Volume: 3 **Issue:** 3
Year: 2022
DOI: 10.53811/ijtcmr.1121162

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

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

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Received: 25.05.2022 Accepted: 05.09.2022

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.

ACKNOWLEDGEMENTS

This study has been supported by Naturin Natural Products Pharmaceuticals and Pharmaceutical Raw Materials Industry and Trade Limited Company.

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

Author contributions: Conceptualization: [MY, GS]; Design: [AG, NS, AFA]; Writing: [AG, NS, AFA]; Investigation/Data collection: [MY, GS, AG, NS, AFA]

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

REFERENCES

1. Lev E, Amar Z. Ethnopharmacological survey of traditional drugs sold in Israel at the end of the 20th century. *Journal of Ethnopharmacology. 2000;72(1-2):191-205.*
2. Yasar M. The remember regeneration therapy method: An overview of new therapy protocol to approach diseases. *Journal of Complementary Medicine Research. 2019;10(1):68-80.*
3. Bayram D. Silibinin İn Vitro Ortamda CCL-228-SW 480 Kolon Kanseri Hücre Hattı Üzerine Apoptotik Etkisinin Araştırılması. *SDÜ Sağlık Bilimleri Dergisi. 2017;8(2):1-7.*
4. Mascolo N, Jain R, Jain S, Capasso F. Ethnopharmacologic investigation of ginger (Zingiber officinale). *Journal of ethnopharmacology. 1989;27(1-2):129-140.*
5. Al-Snafi AE. Medical importance of Cichorium intybus–A review. *IOSR Journal of Pharmacy. 2016;6(3):41-56.*
6. Sharma UR, Surendra V, Jha SK, Chauhan NS, Prakash T, Goli D. Evaluation of Ant-inflamatory Activity of Fumaria Officinalis Linn. Herb Extract on Experimental Animal.
7. Joshi BC, Mukhija M, Kalia AN. Pharmacognostical review of Urtica dioica L. *International Journal of Green Pharmacy (IJGP). 2014;8(4).*
8. Takzare N, Hosseini M-J, Hamideh Mortazavi S, Safaie S, Moradi R. The effect of Achillea millefolium extract on spermatogenesis of male Wistar rats. *Human & experimental toxicology. 2011;30(4):328-334.*
9. Tago Y, Wei M, Ishii N, Kakehashi A, Wanibuchi H. Evaluation of the subchronic toxicity of dietary administered Equisetum arvense in F344 rats. *Journal of toxicologic pathology. 2010;23(4):245-251.*
10. Govind P. Active principles and median lethal dose of Curcuma longa Linn. *Internafional Research Journal of Pharmacy, 2 (5): 239. 2011;41.*
11. Gilani AH, Aziz N, Khan MA, Shaheen F, Jabeen Q, Siddiqui BS, Herzig JW. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of Lavandula stoechas L. *Journal of Ethnopharmacology. 2000;71(1):161-167.*
12. Sweeney B, Vora M, Ulbricht C, Basch E. Evidence-based systematic review of dandelion (Taraxacum officinale) by natural standard research collaboration. *Journal of herbal pharmacotherapy. 2005;5(1):79-93.*
13. Escudero NL, De Arellano ML, Fernández S, Albarracín G, Mucciarelli S. Taraxacum officinale as a food source. *Plant Foods for Human Nutrition. 2003;58(3):1-10.*

14. Agrawal OP, Bharadwaj S, Mathur R. Antifertility Effects of Fruits of Juniperus communis. *Planta Med. 1980;40(S 1):98-101.*
15. Neslihan Şirin, Gülşah Aydın, Nisa Sipahi, Tuğba Türken Akçay, Haydar Göksu. Using Propolis as an Antimicrobial Agent in Syrian Rue Herbal Cream. Paper presented at: 3.rd Internatioanl Cosmetic Congress, Main Theme: Minimalist Cosmetic.2019; Antalya, Turkey.
16. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. *Phytotherapy Research. 2003;17(4):299-305.*
17. Miraj S, Rafieian K, Kiani S. Melissa officinalis L: A Review Study With an Antioxidant Prospective. *Journal of evidence-based complementary & alternative medicine. 2017;22(3):385-394.*
18. Shahamat Z, Abbasi-Maleki S, Mohammadi Motamed S. Evaluation of antidepressant-like effects of aqueous and ethanolic extracts of Pimpinella anisum fruit in mice. *Avicenna journal of phytomedicine. 2016;6(3):322-328.*
19. Badgujar SB, Patel VV, Bandivdekar AH. Foeniculum vulgare Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed research international. 2014;2014:842674.*
20. Hassani S, Alipour A, Darvishi Khezri H, Firouzian A, Emami Zeydi A, Gholipour Baradari A, Ghafari R, Habibi WA, Tahmasebi H, Alipour F, Ebrahim Zadeh P. Can Valeriana officinalis root extract prevent early postoperative cognitive dysfunction after CABG surgery? A randomized, double-blind, placebo-controlled trial. *Psychopharmacology. 2015;232(5):843-850.*
21. Monga M, Sausville E. Developmental therapeutics program at the NCI: molecular target and drug discovery process. *Leukemia. 2002;16(4):520-526.*
22. Saygı Ş, BD Gat. Deneysel Toksikolojide Toksisite Testleri Ve Test Sonuçlarının Önemi. *Gülhane Tıp Dergisi (GTD) Gülhane Medıcal Journal (GMJ). 2003:291.*
23. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. Journal of Basic and Blinical Pharmacy. 2016;7(2):27.
24. Rehan T, Tahira R, Ullah H, Tareen U, Rehan T, Anees M, Murtaza I, Sultan A. In vitro bioactivities and subacute toxicity study of O. basilicum, T. vulgaris and R. officinalis. *Turkish Journal of Biochemistry. 2018;43(4):447-455.*
25. Abdel-Moneim A, Morsy BM, Mahmoud AM, Abo-Seif MA, Zanaty MI. Beneficial therapeutic effects of Nigella sativa and/or Zingiber officinale in HCV patients in Egypt. *EXCLI journal. 2013;12:943.*
26. Yassine EZ, Dalila B, El MansouriLatifa BS, Lebtar S, Sanae A, Abdellah F. Phytochemical Screening, Anti-inflammatory Activity and Acute Toxicity of Hydro-ethanolic, Flavonoid, Tannin and Mucilage Extracts of Lavandula stoechas L. from Morocco. *Int J Pharm Phytochem Res. 2016;8(1):31-37.*
27. Komeili G, Hashemi M, Bameri-Niafar M. Evaluation of Antidiabetic and Antihyperlipidemic Effects of Peganum harmala Seeds in Diabetic Rats. *Cholesterol. 2016;2016:7389864-7389864.*
28. Karakuş A, Değer Y, Yıldırım S. Protective effect of Silybum marianum and Taraxacum officinale extracts against oxidative kidney injuries induced by carbon tetrachloride in rats. *Renal failure. 2017;39(1):1-6.*
29. Ghaffari A, Rafraf M, Navekar R, Asghari-Jafarabadi M. Effects of turmeric and chicory seed supplementation on antioxidant and inflammatory biomarkers in patients with non-alcoholic fatty liver disease (NAFLD). *Advances in Integrative Medicine. 2018;5(3):89-95.*
30. Di Pierro F, Bellone I, Rapacioli G, Putignano P. Clinical role of a fixed combination of standardized Berberis aristata and Silybum marianum extracts in diabetic and hypercholesterolemic patients intolerant to statins. *Diabetes Metab Syndr Obes. 2015;8:89-96.*