprofloxacin
GEN: Gentamicin
TET: Tetracycline
CRO: Ceftriaxone
CN: Cefalexin
FEP: Cefepime
SAM: Ampicillin + Sulbactam
ATM: Aztreonam
AZM: Azithromycin
CAZ: Ceftazidime
FOX: Cefoxitin

Determination of antimicrobial efficacy

In vitro antimicrobial efficacy of extracts was evaluated by agar well diffusion method, as described by Ali *et al*.[33] and Rajput and Kumar[34] with minor modifications. Ciprofloxacin disc (Oxoid) was used as positive control while 40% (v/v) methanol served as the organic diluent and negative control[35] as it was expected to be inactive against the isolates.

Standardization of inoculum

McFarland standard was used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms will be within a given range (1.5×10^8 CFU/mL). The standard was prepared as previously described by Andrews[36] and Cheesbrough[37]. The test isolates were resuscitated from agar slant using a sterile wire loop to touch the surface and streaked on a nutrient agar plate. Upon 18-24 hours of incubation period, an inoculum suspension equivalent to 0.5 McFarland standards was prepared.

Preparations of test samples

The test extracts were prepared by dissolving the crude extracts in sterile distilled water (aqueous extract) or 40% methanol (v/v) (organic extract). Forty percent (40% v/v) methanol was prepared by measuring 40 mL of methanol and dispensed into 60mL of sterile distilled water. Four different concentrations were prepared which include 1000 mg/mL, 500 mg/mL, 250 mg/mL and 125 mg/mL for each extract.

Performance of test assay

After preparation and standardization of inoculum and test extracts, the bioassay was conducted to determine the antimicrobial activity of the extracts. Accordingly, a sterile swab stick was dipped into the prepared inoculum suspension to take up the cells. The swab stick was used to spread (lawn) the cell suspension evenly on the entire surface of the Mueller Hinton agar (Oxoid) plate from edge to edge, turning the plate at every 60^o between streaking while turning the swab stick, too. Then, using a sterile cork borer, a well of 8mm diameter was aseptically bore on the Mueller Hinton agar plate and labeled appropriately. Thereafter, an aliquot of 100µL of each test sample of varying concentrations was carefully pipetted into each well. Plates were left to diffuse at room temperature for 1-2 hours, and they were subsequently incubated at 37^{o}C for 24 hours. Ciprofloxacin disc served as the positive control while 40% methanol as the negative control. Results of zone of inhibition were read and reported in millimeters. The mean and coefficient of variation of replicate values were recorded.

Statistical analysis

The raw data of replicate values of the zones of inhibition were computed using Microsoft Excel (2010). Data were then exported to IBM SPSS Statistical Package (25.0 version) for statistical analysis. The effects of all the four extracts on the isolates, Multiple Comparison Tests within each test sample and concentration against each isolate were analyzed using two-way analysis of variance (ANOVA) adopting Univariate General Linear Model at significant value P <5%.

Quantitative estimation of antimicrobial potency

The following quantitative estimate was performed on the antimicrobial susceptibility testing to determine the percentage and activity index of the extracts, and bacterial susceptibility index of the isolates, as adopted by Rajput and Kumar[34].

Percentage activity

Percentage activity (PA) shows the total antimicrobial potential of a particular extract on test microbial strains or, it demonstrates the number of test isolates susceptible to all concentrations of a particular extract. This ranges from 100 (where all the concentrations of extract tested were effective against all test isolates) to 0 (where all tested concentrations of a particular extract did not exhibit any inhibitory activity against all test isolates).

Mathematically expressed as:

$$PA = \frac{\text{No of isolates susceptible to all tested concentration of a specific extract}}{\text{Total number of concentrations of specific extract tested against each isolate}} \times 100$$

Activity index

The activity index (AI) was calculated to express the relationship between the zones of inhibition of the extract to that of reference antibiotics. When AI is greater than 1, the test extract is better in activity than the reference antibiotic; however, where the result is less than 1, it reveals that the reference antibiotic is better. This is to determine how efficacious the test extracts are in inhibiting test bacteria based on zones ratio.

$$AI = \frac{\text{Mean of diameter of the zone of inhibition with regards to each concentration of extract}}{\text{Diameter of the zone of inhibition of the reference antibiotic}}$$

Bacterial susceptibility index

Bacterial susceptibility index (BSI) is used to compare the relative susceptibility pattern between all isolates tested against each extract. The value of BSI may range from 0 (resistance to all extracts) to 100 (susceptible to all the tested concentrations of extracts). It estimates how susceptible the test bacteria are to the test extracts.

$$\text{BSI \%} = \frac{\text{Number of concentration of extracts effective against each isolate}}{\text{Number of concentration of specific extract tested}} \times 100$$

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) was employed to determine the lowest concentration of an extract that will inhibit the visible growth of an organism after incubation. The MIC was determined using the broth macrodilution method as described by Andrews[36] with slight modifications.

Inoculum suspension and standardization for MIC

Isolates were resuscitated from agar slant as described above. After 18-20 hours of incubation, a loopful of inoculum was transferred into Mueller Hinton broth (Oxoid) and incubated to match 0.5 McFarland standards.

Preparation of test extract for MIC

The test extract was prepared by a double (two-fold) serial dilution in Mueller Hinton broth for a range of 10 concentration gradients (1000 to 1.95 mg/mL). A concentration of 20% (v/v) of methanol was used to prepare the stock solution of organic diluent (methanol extract) which also serve as negative control while sterile distilled water was used as aqueous stock solution diluent. The 20% methanol was used here because of its inability to interfere with the result in MIC, unlike the 40% in agar well diffusion.

Determination of MIC

One milliliter of each test extract was pipetted into sterile test tubes followed by the addition of an equal volume of test isolates. The content of the tubes was thoroughly mixed to achieve an even distribution. For each evaluation, methanol (20% v/v) plus each test isolate (MI) was used as a negative control to ascertain the influence of methanol on the assay; ciprofloxacin plus each test isolate (CI) serve as the positive control. Additionally, another set of control assays were used which include: test isolates plus broth (BI) in one part (to observe the adequacy of the broth in supporting the growth of the test isolates); and extract alone, sterility test (the lowest, 10^{th} concentration gradient, i. e. 1.95 mg/mL) in another part (to check for the sterility of test extract). All tubes including inoculated and uninoculated extract-free tubes were incubated at 37ºC for 24 hours. The MIC endpoint was read as the lowest concentration of test extract at which there was no visible growth.

Determination of MBC

The minimum bactericidal concentration (MBC) is the least concentration at which there was no obvious growth on the agar plate from MIC tube suspension. This was determined by considering the least inhibitory tube (MIC) and other tubes with a higher concentration gradient (that is, the tubes with no visible growth/turbidity). Specifically, a sterilized wire loop was dipped into each corresponding test tube that shows no turbidity, it was then streaked on nutrient agar plates and they were incubated at 37 ºC for 24 hours. At the end of the incubation period, the plates were examined for the presence or absence of growth. MBC was recorded as the least concentration at which no bacterial growth was observed on the plate.

Brine shrimp lethality assay (Cytotoxicity Test)

The cytotoxicity assay was performed to determine the toxicity profile of the test extracts as previously described by Ojewunmi *et al*.[26] with minor modifications. Seawater was obtained from Lagos Bar Beach and cleared off of any obvious impurities by filtering dirties and sand particles.

Hatching of shrimp eggs

A rectangular container with an unequal-internal-demarcation was procured and perforated for the hatching process. Accordingly, the container was half-filled with seawater, and Brine Shrimp Eggs (*Artemia salina* Leach) was gently sprinkled into the smaller compartment of the container. Then, using a blank sheet, the sprinkled side was covered leaving the other side opened. It was expected that the nauplii, upon hatching, would swim to the other light portion as a result of their phototropicity. This procedure was conducted in an undisturbed, well-ventilated and constantly illuminated environment.

After 48 hours of incubation, there were enough freshly hatched nauplii, and these were used for the bioassay.

Performance of brine shrimp lethality assay

In a sterile bottle, 0.05g of each test extract was weighed and 10 mL of seawater was added to make a stock solution of 5000 µg/mL. Potassium dichromate solution was used as a positive control, while seawater served as a negative control. Various concentrations: 1000, 100 and 10 µg/mL of the test extract was prepared from the stock solution. Each test tube contained a final volume of 5 mL each plus 10 nauplii with the aid of a Pasteur dropper, and was carried out in triplicate. The setup was allowed to stand in a ventilated, undisturbed space for 24 hours under constant illumination. After 24 hours of incubation at room temperature, the survived nauplii in each tube assay was counted with a source of light and the average of each of the three (3) tubes was determined. The percentage (%) mortality of the Brine Shrimp nauplii was calculated for each concentration accordingly using the following formula:

$$\% \text{ Mortality} = N_1/N_0 \times 100$$

Where,

N_1 = Total number of killed nauplii after 24 hours of incubation at room temperature

N_0 = Total number of nauplii transferred.

Probit was calculated using the standard probit table. Median Lethal Concentration (LC_{50}) was computed using probit analysis by plotting the mortality rate against dose.

RESULTS

Plant extraction

The total weight of the plant material used, yields, extraction yields and the morphological characteristics of all the four extracts are summarized in Table 3.

Table 3. Percentage yields and morphological observation of *L. inermis* extracts

Parameters	SA	LA	SM	LM
Weight (g [W_1])	450	321	450	321
Yields (g [W_2])	36.8	82.1	83.5	54.8
% yields	8.18	25.58	18.56	17.07
Consistency	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Texture	Gummy/jelly	Gummy/jelly	Neither gummy/nor jelly	Gummy/jelly
Appearance	Light brown	Yellowish-brown	Light brown	Dark brown

SA: Aqueous extract of *L. inermis* seed SM: Methanol extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf LM: Methanol extract of *L. inermis* leaf

It was observed that, despite the least dry weight of plant material used in leaf extraction (321g) compared to seed (450g), aqueous leaf extract (LA) had highest percentage yield (25.58%), and it was observed to be yellowish-brown, while aqueous seed extract (SA), had the least (8.18%). Methanol leaf and seed extract (LM and SM) had similar yield (with 1.5% differences). The consistency of all the extract remained in semi-solid form till the end of the study.

Phytochemical Screening of *L. inermis* extracts

Of all the eleven phytochemical compounds screened, tannins and phenolic compounds were present while steroids were absent in all (Table 4). All extracts had at least five of the screened phytoconstituents. Terpenoids were only detected in both LM and SM. There was an absence of quinones and alkaloids in all extracts excluding SA which also had the highest screened phytoconstituents (9).

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Table 4. Phytoconstituents present in the aqueous and methanol extracts of *L. inermis* (leaf and seed)

S/N	Screening	Reactions	SM	LM	SA	LA
1	Terpenoids (Salkowski's test)	5mL extract + 2mL Chloroform + 3mL conc. H_2SO_4	+	+	–	–
2	Tannins	Extract + few drops 0.1% $FeCl_3$	+	+	+	+
3	Phenolic compounds	1mL extract + 3 drops 5% $FeCl_3$	+	+	+	+
4	Quinones	1mL extract + 1mL NaOH	–	–	+	–
5	Steroids (Salkowski's test)	1mL extract + 1mL H_2SO_4	–	–	–	–
6	Saponins	Extract + H_2O (Shake vigorously)	+	–	+	+
7	Alkaloids	Extract + Chloroform + HCl + allow to stand + Chloroform layer + Dragendoff reagent	–	–	+	–
8	Flavonoids	1mL extract + 3 drops NH_3^+ + 0.5mL conc. HCl	+	–	+	+
9	Cardioglycosides	5mL extract + (2mL glacial acetic acid + a drop $FeCl_3$) + 1mL conc. H_2SO_4	+	+	+	–
10	Proteins	Extract + few drops conc. HNO_3	–	+	+	+
11	Glycosides	Extract + $FeCl_3$ + $boiled_{5mins}$ + cooled + equal volume of benzene + benzene layer separated + NH_3^+	+	+	+	–

SA: Aqueous extract of *L. inermis* seed
SM: Methanol extract of *L. inermis* seed
+: present
LA: Aqueous extract of *L. inermis* leaf
LM: Methanol extract of *L. inermis* leaf
–: absent

Source and percentage occurrence of the multi-drug resistant bacterial strains

A total number of eight (8) MDR and ESβL-producing clinical bacterial strains were obtained for the present study. The percentage occurrences are as follows: urine with 62.5% and others (amniotic fluid, sputum and wound) only had 12.5% each.

Antimicrobial efficacy of *Lawsonia inermis* extracts

The *in vitro* antibacterial activity of *L. inermis* extracts (leaf and seed) and the reference antibiotics against MDR isolates were evaluated based on the presence or absence of a clear zone of inhibition. This is summarized in Table 5. SM had the highest zone of activity against all tested MDR bacteria. It can be noticed from the results that ESβL-producing *Enterobacter aerogenes* 196 was the most sensitive strain with 30.0±0.00 mm zone of inhibition while *Klebsiella oxytoca* U_{30} was the least sensitive with 11.5±0.06 mm. The SA had the highest zone of inhibition (26.5±0.02 mm) against *Pseudomonas aeruginosa* U_{109}, and the least (10.0±0.00 mm) against each of ESβL-producing *E. aerogenes* 196 and *E. cloacae* 195.

The resistance pattern of the clinical isolates was so pronounced as depicted by the results of reference antibiotics (where 62.5% of the isolates were found to be resistant). The leaf extracts (LA and LM) revealed huge variations in their potency. *Pseudomonas aeruginosa* U_{109} was the only susceptible isolate to all tested concentrations of LM; ESβL-producing *Escherichia coli* 190 on the other hand, was only susceptible to LA at 1000 mg/mL (15.5±0.04 mm). As fathomed from the study, the majority of the clinical isolates showed no activity to LA at 250 mg/mL and below, in fact, none was susceptible at 125 mg/mL. As expected, the 40% (v/v) methanol was not active against all isolates.

All the four extracts at different concentrations tested against all isolates differed significantly at $p < 5\%$. In addition, when multiple comparisons of the activity of the extracts and concentrations against each isolate were analyzed, all were found to be statistically significant at $p < 5\%$.

Quantitative estimation of antimicrobial efficacy

As observed from Table 5 below, the percentage activity reveals the totality or effectiveness of an extract to all tested MDR microbial strains. SM showed the most efficacious antibacterial activity against all the multiple drug-resistant and ESβL-producing isolates. That is, the isolates were 100% sensitive to all the tested concentrations of the crude extract. SA gave the second maximum activity (93.75%) against all strains followed by LM (71.88%) while LA was found to possess the least (56.25%) but still better than the reference antibiotic (37.5%).

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Table 5. Antimicrobial Efficacy of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	Conc. tested (mg/mL)	Diameter of zones of inhibition (in mm)* on test isolates								Percent Activity (%)
		ESβL-Producing isolates								
		196	**129**	**190**	**195**	**U_{109}**	**U_9**	**U_{30}**	**U_{87}**	
SA	1000	13.5±0.15	15.0±0.09	21.0±0.06	20.0±0.07	26.5±0.02	22.5±0.03	18.0±0.00	20.0±0.20	93.75
	500	15.0±0.18	14.0±0.10	17.5±0.04	16.5±0.12	21.0±0.06	19.5±0.03	13.5±0.05	11.0±0.12	
	250	11.0±0.00	10.5±0.06	13.5±0.05	10.0±0.00	16.0±0.00	17.0±0.00	13.0±0.00	12.0±0.23	
	125	10.0±0.00	—	13.0±0.00	—	14.5±0.04	13.0±0.10	12.0±0.00	11.0±0.00	
LA	1000	11.5±0.06	14.0±0.00	15.5±0.04	13.0±0.00	13.5±0.05	12.0±0.11	13.0±0.10	11.0±0.00	56.25
	500	11.0±0.00	12.5±0.16	—	10.5±0.06	14.0±0.10	12.0±0.11	11.5±0.06	11.0±0.00	
	250	—	—	—	—	11.0±0.00	11.0±0.12	11.0±0.00	—	
	125	—	—	—	—	—	—	—	—	
SM	1000	30.0±0.00	19.5±0.03	20.0±0.00	22.5±0.03	22.0±0.06	21.0±0.00	18.5±0.03	15.5±0.04	100
	500	28.0±0.00	19.0±0.00	15.0±0.00	22.5±0.03	23.0±0.06	17.5±0.04	18.5±0.03	14.0±0.00	
	250	22.5±0.03	19.0±0.00	19.0±0.00	24.5±0.02	20.0±0.00	16.0±0.00	14.5±0.04	15.0±0.00	
	125	20.5±0.03	12.0±0.11	12.0±0.00	20.0±0.00	16.0±0.00	15.0±0.00	11.5±0.06	13.0±0.00	
LM	1000	14.5±0.04	15.0±0.00	14.5±0.04	16.5±0.04	13.0±0.00	14.0±0.00	16.5±0.12	14.0±0.00	71.88
	500	11.0±0.00	12.0±0.11	15.0±0.00	15.5±0.04	13.0±0.00	15.0±0.18	16.5±0.12	11.5±0.06	
	250	11.0±0.00	10.0±0.00	11.0±0.00	—	14.0±0.00	12.5±0.28	15.0±0.00	—	
	125	—	—	—	—	14.5±0.04	—	—	—	
Control	Cipro (5µg)	7	12	—	18	—	—	—	—	37.5
	Methanol (40% v/v)	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	massive growth	0.0

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L.* inermis
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity

196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
(in mm)*: Mean of replicate value ± coefficient of variation

U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

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The antimicrobial susceptibility patterns of the isolates are presented in Table 6 and 7. Activity index reveals the efficacy of plant extract against MDR bacterial strains in comparison to the reference antibiotic. From Table 6, SM showed a very robust activity index of 4.28 against the ESβL-producing *E. aerogenes* 196 that was isolated from wound, which means that the extract is above four times more effective than the reference antibiotic (1.0). In addition, it was astonishing to find out that all the other extracts (SA, LM and LA) had the highest activity index against the same ESβL-producing *E. aerogenes* 196 that was isolated from wound (2.14, 2.07 and 1.64, respectively). More than 60% of the clinical isolates were resistant to the reference antibiotic, hence difficult to estimate their AI as the ratio of extract to zero (0) will result in a mathematical error, not determinable, thus denoting resistant with 'R'.

The result of the bacterial susceptibility index (BSI) is shown in Table 7. BSI indicates how susceptible an isolate is to all tested treatments. None was 100% sensitive; *P. aeruginosa* U_{109} however, was the most susceptible (93.75%) of all the isolates, followed by *K. oxytoca* U_{30} and *Acinetobacter baumannii* U_9 (87.50%) and ESβL-producing *E. aerogenes* 196 and *E. cloacae* 195 exhibited the least (68.75%).

Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and methanol extracts are presented in Table 8. The least MIC value was found with LM within the range of 1.95-31.25 mg/mL. While it has the least value against *K. oxytoca* U_{30} and the ESβL-producing *E. aerogenes* 196, the highest are against all the other ESβL-producers; and its MBC range from 125-500 mg/mL. SM had MIC value within the range of 1.95-62.5 mg/mL, with the least against *K. oxytoca* U_{30} (1.95 mg/mL) and highest against *P. aeruginosa* U_{109} (62.5 mg/mL) and MBC range of 125-1000 mg/mL. Both aqueous extracts (SA and LA) had similar MIC and MBC range (7.81-250 mg/mL; 125-1000 and 125- >1000 mg/mL, respectively): SA had the least MIC against the ESβL-producing *E. aerogenes* 196 (7.81 mg/mL) and highest against the ESβL-producing *K. pneumoniae* 129 (250 mg/mL), while LA had the least against the ESβL-producing *K. pneumoniae* 129 and *K. oxytoca* U_{30} (7.81 mg/mL) and the highest against the ESβL-producing *E. aerogenes* 196 and *P. aeruginosa* U30 (250 mg/mL).

Table 6. Activity index (AI) of aqueous and methanol extracts of *L. inermis* against MDR clinical isolate

Extract	Conc. tested (mg/mL)	ESβL-Producing organisms							
		196	**129**	**190**	**195**	**U_{109}**	**U_9**	**U_{30}**	**U_{87}**
SA	1000	1.92	1.25	R	1.11	R	R	R	R
	500	2.14	1.16	R	0.91	R	R	R	R
	250	1.57	0.87	R	0.55	R	R	R	R
	125	1.42	—	R	—	R	R	R	R
LA	1000	1.64	1.16	R	0.72	R	R	R	R
	500	1.57	1.04	—	0.58	R	R	R	R
	250	—	—	—	—	R	R	R	—
	125	—	—	—	—	—	—	—	—
SM	1000	4.28	1.62	R	1.25	R	R	R	R
	500	4	1.58	R	1.25	R	R	R	R
	250	3.21	1.58	R	1.36	R	R	R	R
	125	2.92	1.00	R	1.11	R	R	R	R
LM	1000	2.07	1.25	R	0.91	R	R	R	R
	500	1.57	1.00	R	0.86	R	R	R	R
	250	1.57	0.83	R	—	R	R	R	—
	125	—	—	—	—	R	—	—	—

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity

196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
R: Isolate resistant to reference antibiotic

U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

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Table 7. Bacterial susceptibility index (BSI %) of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	ESβL-Producing organisms							
	196	**129**	**190**	**195**	**U_{109}**	**U_9**	**U_{30}**	**U_{87}**
SA	100	75	100	75	100	100	100	100
LA	50	50	25	50	75	75	75	50
SM	100	100	100	100	100	100	100	100
LM	75	75	75	50	100	75	75	50
Total BSI	81.25	75	75	68.75	93.75	87.50	87.50	75

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
196: *E. aerogenes*
129: *K. pneumonia*
190: *E. coli*
195: *E. cloacae*
U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
U_{87}: *S. enterica*

For control, as expected, broth plus inoculum (BI); and 20% (v/v) methanol plus inoculum (MI) showed growth, which, respectively, indicated that the broth supported the growth of the bacteria and the organic diluent (20% methanol) is not the acting principle that's inhibiting the organism. Ciprofloxacin plus inoculum (CI) showed variation with susceptibility and resistant pattern. The sterility test (extracts only), despite being the least concentration (the 10^{th} gradient i.e. 1.95 mg/mL) revealed that the test extracts are free from bacterial colonization, hence sterile.

Brine shrimp lethality assay (cytotoxicity test)

Cytotoxicity assay reveal the toxicity profile of the extract (Table 9). It displays the percentage mortality of the shrimp, probit and LC_{50}. The LC_{50} is the least concentration at which 50% of the test organisms die. The LC_{50} of both aqueous extracts (LA and SA) is above one thousand ($LC_{50} > 1000$), which means that the extracts are safe, while the methanol extracts (LM and SM) is less than one thousand ($LC_{50} < 1000$). The positive control ($K_2Cr_2O_7$) had LC_{50} of 10, while the negative control (seawater) did not affect *Artemia salina.*

Table 8. MIC and MBC of aqueous and methanol extracts of *L. inermis* against MDR clinical isolates

Extract	Conc (mg/mL)	ESβL-Producing organisms								
		196	**129**	**190**	**195**	**U_{109}**	**U_9**	**U_{30}**	**U_{87}**	**Sterility**
SA	MIC	7.81	250	15.63	15.63	125	62.5	15.63	62.5	—
	MBC	500	500	1000	1000	500	125	125	500	NG
LA	MIC	250	7.81	15.63	15.63	250	62.5	7.81	31.25	—
	MBC	1000	1000	1000	>1000	1000	500	125	500	NG
SM	MIC	31.25	15.63	31.25	31.25	62.5	7.81	1.95	15.63	—
	MBC	500	1000	500	500	250	125	125	500	NG
LM	MIC	1.95	31.25	31.25	31.25	15.63	15.63	1.95	15.63	—
	MBC	250	250	250	250	250	500	125	500	NG
Control	CI	G	G	NG	G	G	G	G	G	NA
	MI	G	G	G	G	G	G	G	G	NA
	BI	MG	MG	MG	MG	MG	MG	MG	MG	NA

SA: Aqueous extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
SM: Methanol extract of *L. inermis* seed
LM: Methanol extract of *L. inermis* leaf
—: no inhibitory activity
NG: No growth
MI: Methanol + test isolate
196: *E. aerogenes*
129: *K.* pneumonia
190: *E. coli*
195:*E. cloacae*
U_{87}: *S. enterica*
G: Growth
BI: Broth + test isolate
U_{109}: *P. aeruginosa*
U_9: *A. baumannii*
U_{30}: *K. oxytoca*
NA: Not applicable
MG: Massive growth
CI:Cipro + test isolate

Table 9. Brine shrimp lethality assay of aqueous and methanol extracts of *Lawsonia inermis*

Extract	Concentration	Numbers of survived nauplii			No. of Dead nauplii	% Mortality	Probit	LC_{50}
		1st test tube	2nd test tube	3rd test tube				
SM	1000	0	0	0	30	100	8.09	27.799
	100	7	5	6	12	40	4.75	
	10	5	7	3	15	50	5.00	
LM	1000	6	6	2	16	53.33	5.08	942.640
	100	7	5	10	8	26	4.36	
	10	9	8	7	6	20	4.16	
LA	1000	8	7	7	8	26.67	4.36	37735179.299
	100	7	5	6	12	40	4.75	
	10	9	8	7	6	20	4.16	
SA	1000	7	6	3	14	46.67	4.90	8317.419
	100	8	8	7	7	23.33	4.26	
	10	9	6	6	9	30	4.48	
$K_2Cr_2O_7$	1000	0			10	100	8.09	10
	100	0			10	100	8.09	
	10	5			5	50	5.00	

SA: Aqueous extract of *L. inermis* seed
SM: Methanol extract of *L. inermis* seed
LA: Aqueous extract of *L. inermis* leaf
LM: Methanol extract of *L. inermis* leaf
$K_2Cr_2O_7$: Potassium dichromate

DISCUSSION

The emergence of antibiotic resistance has necessitated the continuous search for new and effective antibiotic alternatives to battle the menace of antimicrobial resistance, worldwide. This can be observed in the urge for continuous investigation of traditional medicines to exploit for safe and effective remedies of microbial and non-microbial ailments[9,38]. This study, therefore, elucidated the therapeutic potential of an indigenous plant, *Lawsonia inermis* extracts to combat multidrug-resistant and ESβL-producing clinical bacteria. To benefit from the usage of long-lasting medicinal plants in the treatment of infectious diseases, as experienced in folkloric medicines, it is essential to mimic, to the maximum possible extent, the traditional method employed[8,10]. It is for this reason, therefore, that the present study followed the ethnobotanical survey as documented by Idowu[25] that substantiated that many individuals use water and alcohol with this plant, but majority prefers water. This was supported in the work of many other researchers[21,39-42] that utilised methanol and aqueous extraction; hence, the choice of solvents employed in our study.

Furthermore, documented by Heinrich *et al*.[8], the success rate of extraction depends on the initial preparation process – the size of the biomass particles. With this in mind, the plant materials were milled twice, first by coarse mill and then a fine mill to generate a fine powder as large particles usually result in poor extraction, whereas small particles do have higher surface area and will therefore be extracted more efficiently. Thus, the application of the cold maceration technique in our investigation corroborated the traditional mimicry, as cold maceration, which allows for soft extraction, has been found to retain most, if not all of the phytoconstituents present in plant materials[8,10]. The variation in percentage yields as depicted in Table 3 could be attributed to different plant parts and solvents used[8]. The leaf aqueous extract showed the highest extraction yields which demonstrated that its constituents are relatively polar, and buttress the artistic preparation process of the plant, thereby supporting the preference of water as solvent of choice in traditional practice.

Phytochemical screening of *L. inermis* extracts revealed the presence of tannins and phenolic compounds in all test extracts, and this is in harmony with the work of Gull *et al*.[20] who also detected these compounds in their study. However, in contrast to their report and that of Usman and Rabiu[43] who reported non-detection of protein as well as alkaloids

in all of their crude extracts, because proteins and alkaloids were detected in our study. The latter detected steroids in their study and this disagrees with our study as there was absence of steroids in all the extracts. In addition, the report from Ali et al.[33] corroborates our study with the presence of glycosides in three of our extracts. All variations, as observed, are tenable as the extraction of phytochemicals has been reported to be affected by pre-extraction factors: plant part used, its location and particle size, method of drying, diurnal and seasonal variation, degree of processing, among others; and extraction-related factors – extraction method adopted, solvent chosen, solvent-to-sample ratio, pH and temperatures of solvent, and length of extraction[11,44].

The phenolic compounds observed in this study may be responsible for the antimicrobial properties exhibited by *L. inermis* extracts as these compounds have been reported to enhance antimicrobial activity against resistant pathogens through mechanisms of action that are not limited to inhibiting and reducing the activity of the efflux pump and interacting with some crucial enzymes that are precursors of the bacterial cell membrane[45]. Tannins, detected in all the screened extracts, have been documented to bind microbial proteins thereby inhibiting protein synthesis[46]. In addition, tannins are astringent and are used for treating intestinal disorders such as diarrhoea and dysentery thus exhibiting antibacterial activity[47]. Tannins are also widely used in traditional medicine in treating wounds and arresting bleeding[48]. The presence of glycosides moieties like saponins, anthraquinones, cardiac glycosides, and flavonoids are known to inhibit tumor growth and serve also to protect against gastrointestinal infections[47], this supports the ethnobotanical use of *L. inermis* to treat different gastrointestinal diseases. Cardioglycosides are active principle that functions in blocking the channels regulating the electrochemical state of heart muscle cells. One of the effects of this activity is the generation of increased pressure in the heart's pumping ability. Plants that possess these phytoconstituents have been used in the treatment of dropsy, a condition also called oedema[49]. The presence of these secondary metabolites is of pharmacognostic importance and this gives credence to the use of Henna in ethnomedicine.

Antimicrobial efficacy of different *L. inermis* extracts against eight (8) multidrug-resistant and ESβL-producing clinical isolates depict different bioactive compounds, and on that basis, variation in their antimicrobial potency. This variation has been documented by other researchers[20,21,24,50]. The variation in the activity of crude extracts is probably due to the different solvents used as well as plant parts that yield varieties of bioactive compounds. Many previous studies indicated that medicinal plant extract contains several phytochemicals that synergistically show remarkable antimicrobial properties against MDR organisms[9,10,21,34,51]. This might be because of the holistic formation of these complex bioactive compounds that synergistically modulate multiple targets to produce overall inhibitory actions[52]. Oftentimes, the bioassay-led method of investigation narrowing activity to a single compound fails because, often times, activities are lost during fractionation[8,9,10,38]. Therefore, the synergistic combination of different phytocompounds as observed in our investigated plant extracts might be responsible for the antibacterial activity.

Only a study[24] has been documented on antimicrobial activity of *L. inermis* seed, and found minimal activity compared to its leaf. However, we reported the best antimicrobial activity of seed methanol extracts against all the MDR bacteria for the first time. SA, as well as LM showed high antimicrobial potential against the multiple drug-resistant bacterial strains. Although previous studies have documented antimicrobial potential of leaf extract of *L. inermis*; methanol extract showed broad-spectrum antibacterial activity against *P. aeruginosa, E. coli,* MRSA, and MDR *E. coli* [21]. Gull *et al*.[20] employed the cold maceration technique, as used in our study, recorded good antimicrobial activity of all four (4) tested extracts (methanol, chloroform, aqueous, and acetone) against all bacteria strains used in their study. However, Elgailany and Elnin[21] reported inactivity of leaf aqueous extract at all tested concentrations (50, 25, 12.5, and 6.25%) against *E. coli* and MDR *E. coli* strains. In a like manner, Habbal *et al*.[24] documented better activity or higher antimicrobial activity of dry and fresh leaves of *L. inermis* than its seeds. This is not inconsonant with our findings, we revealed that seed extracts, which had the highest numbers of phytochemicals, exhibited the most profound and remarkable antibacterial activity against all tested strains.

The variation reported in the two studies might be as a result of the Soxhlet apparatus and water bath respectively used in extraction procedure that is likely to have denatured the heat-labile active principle that is expected to be present in order to

have antimicrobial effect. Rani and Khullar[39] and Sharmeen *et al*.[53] also reported the ineffectiveness of aqueous extract against all tested strains in their study, but the method of extraction was not disclosed in the latter's report. A similar hot method of extraction, Soxhlet, was employed by Kannahi and Vinotha[41] and Rotary evaporator by Al-Rubiay *et al*.[40] and these researchers also reported inactivity of their aqueous extract against all tested isolates. By cold-macerating and freeze-drying our aqueous extracts, we might have preserved most, if not all of the bioactive compounds, hence evident of robust activity recorded in our study. This therefore suggests that, as much as possible, a mild extraction method should be employed, most importantly, if the crux of the study is to derive and buttress the ethnomedicinal benefit, as demonstrated in an 'AncientBiotic' research[10].

Shahabinejad and Kariminik[42], who employed the cold maceration technique, as done in our investigation, reported good antibacterial activity of *L. inermis* extracts against all fifty (50) uropathogenic bacterial strains. Worthy of note from their study, *Acinetobacter, E. aerogenes*, MDR *E. coli*, MDR *P. aeruginosa*, and MDR *K. pneumoniae* showed varying zones of inhibition which ranged from 10-30 mm. This corroborates our findings that contribute to the robust antibacterial activity of the extracts against MDR pathogens isolated from urine with zone of inhibition ranging from 11±0.00-26.5±0.02 mm. Additionally, Aqil and Ahmad[54] evaluated the antibacterial potency of this plant extract against some standard and MDR bacteria and observed *L. inermis* to possess impressive activity against all the eight tested isolates ranging from less than 10 mm to above 40 mm.

In addition to extraction technique, solvent and part of the plant used outlined above, other factors that might have cumulated to the discrepancy in result include, but not limited to, *in vitro* antimicrobial method employed; variation in phytochemicals of the extract; density and size of the inoculum; concentration of test extract; volume of test extract pipetted in agar well or disk; temperature and diffusion period before incubation, composition of medium and incubation temperature[21,55]. Furthermore, a recent study shows that extracts of *L. inermis* demonstrated interesting antimicrobial activities at increasing concentrations[50] as noted in our investigation. We felt that the multiple drug-resistance properties of the test organisms might have contributed to activity at increase dose because from our preliminary lab demo, we observed that if a plant would not be active, even at a similar increase dose, it would still be ineffective.

The sensitivity of the MDR bacteria to the test extracts differed significantly at $P < 0.05$, which indicates the likely different mode of action in respect to the extract and individual bacterium. Also, the phytocompounds might have acted differently based on the multiple drug-resistant strains as opined by Aqil and Ahmad[54].

It was indeed, in reality, astonishing to figure out that all the four extracts had the highest activity index against the ESβL-producing *E. aerogenes* 196 that was isolated from wound. The AI is a qualitative index to evaluate the efficacy of the test extract to the reference antibiotic. This is to say, when the AI is less than 1, it shows that the reference antibiotic possesses good activity than the test extract. However, when AI is greater than 1, it shows that the extract has better activity against the isolates than the reference antibiotic. All extracts indicated tremendous activity index, in particular, SM was above 4 – which means the extract is more than four times better than the reference antibiotic. It, in addition, suggests that the seed methanol (SM) could be better employed in the treatment of wound and skin-related diseases. Furthermore, our findings elucidate one of the most widely recognized ethnobotanical usages of the Henna plant in the management of wounds and other skin-related diseases. Our report attests to the remarkable wound healing potential of *L. inermis* which has also been mentioned by several other researchers[22,33,40,41,43,50]. Recently, Daemi *et al*.[56] elucidated the wound healing mechanism of *L. inermis*, and observed that their extract healed better than their control group.

The present study recorded some varying MIC and MBC values against the MDR isolates. MIC is generally defined as the lowest concentration of extract that inhibits the growth of the test organism[57]. Majority of the MIC of the four extracts recorded in the present study is within the range of 7.81-62.5 mg/mL for both aqueous extracts (SA and LA) and 1.95-31.25 mg/mL for methanol extracts (SM and LM). We established that the methanol extracts (LM and SM) had a better inhibitory potency on the isolates by exhibiting the least MIC values (1.95 mg/mL). This is in contrast to Al-kurashy *et al*.[58] whose MIC values for aqueous and alcoholic extract are within the range of 8-64 mg/mL and 32-64 mg/mL, respectively, for the following non-resistant organisms – *E. coli, S. aureus, P. aeruginosa* and *E.*

faecalis. The wide variation in the MIC and MBC values as observed in this study might be as a result of the invincible acquired multiple drug-resistance capability of the test isolates. Moreover, the presence of various phytoconstituents and their combined activity as well as the intrinsic tolerance of the individual MDR test bacterium as earlier stated by Aqil and Ahmad[54] might have also played a major part, as different bacterium acts differently to test samples. Thormar[57] postulated that the MIC values of antimicrobial agents attracting the most attention are the ones that inhibit or kill bacteria *in vitro* at concentrations below 1% vol/vol (10000 ppm). However, it has also been put forward that the *in vitro* result does not usually correspond to the *in vivo* situation, in which antibacterial and bacterial concentrations in different body fluids and tissues may fluctuate widely[11], hence not making those values the absolute constant. Besides, being an MDR isolates, higher concentrations of MIC or MBC might not be far-fetched.

Artemia salina has been well established first by Michael *et al*.[59] and Meyer *et al*.[60] and several others later[26,61,62] as a general biological assay, convenient for active plant constituents. Specifically, it was proposed as a simple bioassay for natural product research. In our findings, the Brine Shrimp Lethality assay showed that the aqueous extracts of *L. inermis* (SA and LA) which are greatly above 1000 (LC_{50} > 1000) infer that they are very much safe[60,63]. This report is in unison with Ojewunmi *et al*.[26], in their ethanol extract, who documented that the plant is safe and non-toxic. LM was virtually non-toxic on the shrimp with over 940 as LC_{50}[63]. While the SM was, however, found moderately toxic to the nauplii (LC_{50} = 27.799)[64], it was less toxic compared to the control (LC_{50} = 10). Extracts of alcohol or organic solvent are often seen moving more toward toxicity compare to aqueous that is recounted to be less toxic[38,65], this is demonstrated in our study.

CONCLUSION

With the current increase in hard-to-treat infections due to the global mess of antibiotic resistance, the present study investigates the antimicrobial potential of an indigenous plant, *Lawsonia inermis* (Henna), against multidrug-resistant bacteria including ESβL-producers. Our investigation reveals that Henna seed extracts (SM and SA) exhibited the highest antibacterial activity against all tested MDR bacteria than the leaf extracts, which also had the highest number of screened phytochemicals. In addition, *L. inermis* showed good activity not only against the wound- and UTI-causing bacteria; but also against sputum- and amniotic fluid-implicated organisms. The extracts, aqueous in particular, are non-toxic and very safe. Our findings further demonstrate the potential of *L. inermis* in the treatment of MDR-related infectious diseases and provide scientific rationale for medicinal use of this plant. This therefore suggests that "Laali", as it is commonly called in Yoruba, could be used as a cheap and potential strategy to manage infections, when compared to ineffective conventional antibiotics.

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ORIGINAL RESEARCH

Impact of Lavender Herbal Tea on Sleep Quality in Elderly Patients with Poor Sleep Quality: A Randomized Study

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Abstract

Objective: Aromatherapy has been used as a complementary alternative therapy in elderly adults with poor sleep quality. Lavender has sedative, anxiolytic, and analgesic properties. This study aimed to evaluate effect of lavender herbal tea in different doses on sleep quality of elderly people**.**

Material-Method: This study has been designed as a prospective, randomized study with a two-arm parallel design. There were 94 patients aged between 65 and 75 years with a Richards-Campbell Sleep Questionnaire (RCSQ) score of <75. Patients were sequentially randomized into two groups as 1 g and 2 g lavender tea bags used for three months. Demographic and clinical characteristics were recorded. The RCSQ was administered initially and during the 1st-month and 3rd-month follow-up visits.

Results: There was no significant difference between groups in demographic and clinical characteristics ($p>0.05$) or in terms of baseline RCSQ scores ($p=0.685$). However, 1st-month and 3rd-month RCSQ scores in patients who used 2 g lavender tea bags were significantly higher than those who used 1 g herbal tea bags ($p<0.001$ and $p<0.001$, respectively). Additionally, the 1st-month and 3rd-month RCSQ scores were significantly higher than baseline RCSQ scores in both groups ($p<0.05$).

Conclusion: Our findings revealed that lavender herbal tea improved sleep quality in elderly patients with sleep problems. Consumption of higher doses of lavender tea (2 g vs. 1 g) resulted in significantly higher RCSQ scores. Therefore, use of lavender may be recommended in individuals with sleep problems in form of herbal tea preparations.

Keywords: Aromatherapy, Lavandula/Levander, Herbal Tea, Elderly, Sleep Quality.

INTRODUCTION

Elderly adults with chronic insomnia usually suffer from poor sleep quality[1,2]. There is a direct correlation between age and the prevalence of sleep problems[1]. Deterioration of sleep quality leads to several physical and psychological problems, and different types of treatment modalities, including behavioral and cognitive therapies and sleep hygiene practices, and pharmacological therapy, have been recommended to overcome these problems[1,2]. Traditional and herbal therapies have recently gained popularity in this regard[1,3].

Aromatherapy has been used as a complementary alternative therapy to manage stress, muscle spasms, and sleep disturbances[4,5]. Essential oils of the aromatic plants can be produced via the steam distillation of their flowers' heads and leaves[6]. Inhalation, massaging, and bathing in the extracted essential oils are the frequently used aromatherapy methods[4]. Lavender has been used due to its sedative, anxiolytic, and analgesic properties[2,7-11]. Previous studies reported improvements in mood and sleeping problems after lavender use[12,13]. Lavender is also used to treat depression and anxiety[14]. There are different approaches regarding lavender use, utilizing respiratory, gastrointestinal, or cutaneous routes[7]. The use of its inhalable forms has reportedly improved sleep quality and increased sleep duration[6]. The use of lavender was also associated with a reduction in depression and insomnia, relief of anxiety, and calming of the mind[2,7]. It is believed that herbal tea exerts its effects in several psychological and physiological ways[7]. The scent of lavender herbal tea activates the limbic system, promoting the release of different types of neurotransmitters such as encephalin, endorphin, noradrenaline, and serotonin. These neurotransmitters may trigger changes in human emotions[7]. On the other hand, it has been speculated

that the risk of neurotoxicity and hepatic, renal, and cutaneous toxicity during the application of an essential oil via inhalation, massaging, and bathing can be higher than the risks associated with the consumption of herbal tea of the same aromatic plant[15]. In other words, consumption of herbal tea of any aromatic plant may have lower risks of side effects and allergic reactions and milder effects overall, compared to the methods of aromatherapy involving the administration of the essential oil of the same aromatic plant[2]. The relationship between lavender aromatherapy and sleep quality has been studied in diverse populations[4,16]. However, there is still some controversy on the efficiency of herbal tea in elderly people with sleep disturbances. In this context, the objective of this study is to evaluate the effect of the consumption of lavender herbal tea in different doses on the sleep quality of elderly people.

MATERIALS AND METHODS

Research design

This study has been designed as a prospective, randomized study with a two-arm parallel design to investigate the effect of the consumption of lavender tea in two different dosages on the sleep quality of elderly people. The protocol of this study was approved by the Ethical Committee of Istanbul Medipol University (date:26.10.2021, no:1046). This study was carried out in accordance with the principles set forth in the Declaration of Helsinki. Informed consent of the patients who participated in the study was obtained in advance.

Population and sample

The study population comprised the patients admitted to the outpatient clinics of Internal Medicine and Physical Therapy and Rehabilitation at Istanbul Medipol University Hospital. The study sample consisted of the patients who were a) aged between 65 and 75 years and literate, b) without lack of communication problems, c) with a Richards-Campbell Sleep Questionnaire (RCSQ) score of <75, and d) with normal cognitive functions. Patients, who were allergic to any herbal tea or lavender, had severe sleep disorders and have been receiving treatments for these disorders, have been using anti-depressive and anti-anxiety drugs, had anemia requiring parenteral treatment, severe comorbidities, including coronary artery disease, congestive heart failure, hypo-or hyperthyroidism, and alcohol abuse were excluded from the study. RCSQ was used to identify the patients with sleep problems. RCSQ is a diagnostic tool used to evaluate the quality of sleep. Richards developed this five-item self-report questionnaire in 1987[17]. The questionnaire initially had five items, including sleep depth, latency, frequency of awakenings, time awake, quality of sleep, and subsequently was adapted to include a sixth item, that is, the perceived noise level in the environment during the night, to assess the quality of night sleep. The patients responded to each item using a visual analog scale ranging from zero to 100. Scores less than 75 indicate poor sleep quality[18]. Karaman and Ozer carried out the questionnaire's Turkish validity and reliability studies in 2015[18].

Sample size

A pilot study was performed with 20 people who were divided into two groups based on the use of 1 g and 2 g lavender tea bags. The analysis of the RCSQ scores of these 20 participants revealed a 33% difference between the groups in the percent changes between the baseline and 3rd-month RCSQ scores. Accordingly, the sample size was calculated as42±39.6 for Group 1 and 42±63.9 for Group 2. The type I error (α value) was 0.05, and the power of the study (1-β) was 80%. A 10% drop-out rate was factored in, resulting in 47 participants per group (94 participants in total). The sample size calculation was performed using MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Consequentially, patients (n=94) were sequentially randomized into two groups, with 47 patients in each group. Patients in Group 1were provided 1 g lavender tea bags, whereas the patients in Group 2 were provided 2 g lavender tea bags.

Interventions

All patients were instructed to drink one cup (200 ml) of lavender tea preparations prepared using 1 g lavender tea bags in Group 1 and 2 g lavender tea bags in Group 2within the last hour before going to sleep for three months. The patients were also advised to inhale the scent of lavender. The herbal teabag preparations contained the flowers of *Lavandula intermedia* and were steeped for 10 minutes before drinking. A total of 90 teabags were provided to each participant, and their consumption of the tea bags was checked at one-month intervals.

Variables

The patients' demographic (age, gender) and descriptive characteristics (educational and marital status, comorbidities) were obtained during the first time they were interviewed face-to-face. The RCSQ

was administered to the patients in a quiet and comfortable room a total of three times; at the start of the study (RCSQ-baseline) and one month (RCSQ-1), and three months after the start of the study (RCSQ-3).

Blinding

The patients and the researcher who assessed the questionnaires were blind to the groupings.

Statistical analysis

The RCSQ-1 and RCSQ-3 scores were the primary outcomes of the study. The secondary outcome was the percent (%) changes observed between the RCSQ-1 and RCSQ-3 scores and the RCSQ-baseline scores.

Descriptive statistics were expressed as mean ± standard deviation values in the case of continuous variables that were determined to conform to the normal distribution, and as median and minimum-maximum values in the case of continuous variables that were determined not to conform to normal distribution. Categorical variables were expressed as numbers and percentages. The normal distribution of the numerical variables was analyzed using the Shapiro-Wilk test. The student's t-test was used to compare two independent groups with numerical variables that conform to the normal distribution, whereas the Mann-Whitney U test was used to compare two independent groups with numerical variables that do not conform to normal distribution. The Pearson's chi-squared test was used to compare the differences between categorical variables in 2x2 tables. The Fisher's Exact test with Yates continuity correction was used in the analyses where the Pearson's chi-squared test could not be used. The Friedman test was used to analyze more than two continuous variables that do not conform to normal distribution. In the next step, the Post-Hoc analysis was performed using the Wilcoxon-Signed Rank test with Bonferroni correction to uncover the significant differences between the variables.

MedCalc® Statistical Software version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021) was used in all statistical analyses. Probability (p) values$\leq$ 0.05 were deemed to indicate statistical significance.

RESULTS

The mean ages of the patients in Groups 1 and 2 were calculated as 68.9±2.9 and 68.9±3 years, respectively (p=0.985). There was no significant difference between the groups in gender (p=0.999) or in other demographic and clinical characteristics (p>0.05) (Table 1).

Table 1. Demographic and clinical characteristics of the study groups.

	Group 1 (n=47)	**Group 2 (n=47)**	**p-value**
Age (year)	68.9±2.9	68.9±3	0.985*
Sex			
Female	28 (59.6)	27 (57.4)	0.999**
Male	19 (40.4)	20 (42.6)	
Marital status			
Married	31 (66)	29 (61.7)	0.830**
Single	16 (34)	18 (38.3)	
Educational status			
Primary	12 (25.5)	11 (23.4)	0.914***
College	17 (36.2)	16 (34)	
University or higher	18 (38.3)	20 (42.6)	
Comorbidities			
Hypertension	24 (51.1)	23 (48.9)	0.999**
Diabetes mellitus	16 (34)	17 (36.2)	0.999**
Gastritis	20 (42.6)	22 (46.8)	0.836**
Osteoporosis	21 (55.3)	19 (40.4)	0.835**

*: Mann-Whitney U test, **: Yates's continuity correction test, ***: Chi-square test

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The mean RCSQ-baseline scores were calculated as52.5±7.5 and 53.1±7 in Groups 1 and 2, respectively. The difference between the mean RCSQ-baseline scores of the groups was insignificant (p=0.685). However, there were significant differences between the RCSQ-1 and RCSQ-3 scores of the groups (p <0.001 and p<0.001, respectively). The RCSQ-1 and RCSQ-3 scores of Group 2 were significantly higher than those of Group 1 (Table 2).

Table 2. The Richards-Campbell Sleep Questionnaire scores and their changes during the study.

	Group 1 (n=47)	**Group 2 (n=47)**	**p-value**
Baseline	51.7 [40.0- 65.8]	51.7 [40.8- 65.8]	0.685*
1st month	58.4±6.9	66.7±7.5	<0.001*
3rd month	66.8±7.8	76.4±10.5	<0.001**

*: Mann-Whitney U test, **: Student t test

There were also significant differences between the groups in percent (%) changes observed between the RCSQ-1 and RCSQ-3 scores and the RCSQ-baseline scores. Significant increases were recorded in the RCSQ-1 and RCSQ-3 scores compared to the RCSQ-baseline scores in both groups (p<0.001) (Table 3) (Figure 1).

Table 3. Comparison of the percent (%) changes in the Richards-Campbell Sleep Questionnaire scores between different study intervals

	Group 1 (n=47)	**p-value**	**Group 2 (n=47)**	**p-value**
1st month-baseline	9.4 [-6.3-41.9]	<0.001	25.8 [-2.6-70]	<0.001
3rd month-1st month	13.6 [-4.7-35.4]	<0.001	13.6 [-3.5-50]	<0.001
3rd month-baseline	14.9 [-15.1-54.7]	<0.001	28.3 [-14.7-97.6]	<0.001

§: median [min-max], Friedman test with Bonferroni correction

The most significant change was recorded in Group 2 between the RCSQ-3 and RCSQ-baseline scores. The patients in the study groups reported no side effects related to lavender herbal tea usage.

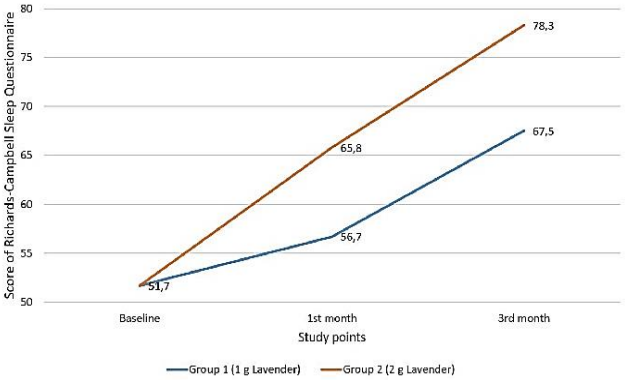

Figure 1. Trends of Richards-Campbell Sleep Questionnaire with 1 g and 2 g of lavender tea.

DISCUSSION

The findings of this study revealed that lavender herbal tea improved sleep quality in elderly patients with sleep problems. The consumption of a higher dose (2g) of lavender tea resulted in more improvements in sleep quality compared to the consumption of a lower dose (1g).

It is a known fact that anti-depressant and anxiolytic medications administered for sleep problems have considerable side effects. The effects of aromatherapy have been studied previously in the context of depression, anxiety, and sleep problems, taking the detrimental effects of such therapies into consideration[7,8]. Although the relevant outcomes show variations depending on the types of aromatic plants studied, the application routes utilized, the sleep-quality measurement tools used, and the characteristics of the study groups, there is a widespread belief that aromatherapy causes relief in the symptoms of depression, anxiety, and poor sleep quality and makes the patients feel good[2,19].

Herbal tea is a traditional form of using aromatic plants[20]. Other forms of use include essential or volatile oils, tinctures, liquid alcoholic extracts, capsules, chewing tablets, lozenges, lollipops, and creams. Although the stability of each form has not been studied in detail, herbal tea bags were preferred as the form of aromatic plants in this study merely based on convenience, considering that they are both inexpensive and easy to use[20].

Previous studies revealed the beneficial effects of lavender preparations, including herbal tea, on depression and poor sleep quality. These effects

were attributed to the ingredients of lavender that act on various neurotransmitters[6,7,21,22]. Several studies reported significant improvements in sleep quality with the use of different Levander preparations[3,10,16,23-30]. However, there are only two studies that investigated the effect of lavender herbal tea on sleep quality[2,7]. In one of these two studies, Bazrafshan et al.[7] investigated the effect of lavender herbal tea on depression and anxiety scores in an elderly group and observed significant improvements after consumption of 2 g lavender herbal tea bags for two weeks. In the other study, Chen et al.[2] investigated the effect of using 2 g lavender tea bags for two weeks on fatigue, depression, and sleep quality in women with sleep disturbances during the postpartum period, yet did not observe any improvement in the sleep quality of the participants. In Chen's study, the positive effect of lavender herbal tea initially observed on postpartum depression was short-lived and became insignificant after four weeks[2]. In comparison, in this study, two different doses of lavender tea were used (1 g and 2 g tea bags) and for a more extended period (three months). Consequentially, significant improvements were observed at the end of three months with the use of both 1 g and 2 g doses. From among the two doses, the use of 2 g lavender tea bags resulted in higher increases in the RCSQ scores compared to the use of 1 g lavender tea bags. Jager et al.[31] did not detect lavender in the blood after 90 minutes of the consumption of the lavender tea. Based on this result, they concluded that the metabolic effect of the herbal tea form of the lavender might be less than its essential oil form given the trace amount of the aromatic molecules in herbal tea preparations. Therefore, multiple daily consumptions of lavender tea are needed to achieve a long-lasting effect.

In addition to studies in which a positive relation was found between the use of lavender preparations and the relief observed in the symptoms of depression and anxiety scores, there are also studies that reported no improvements in the anxiety levels with the use of lavender tea[6]. To give an example, Seifi et al.[11] performed a 2-day intervention using lavender essential oil inhalation in patients who underwent coronary artery bypass graft surgery and found no improvement in anxiety scores three days after the surgery. Hence, this study's authors believe that the duration of the intervention and the route of lavender application is of primary importance in achieving desired outcomes.

Limitations of the study

It is known that there are reciprocal relationships between depression, anxiety, and poor sleep quality; however, only sleep quality was assessed in this study. Secondly, the sleep quality measurements of the patients were carried out right after the patients finished using the lavender tea. If measurements could be repeated after a certain period, it could have been possible to assess how long the effects of the lavender tea have lasted. Selecting the patients from a single center was another limitation. Additionally, patients' adherence to herbal tea consumption was not measured, and it was assumed that the patients used lavender tea as instructed. It is clear that any non-adherence might have negatively affected the results.

CONCLUSION

In conclusion, the findings of this study revealed that lavender herbal tea improved sleep quality in elderly patients with sleep problems. Consumption of higher doses of lavender tea (2 g vs. 1 g) resulted in significantly higher RCSQ scores. Therefore, the use of lavender may be recommended in individuals with sleep problems in the form of herbal tea preparations.

Author contributions: Conceptualization, Writing: [EY]; Investigation, Data collection: [SY]

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ORIGINAL RESEARCH

Investigation of the Acute Subacute Toxicity of KL$^{21®}$ Supplementary Food Product in Rats

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Abstract

Objective: KL$^{21®}$; It is a food supplement using remember regeneration therapy method (RTM). KL$^{21®}$. Whereas products that containing combined medicinal plants are expected to be beneficial for health, on the other hand, their toxic properties are able to potentially increase due to the interactions of the active ingredients in the plants. With this study, it was aimed to investigate the toxic effect of KL$^{21®.}$

Material-Method: In the study conducted with control (n: 8), acute (n: 8), subacute (n: 8) and post-subacute (n: 8) groups; the daily dose of the product was tested by gavage in 8-week-old female Wistar rats. Biochemical parameters P, Ca, ALB, TG, TP, TC, Creatinine, Bilirubin, GGT, ALP, AST and ALT were analyzed. In hematological parameters; WBC, RBC, PLT, HCT, HGB were examined. Liver, lung, spleen, kidney and heart tissues were investigated histopathologicaly. Clinical observation was made throughout the entire process.

Results: When the acute, subacute and post-subacute groups were compared to control, it was observed that there was no significant difference in biochemical, hematological, histopathological terms. No toxicity-related side effects were found in clinical observations.

Conclusion: The potential toxic effect of daily use dose of KL$^{21®}$ containing the combined medicinal plant was investigated in vivo. According to the hemogram, biochemistry and pathology tests, it was determined that it does not show acute and subacute toxicity.

Keywords: KL$^{21®}$, Food Supplement, Acute, Subacute, Post-Subacute Toxicity

INTRODUCTION

The interaction of humanity with plants continues from the existence to the present day. In numerous myths cited by many written or verbal sources, plants appeared to be frequently involved in a wide variety of uses. According to the archaeological findings from the early ages, humans primarily used plants to obtain nutrients and troubleshoot health problems. These experiences, which have been obtained through trial and error, have reached today with some changes and developments in the usage patterns throughout the ages[1].

Traditional methods applied to protect health and treat diseases among the people throughout the ages are called folk medicine/folk medicine/folkloric medicine/medical folklore. When modern medicine is researched up to date, it merges with folk medicine and primitive treatment methods constitute the folkloric side of modern medicine. The interactions of humans with therapeutic properties of plants have evolved from folk medicine to modern medicine and turned into Phytotherapy. In many parts of the world and in Anatolia, miraculous healing power of plants has been used in the fight against diseases throughout the history. Drugs were obtained from plants alone or in combinations.

Remember regeneration therapy method (RTM) deals with the whole of combination complementary-traditional medicine and is not just a part of method[2]. KL$^{21®}$ herbal extract that is used

for remember regeneration therapy method (RTM) includes thistle seeds (*Silybum marianum* (L.) Gaertn.), rosemary (*Rosmarinus officinalis L.*), ginger rhizome (*Zingiber officinale* Roscoe), fumitory (*Fumaria officinalis* L.), chicory (*Cichorium intybus* L.), nettle (*Urtica dioica* L.), yarrow perch (*Achillea millefolium* L.), thyme (*Thymus vulgaris* L.), horsetail (*Equisetum arvense* L.), turmeric (*Curcuma longa* L.), blackhead (*Lavandula stoechas* L.), dandelion (*Taraxacum officinale* (L.) Weber), juniper (*Juniperus communis* L.*)*, syrian rue (*Peganum harmala* L.), black cumin (*Nigella sativa* L.), lemon balm (*Melissa officinalis* L.), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) , anise (*Pimpinella anisum* L.), fennel (*Foeniculum vulgare* Mill.), St. John's wort (*Hypericum perforatum* L.) and valerian (*Valeriana officinalis* L.).

Some academic information about the activity areas of these plants (*Zingiber officinale, Silybum marianum, Rosmarinus officinalis)* have been used for 2000 years liver diseases, toxin and fungal poisoning, antiviral, antibacterial, antioxidant, and immune boost[3,4]. *Cichorium intybus* has hepatoprotective, gastroprotective, cardiovascular, antioxidant, hypolipidemic, anticancer, reproductive, antidiabetic, anti-inflammatory, analgesic, sedative, immunological, antimicrobial, anthelmintic, anti-protozoal, wound healing and many other pharmacological effects[5]. *Fumaria officinalis* has been reported to be laxative, diuretic, antispasmodic, chronic eczema and antileprotic[6]. *Thymus vulgaris* has antimicrobial, antitussive, expectorant, antispasmodic effects. *Urtica dioica* has been reported to have several pharmacological activities, including antibacterial, antioxidant, analgesic, anti-inflammatory, antiviral, immunomodulatory, hepatoprotective, anti-colitis and anticancer effects[7]. It has been reported to be used to treat *Achillea millefolium* spasms, digestive complaints, menstrual disorders, urinary infections, anti-inflammatory, spasmolytic, hemostatic, diarrhea, abdominal pain and other ailments[8]. *Equisetum arvense* has anticonvulsant, sedative, antioxidant activities[9]. It has been reported that *rcuma longa* has carminative, stimulating, antitumor and antioxidant effects[10]. *Lavandula stoechas* has been used for a long time in traditional medicine as an anticonvulsant, antispasmodic, analgesic, hypnotic, sedative and tranquilizer, antibacterial[11]. *Taraxacum officinale* has been used by different continental peoples in diseases of the gastrointestinal tract and respiratory system[12]. It is also used in diet due to the nutritional elements it contains[13]. There are studies on the contraceptive, antimycobacterial, and antibacterial activities of the *Juniperus communis. Peganum harmala*, which is a specific plant to the eastern Mediterranean region, has many different uses such as cardiovascular, gastrointestinal, nerve, endocrine and it also uses cosmetics[14,15]. The areas of use of *Nigella sativa* include analgesic, antipyretic, asthma, diarrhea and dyslipidemia[16]. *Melissa officinalis* is a type of plant whose activity is investigated in chronic angina as well as antiviral and antioxidant activities[17]. *Syzygium aromaticum* is known to have antibacterial and antinociceptive properties. There are datas indicating that *Pimpinella anisum*, which is used food industry, is also used in wound healing and antidepressant[18]. *Foeniculum vulgare* has wide usage in traditional medicine and as natural conservation of food[19]. *Hypericum perforatum* has so many using field in medicine. Studies investigating the effect of *Valeriana oficinalis* on nervous system disorders are quite high[20].

Medicinal plants, besides all these positive effects, can show toxic effects directly from plant origin or depending on usage. Using the wrong doses or interactions of plants in plant mixtures with each other can have a toxic effect[21]. In the toxicity tests; possible toxic symptoms, the degree of effectiveness in organ functions and the lethal dose are determined as a result of exposure to xenobiotics[22].

With this study, we investigated the acute subacute toxicity potential of KL[21®], a herbal mixture extract, on rats.

MATERIALS AND METHODS

Animals

The experimental animals used in the study were obtained from Düzce University Experimental Animals Application and Research Center (DUHAM). In the laboratory, Wistar 8 weeks old, 250-300 g female rats (n:24) were kept at 20-25 ºC room temperature, 55 ± 5% humidity and 12:12 light-dark cycle, with optimal food and water intake free. The experimental protocol was approved by Duzce University Animal Experiments Local Ethics Committee (Decision Number: 2020.4.1.). This study was conducted in accordance with the Declaration of Helsinki Principles.

Materials

The food supplement used in the study was supplied

from Naturin in Turkey. Commercial name is KL[21®] which includes components in table 1. One KL[21®] capsule consists of 640 mg herbal mixture. Dosage to be applied to animals; It was calculated by proportioning[23] to the weight of the animal from the recommended daily use dose for human and 1.2 mg mixture was prepared in 1ml saline as a single dose.

Table 1. Commercial name is KL[21®] which includes components.

Components	Daily dose (mg)
Thymus vulgaris	198
Achillea millefolium	126
Urtica dioica	126
Equisetum arvense	126
Silybum marianum	100
Juniperus communis	54
Lavandula stoechas	54
Zingiber officinale	54
Fumaria officinalis	54
Taraxacum officinale	54
Rosmarinus officinalis	54
Cichorium intybus	54
Curcuma longa	54
Peganum harmala	26
Nigella sativa	26
Melissa officinalis	26
Syzygium aromaticum	26
Pimpinella anisum	26
Foeniculum vulgare	26
Hypericum perforatum	8
Valeriana officinalis	8

Methods

In this experimental study, four different groups were formed. These; control group, acute toxicity group, subacute toxicity group, post-subacute toxicity group. The total test period is 14 days.

Control group (n:8); One ml of saline was given by oral gavage to the control group. Blood was drawn the heart after administration. Animals were sacrificed under anesthesia for histopathological evaluation. Heart, lung, liver, kidney and spleen tissues were taken.

Experimental acute group (n:8); The animals in the acute toxicity group were given 1.2 mg/ml KL[21®] product at one time by oral gavage. Clinical observations were made. Acute group animals with heart blood drawn 24 hours after administration were killed under anesthesia. Heart, lung, liver, kidney and spleen tissues were taken for histopathological examination.

Experimental subacute group (n:8); The subacute group animals were given KL[21®] product by 1.2 mg/ml oral gavage at the same time every day for 7 days. Clinical observations were made during the application. On the 8th day, animals whom blood samples drawn from the heart were killed under anesthesia. Heart, lung, liver, kidney and spleen tissues were taken for histopathological examination.

Experimental post-subacute group (n:8); Post-subacute animals were given KL[21®] product by 1.2 mg/ml oral gavage group at the same time every day for 7 days. The application was stopped after 7 days. Observations were made in the following seven days without application. At the end of the 14th day, blood was taken from the hearts of the subacute group animals. Animals were sacrificed under anesthesia and heart, lung, liver, kidney and spleen tissues were taken for histopathological examination.

Biochemical and Hematological analysis; All blood were taken into biochemistry and hemogram tubes. In Midray BS-120 biochemistry device; inorganic phosphorus (P), calcium (Ca), albumin (ALB), triglyceride (TG), total protein (TP), total cholesterol (TC), creatinine (CRE), bilirubin (BIL), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (UREA) from biological parameters were examined. Midray BC-5000 Vet hemogram device; hematocrit (HCT), hemoglobin (HGB), platelet (PLT), red blood cell (RBC), white blood cell (WBC) from hematological parameters were examined.

Pathological analysis; Heart, lung, liver, kidney and spleen tissues were fixed in 10% formaldehyde for histopathological examination. After fixation; dehydration, paraffinization, blocking, sectioning, painting steps applied. Sections were taken on the slide from the perforated blocks, stained with hematoxylin-eosin and histopathological examination was done under a microscope.

Clinical observation; Animals were routinely observed during the experiment in accordance with the clinical parameters criteria given in Table 2.

Statistical analysis

The study was analyzed using the one-way ANOVA test using the IBM SPSS Statistics 23 program. Statistical significance was determined by post hoc Dunnett's T3 test. $P<0.05$ was accepted as the statistical significance level. Results were expressed as means ± SD.

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Table 2. The Clinical Parameters Criteria

Clinical Observation	Other Observations	Systems to Follow
Respiratory	Dyspnea (Abdominal Breathing), Apnea, Eupne, Tachypnea	Central Nervous System (CNS), Circulatory Cardiac, Respiration
Motor Activities	Decreasing / Increasing, Indeterminate Positions, Tremor	CNS, Somatomotor, Sensory, Autonomous Nervous System (ANS), Muscular-Nervous Systems (MNS)
Convulsion	Clonic, Tonic, Tonic-Clonic Symptoms	CNS, Respiration, MNS, ANS
Reflexes	Initial Reflex	Reflexes CNS, Sensory, ANS, MNS
Ocular Signs,	Lacrimation, Miosis, Mydriasis	ANS, Irritation
Cardiovascular Signs	Bradycardia, Tachycardia, Arrhythmia, Vasodilation, Vasoconstriction	CNS, ANS, Cardiac, Circulatory System
Salivation	Quantity	ANS
Piloerection	Coarse Hair	ANS
Analgesia	Decreased Analgesia	CNS, Sensory
Muscle Tone	Hypotonia, Hypertonia	ANS
Gastrointestinal	Defecation	CNS, ANS, Kidney, Motility
Skin	Edema, Redness	Tissue Injury, Irritation

RESULTS

In our study, which investigated the acute toxicity, subacute toxicity and post-acute toxicity of KL$^{21®}$, compared to the control group, clinical parameters (respiratory, motor activities, reflexes, derivative, defecations, etc.) and tissue parameters (lung, heart, heart, kidney , liver and spleen) were not different. The biochemical parameters shown in Table 3.

Table 3. Biochemical data of control (0th, 1st, 7th and 14th) and KL$^{21®}$ groups (1st, 7th and 14th).

	Control 0th	Control 1st	Control 7th	Control 14th	KL$^{21®}$ 1st	KL$^{21®}$ 7th	KL$^{21®}$ 14th
UREA (mg/dL)	69.53±9.61	83.35±3.11	71.7±4.49	72.53±5.82	75.28±5.67	75.29±8.24	64.77±1.04
ALB (g/L)	56.42±3.18	46.96±5.29	50.63±2.06	48.79±1.83	52.57±3.88	51.94±5.07	52.8±8.1
ALP (U/L) X10	24.34±8.34	26.07±7.83	22.02±7.56	24.05±2.02	22.04±4.27	22.02±4.60	22.57±1.17
AST (U/L) X10	22.81±3.39	31.95±13.54	27.09±13.52	29.52±2.43	18.41±2.63	15.44±3.97	20.67±8.25
TG (mg/dL)	14.38±4.73	9.65±5.50	13.99±2.49	11.82±2.16	18.07±6.84	13.53±1.78	31.73±12.98
Ca (mg/dL)	13.55±0.67	12.08±0.62	13.5±0.19	12.79±0.70	12.96±0.40	13.58±0.83	13.74±0.64
ALT (U/L) X10	14.44±3.73	15.37±5.72	19.44±9.26	17.41±2.03	10.1±2.03	10.55±2.42	11.76±1.05
P (g/mL)	6.22±0.39	6.92±1.32	6.71±0.75	6.82±0.10	6.05±0.91	5.39±0.62	5.43±1.24
TC (mg/dL) X10	12±2.60	9.94±1.95	10.19±2.84	10.06±0.12	9.84±4.84	9.9±2.48	11.78±2.33
TP (g/L)X10	11.53±0.44	9.87±0.55	10.12±0.38	9.99±0.12	10.53±0.49	10.2±0.78	10.36±0.94
GGT (U/L)	4.24±0.10	5.52±1.98	4.98±0.60	5.25±0.26	5.12±1.44	4.46±2.15	3.45±0.45
CRE (mg/dL)	0.12±0.04	0.21±0.10	0.11±0.03	0.16±0.05	0.11±0.09	0.11±0.02	0.06±0.01
BIL (mg/dL)	0	0	0	0	0	0	0

The hematological parameters shown in Table 4.

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Table 4. Hematological data of control (0^{th}, 1^{st}, 7^{th} and 14^{th}) and $KL^{21®}$ groups (1^{st}, 7^{th} and 14^{th}).

	Control 0^{th}	Control 1^{st}	Control 7^{th}	Control 14^{th}	$KL^{21®}$ 1^{st}	$KL^{21®}$ 7^{th}	$KL^{21®}$ 14^{th}
WBC (10^9/L)	6.77±1.40	6.67±2.48	3.64±1.75	5.15±1.51	5.61±3.25	5.09±1.70	4.62±0.07
RBC (10^12/L)	7.78±0.30	7.58± 0.68	6.61±1.75	7.10±0.48	7.29±0.67	7.91±0.40	7.08±0.66
HGB (g/L) X10	14.51±0.64	14.44±1.19	12.51±1.79	13.47±0.96	13.76±1.14	15.06±0.37	13.4±0.6
HCT (%)	44.08±2.08	43.54±3.19	37.68±5.06	40.61±2.92	40.9±3.51	45.92±1.70	42.1±2.4
PLT (10^10/L)	93.1±12.62	87.4±13.59	43.12±22.72	65.26±22.14	78.57±38.23	94.04±14.80	55.4±33.8

DISCUSSION

In the study of *Equisetum arvense* powder's sub-chronic toxicity in rats, the researchers observed that there were no considerable changes in biochemical (TP, ALB, ALT, AST, ALP, GGT, CRE, TG, TC, Ca, P, UREA) and hematological (WBC, RBC, PLT, HCT, HGB) parameters and no toxic clinical signs in kidney tissue examinations[9]. Similar to the non-combined *Equisetum arvense* study, the plants used in combination inside the $KL^{21®}$'s acute/subacute study have been shown to be clinically, hematologically and biochemically no toxic.

Rehan et al. (2018) reported that *Thymus vulgaris* extract caused liver hypertrophy and *Rosmarinus officinalis* extract caused splenic atrophy but did not show any significant histomorphological changes in subacute research in rats. Also in mention study, when hematological data of the control and experimental groups are compared, there is no notable change in WBC, RBC and PLT data intervals[24]. The absence of any trophic changes in histopathological evaluation in $KL^{21®}$ suggests that different plant parts in the $KL^{21®}$ may interact with each other, causing organ activity to stabilize. However, the mean of the hematological parameters RBC, WBC and PLT throughout the process showed parallelism with the control and experimental acute/subacute groups. This indicates that $KL^{21®}$ does not produce toxic effects on the hemogram values of animals under the same conditions. Researchers who report that the high values of ALT, AST, GGT and ALP, which are the liver enzymes of patients with hepatitis C virus, decreased significantly by consuming *Nigella sativa* and *Zingiber officinale* mixture, suggesting that the mixture can provide clinical improvement and is reliable[25]. The fact that $KL^{21®}$ does not have a toxic effect on liver enzymes indicates that it can be clinically reliable and effective as well.

In one study, the phytochemical screening, the anti-inflammatory activity and the sub-acute toxicity of the hydroethanolic, flavonoid, tannin and mucilage extracts of the aerial part of *Lavandula stoechas* were investigated. As a result of the studies, no remarkable change was observed in the control and the treated group by organ functions and biochemical values (AST, ALT, ALP, UREA, CRE, TP, TG, Cholesterol)[26]. In the same way, in the administration of $KL^{21®}$, no difference was observed in the histology and biochemistry results of compared rats. Although it is combined with *Lavandula stoechas* in $KL^{21®}$, it suggests that it may produce similar results with its single application.

Komeili et al. investigated the antidiabetic and antihyperlipidemic effect of the *Peganum harmala* plant on rats and found a therapeutic effect on ALT, AST, GGT, BIL, TC and TG parameters[27]. Studies with plants in $KL^{21®}$ show that it does not cause toxic and side effects on blood parameters and enzymes, but rather creates a therapeutic effect.

Looking at the effect of *Taraxacum officinale* and *Silybum marianum* on damaged kidney, researchers found that they had therapeutic effects on serum Ca, P, ALP, GGT, UREA and CRE values. Therefore, they put forwarded that *Silybum marianum* and *Taraxacum officinale* extracts could be used as protective[28].

Ghaffari et al. who investigated the effect of *Curcuma longa* and *Cichorium intybus* seeds on non-alcoholic fatty liver disease (NAFLD); reported that there were no clinical side effects in ALT and AST, and there was potential to reduce the risk of NAFLD and so the mixture could be effective[29]. Likewise, its non-toxic potential of $KL^{21®}$ suggests that it can be used in related diseases.

The study of the effects of different herbal-drug mixtures prepared with *Syzygium aromaticum* and *Rosmarinus officinalis* on pain has been concluded that the plants may have a positive effect if used in combination with drugs. In clinical study investigating the effectiveness of *Achillea millefolium* and *Hypericum perforatum* ointments, it has been reported that herbal blended ointment

reduces perineal pain level, redness, edema, and episiotomy wound ecchymosis, so consuming them may be beneficial for the treatment of episiotomy. The another clinical study, in which a polyherbal sedative (containing *Valeriana officinalis, Passiflora incarnate* and *Humulus lupulus* extracts) was compared with a modern sedative-hypnotic drug, suggested that the herbal mixture may be an alternative to modern medicine despite its minimal side effects in sleep related problems. The efficacy and lack of side effects of *Melissa officinalis* and *Valeriana officinalis* herbal supplement, which are found to be beneficial for sleep disorders in the menopause, suggest that they may be an alternative to modern hypnotics. According to the study investigating the effect of a plant mixture containing *Silybum marianum* and *Berberine aristata* on lipidic and glycemic profiles of diabetes and hyper cholesterol patients, datas were obtained that consumption of the herbal mixture with the drug or as a single use could be effective[30]. All of these studies indicate that plants used in various diseases in combination can show synergistic effects and positive responses can be obtained in related illnesses.

CONCLUSION

In this study, we investigated the acute, subacute and post-subacute toxicity of KL[21®] herbal food supplement on rats. There were twenty one different plant extracts in each capsule of KL[21®], and the toxic effect of the product was not observed at the administered dose. When we look at our study with KL[21®] herbal supplement and the studies made with herbs in KL[21®], it is concluded that herbal food supplements will not produce toxic and side effects if they are used either individually or as a combination, in appropriate doses.

In conclusion, thanks to the synergistic or similar effects of the plants, the therapeutic effects may occur when used in combination. The therapeutic effect of KL[21®] should be supported by carrying out studies at clinically.

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MINI REVIEW

Cupping Therapy and Scientific Basics

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Abstract
Cupping therapy is a traditional and complementary medicine practice that has a history of 5000 years and is used in many societies. One of the 15 complementary medicine methods accepted in the Regulation on Traditional and Complementary Medicine Practices published by the Ministry of Health in Turkey is cupping. In this article, cupping therapy application and studies on cupping therapy will be presented.
Keywords: Cupping Therapy, Traditional Medicine, Cupping Therapy Studies

INTRODUCTION

Cupping therapy overview

Among the traditional and complementary medicine (T&CM) applications, one of the most frequently used methods in our country and in the world is cupping. Cupping is a procedure performed on various parts of the body with the help of regional vacuum tools. In this process, the process of taking blood safely by creating superficial skin incisions is called wet cupping. The wet cup application is also called hijama.

In dry cupping application, as a result of the vacuum process created by placing cups on certain points on the skin, swelling, redness of the skin, and dilatation of the subcutaneous tissue and blood vessels occur in that area. As a result of this procedure, blood circulation and circulation in the relevant area increase due to the increase in interstitial fluid in the subcutaneous tissues and local temperature increase.

After the dry cupping application, the process of removing the cups and making very superficial and short scratches on the skin within the borders of the epidermis layer, closing and vacuuming the cups again, waiting for a while, removing the cups again and removing the accumulated residual materials in a hygienic way is called wet cupping (Hijama)[1].

The cup practice, which is thought to have a history of 5000 years, was also frequently applied in the Ottoman period. Also, It is known that the Prophet Muhammad (PBUH) personally had received cupping therapy and highly recommended it. Its widespread use increased in the 18th and 19th centuries in Europe and is now used in many clinics[2].

In the Regulation on Traditional and Complementary Medicine Practices published by the Ministry of Health in our country, the definition of cup application, the personel authorized to practice, the situations in which cups can be applied and not, and the devices and materials that should be kept in the practice units are given widely.

There are studies investigating its effectiveness in diseases such as disc herniations, herpetic lesions, spondylosis, chronic low back pain, carpal tunnel syndrome, osteoarthritis, fibromyalgia, chronic neck pain, neuralgia, migraine, and some other headache syndromes. It has also been applied in hypertension and some neurological diseases.

Cupping therapy indications

Cupping therapy indication spectrum is quite wide. Some of these indications have been supported by clinical studies. Others have come from traditional practices and experiences. Here, primary prevention, that is, preventive medicine, has come to the fore. It is used in chronic diseases for its therapeutic and symptom-reducing effects.

The indications specified in the T&CM regulation of the Ministry of Health are given as follows[3].

- Primary Prevention; Strengthening the immune system in patients who do not identify an organic disorder

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- Accompanying rheumatic diseases; Chronic pain, limited range of motion, morning stiffness, fatigue
- Fibromyalgia
- Musculoskeletal system mechanical pains
- Knee pain
- Non-organic Chronic headaches; Migraine, Tension headache, etc.
- Sleeping disorders
- GI disorders; nausea, vomiting, constipation
- In addition in the T&CM centers; Neuralgia-related pain, Stroke-related; Hiccups, aphasia, fatigue, etc symptoms.

Contraindications

- The situations in which cupping treatment should not be performed are given as follows[4].
- Thrombophlebitis
- Active wounds
- Surgical wounds
- Decompensated heart disease
- Anemia (hemoglobin below 9.5mg/dl)
- Hemophilia
- History of bleeding/coagulation disorder
- Antiaggregant drug use
- Cups are not applied directly to the varicose veins.
- Practically; under 2 years old, over 70 years old
- Partial Contraindication: Menstrual Period

Side effects

Side effects of cupping therapy can be listed as syncope, hypoglycemia, infection, and scar formation. Moreover, in a systematic review examining the side effects of cupping therapy, the WHOUMC causality scale was used; they were classified as definite, probable, and possible side effects[4,5]. 572 articles were reviewed and 16 studies were included in the review; Five cases of iron deficiency anemia were detected. In other studies examined; Side effects such as dermatitis, herpes infection, skin pigmentation and laceration, cervical epidural abscess, cardiac hypertrophy, and increased pain have been reported. Methodological differences in practices affect the frequency of side effects according to regions[4]. No serious adverse events were reported in any of the 135 randomized controlled studies reviewed in another review[6].

Although it is rare, the most serious side effect of cupping therapy is vaso-vagal syncope. After the application, there may be a risk of infection such as hepatitis B, C, and HIV. However, when cupping is performed by certified physicians by taking the necessary precautions, the existing side effects will also decrease. Therefore, physical examination, anamnesis, and laboratory evaluation of patients should be performed before treatment. In the early period after cupping, erythema, circular ecchymosis, swelling, bleeding, discomfort, pain, mild headache, sweating, pressure sensation, and tingling can be observed; Scarring, bruising, and hyperpigmentation in the incision area are the changes observed later[7,8].

Effect mechanism

Different theories have been proposed regarding the mechanism of action of cupping therapy[9]. According to the cutivisceral reflex theory, which is one of the theories related to the neuronal system, there are connections with the internal organs in the relevant skin areas along the segments formed by the spinal nerves. These connections are defined as the cutivisceral/viscerouteneal reflex. In the pathological condition occurring in the organ, skin changes, or pain may occur in this area with the signal going to the relevant skin area. According to this theory, it is possible to contribute to the treatment of organs with cupping therapy to the relevant skin segment. The incision and vacuum created in the wet cup application stimulate the thick unmyelinated A-delta fibers with the door control theory and provide the closure of the entrance gates of the pain signals reaching the spinal cord with group C thin myelinated nerve fibers in the substantia gelatinosa[10,11]. In addition, with the stimulation of mechanoreceptors, other pain stimuli are inhibited via nociceptive afferent fibers and their uptake is prevented[10].

With all these theories, the mechanism that best explains the effect of cupping is ensuring microcirculation by bloodletting; as a result of this, it is the detoxification of the connective tissue and the homeostasis of the relevant part of the body. In addition, some of the toxins accumulated in the body can be taken out with the blood. Cupping emerges as a successful treatment method if it is applied according to the basic principles of acupuncture[12].

Studies on cupping therapy

There are a limited number of scientific studies on cupping therapy and cupping. There are studies investigating its effectiveness in diseases such as disc herniations, herpetic lesions, spondylosis, chronic low back pain, carpal tunnel syndrome, osteoarthritis, fibromyalgia, chronic neck pain, neuralgia, migraine, and some other headache syndromes. It has also been applied to hypertension and some neurological diseases[12].

It is also observed that there are some methodological limitations in the studies carried out. These studies

will be presented below in the form of preclinical observational studies, case reports, clinical studies, meta-analyses, and cases indicating complications, especially by examining the Pubmed database.

Preclinical and observational studies and cases

In another study, it was shown in mice that cupping increased pain mediators such as beta endorphin[13]. In a study conducted on mice in China, an increase in lymphatic vascularity was detected with cupping therapy[14]. In another study, cupping therapy was shown to reduce fibromyalgia symptoms[15]. In some preclinical studies, it was stated that cupping therapy increased flap blood flow[16]. In a study, it was shown that both symptoms and EMG findings of carpal tunnel syndrome regressed with cupping therapy[17,18]. In some studies, it has been stated that cupping therapy can activate and increase diseases such as eczema and psoriasis[19].

Clinical trials

In a randomized controlled study, it was shown that cupping reduces migraine pain. It is stated that the effectiveness of the treatment continues to increase in reducing migraine pain and increasing the quality of life if the cupping continues[20]. There are also studies showing that cupping therapy is beneficial in chronic insomnia[21]. There are also studies conducted on some menstrual disorders[22]. There have been some randomized controlled clinical studies showing the reduction of heavy metals in the body. Some studies have reported that cupping therapy reduces blood lipid levels[9,23]. There are studies reported to control hypertension and decrease systolic blood pressure[24]. In addition to these, there are also studies applied to trigger point pain, chronic low back neck pain and osteoarthritis. There are several studies showing that it improves the quality of life[25].

Meta-analysis

A meta-analysis study conducted between 1992 and 2010 reviewed hundreds of studies. In this meta-analysis study, cupping therapy was examined as a randomized study, and it was reported that when used together with other treatment methods, it contributed to a significant increase in recovery and did not have significant side effects[26]. Meta-analyses were conducted on chronic low back pain, musculoskeletal pain and neck pain. It has been stated that it can be used in the treatment of pain[27].

Cases indicating complications

As with any medical intervention, complications may develop during and after cupping therapy. However, no serious complications were described in the studies. More localized complications were noted. There are cases where it may increase eczema and psoriasis[28].

In a study showing that skin interventions should be handled sensitively during cupping treatment, cupping was applied to the abdomen and a case of widespread skin infection was reported afterward[29]. There are also cases showing localized bulla formation and infection after cupping[30]. Continuing severe bleeding in patients with a diagnosis of hemophilia has also been reported in cases[31].

Conclusion

We have seen that there are limited number of scientific studies on cupping therapy. It has been stated in most of the studies that there are especially methodological limitations in the studies. We have seen that there are studies in which cupping therapy is frequently used, especially in chronic low back pain, osteoarthritis, fibromyalgia, chronic neck pain, migraine and hypertension. There is a need for more studies with large participation in cupping therapy.

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REVIEW

Popular Complementary and Alternative Therapy Methods in Different Conditions

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Abstract
Complementary and alternative therapies (CAM) are methods for the prevention, diagnosis and treatment of diseases based on various cultural beliefs and experiences that are not currently considered part of modern medicine. In recent years, the integration of CAM applications into healthcare systems all over the world has led to an increase in their use and frequency, and it has also increased the necessity and expectation of evidence-based practices. In this review, it was aimed to examine the alternative treatment methods that are frequently used in different conditions, their mechanisms of action, and their application within the framework of scientific evidence. For this purpose, popularly used complementary and alternative therapies for musculoskeletal conditions (dry needling, instrument-assisted soft tissue mobilization, dry cupping), neurological conditions (acupuncture, reflexology), and other conditions such as cancer and metabolic diseases (yoga) were examined.
Keywords: Dry Needling, Instrument-Assisted Soft Tissue Mobilization, Dry Cupping, Acupuncture, Reflexology

INTRODUCTION

Complementary and alternative therapies (CAM) are methods based on cultural beliefs and experiences that involve preventing, identifying, and treating diseases to protect physical and mental health. Complementary therapies refer to treatments that complement traditional western medicine, while alternative therapies refer to treatments that are used in instead of others[1,2]. Although CAM and western medicine are considered separate from each other, the boundary between them is not always sharp or fixed. CAM encompasses current health system practices and accompanying theories and beliefs. Therefore, the definition of treatment as a CAM application depends not only on scientific but also on social and cultural evidence[3,4]. CAM has been applied in Turkey for centuries as in the rest of the world, but the legal process regarding the implementation is quite new. The most comprehensive regulation regarding CAM applications is the Regulation on Traditional and Complementary Medicine Practices issued in 2014 and in this way, CAM is included in the legal medicine system[5].

In recent years, the number of people who think that the use of CAM applications is appropriate in Turkey has increased, and those who think that they should not be used have decreased. This may be because preferred CAM practices have changed over time, especially for social reasons, and changes in health policy may have made practices more accessible[6]. In recent years, the increase in interest in CAM applications all over the world has increased the necessity and expectation that applications should be evidence-based. However, it is not easy to conduct and implement evidence-based research on CAM applications. The obstacles faced by such studies are compliance with the norms and values of society, physical and mental dimensions of CAM applications, access difficulties, inadequacies in the competency of the practitioners, prejudices in the society, the duration of the applications, and insufficient incentives[7]. Despite these difficulties, there are different studies in the literature. In addition, the integration of CAM modalities into the healthcare systems has led to an increase in usage areas and frequency. In this review, it was aimed to examine the alternative treatment methods that are frequently used in different conditions, their mechanisms of action, and their application within the framework of scientific evidence.

Complementary and alternative therapies for musculoskeletal conditions

Musculoskeletal system diseases are characterized by chronic joint pain/damage, muscle pain/tenderness, and inflammation. This may be

accompanied by cardiovascular diseases and/or depression[8]. In the management of the disease, it is aimed to maximize the individual's physical, functional, psychological, and social[9]. For this purpose, alternative treatments are integrated into medical treatments, especially in pain relief[10]. Dry needling, dry cupping, and instrument-assisted soft tissue mobilization are frequently used in chronic low back and neck pain, tension-type headache, tendinitis, and widespread muscle pain[11-16].

Dry needling

Dry needling is a skilled alternative treatment method that uses a thin filiform needle to penetrate the skin, with the intent to mechanically disrupt tissue without the use of an anesthetic[17]. The origin of the term "dry needling" is attributed to Janet Travell, M.D[18]. The solid filiform needle used in dry needling is regulated by the FDA as a Class II medical device described in the code titled "Sec. 880.5580 Acupuncture needle, an acupuncture needle is a device intended to pierce the skin in the practice of acupuncture"[19].

In myofascial trigger point therapy, the neural processes of acupuncture and dry needling treatments and the localization of trigger points and classical acupuncture points are highly compatible. However, the difference between dry needling and traditional acupuncture is that acupuncture does not use all the theories of Traditional Chinese Medicine[20-22]. Presence of needle phobia, a history of an abnormal reaction to needling or injection, the use of anticoagulants, and lymphedema dry needling is an absolute contraindication of dry needling. Bleeding disorders, immune system diseases, vascular diseases, diabetes, pregnancy, cachexia, epilepsy, and metal and latex allergy are relative contraindications[23].

Dry needling is often used to treat myofascial trigger points, which are defined as discrete, focal, hypersensitive spots located in a palpable taut muscle band. These extremely irritable points are classified as active trigger points that produce spontaneous or severe pain when palpated, and hidden trigger points that do not produce spontaneous pain and only produce pain on palpation. Trigger points can occur in a muscle anywhere in the body in response to sudden or overuse injury. It is hypothesized that the damaged muscle fibers shorten (form taut bands) either in response to an excess of calcium ions released from the damaged fibers or in response to the respective motor endplate releasing an excess of acetylcholine. Prolonged release of acetylcholine, when combined with chronic shortening and sarcomere contractures, may lead to hypoxia and local ischemia with decreased circulation[24,25]. Local tenderness and referred pain occur in response to low oxygen levels and increased inflammatory chemicals (prostaglandins, bradykinins, cytokines, and histamine) at the injury site. Moreover, bombardment of nociceptors by endogenous chemicals often produces an increase in central sensitivity in dorsal horn neurons. All these predispose to the development of chronic muscle hypertonus and trigger points[25].

Although myofascial trigger points are a common cause of pain, they can sometimes be overlooked. Trigger point pain has a significant impact on a person's life and causes a social burden and its management is important. Dry needling is an effective method of reducing pain and is also inexpensive and easy to access. Local twitch responses are expected to occur because of the rapid depolarization of muscle fibers with needling. When muscle twitching ends, spontaneous electrical activity decreases, and pain and dysfunction may disappear[26-29].

Dry needling techniques are mostly divided into superficial and deep techniques. Superficial needles are inserted superficially (approximately 5-10 mm) into the tissue above the underlying trigger points. After holding it for a short time (about 30 seconds to 3 minutes), the needle is removed, and the pain is expected to be greatly reduced. If the pain persists, the procedure can be repeated two or three more times [17]. Deep needles are placed deeper directly over the trigger points and the needles can be left in place for 10 to 30 minutes. There is no standardization in the frequency of treatment sessions and the number of needles used[28].

Dry needling at trigger points can reduce short-term low back pain intensity (level of evidence: moderate). However, the long-term effects and clinical superiority of dry needling in improving functionality are unclear. Dry needling is not recommended in the short and midterm for the relief of trigger point pain in the neck and shoulders. However, wet needling with lidocaine was found to be more effective in the midterm[11, 12]. Dry needling to trigger points in the head and neck in chronic tension-type headache may be effective in reducing headache intensity, frequency and duration, and improving health-related quality of life[13]. In temporomandibular joint dysfunction, its application to the masseter, pterygoid, sternocleidomastoidus, and trapezius muscles can

be an effective method for short-term relief of pain (level of evidence: low)[14]. Tendon and peritendinous dry needling can be applied in tendinopathies such as lateral epicondylitis, rotator cuff tendinopathy, Achilles tendinopathy, patellar tendinopathy, great trochanteric pain syndrome[30]. Electric dry needling can reduce pain and improve function in plantar fasciitis[31]. It can be applied to lower extremity muscles to reduce pain, increase joint range and functionality in knee osteoarthritis[15,32]. It is also known that dry needle application to the lower extremity muscles reduces pain in patellofemoral pain syndrome[33,34]. Further comparative studies are needed in musculoskeletal conditions[30].

Instrument-assisted soft tissue mobilization

Instrument-assisted soft tissue mobilization is a skilled method that involves the use of special assistive instruments for soft-tissue treatment. Soft tissue manipulation increases the activity and number of fibroblasts and facilitates collagen synthesis and collagen realignment [35]. Instrument-assisted soft tissue mobilization can be an alternative treatment method that accelerates recovery after sports injuries and provides return to sports or daily activities[36,37].

Instrument-assisted soft tissue mobilization has its origins in the small metallic tool known as the "strigil" used for therapeutic purposes in baths in Ancient Greece and Rome, and a traditional Chinese therapy known as "gua sha". The word "gua sha" refers to the formation of red dots on the skin by pushing the skin by tools and providing blood and oxygen flow to the soft tissues[38,39]. The technique is known by different names such as enhanced soft tissue mobilization (astym technique), fascial abrasion technique, Graston technique, and sound-assisted soft tissue mobilization[40,41]. While rocks, wooden sticks, and animal bones were used in the past, various tools made of stainless steel are used today. It is a simple and practical technique. The surface of the instrument maximizes the force transmitted to the tissues while minimizing the force exerted by the therapist. In this way, adhesions in deep areas can be stimulated[35,42,43]. The major goal of instrument-assisted soft tissue mobilization is to remove scar tissue and promote a return to normal function following soft tissue regeneration. Scar tissue mobilization can provide functional normalization around soft tissue[44]. Localized inflammation and microvascular and capillary bleeding occur with the application of appropriate pressure and shear force to the soft tissue. Inflammation releases adhesions and stimulate the healing process. Furthermore, nutrition of the injured area increase. The migration of fibroblasts accelerates, and eventually, new collagen is synthesized and aligned[45]. Bruising and pain are prominent among the side effects, and these effects can be controlled with cryotherapy[46].

The literature describes instrument-assisted soft tissue mobilization as a time-saving technique for both patients and practitioners. It is particularly effective in early rehabilitation. However, there is no consensus on the treatment intensity and its use requires certification. Instrument-assisted soft tissue mobilization can provide significant improvement in pain intensity, function, and range of motion (level of evidence: low and moderate)[47]. It is as effective as scraping massage in improving pain and function in patients with upper trapezius trigger points [48]. There is no strong evidence for its use to improve pain, function, and range of motion in people with and/or without extremity or spinal pathologies [16]. It may be effective in increasing range of motion in the uninjured individuals, while reducing pain and/or increasing patient-reported function in the injured ones. There is low evidence that it enhances short term joint range of motion[49]. Its effectiveness is not fully known due to the lack of evidence and heterogeneity. There is no consensus on the optimal schedule, instrument type, dose duration, and outcome measures. Higher-quality randomized controlled studies with larger sample size and product diversity are needed[50].

Dry cupping

Cupping is an alternative treatment method applied by placing cups on the skin surface with subatmospheric (negative) pressure created by heat or suction. It is applied as a complementary therapy to coping with chronic diseases for which conventional medicine offers no cure but only management[51]. The earliest recorded references to cupping therapy are found in the Ebers Papyrus, written by the Ancient Egyptians around 1550 BC[52]. Cupping therapy is handled in six categories according to the classification updated in 2016. The first category is technical types that include dry, wet, massage, and flash methods. The second category is associated with vacuum power types, including mild, moderate, severe, and pulsatile methods. The third category is associated with the vacuum method types, which include fire, manual vacuum, and automatic vacuum methods. The fourth category is associated with additional treatment types including needle, moxa, herbal,

magnetic, laser, electrical stimulation, and aquatic methods. The fifth category is associated with the treated area types: face, abdomen, female, male, and orthopedic types. The sixth category is other types including sports and cosmetics[53]. Cupping therapy is used for health promotion, disease prevention, and therapeutic purposes. It is often used in the treatment of pain and spasms. It is not recommended for use in patients with bleeding disorders such as hemophilia or on anticoagulant therapy, children, elderly and pregnant or menstrual women. It is also contraindicated for use on skin areas with active inflammation, burns, infections, and open wounds. Hematoma, burn, abscess and irritation are local adverse effects, while dizziness, insomnia, headache and nausea are systemic effects[53,54]. Each cupping therapy session takes approximately twenty minutes. The first step involves the vacuum. At this stage, the practitioner identifies specific spots or areas for cupping and disinfects these areas. Massage oil can be used to slide the cup over the skin in the treatment. A cup of suitable size is placed on the selected area and the air in the cup is sucked by fire, electric or manual vacuum and left on the skin for 3-5 minutes. Then it moves on to another skin region[53,54].

Many hypotheses have been proposed regarding the mechanisms of action of cupping therapy. The main ones of these hypotheses are neural, immunological, metabolic, and psychological hypotheses [55]. According to the neural hypothesis, it is thought that cupping therapy is effective in chronic pain by changing the signaling process at the nociceptor, spinal cord, and cortex levels, and affects pain perception by stimulating Aδ (delta) and C fibers in the spinothalamocortical pain pathways. In addition, it inhibits pain transmission by increasing the release of opioids such as endorphins and enkephalin at the level of the spinal cord and cerebral cortex, resulting in an analgesic effect[55,56]. Immunological theory argues that inflammation occurs in the cupping area and there is an increase in inflammatory markers such as TNF and interferon, substance P, and other inflammatory mediators. This inflammatory process can create an immunomodulatory effect[57]. The metabolic hypothesis emphasizes that cupping therapy decreases the increased muscle activity and muscle tone and increases the microcirculation of the region by creating local vasodilation. Thus, an analgesic effect may occur[58]. The decrease in the perception of pain with the effect of physical touch on the limbic system is based on psychological theory[59].

Dry cupping may be effective in reducing pain in chronic neck pain and nonspecific low back pain (level of evidence: high). However, there are no definitive conclusions regarding the effectiveness and safety of dry cupping for musculoskeletal pain and range of motion (level of evidence: low and moderate)[60]. Dry cupping can improve pain and joint stiffness in knee osteoarthritis, but its effect on physical function is insufficient (level of evidence: low) [61]. Combined application with acupuncture in migraine may be more effective than acupuncture alone (level of evidence: low)[62]. Dry cupping therapy can be as effective as electrical stimulation on pain and function in plantar fasciitis[63]. In the first-line treatment of carpal tunnel syndrome, cupping therapy as an adjunct to medical management can reduce pain and tightness (level of evidence: low)[64]. There is a need for studies with a high level of evidence regarding the effectiveness of dry cupping in musculoskeletal system conditions.

Complementary and alternative therapies for neurological conditions

The fact that neurological diseases are usually chronic, and the treatment process is long and difficult, affects patients and caregivers negatively**[65].** Medical therapy is recommended for the initial treatment but side effects of drugs, concerns about substance abuse, and high treatment costs increase the interest in alternative treatment methods[66]. The fact that these treatments are more compatible with values, beliefs, and philosophical orientations related to life and health and offer a holistic approach also affects patient preferences[67]. Alternative therapy is often both easy and inexpensive to administer[68]. Acupuncture and reflexology applications are frequently used in the treatment of symptoms of many neurological diseases such as pain, spasticity, emotional and urinary dysfunction[69].

Acupuncture

Acupuncture is a therapeutic treatment that involves inserting solid filiform needles into specific areas of the body. The acupuncture has been practiced in China for over 3000 years. Scientific research began in the 18th century and the possible properties of acupuncture points and meridians, the physiological and biological mechanisms underlying acupuncture, and its use in clinical practice were examined[70]. Acupuncture stimulation includes sensory-discriminative and affective-social touch dimensions rather than needle stimulation alone. The sensory-discriminative dimension is a

combination of sensations associated with needling, such as pain and numbness. The affective-social touch dimension includes gentle manual touch stimulation by activating C tactile afferent fibers and can induce a limbic touch response resulting in hormonal reactions[71]. The areas stimulated by the needle in acupuncture are the points on the meridians where chi energy is thought to flow. Acupuncture theory believes that the irregularity or obstruction of the flow in the meridians causes diseases and that the flow is restored with the application of acupuncture[72]. Needle acupuncture is the most widely used method since ancient times, but acupressure (pressure stimulation) and electroacupuncture are also frequently used. Techniques such as stimulating the acupuncture points with the effect of heat (moxibustion), using laser diode devices (laser acupuncture) and injecting sterile water-soluble substances into the acupuncture points (aqua acupuncture) are also used[73].

Gently manipulating the needle may be the mechanism underlying the immediate relief of pain in patients with acupuncture treatment. However, different mechanisms have been proposed to explain the immediate effects of acupuncture. The possible mechanism to explain the immediate suppression of pain by stimulation is diffuse noxious inhibitory controls. According to this theory, a noxious stimulus applied to any part of the body can immediately suppress pain transmission in trigeminal caudal and/or spinal dorsal horn neurons[74]. Electroacupuncture analgesia has been hypothesized to generate an increased release of endogenous opioids in plasma or cerebrospinal fluid, and the type of secreted peptide changes according to the available frequencies[75,76].

Acupuncture points are considered essential components of acupuncture practice for diagnosis and treatment. Although there is no conclusive evidence for the existence of these points, the sensitivity of acupuncture points, the firmness felt by palpation, the sensitization of nociceptors, and their effector (visceral organs) functions are partially accepted. The pathophysiological mechanisms underlying the formation of tender points are not clear, but the possible mechanism may be the presence of sensitized nociceptors at these points[77]. In addition, under these points (active trigger points), there are regions rich in bradykinin, inflammatory cytokines (IL-6, TNF-alpha), substance P, and calcitonin gene-related peptide (CGRP). Acupuncture application to these points creates a healing reaction with a similar mechanism of action of dry needling[78]. Moreover, disorders in the visceral organs may cause pain and tenderness at areas or points on the skin through the viscero-cutaneous reflex, and it is emphasized that acupuncture applied to these points can regulate organ dysfunction through the cutaneous-visceral reflex[79].

Acupuncture treatment in Parkinson's patients showed positive improvements in the Unified Parkinson's Disease Rating Scale (UPDRS) scores. An increase in neural responses was also found in many brain regions after treatment[80]. Laser acupuncture can reduce spasticity in children with spastic cerebral palsy. The action mechanism of laser acupuncture in spasticity may be as follows: stimulation of acupuncture points 1) may also cause activity changes in parts related to the sensorimotor area 2) can increase parasympathetic activity by inhibiting sympathetic nerve fibers. The increase in the secreted inhibitory neurotransmitters may lead to inhibition of alpha motor neuron and spasticity may be alleviated[81]. Acupuncture is also widely used in symptom management of patients with Multiple Sclerosis (MS). Traditional Chinese acupuncture and scalp acupuncture can improve MS symptoms (fatigue, neural functional disorders, pain, gait disturbances, and bladder dysfunctions) and reduce attacks (level of evidence: moderate)[82]. Experimental studies on ischemic stroke explain the action mechanism of acupuncture in five different ways: promotion of cell proliferation in the central nervous system, regulation of cerebral blood flow through modulation of angiogenesis and vasoactive mediators, anti-apoptosis, regulation of neurochemicals, and strengthening and recovery of memory and learning processes[83]. The outcomes of ongoing randomized controlled prospective studies in stroke patients are eagerly awaited[84,85].

Reflexology

Reflexology is a CAM method that involves applying pressure to any certain areas in the feet, hands, and ears and aims to increase blood and energy circulation, give a feeling of relaxation, and maintain homeostasis[86]. The feet, hands and ears are a mini map and a mirror of the body, and all organs and glands are associated with reflective points in these areas. Applied pressure to these areas provides relief and healing to the corresponding part of the body[87,88]. There is evidence that some form of reflexology was practiced in Egypt around 2000 BC. The development of modern reflexology was initiated in the late 19th century with the definition

of the concept of Zone Therapy by Dr. Fitzgerald. Eunice Ingham (physical therapist) also discovered reflex zones (mirror reflections) of organs and glands in the feet in the 1930s and began to develop a map of the whole body[89].

The exact mechanism of action of reflexology has not yet been confirmed, but the possible mechanism is that it re-establishes a state of balance by making the autonomic nervous system more regular. The downregulation in the nervous system has been adversely affected by the modern lifestyle that has profoundly changed human resting habits. The lack of adequate regulation may create stress in the body and especially affects the hormonal balance[90]. Especially in women, hormonal imbalance can cause menstrual irregularities and cortisol secretion can lead to syndromes such as polycystic ovary. Based on the evidence showing that reflexology can have a positive effect on menstrual irregularities[91,92], it can be said that it has the potential to restore hormonal balance through a mechanism such as mindfulness and exercise. The therapeutic approach (communication between the patient and the clinician) and the therapeutic touch during the reflexology session also have positive effects on the patient. The mechanism underlying reflexology to provide relaxation and improve sleep patterns may be the properties of reflex points as well as the effect of the ambient features (lighting, temperature, scent, sound etc.) or placebo. Besides, improvement in the musculoskeletal system can be explained by applying pressure to specific reflexology points that match the fascial planes (such as the reduction of pain with the sensory and emotional effects of touch, reorganization of fibroblasts, and spread of the mechanical effect to neighboring tissues). The effect on organs can be attributed to the spread of mechanical forces in the fascia[93]. The action mechanism of reflexology is also explained by the theory of energy channels, which is believed to eliminate the irregularity in energy channels such as acupuncture[94]. The lactic acid theory [95] is based on the accumulation of lactic acid below the reflex points and that they are broken down (detoxification) by reflexology.

Foot reflexology practice may have positive effects on central pain, fatigue, and sleep disorders in patients with stroke[96,97], and it can also significantly reduce the mean systolic and diastolic blood pressures of these patients[98]. Although the application of reflexology in patients with Parkinson's varies according to the patient profile (the severity of the disease, attitude towards reflexology etc.), it may increase the well-being of the patients in general[99]. In MS patients, it may have positive effects in symptom relief such as spasticity, pain, decreased quality of life, fatigue, sleep disorders, bladder-intestinal dysfunctions, anxiety, and depression (level of evidence: low)[100-104]. It may also have positive effects on motor performance and constipation in children with cerebral palsy[105].

Complementary and alternative therapies for other conditions

In the treatment of metabolic diseases such as cancers with a long and difficult treatment process or diabetes that require chronic care, drug therapy is integrated with other treatment methods. Healthy behavioral profiles such as regular physical activity, healthy eating, and stress management should be established in the treatment process and disease management. Yoga includes relaxation, meditation and deep breathing and is an alternative therapy that can provide stress management for both cancer patients and diabetics. It also helps them perform self-care tasks more easily[106,107].

Yoga is a mind-body exercise based on mindfulness which originated in ancient India that use postures (asana), breath regulation (pranayama), withdrawal of the senses (pratyahara), concentration (dharana), meditation (dhyana) and absorption into the supreme (samadhi). Relaxation, meditation and breathing exercises make the person aware of his body and the moment[108]. It is known that mindfulness-based approaches increase one's ability to recognize emotional stress, respond skillfully, and develop responses to cope with stress effectively[109]. Yoga, one of the mindfulness-based approaches, can reduce stress by decreasing sympathetic activation and increasing parasympathetic activity[110], and by providing stress control, body cortisol levels decrease, or the increase becomes controllable. Yoga also prevents stress-induced responses (such as increased inflammatory responses, telomere shortening, and decreased cell-mediated immunity) and bad food choices (unhealthy foods high in sugar, fat, and salt) are also prevented[111-113]. Considering these effects, it is a strong candidate to be included in the treatment programs of both cancer and diabetes patients.

Yoga can be a safe and interesting method to help improve the mental health, fatigue, sleep quality, and quality of life of cancer patients. However, apart from the theoretical elements of yoga, with its broad philosophy and approach, the patient can create a wholly healthy life profile (such as the

healthy food choices, exercise, definition of life purpose, and right relationships)[114]. The latest scientific evidence reveals the potential role of the yoga approach in the management of type 2 diabetes and associated risk factors. It is thought that yoga has effects on diabetes control with psychological, neural, endocrine, and immunologic mechanism. Decreased sympathetic system activation, increased parasympathetic activation, and related anti-stress mechanisms improve both the metabolic and psychological profiles of patients. Thus, it increases insulin sensitivity and improves carbohydrate and fat metabolism. Yoga practices can lead to significant positive clinical outcomes as they help manage diabetes-related comorbidities by reducing blood glucose levels[115].

CONCLUSION

As a result of examining the complementary and alternative treatments that are popularly used in different conditions: dry needling, instrument-assisted soft tissue mobilization, dry cupping in musculoskeletal conditions; acupuncture and reflexology are used in neurological conditions, and yoga is used in other conditions such as cancer and metabolic diseases. The limited number of studies in the literature are insufficient to establish clinical practice guidelines. The effectiveness of complementary and alternative treatment methods in the different conditions needs to be supported by studies with high levels of evidence.

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