INTERNATIONAL JOURNAL OF TRADITIONAL AND COMPLEMENTARY MEDICINE RESEARCH

August 2020 **VOL** : 01 **ISSUE** : 02 **ISSN** : 2717-7491

ORGANIZATION, PREPERATION AND CORRESPONDENCE

Duzce University Traditional and Complementary Medicine Application and Research Center, Duzce, TURKEY

Date of Issue: 27.08.2020

• **International Journal of Traditional and Complementary Medicine Research** is an international peer-reviewed journal and is published three times a year. The responsibility of the articles published belongs to the authors.

Volume: 1 Issue: 1
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

CONTENTS

ORIGINAL RESEARCHES

CASE REPORT

REVIEWS

Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Antiproliferative Effect of IST-GLIO® Supplement Food Product on C6 Glioma Cells

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Received: 29.05.2020 Accepted: 04.08.2020

Abstract

Objective: Cancer is one of the leading causes of death worldwide and is characterized by the proliferation of abnormal cells. In recent years, as in the treatment of many diseases, there is also a return to nature in the treatment of cancer. In developing societies, the concepts such as prophylaxis of disease prevention rise to prominence in order to reduce the material and moral losses caused by disease treatment. Consequently, the use of supplementary food products occupies more and more space in daily life. In this study, it was aimed to investigate the antiproliferative properties of IST-GLIO® food supplement, which is one of these products and used in the Remember Regeneration Therapy Method (RTM), on C6 (rat glioma) cells.

Material-Method: WST-1 cell proliferation test protocol was applied to examine the antiproliferative effect of IST-GLIO® and the results were evaluated according to ELISA microplate reading data. The product was tested at concentrations ranging from 1 ng/mL to 100 ng/mL.

Results: When the results were evaluated, it was appeared to be effective in the concentration range of 6-20 ng/mL on the C6 glioma cell culture; it was found that the efficacy didn't occur through general toxicity, specifically reducing the reproduction of the cancer cells in question, since food supplement did not inhibit cell division further at higher doses.

Conclusion: As a result, non-specific toxicity is observed in many cultures that are similarly investigated in cell cultures, depending on concentration as high doses are obtained. The IST-GLIO® product does not show this feature is considered as promising.

Keywords: C6 Glioma, Antiproliferative, Cancer

INTRODUCTION

Cancer is used to describe neoplasia characterized by the uncontrolled proliferation of cells in a particular area of the organism due to the effect of epigenetic factors or a number of genetic changes[1]. According to the Turkish Cancer Research and War Agency, deaths from cancer rank second after cardiovascular diseases in the most common deaths list. Primary brain tumors account for 2% of all cancer-related deaths[2]. Although there are many kinds of treatment methods and application, the average life span of brain tumor patients is one year[3,4]. Due to prognosis of the tumor and the limited success of surgery and cytotoxic therapy in brain tumors, agents that are non-toxic and susceptible to brain tumor cells have been a source of hope for the development of new treatment methods. In the recent studies for this purpose, the anticancer effects of synthetic, herbal and fungal drugs against various types of cancer have been investigated.

Various herbal substances used in cancer treatments are used because they inhibit cell proliferation and in turn trigger apoptosis. Medicinal plant extracts and secondary metabolites obtained from these extracts; Investigation of antioxidant, free radical scavenging and anticancer activities is one of the widely studied topics today[5, 6, 7, 8]. Today, 20% of sentetic drugs used in Germany, more than 1/3 of the drugs used in Russia are herbal drugs[9]. Many medicinal plants have been studied extensively in cancer studies in vitro.

In the light of this information, the studies of plants are remarkable, but the combined studies are almost nonexistent. Therefore, in this study, IST-GLIO® food supplement with combined plant content and also used in the treatment of remember regeneration was used[10]. In our study, we investigated the in vitro effects of IST-GLIO® fortifying food product in rat C6 glioma cell line on cell proliferation in a dose-dependent manner.

MATERIALS AND METHODS

Materials

The food supplement used in the study were supplied from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). The product content is shown in Table 1. The doses used are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppb.

Table 1. IST-GLIO® supplement food supplement content

No	Product content	Quantity in 1 capsule
1	*Urtica sp.* seed	237 mg
2	*Peganum harmala* (L.)	133 mg
3	*Silybum marianum* (L.)	89 mg
4	*Zingiber officinale*	74 mg
5	*Nigella sativa* (L.)	68 mg
6	*Curcuma longa* (L.)	67 mg
7	*Juniperus communis* fruit	44 mg
8	*Thymus sp.*	15 mg
9	*Foeniculum vulgare*	4 mg
10	*Pimpinella anisum*	3 mg
11	*Cassia acutifolia*	3 mg
12	*Syzygium aromaticum*	3 mg

Cell culture

Experimental studies were carried out at Duzce University, Traditional and Complementary Medicine Application and Research Center, Cell Culture Laboratory. In the study, C6 cell line was obtained from Nevsehir University. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma) with 10% Fetal Bovine Serum (Sigma) and 1% penicillin + streptomycin (Sigma) broth containing mixture, 37 °C in medium containing 5% CO2 and 95% humidity 25 cm^2 incubated in flasks.

Antiproliferative assay

Anticancer activities of plant extracts were performed according to the sodium salt of 4-[3-(4 iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3-benzene disulfonate (WST-1) Cell Proliferation Assay System (Takara Bio Inc., Shiga, Japan). The WST-1 test used to determinacy to toxic effect and metabolic activity is a non-radioactive, spectrophotometric, colorimetric test based on the separation of tetrazolium salts from living cells. Tetrazolium salts are converted to formazan salts by cellular enzymes. An increase in the number of live cells leads to an increase in mitochondrial dehydrogenase activity in the samples. With the increase of enzyme activity, there is an increase in the form of cells stained with formazan in correlation with the amount of cells that are metabolically active in culture cells. Formazan dye is produced by cells that are metabolically active, and the absorbance of the cells stained at the wavelength specified by the spectrophotometer is measured[11].

According to this method, when the cells reached the appropriate concentration, the cells were seeded in a 96-well plate at a density of $5x10^4$ cells/well. Solutions of IST-GLIO® food supplement ranging from 1ng / ml to 100 ng/ml were prepared and added to the medium. All experiments were performed in triplicates. It was also created in the negative group with no products applied. The product was incubated with cells for 24 hours.

After incubation, WST-1 solution was added to each well. The plate was incubated at 37 °C for 4

hours. At the end of incubation, the absorbance value (OD) of each well was read in the plate ELISA (Cytation ™ Biotek, USA) at 490 nm wavelength and 630 nm reference range. Cell viability percentage was calculated by dividing the optical density value measured in each well by the control optical density value and multiplying by the face.

RESULTS

Cell viability on the C6 glioma cell line treated and untreated with the IST-GLIO® food supplement was shown in graphic 1. According to ELISA microplate reading results, the group classified as negative control without any product was evaluated as 100% alive and the results given in Table 1 were obtained by calculating the % viability in the test concentrations.

Table 1. Cell viability rates

Concentrations	% Viability
1 ppb	97
2 ppb	96
3 ppb	96
4 ppb	93
5 ppb	93
6 ppb	78
7 ppb	78
8 ppb	78
9 ppb	77
10 ppb	76
20 ppb	72
30 ppb	71
40 ppb	72
50 ppb	72
60 ppb	70
70 ppb	70
80 ppb	70
90 ppb	70
100 ppb	70

According to the results of the analysis, the effectiveness up to 90% cell viability was not considered in terms of data security interval. It was assumed that there was no efficacy accordingly at concentrations between 1-5 ng/mL doses applied to the C6 glioma cell line. It was understood that the cell viability rate in the concentrations between 6-20 ng/mL was in the range of 70-80%, thus inhibiting moderate to strong cell growth. Since the 70% viability rate was maintained between 20-100 ng/mL concentrations, the maximum effective concentration was thought to be achieved.

DISCUSSION

Plants have played an important health role since ancient times. Traditional plant-based medicines are still of great importance to people living in developing countries. It is also a resource for the discovery of new drug candidates. However, it needs to be scientifically evaluated to confirm the use of medicinal plants. Mortality rates in cancer patients are increasing day by day. It is an indication of the limited efficiency of current treatments used in cancer treatment. The search for cancer treatment for many years has focused on chemically synthesized compounds. Over the past few decades, research has focused on the use of natural products, such as raw plant extracts or a combination of different phytochemicals[12].

The IST-GLIO® food supplement used in the study contains different plant combinations. Anticancer properties of most of these plants have been proven by studies. The anticancer effect of *Urtica dioica* has been investigated in breast cancer cells and it has been revealed that its use with cancer drug paclitaxel may have therapeutic potential. In another study, it was revealed that the apoptotic effects of nettle extract of dichloromethane extract were examined, prostate cancer had positive results[13]. In another study, it showed the inhibitory effect of cell proliferation on prostate cancer cells (LNCaP and hPCPs) by the plant's aqueous and ethanol extracts[14]. Also in a report, the anticancer effects of this plant against esophageal cancer are mentioned. In another study, the anti-proliferative effect on human prostate cancer cells with nettle root extract has been proven[15]. They proved that the seed extract MDA-MB-231 (breast cancer) of *Peganum harmala* L. induces apoptosis and inhibits the growth of its cells[16].

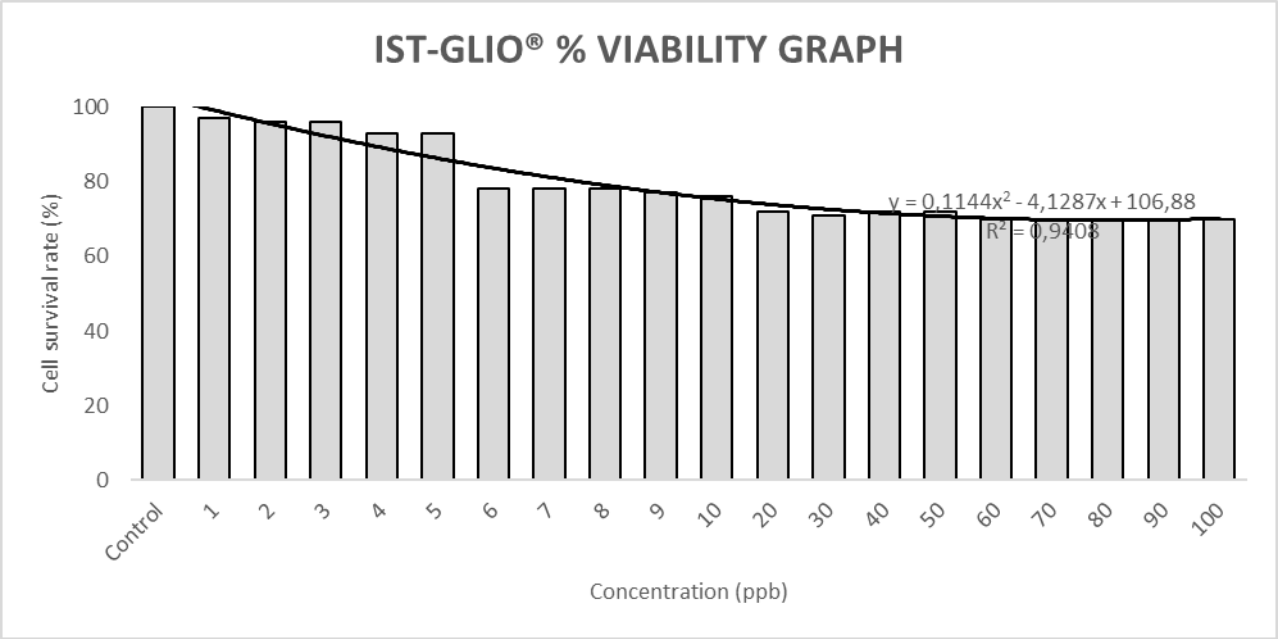

Figure 1. Effect of different doses of IST-GLIO® on C6 cell survival rates. The control group was accepted as 100%

Many studies on *Silybum marianum* are available in the literature. Some concentrations of silybin have been found to inhibit growth in human prostate cancer cells and reduce tumor volume by 53-64%. It has also been suggested that silymarin causes regression of skin tumors. In another study, it has been revealed that it decreases hyperplasia and proliferation index. In another study, it was revealed that Silymarin both inhibits cell growth and inhibits DNA synthesis in different breast and cervical human-carcinoma cells[17]. Aqueous extract of *Zingiber officinale* acts on breast cancer cells (MCF7 and MDA-MB-231). It has been demonstrated that it inhibits the growth of cancer cells and induces cell death[18]. *Nigella sativa* extracts have been evaluated for their anticancer properties on the MCF-7 cell line and have been shown to be a potential therapeutic agent for cancer[19]. In another study, when the effects on kidney cancer cells (ACHN) were evaluated, apoptotic effect was seen on the cells. In another study, it has been shown to inhibit cell growth, apoptosis and increased cell morphological changes on colorectal cancer cells. It has also been shown to induce programmed cell death and anticancer activity is observed in an alcoholic extract of *Nigella sativa*[20]. The anticancer effect of *Curcuma longa* aqueous extract on sarcoma and breast cancer has been demonstrated[21]. Curcumin has demonstrated significant anticancer effects against many different types of cancer, including in vitro and in vivo prostate cancer, MCF7 (human breast adenocarcinoma), colorectal cancer, pancreatic cancer, and head and neck cancer. Investigation of the cytotoxic properties of turmeric on liver cancer cells (HepG-2) showed that curcumin-mediated cytotoxicity caused apoptosis of cancer cells via mitochondrial route[22]. Cytotoxic activities were shown in *Juniperus communis* leaf extracts EJ138 (human bladder), HepG-2 (human liver hepatocellular carcinoma), A549 (human lung carcinoma) and MCF7 (human breast adenocarcinoma) cell lines[23]. *Thymus sp.* among its active ingredients, thymol and carvacrol are the most important plant phenol compounds that are useful in the treatment of breast cancer and colorectal cancer. In one study, *Thymus sp.* has been shown to inhibit the growth, proliferation of human colorectal cancer cell[24]. Cell cycle arrest, which can occur through synergistic effects

between the active ingredients of fennel (*Foeniculum vulgare*) and clove (*Syzygium aromaticum*) oils, and its effect on Caco-2 cells by apoptosis has been demonstrated[25]. In addition, the acute toxicity of the IST-GLIO® food supplement product in experimental animals was examined and no toxicity was found[26].

Looking at the literature, almost every plant in the IST-GLIO® food supplement product has been studied in different cancer cell lines alone. As a result of the studies, it has been revealed that these plants inhibit growth and proliferation in different cancer cell lines.

In our study, these plants were combined and studied in different cancer lines (C6-glioma) by making use of the synergistic effects of herbal mixtures. In our study, 19 different concentrations were applied and in the cell culture of the product; It appears to be effective in the concentration range of 6-20 ng/mL. It appears to be effective by blocking cell reproduction. It is understood that the efficacy is not through general toxicity, specifically reducing the reproduction of the cancer cells in question, since higher doses do not inhibit cell division more. Many products that are similarly investigated in cell cultures show non-specific toxicity depending on concentration as high doses are obtained. The fact that the IST-GLIO® product does not show this feature has been considered as promising. Although successful results are obtained in cell culture, any product must be supported by clinical research in order to talk about anti-carcinogenic activity.

CONCLUSION

In this study, the antiproliferative efficacy of the IST-GLIO® product, which is used as a food supplement product, in the concentrations determined by considering the daily usage amount, was studied in the C6 glioma cell line. According to the results of the study, it is understood that the product is effective in the concentration range of 6-20 ng/mL. It is thought to show non-specific toxicity as the product retains its effectiveness as higher doses are reached. This research supports the effectiveness of IST-GLIO® food supplement product in glioma cell culture and the level of evidence should be increased by clinical research.

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Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Separating Mad Honey from Other Honeys with Grayanotoxin Analysis in LC-MS/MS

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Received: 07.07.2020 Accepted: 21.08.2020

Abstract

Objective: Honey samples were collected from 82 different pieces during the season when rhododendron flowers bloomed in Duzce areas in 2017. Since the majority of these honeys collected are sold as mad honey by local people, has been determined that it is the most suitable period to make their analysis.

Material-Method: To apply our sample preparation method, honey samples were prepared to be analyzed on the LC-MS/MS. The accuracy, repeatability and reliability of the analysis method were provided by us.

Results: The obtained results were calculated with the calibration plot drawn at ppb (ng/ml) level in LC-MS/MS. The grayanotoxin -III levels in mad honey were found to be % 36 for 0 ng/ml, %43 for 0.5-10 ng/ml, %15 for 10-50 ng/ml and %6 for 50≥ ng/ml.

Conclusion: Most of honey samples do not contain toxins. Some of them are safe to use in middle proportions and less of them dangerous to consume.

Keywords: Grayanotoxin-III, Mad Honey, LC-MS/MS

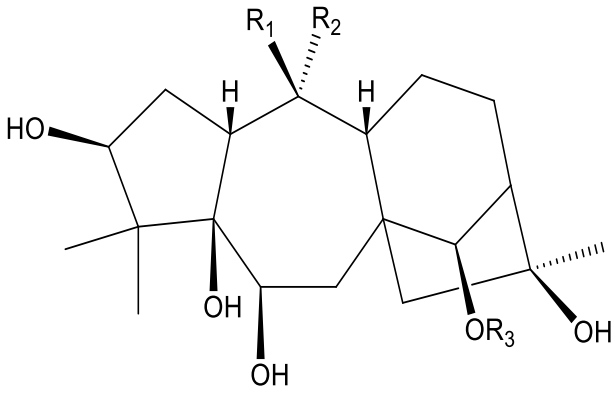
INTRODUCTION

Honey is one of the most important nutrients, which is consumed by people from past till today. Honey, which is among the rare nutritional source that is naturally sweet, has a wide range in taste depending on the nectars collected by the bees. As the multi-floral honeys, which are collected from the different kinds of flowers, are general, also the mono-floral honeys, which are formed by mainly collecting nectars from a single kind of flower, are known and consumed consciously. As an example to the mono-floral honeys, one of them is known as mad honey or bitter honey, which is both attention-grabbing and dangerous[1].

Mad honey is a natural nutrient, which is frequently, consumed in certain regions in our country, with its specific odour, bitterly – harsh taste, which causes a sense of slightly burning in throat, with its yellow colour, that is lighter than the other honeys, and late crystallization feature. Even though the poisoning due to andromedotoxine are phenomenal cases reported from the different countries world-wide, it is seen more frequently particularly in the settlements on the Black Sea coast of our country. It is consumed in small quantities in our country, generally the aim of self-medication for some medical effects of it[1, 2].

In some studies, it was suggested by the researchers that it may be related with Ericaceae family, through determining acetylandromedol (andromedotoxine), andromedenol, and andromedol, which are isolated from some species of Ericaceae plant family, in also andromedotoxine. The toxins, which are extracted from these plants by the bees, are mixed into the honey directly and causes poisonings, as they cannot be detoxified. Rhododendron, which is the scientific name of the forest roses within this plant family, means rose tree (In Greek rhodon = rose, Dendron = tree)[3, 4]. Meantime there are great number of and may different types of rhododendron in the world, particularly yellow rhododendron (*Rhododendron luteum*) and

rhododendron with purple flowers (*Rhododendron ponticum*) are very common in the forests on the north coasts of our country[4]. Acetylandromedol (andromedotoxine), andromedenol, and andromedol, which are the toxic compounds that are isolated from these species, are known also as grayanotoxin-I, grayanotoxin-II, and grayanotoxin-III, respectively. The grayanotoxines, which forms a group of toxic diterpens, are consisted of non-nitrogenous polyhydroxylecyclic hydrocarbons[5]. They are located in the nectar, flower, pollen, and leaf parts of the rhododendrons. Poisoning mostly occurs with the honeys produced from the nectars containing toxin. The type and amount of grayanotoxin, which is contained by the nectar, variety from one type to another. Today, more than fifty types of grayanotoxin are determined (Figure 1) and grayanotoxin-I, III, and IV are those having toxic characteristic compare to less toxic one is grayanotoxin-II. Grayanotoxin-III is the mostly found in the plant and honey, among those toxins[6].



Grayanotoxin	R_1	R_2	R_3	R_1=R_2
GTX I	-OH	CH_3	-H	-
GTX III	-OH	$-CH_3$	-Ac	-
GTX IV	-	-	-Ac	$=CH_2$

Figure 1. Structure of Grayanotoxins.

The toxic impacts of grayanotoxin in the cell emerge through voltage-dependent Na channels. They increase the permeability of the sodium ions in excitable membranes and facilitate the entry of calcium into the cell. Initially toxin is linked to these channels in the opening stage of voltage-dependent channels. Then, the channels are modified and activation is hindered. Finally, the activation potential of the modified Na channels leads to the hyperpolarization of the channel. The excitable cells (nerve and muscle) remain as depolarized[7]. Although there may be cases, in which one teaspoon of honey may cause poisoning, the amount of the consumed honey may vary between 5 – 30 g in most of the poisoning cases, however same poisoning indications may not be present in everyone tasting the honey[8]. Toxic effects of the poisoning are rarely fatal, and mostly it does not exceed 24 hours. Depending on the amount of consumed honey, the symptoms may get more significant, and starts within average 1.5 – 3 hours after taking the honey[9]. The mostly seen symptoms are significant hypotension and bradycardia, which is seen approximately in more than %90 of the patients. Sweating, dizziness, and mental status alterations follow this. Syncope, diplopia, blurred vision, and hypersalivation are the other seen symptoms.

Due to the rapid development in tourism and trade today, it is possible to come across poisonings of this natural food in many parts of the world. Also in the early 1800s, Barton first described the symptoms of poisoning for a patient poisoned after mad honey in a published study[9, 10]. Bucak et al., one of the first researches conducted in our country about this nutrient called mad honey, holding honey, black net or bitter honey among the people[10]. Iberoglu et al., which examined 16 cases poisoned from mad honey and grayanotoxin was determined in these honeys between 1984 and 1986[11]. In the following years, although many cases of poisoning continue to be reported from Turkey, applications are considered to be much higher than those published due to mad honey poisoning.

Because of its widespread production and consumption in the Black Sea region, the occurrence of mad honey poisoning or the so-called popular involvement is common. Some of the honey produced in Duzce province was defined and sold by producers as mad honey. It is dangerous to

use because the amount of grayanotoxin is unknown. For these reasons, samples of honey produced in 2017 in Duzce province were collected and their grayanotoxin contents were analyzed[12].

MATERIALS AND METHODS

Chemicals and instruments

GTX-III standard was supplied as grayanotoxin III Hemi (ethyl acetate), MS grade methanol and acetic acid solvents obtained from Sigma-Aldrich (St. Louis, MO, USA). High quality ultra-pure water was supplied by Human Zeneer Navi Power I Integrate (Human Corporation, Korea). The grayanotoxin content and composition of mad honey were determined by using LC-ESI-MS/MS (Shimadzu, Kyoto, Japan).

Preparation of standards and samples

250 mg honey sample taken and added 2,5 ml dilution solution (methanol/water 1:4 + 0,1% acetic acid). Vortexed 2 minutes. Then diluted with same solution to 1 to 10. Vortexed 2 minutes and filtered with 0,45 µm filters. Filtered solution used to injection.

For calibration grayanotoxin standard solutions prepared concentrations between 1-200 ng/ml. Nine point of these concentration used for calibration (1, 2,5, 5, 10, 25, 50, 100, 250 and 500 ng/ml). The linearity of the method was confirmed by linear correlation which is R^2= 0,9999

Analysis method

We used linear gradient LC-MS/MS method for all grayanotoxins analysis. Analysis performed 100-mm x 4,6-mm, 5-mm particle C18 column. Column oven set to 45 °C. Mobile phases (A) water +0,1% acetic acid and (B) methanol +0,1% acetic acid. 0,3 ml/min flow and starting conditions with mobile phases %70/%30 respectively. From start to 2 min B was used %30; from 2 to 4,5 min B linear gradient to %52; from 4,5 to 7 min B linear gradient to %90. Thereafter, a linear gradient back to %30 for 3 min to equilibrate column for next injection. Injection volume was 10 µl.

ESI-MS/MS analysis was performed using multiple reaction monitoring (MRM) to detect the major product ions from the protonated molecules of Grayanotoxin III-3:25/4:76 (m/z 335.2→299.1, 317.1 and 91.1). The MS conditions were: nebulizer gas 15 psi, temperature 450 °C and collision energy −30 V.

RESULTS AND DISCUSSION

In LC-MS/MS MRM (Multi Reaction Monitoring) mode was used for analysis. Grayanotoxin-III peaks were detected nearly 4th minutes of analysis method (Figure 2). From these knowledge, 9 different concentrations of grayanotoxin-III standard were prepared, analyzed and calculated for calibration (Figure 3).

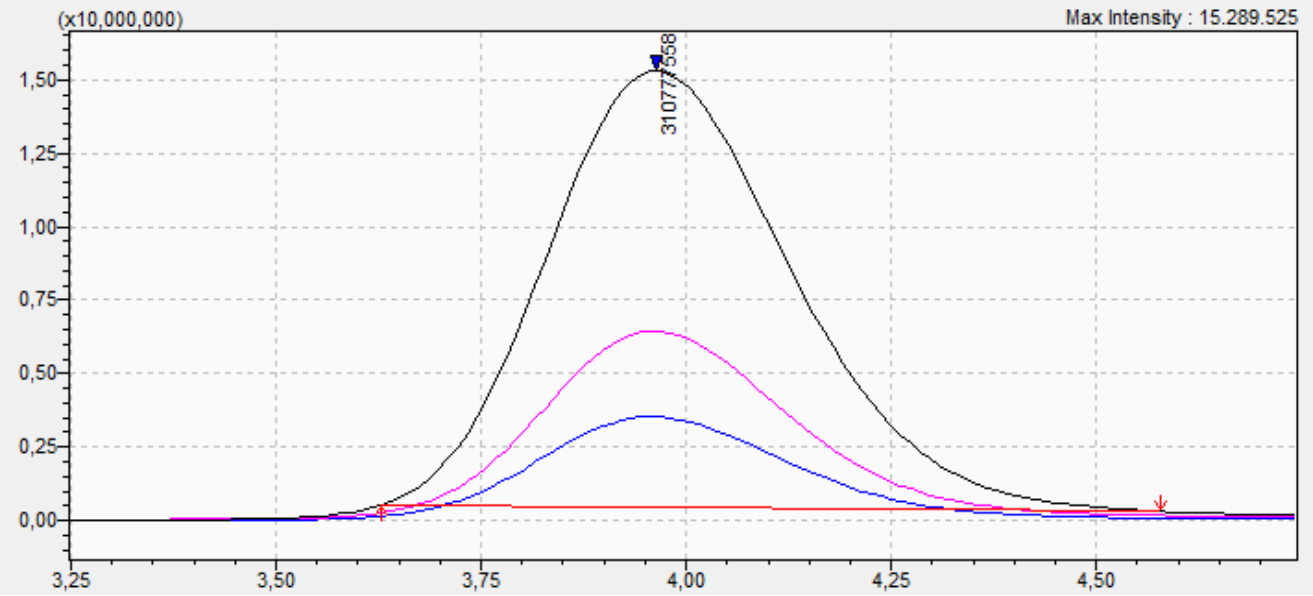


Figure 2. Peak of the grayanotoxin-III in LC-MS/MS MRM mode.

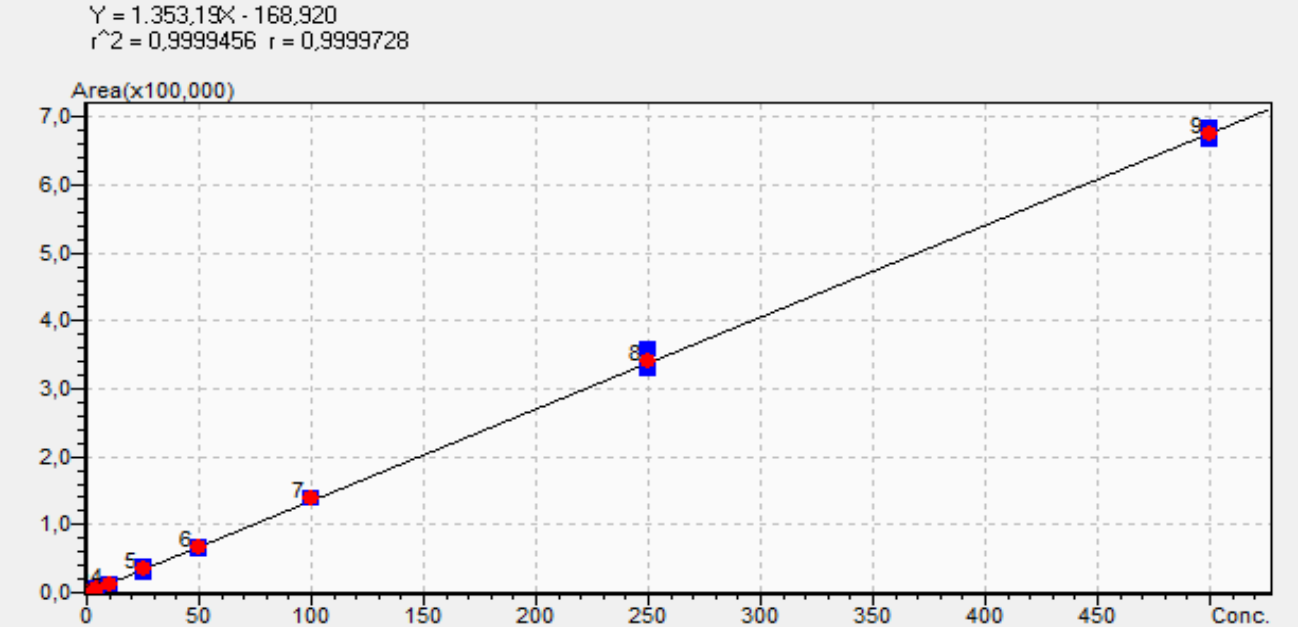


Figure 3. Nine point of calibration curve with linear correlation.

Upon the results of honey samples we came across different concentration of grayanotoxins. Nearly half of the samples have none toxins even the physical properties are pointed to mad honey. Other half of the samples have low and safe to eat concentrations of toxins. In 5 of the honey sample we calculated high amount of grayanotoxin-III and those are very dangerous to consume (Figure 4). Concentrations of grayanotoxins depends on *Rhododendron* blossoms. It affected by weather conditions such as cold and rainy weathers. If spring season become hot and dry, more *Rhododendron* blossoms appear and bees collect more nectar from them. For these reasons grayanotoxins levels in honeys changing every year. Due to the lack of standardization and changing conditions every honey samples should analyze before consumption. Most of beekeepers looking for strong odor and light yellow colour for mad honey but in some samples they need more informations. For measured samples contains different amounts of grayanotoxins between 0 – 70 ng/ml (Table 1).

Looking at the different analysis methods in the literature, the sample preparation part of our method takes less time and eliminates the preparation steps such as cartridges and similar with less amount. This situation can be called more useful as it shortens the preparations before analysis. The results we obtained match the literature and confirm the ranges determined as dangerous doses. Even if the results obtained from honey samples differ depending on the season, a common value can be revealed by contributing to such studies. Due to recent updates for the sample preparation and method section, it has seen that our method is more advantageous than other methods in order to get more practical and short results in poisoning cases[13-16].

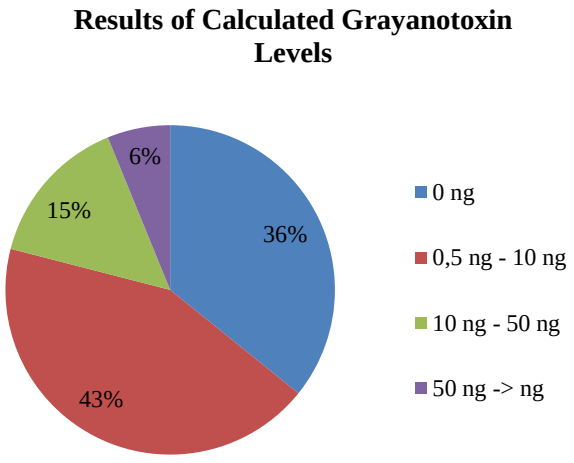


Figure 4. Percent representation of results.

Table 1. Concentration results of collected honey samples.

Sample	Concentration (ng/ml)	Sample	Concentration (ng/ml)	Sample	Concentration (ng/ml)
1	9.028	28	0.915	55	0.893
2	28.256	29	1.436	56	0
3	38.248	30	3.496	57	0
4	44.526	31	0.963	58	54.875
5	32.289	32	2.473	59	51.868
6	48.520	33	0	60	0
7	33.489	34	0	61	0
8	26.597	35	0	63	0
9	66.498	36	0	64	2.197
10	16.259	37	61.169	65	3.497
11	24.596	38	0	66	1.178
12	4.267	39	0	67	0.924
13	2.462	40	0.983	68	0
14	1.637	41	1.455	69	0
15	5.420	42	2.784	70	0
16	3.196	43	2.258	71	0
17	0	44	0	72	1.909
18	0	45	0	73	2.478
19	0	46	0	74	0
20	2.268	47	0	75	57.329
21	1.267	48	0	76	0
22	1.479	49	0	77	0
23	4.563	50	0	78	0
24	5.839	51	27.938	79	3.407
25	9.120	52	22.289	80	6.316
26	7.427	53	1.258	81	6.489
27	5.249	54	11.256	82	8.159

In conclusion, they seem physically same but in chemical composition some of them are not mad honey. Local people use mad honey for medication and they take grayanotoxins unknowingly. Calculated grayanotoxin amounts define of the purposes of their honey usage. In this study we collect samples from Duzce and near villages in the mad honey season. Some samples do not contain grayanotoxins even though strong odor and light yellow colour. Some samples contain high values of grayanotoxins even without physical differences. Mad honey usage is highly risky and not recommended for consuming but for medication purposes its promising for hypertension.

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Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

Evaluation of Acute and Subacute Toxicity of ISY-CP® Food Herbal Mixture in Rats

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Received: 27.05.2020 Accepted: 04.08.2020

Abstract

Objective: Recently, there is a growing interest in medicinal and aromatic plants also the treatment methods by using these plants in traditional and complementary medicine. On the other hand, possible toxicities of medicinal and aromatic plants has lately begun to be researched and it is extremely important to cognize these possibilities for human consumption. Therefore, a research has been conducted to understand the acute/subacute toxicity characteristics of a plant based herbal mixture, ISY-CP® used in remember regeneration therapy method (RTM) in rats. The product contains a mixture of nettle leaves (*Urtica dioica*), yarrow (*Achillea millefolium*), thyme *(Thymus vulgaris*) and horsetail (*Equisetum arvense*) plants. The aim of this research was to examine the effects of ISY-CP® herbal mixture in term of toxicity.

Material-Method: In the experimental design, 32 female rats were divided into 4 groups (control, acute, subacute and post-subacute). In order to observe acute and subacute toxicity, clinical observations were performed and the biochemical, hematological and histopathological parameters of the animals were evaluated at the end of the application.

Results: According to the results obtained from the study, there were not found significant differences in biochemical, hematological and histopathological evaluations between the control and the application groups. Only phorphorus data is statistically different between subacute toxicity group and control group.

Conclusion: In our study, the acute and subacute toxic effects of ISY-CP® herbal mixture's doses used in this study were not observed.

Keywords: Traditional and Complementary Medicine, Acute Toxicity, Subacute Toxicity, *Urtica dioica, Achillea millefolium, Thymus vulgaris, Equisetum arvense*

INTRODUCTION

The use of traditional and complementary medicine practices has increased significantly in recent years in the prevention of diseases and sustaining health worldwide. Although the usage rates reported in different sources vary, they have usage ranges between 40% and 90% worldwide; 42% in America; 70% in Canada; 50% in France; 48% in Australia and 90% in Asian and African countries [1]. Many different purposes and treatments have been used in traditional and complementary medicine products. According to Yasar et al. (2019), some plants thought to be modulators in epigenetic mechanisms may exhibit anticancer features with phytotherapy approach [2].

The type and amount of synthetic compounds used in the health, cosmetics and food industries are increasing, threatening the environment and human health, affecting the country's economy negatively every year [3]. Some types of synthetic drugs accumulate biologically in the environment and cause serious environmental damage [4,5]. In this respect, the use of herbal products has increased

worldwide and has highlighted the "safety" issue of these products. Herbal products are considered to be natural; some perceptions that "It does not contain toxins; it does not have undesirable effects; it can be used for a long time and it is safe" is misdirecting. Herbal products are usually in the form of mixture and contain many substances. For this reason, it may have unknown features and effects.

Toxicity may depend on the natural chemical composition of the plant, or it can occur due to possible contamination, adulteration or misidentification of the plant. Dosage and duration of use are extremely important in monitoring toxicity of herbal products. High dosage and long-term use of these products can cause side effects. Also, Toxicity may depend on the active ingredients or dosage in herbal mixture, as well as on user-related factors such as age, genetics, other diseases, and other drugs used. Some plants have their own toxicity at normal therapeutic doses or overdoses [6]. Some substances in the components of some plants, such as ephedra, archtolochic acid, and aconitum, can directly generate toxicity. In some cases, external toxicity can be mentioned [7].

All medicines, cosmetics, pesticides, food additives and chemicals used in industry are evaluated for their toxic potential before they are presented to human use. The effects of these substances on human health are determined by designing toxicity tests by considering the exposure routes and durations. Toxicity tests are also carried out to determine the safe dose values of these substances[8]. One of the most commonly used acute toxicity tests is the 'lethality' test in lots of studies. This test is carried out to determine the toxic symptoms that may occur as a result of interaction with a chemical substance, lethal dose (lethality) value or the degree of influence of certain organs such as brain, kidney, liver. The lethal dose value is considered as an indicator of how safe chemical can be used for human health.

While there are many studies on the benefits of the ingredients of plants, there are a limited number of studies investigating the negative effects of their ingredients. Toxicity studies must be carried out before the products consisting of plants or their ingredients are presented for consumption. Preclinical studies provide detailed information about the effects of toxicity [9]. While any beneficial effects of any substance contained in plants can be seen in some doses, they may show toxic effects or be lethal when combined with other doses or other substances[10].

Toxicity studies are basically divided into 4 parts as acute, subacute, chronic and subchronic [11]. However, there are also special toxicity tests such as immunotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity[12]. These tests provide us with information about the toxicities of the substances contained in the product[13]. The use of products that have been tested for toxicity will make an important contribution in preventing negative effects.

In the current study the acute toxicity and subacute toxicity properties of herbal mixture called ISY-CP®, were investigated.

MATERIALS AND METHODS

Herbal mixture content

ISY-CP® contains a mixture of Nettle Leaf (*Urtica dioica*), Yarrow Perch (*Achillea millefolium*), Thyme (*Thymus vulgaris*) and Horsetail (*Equisetum arvense*) plants. The herbal mixture were supplied from Naturin (Natural Products Pharmaceutical and Pharmaceutical Raw Materials Industry Trade Limited Company). The animals in the acute and sub-acute toxicity groups were administered at 11.8mg/ml ISY-CP® by oral gavage, with the recommended daily use of the ISY-CP herbal mixture (contents is shown in table 1.) adapted to the rats. Herbal mixture was dissolved 1 ml water before gavage. Doses to be given daily were prepared freshly. The control group was given 1 ml water daily.

Table 1. ISY-CP® contents

Herbs	Quantity (1 Capsul)
Achillea millefolium	*184 mg*
Urtica dioica	*92 mg*
Thymus vulgaris	*92 mg*
Equisetum arvense	*92 mg*

Experimental animals

The animals to be used in the study were obtained from Düzce University Experimental Animals Application and Research Center. Wistar Albino 8 weeks old, 250-300 g female rats were used in the laboratory at 20-25 ºC room temperature, 55 ± 5% humidity and 12:12 light-dark cycle, with optimal food and water intake. Animals were provided with commercial food pellets and water *ad libitum*. Experiments were carried out with the approval of the Düzce University Animal Experiments Local Ethics Committee (2020.4.3).

Acute and subacute toxicity study procedure

In the experiment to be carried out using the ISO 10993[11] toxicity protocol with minor modifications, 32 animals were randomly divided into 4 groups.

1 ml of water was given to the control group by gavage for a 7 days. 24 hours after application, blood was taken. At the end of administration, the control group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The 2nd group was designed as an acute group. They were administered content with once oral gavage. Clinical observations were made. 24 hours after administration, the acute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The 3rd group was subacute group. They were administered once time content for 7 days with oral gavage. Clinical observations were made. At the end of 7 days, the subacute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

The final group was post-subacute group. They were administered once time content for 7 days with oral gavage. Content delivery was stopped after 7 days. Aftewards, Clinical observations were contiuned additional 7 days. At the end of 14 days the post-subacute group animals were sacrificed under anesthesia. Liver, kidney and spleen tissues were taken for histopathological examination. Blood was collected from heart.

RESULTS

Clinical observation parameters

In all groups, clinical observation was performed to obtain the data of toxicity at 0. min, 30 min, 60 min, 120 min, 240 min, 480 min and 1440 min during the administration. In this observation, the parameters given in Table 2 were evaluated.

Biochemical and haematological parameters

As seen in Table 3, no statistical difference was found between the control and administration groups in terms of biochemical parameters except 3 parameters.

Table 2. Observation criteria

Clinical Observation	*Observations*	*Systemic Observation*
Analgesia	Decreased analgesia	CNS, sensory
Cardiovascular observation	Bradycardia, tachycardia, arrhythmia, vasodilation, vasoconstriction	MSS, Autonomous SS, cardiac, circulatory system
Gastrointestinal	Diuresis	MSS, autonomous SS, kidney, motolite
Motor activities	Descending / increasing, Indeterminate positions, tremor	MSS, Samatomotor, sensory, autonomous, muscular-nervous systems
Muscle tone	Hypotonia, hypertonia	Autonomous SS
Oculer observation	Lacrimation, miosis, mydriasis	Autonomic nervous system, irritation
Reflexes	Initial reflex	MSS, Sensory, automic, muscular-nerve
Respiratory	Dyspnea (abdominal breathing), apnea, eupne, tachypnea	Central nervous system (CNS), circulatory cardiac, respiration
Salivation	Quantity	Autonomous SS
Skin	Edema, rash	Tissue injury, irritation
The convulsion	Clonic, tonic, tonic-clonic symptoms	CNS, respiration, muscular-nervous, automic
The piloorection	Coarse feathers	Autonomous SS

Table 3. Groups' biochemistry mean and standard error values

GROUPS	P MEAN ± SE	CA MEAN ± SE	ALB MEAN ± SE	TG MEAN ± SE	TP MEAN ± SE	TC MEAN ± SE	CREA MEAN ± SE	GGT MEAN ± SE	ALP MEAN ± SE	AST MEAN ± SE	UREA MEAN ± SE	ALT MEAN ± SE
CONTROL	6.71 ± 0.34	13.5 ± 0.09	50.63 ± 0.92	139.92 ± 11.15	101.2 ± 1.71	101.9 ± 12.71	0.11 ± 0.01	4.98 ± 0.27	220.90 ± 33.83	270.90 ± 60.51	71.71 ± 2.01	194.46 ± 46.35
ACUTE	5.95 ± 0.43	12.81 ± 0.2	49.81 ± 1.79	145.65 ± 25.84	104.3 ± 1.78	121.46 ± 17.41	0.11 ± 0.02	4.41 ± 0.19	210.17 ± 16.20	289.66 ± 52.79	78.42 ± 6.28	140.96 ± 13.39
SUBACUTE	10.45 ± 1.93*	12.27 ± 0.72*	46.07 ± 5.60	72.38 ± 22.41	90.4 ± 1.65*	77.61 ± 3.32	0.11 ± 0.07	5.50 ± 0.1	228.33 ± 40.85	365.27 ± 141.86	72.24 ± 4.63	178.27 ± 62.63
POST-SUBACUTE	8.98 ± 0.26	13.31 ± 0.42	50.15 ± 0.45	154.18 ± 20.97	104.7 ± 5.9	98.25 ± 7.13	0.10 ± 0.10	6.10 ± 0.0	161.90 ± 14.80	222.90 ± 41.80	68.61 ± 7.54	108.40 ± 15

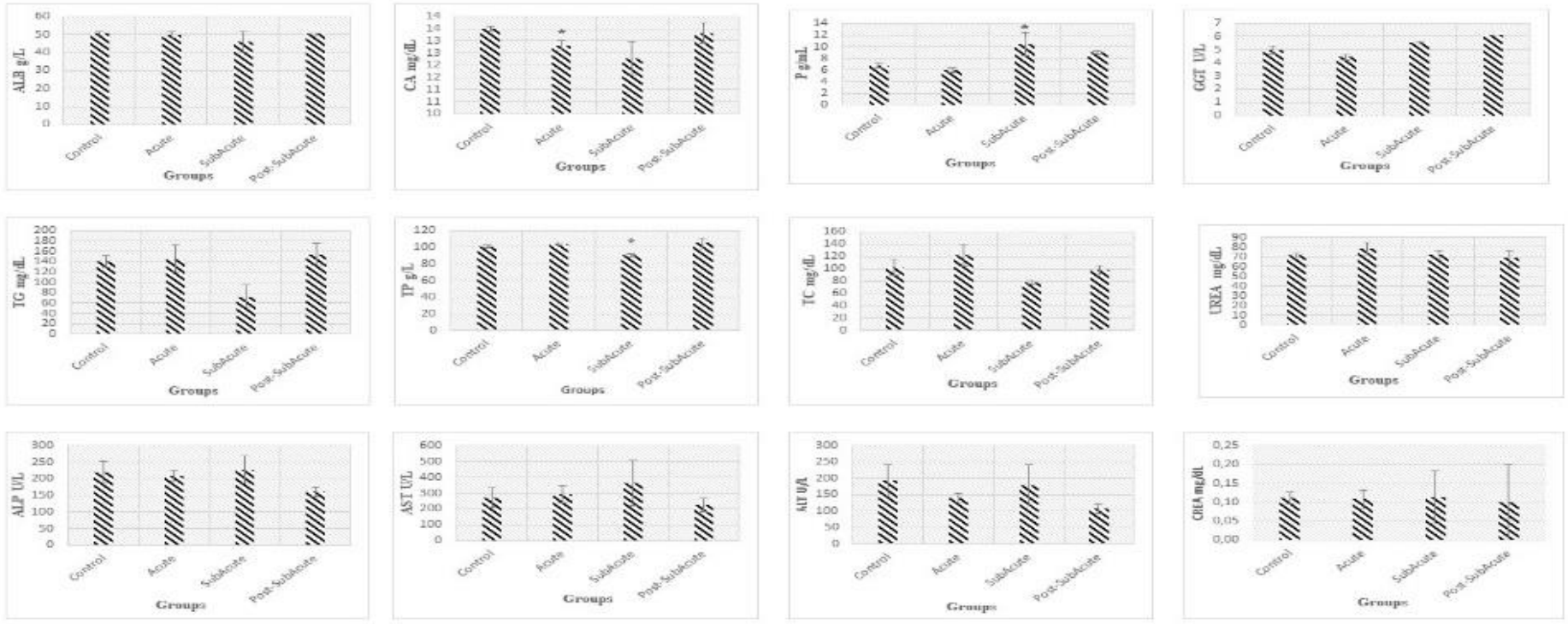

Figure 1. Groups' biochemistry parameters
* significant differences with control group≤0.05

As seen in Table 4, there was no statistically difference between the control and application groups in terms of 5 haematological parameters.

Histopathological evaluation

For histopathological examination, the histopathology of organs taken by appropriate methods from each animal in each experimental group was generally evaluated. When the results of lung histopathology of the herbal mixture group animals and control group animals given ISY-CP® herbal mixture were evaluated in terms of interstitial and bronchointerstitial pneumonia, degeneration, hyperemia and necrosis, there was no difference between the groups.

For the liver, kidney, spleen histopathology results of the animals in the application group and the control group were evaluated in terms of pigmentation, degeneration, hyperemia, bleeding and necrosis, there was no difference between the groups.

Histopathologically, no comparison was observed between the animals of the herbal mixture group that was given ISY-CP® herbal mixture and the animals of the control group.

Table 4. Groups' haematological parameters mean and standard error values

Groups	WBC Mean ± SE	RBC Mean ± SE	HGB Mean ± SE	HCT Mean ± SE	PLT Mean ± SE
Control	5.64 ± 0.60	7.31 ± 0.22	13.79 ± 0.40	41.66 ± 1.18	721.88 ± 82.24
Acute	8.03 ± 1.12	8.24 ± 0.42	14.93 ± 0.60	45.83 ± 1.86	908.71 ± 68.84
SubAcute	5.64 ± 0.43	6.66 ± 0.21	12.33 ± 0.09	37.7 ± 0.38	966.67 ± 221.40
Post-SubAcute	4.27 ± 0.69	7.09 ± 0.28	13.7 ± 0.70	41.8 ± 2.30	460.5 ± 438.50

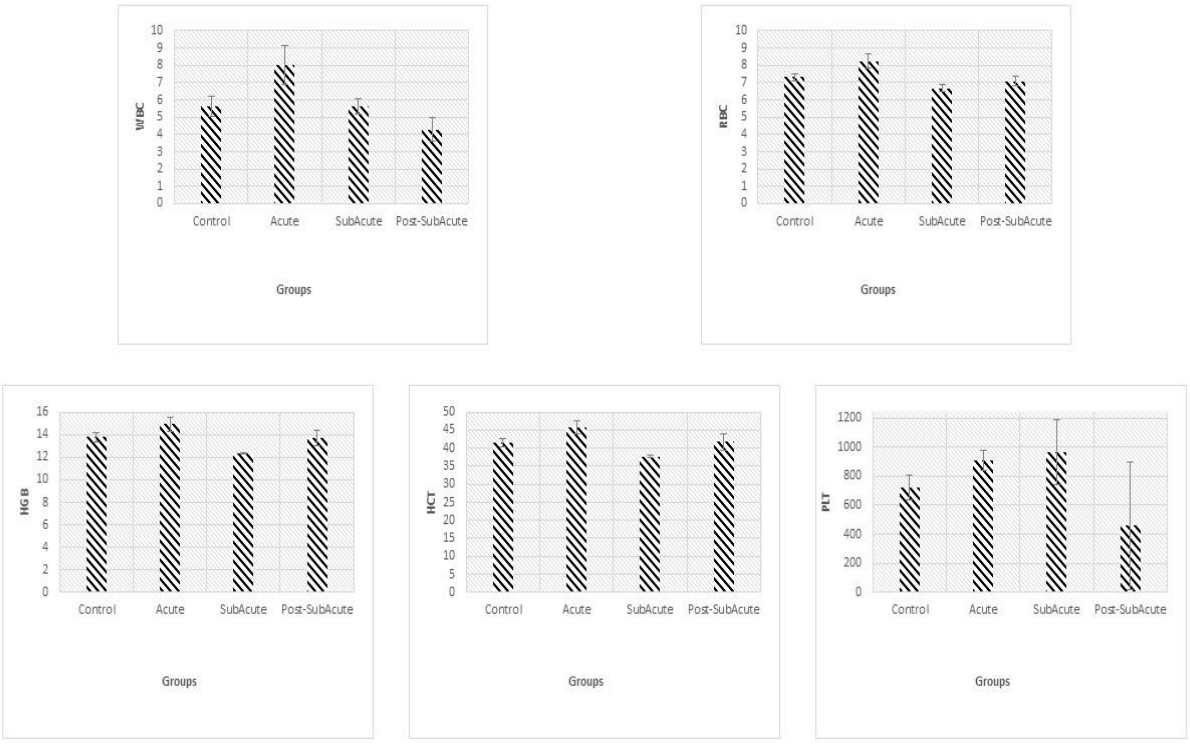

Figure 2. Groups' hemogram parameters

* significant differences with control group≤0.05

DISCUSSIONS

In different studies , it was observed that different target organs were affected by the active substances in herbal mixture. Taylor et al. (2004) observed hepatotoxicity with plants containing senna alkaloids; dermatitis and nephrotoxicity caused by heavy metals such as lead and mercury [14]. So, investigating effects of herbal supplement is important for toxicity.

In a study conducted by Dar et al. (2013) on nettle leaf (*Urtica dioica*), it was found that toxicity tests showed higher safety margins of all solvent extracts with an LC_{50} of 1000 µg/ml on *A. salina* [15]. *Urtica dioica* contains different components such as lignans, polysaccharides, and lectins which prevent prostate enlargement. It prevents cell growth and has anti-inflammatory properties. Nettle leaf has many uses such as stopping bleeding, relieving anemia, sciatica, urticaria, psoriasis, diarrhea, rheumatism, prostate and mouth sores etc.[16]. The LD_{50} dose detected in mice was calculated to be 3625 mg/kg[17].

Yaeesh et al. (2006) were investigated hepatoprotective effects of yarrow (*Achillea millefolium*). D-galactosamine and lipopolysaccharide were administered to mice. All mice were dead. Mortality is 100%. After pre-treatment yarrow crude extract to mice, mortality decreased to 40%. In the light of the obtained data, the extract of yarrow was significantly lower in the alanine aminotranferase (ALT) and aspartate transferase (AST) levels. these results make us think that yarrow may have a liver protective effect[18]. Hasheminia et al. (2011), in a study examining the toxicity properties of the yarrow, LC_{50} and LC_{25} values were found to be 4.19% and 1.69%, respectively[19].

Basch et al. (2009) suggested that not to take oral doses of 10 grams of dried leaves with 0.03% phenol (calculated as thymol) in thyme. Oregano oil might be very toxic. *In vivo* studies revealed that toxicity symptoms may include nausea, tachypnea and hypotension [20]. LD_{50} of thyme essential oil is 2.84 g / kg body weight in rats [21]. Another study by Fakılı (2010) on thyme was found that oregano underground water had neither acute nor chronic toxic effects and was effective on the digestive and cardiovascular system[22].

Equisetum arvense (horsetail) contains abundant calcium and silicon elements[23]. Due to the active ingredients contained in horsetail, it is used in the treatment of ulcers, stopping bleeding, healing wounds, kidney diseases. It also shows antioxidant properties. In addition, there are anticonvulsant, sedative and antioxidant activities in the studies. In addition, there are anticonvulsant, sedative and antioxidant activities [24,25]. In a single-dose toxicity study to determine the LD_{50} value in rats, the toxic dose was found to exceed 5000 mg / kg [26].

Considering the results obtained in our study, post, subacute group ALT, AST and ALP values were not statistically significant, but decreased compared to the control group. These results are Yaeesh et al. (2006) is similar to the results. It is seen that the toxic doses of the plants used are high. The toxic effect of plants was not observed in the ISY-CP® mixture we used in our study.

There was no difference in clinical observation between the control group and the application groups performed in the experimental process. These results are supported by biochemical and histopathological evaluations. In terms of biochemical parameters, no statistically significant difference was found between the acute, the post-subacute groups with the control group. But subacute phosphorus, calcium and total protein values are statistically lower than control group values. Considering the toxicity parameters (eg AST, ALT, ALP etc.), there is no significant difference between the subacute group and the control group. Therefore, when all the data are evaluated together, it is believed that the ISY-CP® product has no toxic effect at the doses determined in this study. According to the data obtained during the experiment and after the experiment, acute and subacute toxicity effects of the ISY-CP® herbal mixture were not found clinical symptoms observed, biochemical and haematological parameters and pathology findings. Based on our data, there is no toxicity effect. It is necessary to plan new studies to identify ISY-CP® positive effect with physiologically.

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Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

ORIGINAL RESEARCH

The Profile of the Patients Who Consulted to the Conventional and Complementary Medical Centre in Duzce University

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Received: 04.08.2020 Accepted: 25.08.2020

Abstract

Objective: This paper investigates the consulting reasons of the patients, the applied cures to them and also the demographic features (age, gender) of the patients who consulted to the Centre of Conventional and Complementary Medicine, Duzce.

Material-Method: In this paper the patients who consulted to the related centre between the dates 15.05.2019-15.05.2020 were analysed in a retrospective way. The ages, genders, date of consultation, complaints and results of the applied practice were obtained and discussed.

Results: During our researches, when looked over the range of gender, it is stated that 39,8 % of 860 patients were male and 60,1 % were female. If we consider the range of age, it is seen that the most crowded group ranges between 30-49 ages with a percentage of 45%. This group is respectively followed by the ones between 50-65 with a percentage of 39 % and the ones over 65 as 10 %, 18-29 ages (4%), 0-17 ages (2 %). If we consider the range of the patients according to the applied cures, we see that the most common practice is ozone therapy as 42%. It is respectively followed by cupping therapy as 19%, mesotherapy as 15%, acupuncture as 11%, hirudothearpy as 10%, hypnotherapy as 2% and lastly the apitherapy as 0,4%.

Conclusion: Consequently, it is obvious that the use of conventional and complementary medicine is more common among females than males and it is preferred more when the age gets older. Also, it is preferred

Keywords: Conventional and Complementary Medicine, Acupuncture, Hirudo Therapy, Cupping Therapy, Ozone Therapy

INTRODUCTION

The definition of conventional and complementary medicine by The World Health Organisation (WHO): It is a whole of the data, skills and practices –accountable or not- , which are based on the theories, beliefs and experiences peculiar to different cultures[1]. It aims defence against the physical and mental illnesses, diagnosing these in addition to healing or remediation to maintain wellness. Familiarity to and use of conventional and complementary therapies has recently increased both in our country and around the world. The supreme issue about this topic is the fact that this kind of therapies are administered as uncontrolled; depending on that serious complications may occur[2]. To prevent this kind of situations, conventional and complementary medicine was legitimised on the official journal on 27.10.2014 by the regulation named the same and numbered as 29158[3]. In our country, only the medical doctors and the dentists have been legally licensed and allowed to administer in 15 different fields. These are acupuncture, apitherapy, phytotherapy, hypnosis, hirudotherapy, homeopathy, chiropractic, cupping therapy, maggot debridement therapy, mesotherapy, prolotherapy, osteopathy, ozone therapy, reflexology and musicotherapy[4].

In the relevant regulation, these therapies are defined as:

- Acupuncture means the application by means of

stimulating the particular points stated on the human body using the stimulation procedures likewise the needle, laser beams, electrostimulation, cupping, ear seeds, magnetic massage balls, thermic stimulation, acupress and audio or magnetic resonance.

- Apitherapy is the use of bees and bee products to cure some illnesses as a complement and promoter.
- Phytotherapy is a way of treatment using the conventional herbal medical materials and herbal remedies.
- Hypnosis is the process designed to obtain or reveal a change on someone's conscience, awareness, body, emotions, feelings, thoughts, mind or manners.
- Hirudotherapy is the use of sterilised leeches.
- Homeopathy is an integrative practice that aims to heal the patient using homeopathic drugs that are specifically chosen for that person.
- Chiropractic is a promoter field concerning the prevention of bio-mechanic disorders and their effects on the nervous system by the musculoskeletal system.
- Cupping therapy has two types: dry cupping therapy is the treatment method that aims a local vacuuming in order to boost the bloodstream. On the other hand, wet cupping(hijama) is a method that involves puncturing the skin to remove the blood after vacuuming on the specific parts of the body.
- Maggot debridement therapy is the use of Lucilia Sericata sterile maggots with the aim of bio debridement on the chronic lesions.
- Mesotherapy is the local and intradermal injection of herbal and pharmacological drugs that aims to cure the organ pathologies originating from mesoderm by minimal doses, specific needles and techniques.
- Prolotherapy is the intraligamentary injection of proliferative and irritant solutions.
- Osteopathy is a non-invasive conventional and complementary medical treatment that helps strengthening the musculoskeletal system involving the diarthrosis, muscles, connective tissues and spine. It focuses on the total body weight and the effectiveness of musculoskeletal system on illnesses.
- Ozone therapy is the local or systematic use of ozone-oxygen mixture.
- Reflexology depends on the basis of the presence of the collimator reflex areas on hand, sole and ears related to whole body parts, organs and glands. Without the use of any tools, materials, cream or lotion, these reflex areas are only exerted pressure. Reflexology doesn't involve diagnosing and healing specific illnesses or mobilisation and manipulation of diarthrosis.
- Musicotherapy is the clinical and evidence-based use of music and its implementations by a professional licensed in musicotherapy in order to meet one's physical, psychological, social and mental needs[4].

Cupping therapy is a conventional and complementary medicine type whose background dates back to old times. Cupping therapy, which was thought to have a history of 5000 years, was extensively used in Muslim socieities and Ottoman Empire. It boosts subcutan blood build up by local vacuuming on body. The type cupping which is conducted only by local vacuuming without puncturing is called as dry cupping. After vacuuming, revealing the accumulative blood hygienically is called as wet cupping (hijama). Nowadays, cupping therapy has been used to cure many illnesses as a complementary medical practice. Some of these are musculoskeletal system diseases, hematologic diseases such as iron overload, migraine type headache, rheumatic disorders and gastrointestinal system disorders[5].

Apitherapy is type of conventional and complementary medicine in which bees and bee products are used aiming the wellness and treatment. When we gaze at its history, the oldest chronical is a Sumerian tabloid belonging to 5000 years ago[6]. The mostly used bee products are honey, pollen, bee gum, royal jelly and bee venom. Thanks to the researches, it is obtained that these products have anti-inflammatory, antimicrobial, anticancer, antioxidant and immunomodulator effects[7]. Those kind of products like royal jelly,

pollen, honey and bee gum are used to strengthen the immune system as a complement. When it comes to bee venom, it is used for the purpose that decreasing the pain experienced in musculoskeletal system diseases and as a supporter against myasthenia. Apitherapy is never administered on the patients who suffer from bee sting allergy.

Ozone therapy is the practice of mixing certain amount of blood taken from the body with ozone gas and then transferring it back to the patient intravenously. This practice is called as major autohemotherepy. Taking less amount of blood (2-10 cc) from the body and intramuscular transfer of it back to the patient after mixing ozone gas is called as minor autohemotherapy. The history of ozone therapy goes back to 1840s and the German Chemist Christian Friedrich Schönbein is accepted as the father of ozone therapy. The situations in which it is primarily used are arterial circulation disorders, dentistry practices, rheumatic arthritis, decubitus ulcer, diabetic ulcer, muscle and diarthrosis disorders and much more[8].

Mesotherapy is the injection conducted with specific needles of 4.6 or 13 mm into the tissues which are based on mesoderm. For this procedure the materials varies in compliance with the aim of treatment. Dr. Michel Piston practiced Mesotherapy for the first time in history in France in 1952[9]. It is used in two different fields as cosmetics and medicine. In cosmetics, it is used for such treatments that losing weight, alopecia, cheloid and acne. In medicine, it may be preferred to strengthen the immune system or to treat migraine type headache, sports injuries on soft tissues, arthritis, trigeminal neuralgia, arteritis, vasculour diseases and much more[4].

Acupuncture is a conventional and complementary curing type that dates back to 5000 years ago. In China, proofs on acupuncture practice by using stone needles were obtained. The internal diseases book written in Chinese and in 200 B.C. is the oldest source on acupuncture. The Turkish doctor İbn-i Sina referred acupuncture in his works in 1100s[10]. Acupuncture is stimulating certain areas of the body, which are called as acupuncture points, by the way of dry needling. It is used as a complementary treatment method against such diseases that musculoskeletal disorders, arthralgia, migraine and other headache types that are nonorganic, accordance to regime in exogen obesity, anxiety, nausea and vomiting based on the chemotherapy and actinotherapy, sleep disturbances based on nonorganic reasons, dysmenorrhea, infertility, polycystic ovaries and much more[4].

Hirudotherapy is the use of medical hirudos to cure. In this way, the hirudos are fed with the blood of host; meanwhile, their function of secreting many substances that have anticoagulant, anti-inflammatory, anaesthetic features is utilised. It has been used since from very old times. In Egypt, demonstrations of the use of medical hirudos were found on a tomb which was thought to date back to 1500s. In Kanun Fit-Tıp, the work of the important medical scientist İbn-I Sina, it is mentioned how and against which diseases the hirudos were use.[11] The indications in which hirudotherapy is used are degenerative arthropathies like osteoarthritis, venous dysfunction post flep surgery, also, it is used to decrease the pain in varicose venous diseases and such diseases that lateral epicondylitis.

- Definition of hypnotherapy in words of American Psychology Union- Hypnosis Department: Hypnosis is the consciousness in focus and minimised environmental awareness and it is characterised by a rise on the capacity of replying the suggestions[12]. Within the Regulation of Conventional and Complementary Medical Techniques, medical practice fields of hypnosis are stated as: Conquering pre-op fear before the operations,
- coping with the anxiety and intra-op pains,
- during whole diagnostic and interventional operations, quelling and supporting the accordance to treatment in emergency,
- in the process of infertility treatment,
- during pregnancy and the moment of birth,
- during treatment of gynaecological diseases, obesity, eating disorders, smoking cessation, alcoholism, stress disorder, nonorganic sleep disturbances, depression, functional intestinal

disorders, acute and chronic pains, allergic rhinitis and allergic asthma;

- strengthening the immune system,
- reducing the pain and anxiety in treatment of ambustion,
- narcotising,
- coping with the pain, vomiting, anxiety and side effects of the drugs seen on the ones who suffer from cancer

Apart from these, hypnosis practices in dentistry are stated as[4]:

- Phobias on dentistry operations
- Removing pain and during anaesthetic procedure
- In treatment of bruxism, temporomandibular joint disorders, trigeminal neuralgia, oral disorders
- Increasing compatibility to treatment or denture prosthesis

Maggot debridement is the use of Lucilia Sericata maggots in medicine. It is preferred to cure purulent or scabbed lesions. The enzyme released by the maggot helps to remove and disinfect the infarct on the lesion[13]. Also, it helps stimulating the creation of scar tissue[13].

In this paper, we aim to analyse the number of the patients consulted to the conventional complementary medical centre in Duzce University, the complaints and demographic features of the consultants, which conventional and complementary medical practices are carried out against which situation.

MATERIALS AND METHODS

In this study the patients who consulted to the Conventional and Complementary Medical Centre of Duzce University between the dates 15.005.20019 and 15.05.2020 were analysed retrospectively. Within this period the total number of the consultants is 860. On the other hand the ages, genders, application dates, complaints of the patients and the results of the administrations were collected.

With the help of the collected data demographic feature range of the patients, the ratio of the consultant number by months, by complaints and finally by the applied treatments were analysed.

Our medical centre is licenced to practice acupuncture, cupping therapy, ozone therapy, hirudotherapy, hypnosis, mesotherapy, apitherapy, maggot debridement, phytotherapy and music therapy.

Microsoft Excel XP and SPSS were used to analyse the data. The values were given in numbers and percentage.

RESULTS

When we consider the range between the genders of 860 consultants in the mentioned period, we see that 39.8% (n=343) of the total number is male, 60.1% (n=517) is female. When the range of age is considered, the most crowded group is between 30-49, that covers 45 % of the total number. It is respectively followed by the ages 50-65 (39%), >65 (10%), 18-29 (4%), 0-17 (0.2%) (Figure 1).

The average of age for the target population of the study is 49.7(±12.7). The age average of the male ones is 49(±14.8), only the female consultants' age average is 50.2(±11.4).

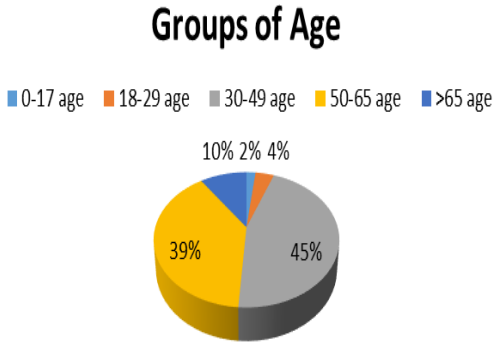

Figure 1. Age Division of the Patients Consulted to Conventional and Complementary Medical Centre of Duzce University

When we consider where the patients come from, the common answer is Duzce with a percentage of 97.6% (n=840). The rest of the total number (2%) is from other cities. When we consider the range of the patients according to the treatments they got, the most common practice is ozone therapy as 42%. It is followed by cupping therapy (19%), Platelet Rich Plasma (15%), acupuncture(11%), hirudotherapy (10%), hypnosis (2%), apitherapy (1-0.4%) (Figure 2).

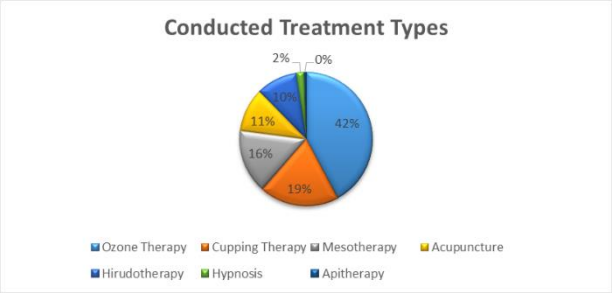

Figure 2. The range of the treatment types administered on the patients

When we consider the distribution of the patients according to their genders in terms of the treatment types, it is seen below (Figure3).

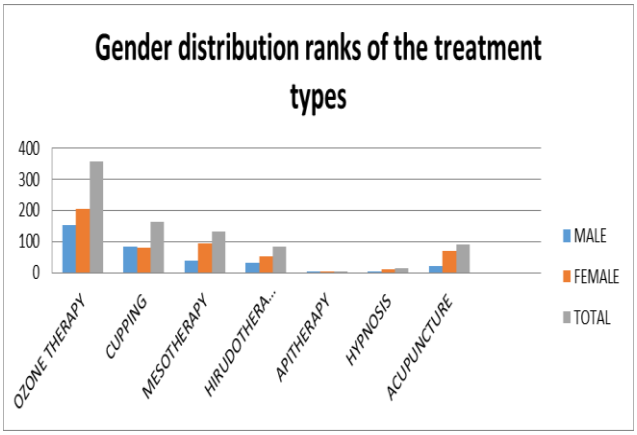

Figure 3. The graphic monitoring the gender distribution ranks of the treatment types

When the treatment types were analysed in accordance with the complaints, we see that 49.2% of the patients who demanded general well-being got ozone therapy, 43.8% of them got cupping therapy. 74.2% of the patients who complained knee pain got Platelet Rich Practice, 23.4% of them got hirudotherapy.

According to the treatment indications data;

- For ozone therapy, general well-being demand takes place on the top as 37% of the total number. Diabetic foot takes second place as 16%. Pain complaint comes then as 8%.
- For cupping therapy, general well-being demand comprises 73.7% when the pain complaints comprise 13.4%.
- For hirudotherapy, the most common consulting reason is knee pain as 36.4%. Leg pain based on the peripheral venous statis follows it as 20%.
- For acupuncture, the most common reasons to consult are obesity (64.4%) and being overweight. Alcohol withdrawal syndrome follows them as 13.3%.
- For Platelet Rich Plasma, 74.2% of all the indications to consult is knee pain as the most common one.

DISCUSSION

Use of the conventional and complementary treatments has recently increased in our country; yet, the number of academic studies in this field is not satisfying. For this reason, we expect more studies will be carried out on the topic.

In this study, ozone therapy is the most common conventional and complementary treatment type among all. Oral and her colleagues conducted a study to detect the notion, manners and actions of the ones who consulted to the primary care clinic in relation to conventional and complementary treatment types. They reached that the most common method is thermal spring; on the other hand herbal products and remedies follow it[14]. Again, in the study written by Kav and colleagues to analyse the frequency of occurrence of the complementary and alternative medicine, the most common method is found as herbal remedies and products[15]. These data conflicts the data that we obtained in our study. The reasons of this conflict are the different complaints and demands that the patients have. In the lastly mentioned study, all of the patients suffer from cancer; however, in our research, the number of the cancer sufferers is limited. In our study, whereas the least common methods are hypnosis and apitherapy, Oral and her colleagues stated ozone therapy and hypnosis as the least common[14]. Hypnosis is the method that has been practiced rarely according to both results.

We can easily detect that the quantity of female patients is more than male ones. Similarly, oral and her colleagues reached the same conclusion in their study[14]. Considering the ages of the consultants in our medical centre, the most frequent ages change between 30-49; the secondary group ranges between 50-65. Again, Oral and her colleagues reached the information that the ones over 30 prefer

the conventional and complementary treatments more[14]. Seeing the same results in both studies may make us think that the given medical methods are preferred mostly by the ones who are older than 30. In our study, the rate of the ones who consulted for the general well-being without any complaints is less than the rate of the ones who have a complaint. Similarly, in Oral and her colleagues' study, the practice of conventional treatments based upon a complaint is on the top rank[14]. This data obviously reveals that the patients prefer conventional and complementary treatments mostly because they suffer from a disturbance. However, some of the consultants prefer these kind of treatments even though they have no complaint.

Restriction of the Research

This paper frames only the consultants who consulted to The Conventional and Complementary Medical Centre of Duzce University.

In conclusion, this study claims that conventional and complementary treatments are more popular among females and older groups; they may be preferred just for the general well-being apart from a complaint also.

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Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

CASE REPORT

Acupuncture Method in the Treatment of Idiopathic Abdominal Pain

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Received: 25.08.2020 Accepted: 25.08.2020

Abstract
Acupuncture is an ancient treatment method with a history of three thousand. In this study, we performed acupuncture treatment for a 49-year-old patient with no additional disease and an unknown cause of abdominal pain. We applied 12 sessions of body acupuncture in total. After the end of the treatment, we called for 1 and 3 month controls. After twelve sessions, a significant improvement was achieved in the patient's clinic. We observed that his well-being continued in his controls. We think that it should be among the treatment options in acupuncture in the treatment of idiopathic abdominal pain.
Keywords: Acupuncture, Pain, Abdominal Pain

INTRODUCTION

Acupuncture consists of two Latin words (acus: needle and puncture: to needle). Traditional Chinese Acupuncture has a 3000-year history[1]. Various points on ear, head and body were determined and it was thought that each point was lined up on imaginary lines named meridians[2]. Acupuncture points have low electrical resistance. 12 pairs and 2 single meridians were defined by combining the acupuncture points. Acupuncture is applied by inserting steel, silver and gold needles are inserted into acupuncture points[3]. During the Han dynasty (206 BC-220 AD), the principles of both theoretical and practical applications of Chinese medicine were determined. During this period, Ying and Yang, five elements, channel theories and various needling methods were explained[4]. The World Health Organization published a book on acupuncture as a result of examination of clinical studies in 2003. The indications for acupuncture threatment are also specified in this report. Some of the diseases, symptoms and conditions that can be effectively treated with acupuncture were defined as: dysmenorrhoea, peptic ulcer, acute and chronic gastritis, gastrospasm pains, facial pain, migraine and other headaches, knee pain, low back pain, morning fatigue, neck pain, dental related pain, shoulder joint pain, postoperative pain, rheumatoid arthritis, tennis elbow[5, 6]. In our study, we applied acupuncture treatment for abdominal pain whose cause was not found.

CASE

A 49-year-old married, healthcare worker (audiometrist), female patient. There is no known history of additional illness. There is no continuous medication. She did not have any surgery. There is an abdominal pain that has been going on for six

months, comes in attacks on average 4 times a week. Her pain does not respond significantly to analgesic drugs and can last for an average of 4-6 hours. No pathology was found in examinations, tests and imaging methods performed by general surgery, gastroenterology and gynecologists. When she applied to our clinic, we planned 12 sessions of acupuncture treatment as twice a week for the first 4 weeks, then once a week for the next 4 weeks. Informed consent was obtained from the patient and treatment was initiated. We chose Yintang, Du20, St36, St25, Li4, Li11, Ren 12, Ren 6, Sp 6, Liv3 as acupuncture points. Session duration time was 20 minutes on average and we preferred sterile needles of 0.25*25 mm as acupuncture needle. After 12 sessions, it was determined that the pain of patient was an average of once on every ten days, was less than before and resolved with analgesic drugs containing paracetamol. It was stated that the pain did not start in one month and three months controls.

DISCUSSION

Versatile researches on acupuncture began in China after the cultural revolution in 1965. Today, acupuncture training is provided within the Medical Faculties in many western countries, especially France, Germany, England and Austria. Among these, Acupuncture Institute within the Vienna Medical Faculty, founded by prof.dr Johannes Bischko, is the best known[7]. It demonstrated that autonomic dimension of acupuncture stimulation is generated by a mesencephalic and brain network, in which areas hypothalamus, medulla oblongata, ventrolateral peri-aquaductal gray and dorso-medial pre frontal cortex are involved. All these areas require autonomic regulation[8]. When the acupuncture needle is inserted into body, the impulses initiating from the nociceptors activates the analgesic system by stimulating the enkephalinergic and serotoninergic neurons in the mesencephalon on the way from medullaspinalis to cortex. At the end of this, beta-endorphin, enkephalin, serotonin and norepinephrine increase in central nervous system and plasma, analgesic, anti-inflammatory and immunomodulatory effects occur. Enkephalins have antidepressant, anxiolytic and anticonvulsive effects on bulbus, pons and medullaspinalis. Beta endorfin has analgesic and anti-inflammatory activity. Serotonin is effective on appetite, libido, body biorhythm, and the psychomotor system, and also has an analgesic effect[9]. Wu and colleagues evaluated the effect of acupuncture in treating visceral hyperalgesia in an animal model and with electroacupuncture (EA), visceral hyperalgesia was attenuated by downregulation of central serotonergic activities in the brain-gut axis[10]. Modulation of the dorsal column medial lemniscus pathway and partly the regulation of visceral functions is regulated with stimulation of Zusanli (ST 36) point, this is the acupuncture effect. This mechanism is the main known pathway of dorsal column path activation[11]. Stimulation of ST 36 and LIV3 points with acupuncture contributes to the relief of abdominal pain by downregulating the levels of substance P, vasoactive intestinal peptide and somatostatin[12]. 5-hydroxytryptamine (5-HT) concentrations, 5-HT3 receptor (5-HT3R) and 5-HT4 receptor (5-T4R) in colon tissue were quantitatively analyzed by enzyme-linked immunosorbent method with stimulation of ST 25 point. However, it did not have an effect on the 5-HT3R concentration[13]. In another study, while the electroacupuncture stimulation of ST 25 effectively reduce gastric motility in the abdomen, stimulation of LI 11 with electroacupuncture had a stimulating effect on gastric movement. When it is applied in form of pairs; ST25 shows suppression on gastric motility, li11 promotes gastric activity. This suggests the specificity of the stimulation effect of different acupuncture points. In our study, we chose Yintang, Du20, St36, St25, Li4, Li11, Ren 12, Ren 6, Sp 6, Liv3 as acupuncture points. We thought that by stimulation of acupuncture points, neuroactive components in local and systemic nerves and skin, muscle, connective tissues, brain and internal organs were regulated by stimulation and helped the treatment. The use of acupuncture for pain relief is increasingly common. Awareness in society and physicians is not yet at a sufficient level. Acupuncture has few side effects and low

cost. The positive results seen in literature reviews, personal experiences and feedback show that the use of acupuncture in the treatment of pain is effective. The number of randomized controlled studies is few. It is observed that the studies conducted do not have enough features such as multi-centered and large number of patients and long follow-up period. There is a need for scientific studies using internationally valid evaluation criteria and have these features. Acupuncture can be safely used alone or as a combined treatment method in pain management.

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Volume:1 Issue:2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

REVIEW

Use of Macrofungi in Traditional and Complementary Medicine Practices: Mycotherapy

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Received: 01.07.2020 Accepted: 04.08.2020

Abstract
Modern people have increased their living standards due to the development of technology. However, health problems have increased with increasing living standards. Efforts to return to nature and natural products such as herbal drugs, medicinal mushrooms and traditional medicine practices have become popular. Medical mushrooms, which have an important place for human beings at every stage of history; in recent years, has succeeded to take its place from traditional medicine applications by separating itself from phytotherapy applications. The aim of our research is to raise awareness about this value, which is present in our country, which has a rich mycota, and to make determinations regarding its usage areas in the world and its importance in the history. Our study could be regarded as a resource for the practices and future studies in our country.
Keywords: Medicinal Mushrooms, Macrofungi, Tradional and Comlementary Medicine

INTRODUCTION

According to the World Health Organization (WHO); "Traditional medicine dates back to an ancient time. It is a collection of knowledge, skills and practices based on theory, beliefs and experiences of different cultures that are also used in the protection of health such as protection from physical and mental illnesses. It has all along been supportive and complementary methods to modern medicine"[1]. Traditional and complementary medicine practices, which have been used in folk medicine for thousands of years, have been collected under three main headings: 1. Natural products (medicinal plants, macrofungi, probiotics etc.), 2. Mind and body medicine (meditation, acupuncture etc.), 3. Physical applications (massage, osteopathy etc.)[2]. It is known that natural products in medical plants, probiotics and macrofungi have been used by every society in every period of history to protect against diseases, strengthen immunity or treat diseases. Ninova tablets dated back to B.C. 1000; founded in Mesopotamia, Assyrians, Sumerians and Akat proved that herbal treatments have an important place among traditional medicine practices. Approximately one thousand medicinal plants are mentioned in the works of Rig Veda, which is accepted as one of the important representatives of Indian medicine. Nearly 400 herbal drugs have been mentioned in the works of Eskulap, one of the important names of Greek medicine, and Hippocrates, the father of modern medicine[3]. The use of mushrooms for medicinal purposes, like plants, is as old as human history. There is evidence that mushrooms are used both in food and medicine in ancient Chinese, Egyptian, Roman and Greek civilizations. It is known that Hippocrates recommends mushrooms to treat certain complaints[4]. In Egyptian civilization, it is known that people believe that mushrooms are a gift of the god Osiris. In ancient Rome, there are sources indicating that mushrooms were accepted as "the food of the God" and that they were made of lightning thrown from Jupiter to the world during storms[5,6]. In ancient times, humankind separated edible mushrooms from poisonous mushrooms by trial and error, and as a result of these experiences

they distinguished poisonous, hallucinogenic or medicinal mushrooms[7,8]. Thus, macrofungi have played an important role in the history scene for centuries as a delicious food, a healing tea, a magic shaman cure, or a deadly poison responsible for the emperors' death[9]. With all these features, mushrooms have managed to be the passion of people since the early ages. The most important reason for this is that it is a delicious food as well as a deadly poison. Apart from being a delicious food, the magic mushroom of the shamans, the healing source of the Far East, or a deadly poison responsible for the death of emperors, mushrooms is a passion for some people. Every year, these passions bring together thousands of people from all over the world at mushroom festivals (Figure 1)[10]. Mushrooms have long been a tasty food alternative for many societies around the world in the early ages, people discovered which mushrooms are edible, poisonous or halisunogic by

Figure 1. Metropolitan Municipality Wild Mushrooms Training Festival Ida Mountains Mushroom Hunt (2017)

trial and error. Wild mushrooms in many parts of the world are regularly collected and used directly as a primary food source, or soups, stews and it is added to beverages such as tea[8].

The use of mushrooms in traditional medical practice dates back to Neolithic time[11]. Nearby the Bronze Age Ice Man discovered in the Alps in 1991; clothes, equipment and two types of mushrooms (*Fomes fomentarius* (L.) Fr. and *Fomitopsis betulina* (Bull.) BK Cui, ML Han & YC Dai (also known as *Piptoporus betulinus* (Bull.) P. Karst**.**) that he carried with him were found. While *Fomitopsis betulina* is believed to be used for medical purposes, the other species is considered to be used as a matche for making fire [12]. The use of macrofungi in traditional medicine is arguably the most popular in the Far East, especially in China. As a proof of the use of macrofungi in Chinese Traditional Medicine which dates back to a long time, the mention of Lingzhi (*Ganoderma lucidum* Curtis) P. Karst.) (Figure 2) in the 2000-year-old poem of the Chinese Han dynasty can be shown[13]. Today, commercial, ecological, pharmacological, and medical value of macrofungi are increasing. In this study, traditional and medicinal uses of medicinal mushrooms are compiled.

KINGDOM FUNGI

1. What is Fungi?

The common feature of the kingdom fungi, which contains a wide variety of groups, is the eukaryotic cell structure, the presence of chitin on the cell wall and the formation of zygote by undergoing meiosis[14]. The kingdom Fungi contains one of the most diverse groups of organisms on Earth, and they are integral ecosystem agents that govern soil

carbon cycling, plant nutrition, and pathology.

Figure 2. *Ganoderma lucidum*

Macrofungi are specific part of the kingdom fungi that include the divisions *Ascomycota* and *Basidiomycota*[15,16]. They are multicellular or single-celled eukaryotic, high-structure organisms that live as parasites or saprophytes that do not carry chlorophyll[17]. Fungus cells with a cylindrical structure are called hyphae. The intermediate wall between the hyphae is called the septum. Some hyphae are without compartments. Hyphae usually grow from their ends. The wall structures of mushrooms are usually chitin in higher forms but some fungi contain cellulose[18]. Hyphae unite to form micelium, and micelium combine to form masses of micelium called mycelia. The term "mushroom" refers to the fructification body formed by stacking mycelium stacks in appropriate ecological conditions[19,20].

2. Fungal Classification

The kingdom Fungi contains four major divisios *Chytridiomycota* (Chytrids), the *Zygomycota* (conjugated fungi), the *Ascomycota* (sac fungi) and the *Basidiomycota* (Figure 3)[14]. Among them, Basidiomycota and Ascomycota commonly grow to sufficient sizes to be recognized as larger fungi[21].

3. Commercial Value of Mushrooms

There are more than 100.000 identified fungal species[22,23] on earth, but about 2.000 of these are edible mushrooms[24,25]. Wild mushroom gathering, a popular occupation among the Khasi tribe of Northeastern India from the early ages to the present day; became an important income generating activity for gastronomic and medical uses in Japanese community, China, Turkey and more than 85 countries in Eastern Europe. Thus, it has acquired an important commercial value. Organized in various regions of the world and in our country for decades and hosting thousands of mushroom enthusiasts, Mushroom Festivals are the most important evidence of the increasing commercial value of macrofungi[10] (Figure 4-5)[26,27]**.** However, there are dozens of mushrooms, such as truffle mushrooms, morel mushrooms, Reishi mushrooms or Lingzi, which have important commercial value for their world-class taste or medicinal value.

For example; Lingzi (*G.lucidum*) extracts with a wide range of products from cosmetics to food supplements has commercial value exceeding four million dollars[28,29] in the world. Shiitake has been used as a source of medicine, especially in Asian Countries such as China and Japan. It has been cultured in the Far East since ancient times because of its medicinal properties. Today, its culture is done all over the world.

USE OF MUSHROOMS IN TRADITIONAL AND COMPLEMENTARY MEDICINE APPLICATIONS IN HISTORY

In Asian countries (Korea, Japan, China) approximately 300 mushroom species are used as drugs[30].

Olympic athletes in 7000 BC; are known to use stimulants naturally obtained from hallucinogenic mushrooms for fatigue and injury treatments[31]. About 270 mushroom species are considered therapeutic in Chinese Traditional Medicine (TCM). Some traditionally used edible mushrooms *Lentinula edodes* (Berk.) Pegler (Figure 6), *Grifola frondosa* (Dicks.) Gray (maitake), *Hericium erinaceus* (Bull.) Pers *Flammulina velutipes* (Curtis) Singer, *Pleurotus ostreatus* (Jacq.) P. Kumm., and *Tremella mesenterica* Retz. are also a source of pure bioactive compounds; non-edible rechargeable species with medical use such as, *G.lucidum*, *Schizophyllum commune* Fr. and *Trametes* versicolor (L.) Lloyd are used for medicinal properties only. The term "Medicinal Mushroom" today is accepted worldwide[7].

It is known that macrofungi have been used in folk medicine in ancient Chinese, Egyptian, Roman and Greek civilizations throughout history. Recently, as in far east medicine, the use of macrofungi in western countries has increased, especially in allopathic medicine. Europen people

therapeutically used *F.fomentarius*, *Inonotus obliquus* (Fr.) Pilát and *Fomitopsis officinalis* (Vill.) Bondartsev & Singer [32]. In Traditional Chinese Medicine, *H. erinaceus* is used to treat symptoms related to gastric ulcers[33]. In Asia, Russia, USA, Canada, Mexico, and in Venezuela; It is known that mushroom use has a long history in the treatment of various diseases[30,34–36]. According to folkloric reports from Siberia, the Baltic and Finland, a tea prepared from a premature fruiting bodies of a certain fungus were given to cancer patient until a prognosis is achieved.

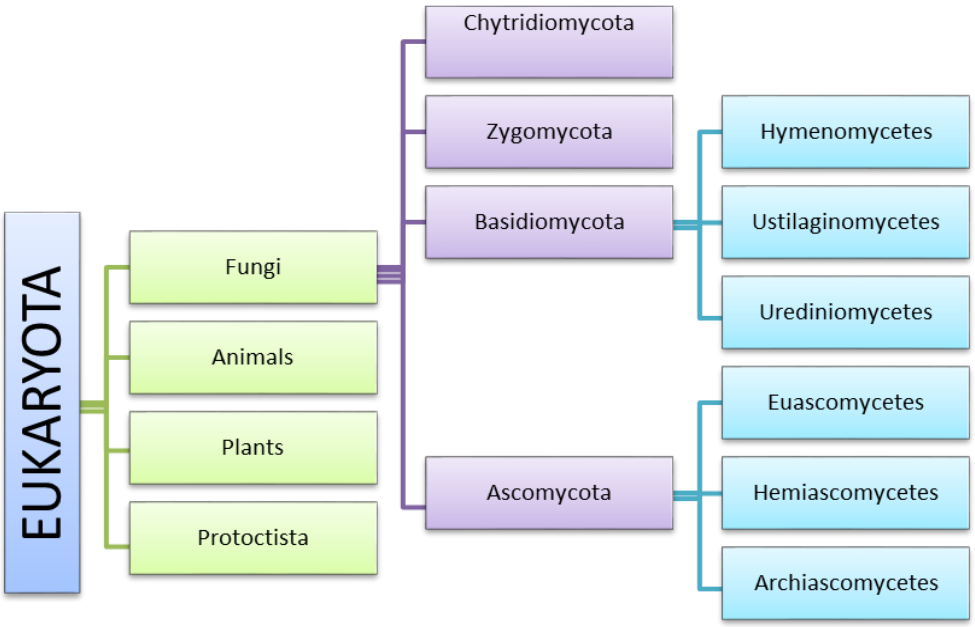

Figure 3. Major kingdoms of the Eukaryota, the four divisios of the Fungi, and the three classes of the Basidiomycota and the Ascomycota [14]

It was considered a tonic, blood purifier and pain reliever and was widely used against cancer in the 1960s in Poland[34]. According to literature, Avicenna recommended truffles, wild boar meat, phlebotomy and balanced nutrition for the treatment of Epilepsy[37].

Figure 4. Mt. Pisgah Mushroom Festival, Eugene Oregon

Figure 5. The Richmond Mushroom Festival (2019)

The blood clotting effect of *Auricularia auricula-judae* (Bull.) Quél. (Figure 7), known as the "ear fungus" among the people, in some communities, the blood stopping effect of *Lycoperdon* Pers.(Figure 8) and some *Polyporus* P. Micheli ex Adans. species in the umbilical hemorrhage of newborn babies and nasal bleedings, in some societies, the weaning effect of *Laricifomes officinalis* (Vill.) Kotl. & Pouzar in controlling the milk secretion and the healing effect of *Lactarius* (L.) Pers. milk in viral wart treatments were all along known by the people.

Figure 6. *Lentinula edodes*

Fomitopsis officinalis (Vill.) Bondartsev & Singer is known to be used in some societies to control milk secretion at the time of weaning and *Lactarius piperatus* (L.) Pers.'s milk is used against viral warts[9]. *Geastrum triplex* Jungh.; it is caustic, detoxic, strengthens the throat and lungs and regulates body temperature and lowers fever [38]. It is also said to reduce respiratory inflammation. According to the literature, laryngitis is boiled together with licorice for sore throat and cough. It is used to stop bleeding and reduce swelling[38]. The yeast used the spores of the *Geastrum* Pers. species to treat wounds[39]. There are sources in North America that Cherokee natives apply the powders of some Geastrum species to the umbilical cord after birth as hemostatic and antibiotic[40]. A mushroom used in the treatment of various cancers in the north-west of Russia and grown on birch is noted in Russian folklore. Being known as Chaga (*I. obliquus*), this fungus is well known in Russia with its anti-cancer properties[41]. *Hericium* species are edible medicinal mushrooms in the *Basidiomycota* class in *Hericiaceae* family types. In China and Japan,it is widely used as a medicine

Figure 7. *Auricularia auricula-judae*

as well as a food source[42,43]. Being known as "Red Immortal Mushroom Lingzi or Reishi"; *G. lucidum* has been used in Chinese and Japanese folk medicine for more than 2000 years.

Figure 8. *Lycoperdon perlatum* Pers.

It is preferred as a natural medicine in the treatment of diseases such as liver disorders, cancer, bronchitis, arthritis and hypertension[9]. It is known that *L.edodes*[44], whose common name is 'Shiitake' in Japan, is used in diseases that suppress the immune system such as AIDS, cancer, environmental allergies, Candida infection, recurrent flu and colds. Shiitake is also known to be good for chronic high cholesterol, where it relieves bronchial inflammations and regulates the problem of urinary incontinence. *L. edodes*, which is among the top five cultivated mushrooms in the world[45,46], is among the important medicinal mushrooms with its lentinan content.

MUSHROOMS AND COSMETICS

Today, cosmetics with commercial value contain products of herbal origin. In addition to the herbal cosmetics, cosmetic products containing fungi include anti-aging, antioxidants, skin care products (Figure 9-10)[47,48] such as skin brightening and hair products[49].

Figure 9. Cosmetic product containing mushrooms

MUSHROOMS AND MEDICINE

1. Poisonous Mushrooms

Although mushrooms are among the foods that are high in nutrients and are a cosmetic raw material with a high commercial value, they come to the fore with their hallucinogenic properties and toxins they carry. Their bad reputation due to their undeserved toxins negatively affects mushroom consumption in our country. Among the more than 22,000 species of macrofungi, the number of species with medical value is considerably higher than that of poisonous species.

2. Medicinal Mushrooms

Mushrooms are thought to possess around 130 medical functions[11]. These medical functions can be listed as antitumor, immunomodulator, antioxidant, radical scavenger, cardiovascular, antihypercholesterolemic, antiviral, antibacterial, anti-parasitic, antifungal, detoxification, hepatoprotective and antidiabetic activity. In addition to these medical effects, psychiatric science and practice also benefited from the discovery and subsequent research of hallucinogenic mushrooms[34].

Figure 10. Cosmetic product containing mushrooms

Fungi contain a wide variety of bioactive molecules, such as polysaccharides, proteoglucans, terpenoids, phenolic compounds, steroids, and lectins. These molecules have therapeutic effects and function as immunostimulating, anticarcinogenic, antiviral, antioxidant and anti-inflammatory agents[50–52]. Barros et al. (2007) *Lactarius deliciosus* (L.) Gray (Figure 11), *Sarcodon squamosus* (Schaeff.) Quél. and *Tricholoma portentosum* (Fr.) Quél. extracts have been reported to inhibit some important microorganisms for medical purposes[53]. A number of controlled clinical trials have been conducted in Japan since 1990 for supportive treatment in hospitals and clinics treatment of patients with various cancers, especially colorectal and stomach cancer, as well as breast and lung cancers[54]. Queiroz et al.(2010) methanolic extract (mainly glucan-type polysaccharides) of *Gymnopus montagnei* (Berk.) Redhead fungus have been shown to exhibit anti-inflammatory effects on the male Swiss mice and male Wistar rats. Glucans significantly reduced the inflammatory infiltrate produced by thioglycollate-mediated peritonitis by 75%. In addition, a significant reduction in nitric oxide levels was observed in exudates[51,55]. Liu et al.

(2016) reported that ethanol extracts of *H. erinaceus* fungus showed *Helicobacter pylori* inhibition[33]. *Terfezia boudieri* Chatin (Scop.) Pers. is a famous mushroom both in our country and in the world with its pleasant aroma and taste.

Figure 11. *Lactarius deliciosus*

Doğan and Aydın (2013) reported that *T. boudieri* mushroom has antimicrobial activity in gram (-) and gram (+), yeast and bacteria and also has high antioxidant capacity[56]. Zhong et al.(2011) reported that inotodiol extracts of the *Inonotus obliquus* (Fr.) Pilát fungus have an anti-proliferative effect on the human lung carcinoma cell line A549[57]. Jayakumar et al.(2006) reported that an extract of oyster mushroom *P. ostreatus* can protect Wistar rats against acute hepatotoxicity induced by CCl4 administration[58]. In 2010, a study by Nitha et al. revealed that morel mushroom (*Morchella esculenta* (L.) Pers.) mycelium is an excellent source of antioxidants that can provide different levels of protection and potential therapeutic use[59]. Based on the results of a study by Jeng-Leun et al.(2006), It can be concluded that upon the consumption of *Cyclocybe parasitica* (G. Stev.) Vizzini, the alleged antioxidant properties may be somewhat beneficial against the antioxidant protection system of the human body against oxidative damage[60]. Canli et al. (2016) reported that *Xylaria hypoxylon* (L.) Grev. (Figure 12) has in-vitro antimicrobial activity[61].

Figure 12. *Xylaria hypoxylon*

CONCLUSION

According to the literature, mushrooms have played important roles in the history scene with people, and have become an integral part of traditional and modern medicine. In the academic studies carried out since 1900s, the medicinal effects of fungi have been investigated, however, the fungal kingdom, which is assumed to be represented by 750.000–1 million species, has very few fungi whose medical properties have been defined. In addition to in vitro and in-vivo studies on mushrooms, clinical studies based on their use in traditional and complementary medicine applications are important. Clinical studies to be performed could be important steps for the solution of many diseases such as cancer.

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Volume:1 **Issue:**2
Year: 2020

International Journal of Traditional and Complementary Medicine Research

Publisher
Duzce University

REVIEW

A Review: *Momordica charantia* L.'s Biological Active Components and Its Potential Use in Traditional Therapies

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Received: 27.05.2020 Accepted: 04.08.2020

Abstract
The use of herbs for therapeutic purposes has been widespread since ancient times. In traditional treatments, plants are applied in a variety of forms, either directly or prepared by isolation and / or enrichment of biologically active ingredients. *Momordica charantia* L. (bitter melon) is a popular bush that has traditional use as a supplement in the treatment of various diseases with its rich bioactive ingredient content. The plant has common pharmacological potential, including antimicrobial, antimutagenic, antifertility, antidiabetic, antioxidant, antilipolytic, hypoglycemic, anticancer, antimicrobial, antiviral and hepatoprotective activities. This review provides a short overview of the in vitro, in vivo and clinical studies conducted to evaluate the therapeutic efficacy and safety of *Momordica charantia*.
Keywords: *Momordica charantia*, Bitter melon, Traditional Medicine.

INTRODUCTION

The use of medicinal and aromatic herbs in traditional treatments dates back to very old years. There are many of traditional and herbal medicinal products that are used as supplements for the treatment of many diseases. Most known drug active substances are molecules that have been isolated from natural products and have been tested for efficacy. The use of molecules isolated from plants as medicine dates back to the 1800s. The vast majority of drugs approved until 1990 are molecules with natural products or derivatives[1]. In addition, the mixture of many active substances obtained by extraction of natural products with various solvents can show a synergistic effect and respond much more effectively to traditional treatment than a single chemical molecule will show. Therefore, extraction, purification and efficacy studies of natural products in the field of traditional medicine is an area that is always explored from ancient times until today without losing its importance. Evidence-based assessment of the efficacy and safety of components isolated from medicinal and aromatic plants is important for traditional medicine to be based on safe foundations. For purposeful safe applications of herbal supplements, it is necessary to conduct experiments to determine the effectiveness of natural products commonly known in traditional therapy with in vitro, in vivo and clinical studies.

All parts of the *Momordica charantia* L. (bitter melon) plant from the Cucurbitaceae family have bitter taste. Members of the Cucurbitaceae family have medicinal and nutritionally beneficial plants that contain many components with pharmacological activity. *M. charantia* is rectangular like and looks like a small cucumber. The young fruit becomes emerald green and turns orange yellow when it ripens. The seeds are reddish in color. Despite being of Asian origin, it has a wide distribution in various parts of the world, including Central and South America and Africa[2]. *M. charantia* contains many biologically active compounds such as glycosides, oils, alkaloids, proteins, triterpenes, saponins and steroids.

Unripen fruits are a storage of vitamin C, but also have phosphorus, Vitamin A, and iron content. Bioactive compounds are isolated from fruit, seeds and leaves of the plant[3,4]. As a result of phytochemical analysis studies on *M.* charantia plant, it was determined that the plant contains cardiac glycoside, alkaloid, tannin, flavonoid, saponin and steroid group chemicals[5,6]. Phytosterol groups with up to 30 carbon atoms in total have been reported to have anticancer, atherosclerotic, anti-inflammatory and antioxidant activities[7-9]. Terpenoid derivatives are known to have anti-inflammatory and anticancer activities[10,11]. Six new triterpenoids isolated from *M. charantia* have been reported to cause up to 72.4% blockade and no cytotoxicity on proliferation of vascular smooth muscle cells. Thus, it has been noted that *M. charantia* is rich in supportive bioactive molecules in the treatment of cardiovascular diseases through inhibition of proliferation of vascular smooth muscle cells[12]. *M. charantia* has high level of fatty acids. It contains fatty acids, that reduce the risk of developing cardiovascular disease such as omega-3[13]. It has been reported that the fatty acid content may act as antimicrobial agents against bacteria[14] and fungi[15]. Phenolic compounds containing coumarins, phenolic acids, lignins, tannins, lignanes and flavonoids are among the secondary metabolites that are abundant in the plant[16,17]. Phenolic compounds in *M. charantia* have important activities such as antimicrobial, antioxidant, anticancer and anti-HIV-1 activities[18-20]. These activities of *M. charantia*, which is a plant species rich in biological activity, are thought to originate from rich complex chemical compounds such as flavonoids, tannins, carbohydrates, resins, terpenoids, anthraquinones, saponins, sterols, phylobatamines, amino acids, glycosides, fatty acids and phenolic compounds[21,22]. Bioactive compounds can be extracted with the help of many different organic solvents using traditional extraction techniques and new technological techniques[23-25]. Polysaccharides are primary active ingredients that perform various pharmacological activities of *M. charantia*. There are studies investigating the blood sugar levels of the polysaccharides isolated from the plant and their biological activities on the regulation of antioxidant, antibacterial, antitumor and immune system functions[26]. MAP30, a 30 kDa protein isolated from seeds, has been proposed as the agent responsible for antiviral and antineoplastic properties[27-30]. *M. charantia*'s chemicals responsible for hypoglycemic action are a mixture of steroidal saponins known as charantins, alkaloids and insulin-like peptides[31] Therefore, it is recommended to consume *M. charantia* fruit extracts for antihyperglycemic activity[32].

It is known that *M. charantia* provides a very good supportive treatment in the treatment of diabetes in lowering blood glucose levels, in the treatment of inflammatory wounds, in nutritive disorders such as stomach and intestines[33]. It is also known to be used as a supportive in the treatment of various infections, in preventing tumor formation, in the treatment of measles, hepatitis, HIV and AIDS viruses, in the treatment of diseases such as infertility, gastrointestinal cramps, cancer, eczema and psoriasis. α- and β- momarcharin are two proteins in the *M. charantia* that are known to inhibit the AIDS virus[34]. It is known to use both internally and externally for the prevention of worms and parasites locally for the treatment of wounds. Ripen fruits are used for traditional therapies for skin wound healing externally and against to peptic ulcer internally in Turkey[35]. Studies on antidiabetic, antitumor, antioxidant, antileukemic, antibacterial, immunostimulant, anthelmintic, antiviral, antimutagenic, hypocholesterolemic, antiulcer, anti-inflammatory, insecticidal, hypotriglyceridemic, hypotensive and antimycobacterial properties of *M. charantia* are common[31,36,37].

POTENTIAL BIOLOGICAL ACTIVITIES OF *M. CHARANTIA*

Hypoglycemic activity

There are numerous in vitro, in vivo and few clinical studies supporting the use of *M. charantia* as whole fruit, extract or dried powder, which can lower blood sugar levels. In line with these studies, it is common to use *M. charantia* as an alternative to decrease blood sugar levels in the treatment of

type 2 diabetes[38,39]. In the component analysis of *M. charantia* extract, it has been shown that the components showing structural similarities to animal insulin are responsible for the hypoglycemic effect [30]. In general, it has been suggested that *M. charantia* polysaccharides have a significant effect on hypoglycaemia[40]. Polysaccharides have been reported to have protective and reparative effects on pancreatic islet tissue and the ability to reduce blood sugar[40]. Polysaccharides are thought to exhibit antihyperglycemic activity by improving glucose tolerance, glucose intestinal absorption, or improving glucose metabolism[41]. In addition, a mixture of polypeptides called "polypeptide p" isolated from seeds and two steroid glycosides specified as "charantin" are other chemical molecules reported to have hypoglycemic properties[42-44]. *M. charantia* aqueous extract has been reported to be a safe alternative for lowering blood sugar levels[45]. Glucosaminoglycans isolated from plant fruits have been found to have beneficial effects on diabetes metabolism[46]. It has been reported that the mixture of maximum molecular weight alkali polysaccharides extracted from *M. charantia* polysaccharides has the best antihyperglycemic effect and can lower blood sugar levels through increased insulin secretion from Beta cells[47].

A water-soluble polysaccharide isolated from *M. charantia* fruits has been reported to reduce blood glucose levels and increase glucose tolerance in alloxane-induced diabetic mice. Water-soluble polysaccharides can increase insulin secretion in streptozotocin-induced diabetic mice by repairing injured pancreatic islet tissues[48]. Another study has shown that by reducing the amount of *M. charantia* polysaccharides, the glucose level increases significantly and *M. charantia* polysaccharides can be a potential antidiabetic agent[49]. *M. charantia* methanol extract has been shown to exhibit hypoglycemic activity in diabetic albino rats due to alloxan [38,50-52]. Various *M. charantia* extracts have been reported to lower high glucose levels in diabetic rats [53-55]. In diabetic rat models, the ethanol extract of fruit pulp at a dose of 500 mg / kg body weight has been reported to reduce glucose levels 1 hour after administration[56]. It has been reported that aqueous extract and alkaline chloroform extract reduce blood sugar levels 1 hour after application[57]. In diabetic rats and glucose-dependent hyperglycemic rats, it has been reported that the blood sugar levels of the fruit of the plant are lowered half an hour after administration of the methanol extract and water extract[58]. It has also been observed that the extract increases thyroxine levels, normalizes glucose levels, and maintains the normal lipid profile in diabetic rats fed a normal diet or fed a high-fat, low-carb diet[59,60]. It has been reported that the glucose level is normalized and the effect is protected for 15 days in diabetic rats fed with the acetone extract of the fruit for a long time[61]. Hypoglycemic effects of the aqueous extract of the whole plant have been reported in rats[62]. The plant reduced plasma and hepatic triglyceride content and tissue fat accumulation [63]. It has been noted that plasma and liver lipid parameters improve in diabetic rats fed a cholesterol-rich diet and a high-fat diet[64]. The possible hepatoprotective effect of *M. charantia* in diabetic rats has been investigated. A group of male Sprague Dawley rats were treated with *M. charantia* ethanol extract after treatment according to known protocols. The liver of the animals was examined at 10 weeks, blood was collected by cardiac puncture and centrifuged to collect the serum. While blood glucose levels increased consistently in all groups without *M. charantia* treatment, the increase in blood glucose levels in the group treated with ethanol extract became close to normal. It has been reported that while the markers of liver damage showed a significant increase in the group that did not receive *M. charantia* treatment, it could be used as a supplement in alleviating liver damage in the treatment group[65]. In another study, the mechanisms of the renoprotective effects of *M. charantia* in diabetic rats were investigated. The application of *M. charantia* extract has been reported to restore kidney function, reduce weight loss and restore blood sugar levels to normal levels[66]. The effect of *Lactobacillus plantarum*-

fermentation on the antidiabetic functionality of *M. charantia* was investigated using a type 2 diabetic rat model. Fermented *M. charantia* juice administration has been reported to reduce hyperinsulinemia, hyperglycemia, hyperlipidemia and oxidative stress well than its non-fermented counterpart in diabetic rats. Diabetic rats treated with fermented *M. charantia* juice showed higher concentrations of acetic acid, propionic acid, butyric acid, total short-chain fatty acids and lower pH values in colonic contents than rats treated with unfermented *M. charantia* juice. These results showed that *L. plantarum*-fermentation increases the antidiabetic property of *M. charantia* juice by disrupting the regulation of the intestinal microbiota and the production of short-chain fatty acids[67,68]. *M. charantia* has been shown to permanently normalize blood sugar levels comparable to healthy rats. In treated rats, the expression of insulin and Pdx1 genes increased, and Glut2 expression decreased. Liver ALT, AST and ALP enzyme activities have fallen to the normal range in the treatment group to support the protective effect of *M. charantia*. These data showed that the plant improves pancreatic function by activating pancreatic beta cells and protecting liver tissue[69].

The juice of *M. charantia* has been reported to improve glucose tolerance in 73% of patients with beginner diabetes, while 27% of patients are unable to respond[70]. The effects of *M. charantia* PEG and *M. charantia* adsorbed with nanofraction extracts on blood rheological parameters in hyperglycemic patients were clinically investigated. Blood samples of 56 normoglycemic individuals and 26 hyperglycemic patients were collected, their general characteristics, rheological parameters and hematological features were determined. Whole blood viscosity was evaluated after treatment with PEG microspheres adsorbed with *M. charantia* extract, PEG and *M. charantia* nanofraction extracts. Although the viscosity of the blood of hyperglycemic patients is higher than that of normoglycemic individuals, it has been noted that the nanoparticles of *M. charantia* extracts reduce blood viscosity equally in normo and hyperglycemic individuals.

It has been noted that PEG microspheres do not reduce blood viscosity in hyperglycemic patients but PEG microspheres adsorbed with plant nanofraction extract reduce blood viscosity. Based on clinical study data, it has been reported that the use of nanoparticle extract of *M. charantia* and PEG microspheres adsorbed with nanofraction extract may play a role in the treatment of blood disorders in diabetic patients[71]. The effects of *M. charantia* on blood sugar levels in patients with type 1 or type 2 diabetes were investigated. For diabetic patients treated with *M. charantia*, an average decrease in serum glucose levels was noted within 30 minutes. At the end of the 4-hour period, the maximum decrease in serum glucose levels was observed with a rate of 49.2%, while after 12 hours, a permanent 28% decrease was observed[72]. A clinical study was conducted with a group of 9 type 2 diabetic patients, 8 of whom received simultaneous sulfonyl urea.

Subjects were initially given 50 ml of *M. charantia* juice and received 0.23 g of *M. charantia* fruit daily over the next 8-11 week period, and then glucose tolerance tests were performed. The test results, which were applied 1 hour after 50 ml of juice consumption, showed a 12% decrease in blood glucose level. The test, performed 1 hour after the intake of 0.23 g of fruit, showed a decrease of about 6% in glucose levels. It has been noted that after consuming 0.23 g of *M. charantia* for 8-11 weeks, an 8% decrease in glucose levels was observed from the first value[54]. In another clinical study, the effect of *M. charantia* juice on blood glucose levels was investigated in a case series of 18 patients diagnosed with type 2 diabetes. Individuals were given 100 ml of *M. charantia* juice 30 minutes prior to glucose loading. When the glucose tolerance test results before supplementation were compared with the results after treatment, it was observed that 13 patients showed a moderate improvement after taking *M. charantia*[70]. A clinical study was conducted with a group of 12 patients with type 2 diabetes who were not treated other than diabetic diets for 3 weeks. 5 patients received 3 g of dried fruit powder 3 times a day. In 7

patients, 100 ml of the extract of 100 g *M. charantia* powder prepared by boiling in water was given once every morning. While a 25% decrease in blood sugar level was observed in the patient group receiving dry powder, a significant decrease was observed in the blood sugar level with an average of 54% in the patient group receiving an aqueous extract[73].

When evaluating in vitro, in vivo and clinical studies, *M. charantia* extracts have been reported to show a moderate hypoglycemic effect. It is reported that the use of as a supportive therapy in diabetic patients may be an important traditional herbal supplement if it is evaluated and adjusted in dosage with co-consumed drugs.

Antibacterial activity

Poultry-related *Bacillus* spp and α-glucosidase activities and antiobesity properties of *M. charantia* fruit extract were evaluated. As a result of the test, it was observed that the ethanolic extract showed pronounced antibacterial properties against *B. licheniformis*[74]. The antiobesity potential of fruit extracts has been shown for porcine pancreatic lipase activity. The ethanolic extract of *M. charantia* fruits has been observed to cause inhibition of the pancreatic lipase enzyme. When the data obtained in the study were evaluated, it was reported that *M. charantia* fruits may be effective in inhibition of bacterial pathogens related to poultry[74].

Polysaccharides isolated from *M. charantia* have been reported to have significant inhibitory effects on bacteria. It has been shown that the main components of *M. charantia* responsible for antimicrobial functions are polysaccharides[26]. It has been reported that *M. charantia* polysaccharides have a good bacteriosis activity in *B. subtilis*, *S. aureus*, *S. typhimurium* and *E. coli* and the most obvious effect is the effect on *S. aureus*[40]. In the study conducted to determine different concentrations and pH values of *M. charantia* polysaccharides acting on *S. aureus*, *A. niger*, *E. coli and A. oryzae*, it was shown that antibacterial effect of polysaccharides was significantly affected by pH value and showed a positive correlation with the concentration[26].

Immunomodulatory activity

In vitro experiments have shown that *M. charantia* olisaccharides can increase immunity by stimulating the activation of lymphocytes and macrophages[75]. In another in vitro study, *M. charantia* polysaccharides have been reported to have significant immunomodulatory activity[76]. It has been reported that the water-soluble polysaccharide isolated from *M. charantia* may increase endothelium-derived relaxing factor production in the cell proliferation, the development of the inflammatory and immune response, and stimulate splenocytes and thymocytes[77].

In immunosuppressed mice from cyclophosphamide, it has been reported that carbolic particle clearance index, production of serum hemolysis, spleen index, thymus index and natural killer cell cytotoxicity can be increased to normal control levels by *M. charantia* polysaccharides. It has been reported that the aqueous extract can increase the phagocytic index and the percentage of adhesion of high doses of neutrophils to nylon buffers increased significantly compared to normal control animals[78].

Anti-inflammatory activity

It is known that oxidative stress and inflammation activate each other and oxidative stress plays a role in chronic infectious diseases[79]. The beneficial properties of *M. charantia* appear to be due to anti-inflammatory and antioxidant activities. *M. charantia* shows anti-inflammatory effects by acting on several important signal pathways involved in inflammation[80,81]. Momordicoside G, one of the bioactive components of *M. charantia*, has been reported to contribute to the repair of lung injuries[82]. The anti-inflammatory properties of BG-4, a new bioactive peptide isolated from *M. charantia* seed, have been evaluated and reported to have an anti-inflammatory effect by reducing the production of intracellular reactive oxygen species[83].

The wound-healing activity of the olive oil macerate of *M. charantia* was investigated in the linear incision and circular excision wound models. It has been noted that *M. charantia* olive oil

macerate shows healing activity in incision and excision wound models and shows 31.3% anti-inflammatory activity. Experimental data have shown that *M. charantia* has wound-healing and anti-inflammatory effects[84]. It is known that *M. charantia* plays a role in wound healing by increasing oxygenation by accelerating the production of growth factors, ensuring the proliferation of fibroblast cells and accelerating capillary circulation. The antioxidant and antimicrobial effects of phytochemicals in the plant content are thought to play a role in accelerating the wound healing process[33]. *M. charantia* supplements are thought to play a role in reducing inflammation, obesity and insulin resistance in obese mice by normalizing the serum levels of cytokines[85]. *M. charantia* powder supplementation has been reported to reduce systemic inflammation in obese mice fed a high-fat diet [86,87].

The therapeutic role of polysaccharides in *M. charantia* and their mechanisms of action against gastric ulcers from ethanol have been investigated and reported to play a role in suppressing gastric inflammation[88]. *M. charantia* fruit extract supplement has been reported to significantly reduce neuro-inflammation and contribute to the improvement of neurodegenerative diseases[89].

M. charantia extracts have been found to alleviate *P. acnes*-induced bacterial skin inflammation in mice. It has been reported that this effect may be due to the anti-inflammatory effects of phenolic compounds[90]. In mice fed high-fat diets for 16 weeks, it was observed that *M. charantia* juice reduced fat inflammation by 60% and prevented inflammation of colon and intestinal microbial dysbiosis. Based on these, *M. charantia* has been reported to have the potential to be supportive in the treatment of inflammatory diseases such as obesity and type 2 diabetes[91].

In a clinical study performed by applying *M. charantia* to 38 patients with primary knee osteoarthritis and placebo supplementation to 37 patients for 3 months, the effect of *M. charantia* on reducing pain in patients with primary knee osteoarthritis was evaluated. After 3 months of supplementation, *M. charantia* group significantly reduced body weight, body mass index and fasting blood sugar, and significant improvements in knee osteoarthritis were observed. The study showed that *M. charantia* can offer an alternative to reducing the need for analgesic drug consumption by reducing pain and improving symptoms in diseased individuals[92].

Antioxidant activity

The antioxidant activities of *M. charantia* polysaccharides have been extensively studied and have been shown to exhibit significant antioxidant activity[77,93,94]. While polysaccharides are reported to be the main source of antioxidant activity, it has been noted that uronic acid and proteins can strengthen antioxidant activity[95]. It has been reported that pectin polysaccharide isolated from *M. charantia* has a significant effect on the removal of hydroxyl radicals depending on the dose and may prevent lipid peroxidation[77]. The hydroxyl and superoxide radical scavenging capabilities of the three water-soluble polysaccharide fractions isolated from *M. charantia* have been evaluated, all of which have been shown to have powerful hydroxyl radical scavenging and weak superoxide radical scavenging effects[93]. Sulfated modification of *M. charantia* polysaccharides has been reported to show better antioxidant activity in vitro. In addition, it has been shown that polysaccharides can lead to improved antioxidant activity by increasing water solubility by chemical modification[96-98].

It has been noted that the treatment of neuroblastoma cells with *M. charantia* extract reduces cytotoxic oxidative stress from H_2O_2 by increasing intracellular cleansing activity[99]. Triterpene glycosides isolated from *M. charantia* stems and fruits have been reported to significantly inhibit xanthine oxidase activity [100]. Triterpenoids isolated from *M. charantia* bodies exhibited an inhibitory effect on cleaning activities and xanthine oxidase activity[101]. The antioxidant compounds in *M. charantia* seed powders have been reported to inhibit lipid peroxidation by demonstrating potential natural antioxidant activity[102]. The antioxidant activity of *M. charantia* against oxidative damage caused by peroxynitritis has been

evaluated in vitro and has been clearly shown to contribute to improvement[103].

There are also studies evaluating the antioxidant effects of *M. charantia* polysaccharides in mouse models. It has been reported in vivo that water-soluble polysaccharides significantly increase GSH-PX activities in superoxide dismutase and liver homogenate and reduce maleic dialdehyde content by 25.6% [104].

In mouse models treated with *M. charantia* polysaccharides for 28 days, it has been reported that the activity of superoxide dismutase and catalase in serum, liver and cerebrum is significantly increased and maleic dialdehyde level is decreased. The in vivo antioxidant mechanism of *M. charantia* polysaccharides has been linked to its contribution to improving antioxidant enzyme activities against damage caused by free radicals[98].

Antitumor activity

M. charantia extract has been reported to have promising potential as adjuvants in traditional anticancer therapies[105]. The effectiveness of *M. charantia* extracts and / or components isolated from *M. charantia* against different tumor cells was evaluated. Anti-proliferative and immunomodulatory effects have been reported in most studies[106]. *M. charantia* has been shown to modulate proteins associated with different cancer pathways[107]. The cell cycle is stopped by modulation of the signal path and cell cycle proteins, and an antitumor effect is shown by inducing apoptosis or other cell death pathways[108,109] *M. charantia* polysaccharides have been reported to inhibit proliferation of human leukemia cell line K562[110]. Sulfate modification of *M. charantia* extract has been shown to significantly inhibit the growth of HepG2 and Hela cells, and it has been reported that sulfated modification may increase anti-tumor activity[111].

The effects of Momordicoside G, an important bioactive component of M. charantia, on lung injury and carcinoma lesion were investigated. It is reported that Momordicoside G induces apoptosis with morphological changes in M1-like macrophages by decreasing the amount of reactive oxygen species and promoting autophagy when applied at a dose that has no effect on cell viability in M2-like macrophages[82].

Three triterpene glycosides isolated from *M. charantia* have been reported to show significant antitumor activity in cell lines derived from liver carcinoma[112]. Given the effect of *M. charantia* extracts on several inflammatory-related signaling pathways, it is thought to play an important role as an anti-tumor agent[107].

The effectiveness of *M. charantia* juice against PanC pancreatic cancer cells has been demonstrated in vitro. Autophagy inhibitors significantly prevent cell death of cancer cells. *M. charantia* juice has been reported to cause cell death by activating the autophagic pathway in drug-resistant pancreatic cancer (AsPC-1) cells[113]. In another study evaluating the effect of *M. charantia* juice on targeting pancreatic cancer-related cancer stem cells (PanC-CSCs), it was reported that the supplement may cause a decrease in the expression of genes and proteins involved in the regeneration and reproduction of PanC-CSC[114]. 9 compounds containing three cucurbitan-type triterpen glycosides isolated from the immature fruit of *M. charantia* L, antihepatic fibrosis activity against murine hepatic stellate cells (t-HSC / Cl-6) and It was investigated for antihepatoma activity against two types of liver cancer cell line (HepG2 and Hep3B). Karavilosid III has been reported to cause inhibition of the t-HSC / Cl-6 cell line and exhibit cytotoxic activity against the Hep3B and HepG2 cell lines. It has also been developed as a chemotherapy agent for the treatment of liver fibrosis or carcinoma[112]. MAP30 has been reported to have in vitro antineoplastic effects. These effects are thought to be due to the ability to reduce the expression of growth factor receptors attached to the breast factor, such as the transmutran tyrosine kinase receptor encoded by the HER2 oncogene[30,115]. It was determined in vivo that tumor growth in S180 sarcoma and H22 liver tumor mice was significantly inhibited by *M. charantia* polysaccharides[116].

In another study, *M. charantia* polysaccharides have been shown to suppress the activity of apoptotic markers Bax and caspase-3 and increase

the level of anti-apoptotic protein Bcl-2 that promotes cell survival[88].

In the study where ribonuclease, which has a molecular weight of 14 kDa isolated from *M. charantia*, is injected daily into mice bearing HepG2 tumors, it was noted that tumor growth was significantly reduced[117]. Lectin isolated from *M. charantia* has been reported to significantly inhibit tumor growth in mouse cells carrying nasopharyngeal carcinoma[118]. In vivo study of a mouse model with PC3 prostate cancer, the leaf extract of *M. charantia* was reported to inhibit tumor growth. Cancer has been observed to metastasize to the lymphatic nodes, but no evidence has been found to metastasize to other organs[119].

The progression of the tumor towards malignancy is strongly associated with chronic inflammation responsible for tumor invasion and angiogenesis of normal tissues in the environment. *M. charantia* components also have the potential to have antitumor effect by modulating the inflammatory state. *M. charantia* extract treatment has been shown to be more effective in inhibiting tumor growth in mouse models with aggressive triple negative breast cancer (TNBC) compared to ER positive breast tumor growth. It has been shown that abnormal irregularity of lipid metabolism is associated with breast cancer progression, and treatment causes decreased esterified cholesterol accumulation in TNBC cell lines compared to control cells. Moreover, expression levels of acyl-CoA and cholesterol acyltransferase 1 (ACAT-1) were evaluated in TNBC cells treated with *M. charantia* extract, and the extract was shown to inhibit ACAT-1 expression in TNBC cells. It has been reported that *M. charantia* therapy may have therapeutic potential in human breast cancer by suppressing TNBC cell growth[120]. Considering the performed in vitro and in vivo studies, it is thought that consumption of *M. charantia* supplement may help reduce the risk of cancer.

Other biological activities

It has been reported that *M. charantia* polysaccharides have a neuroprotective effect that can reduce neuronal death caused by thrombin in primary hippocampal neurons[121,122]. The hepatoprotective effect of *M. charantia* water soluble polysaccharides has been investigated on the CCl4 liver damaged mouse model, and it has been shown that plant water soluble polysaccharides can reduce mouse serum ALT damage to 10.6% and AST damage to 30.7% [104]. In another study, the effects of pectic polysaccharide supplement isolated from *M. charantia* against female reproductive toxicity and infertility triggered by sodium arsenite in Wistar rats were investigated. Pectic polysaccharide has been reported to significantly reduce ovarian and uterine lipid peroxidation and the formation of reactive oxygen species from sodium arsenite by regulating superoxide dismutase, catalase and glutathione peroxidase activities. It has been noted that pectic polysaccharide therapy reduces sodium arsenite toxicity by modulating S-adenosine methionine pool components such as B12, folate and homocysteine. Thus, a successful fertility was reported in rats receiving supplements instead of infertile conditions[123]. MAP30 isolated from *M. charantia* has been reported to inhibit the HIV viral integrase and cause irreversible relaxation of super-helix viral nucleic acids. These changes caused by the MAP30 protein have been reported to prevent viruses from integrating themselves into host cell genomes[29].

TOXICITY

Although there have been many studies on *M. charantia*, strong enough, randomized, placebo-controlled clinical trials are needed to properly determine safety and effectiveness before being routinely recommended. It is likely to cause additional side effects, especially when taken with other glucose-lowering agents. There are no other data on pediatric dosages. Convulsions and hypoglycemic coma were observed in two children, whose glycemia decreased strongly 1-2 hours after *M. charantia* tea was given on an empty stomach[124]. It has been reported as the most serious side effect known to humans. Other known side effects of *M. charantia* are the reduction of fertility in mice, an increase in γ-glutamyltransferase and alkaline phosphatase levels and headaches in

animals receiving oral juice and seed extract[37,125]. It has been reported that favism disease caused by glucose-6 phosphate dehydrogenase enzyme deficiency may develop after consumption of *M. charantia*. The disease is defined by other symptoms such as hemolytic anemia, headache, stomach pain, and coma[31]. It was determined that this was due to the glycosidic compound named "vicin" in the composition of *M. charantia*[126].

It has been reported that the fertility rate of mice fed with M. charantia juice supplement decreased. It has been reported that particular attention should be paid to the use of plants in pregnant women, since the proteins in the composition of *M. charantia* significantly reduce fertility in animal models and pose a risk of losing the baby[127-130]. It has been reported that spermatogenesis is inhibited in dogs fed with *M. charantia* fruit extract[131]. In the study performed with MAP30 protein isolated from *M. charantia*, it was stated that human sperm was not affected[132].

In diabetic animal models, it was reported that hepatotoxicity symptoms were not observed based on histological and biochemical observations after treatment with *M. charantia*[65]. Even if histopathological changes do not occur in animals with liver disease, caution should be exercised as transaminase increase is reported.

Many in vitro and in vivo studies have been conducted in which the beneficial effect of *M. charantia* on the heart has been reported. In a study conducted to investigate the potential developmental toxicity on fetal heart development using zebrafish (Danio rerio) embryos, seed extract was reported to be lethal with LD_{50} values. In addition, multiple anomalies were detected in zebrafish embryos at these concentrations[133]. In the same study, it has been reported that the fruit extract can be used safely without harming zebrafish embryos at a concentration of 200 µg / mL. However, it has been reported that severe cardiac hypertrophy develops in embryos treated with fruit extract over time and the cardiac myoblast specification process is impaired in zebrafish embryos. It was emphasized that the supplement should be used with caution to prevent possible damage to the development of the fetus in pregnant diabetic patients due to the teratogenicity of the seed extract and fruit extract cardiac toxicity[133].

A case of acute interstitial nephritis has been reported in a man with type 2 diabetes and hypertension using the hyponidd drug containing *M. charantia*[134]. Anuria was defined in 2-3 days following edema and urinary reduction in the patient who took one tablet daily for a week. Kidney toxicity has been reported in mice treated with *M. charantia* for more than a week[135]. It has been reported that *M. charantia* seeds and the outer shell contain lectin, which inhibits protein synthesis in the intestinal wall, but this is not related to clinical signs or symptoms in humans[136]. *M. charantia* can cause additional side effects when taken with blood sugar lowering agents. It has been observed that glucose-lowering effects are enhanced in rats supplemented with sulfonylurea tolbutamide together with *M. charantia* juice. Additional glucose-lowering effects were observed in 8 of 9 patients who took *M. charantia* juice or fruit powder with sulfonylurea[54]. In a woman with type 2 diabetes, it has been reported that the use of *M. charantia* and garlic supplements containing sulfonylurea chlorpropamide and curry caused additional glucose-lowering effects[137].

Safety data are derived from animal models rather than clinical studies. Clinical findings indicating that long-term use of *M. charantia* at high doses may cause kidney conditions should be tested by better organized clinical trials. In a meta-analysis study, it was reported that better structured studies are needed by emphasizing the scarcity of clinical data[138]. People who report allergies to other herbs from the Cucurbitaceae family should avoid the use of *M. charantia*.

CONCLUSION

It is known that the majority of the world population prefer traditional folk medicine products to industrial products. One of the main reason for the increased interest in herbal medicinalproducts is that natural products will be considered less toxic, but this is often a false perception. In health problems, many components

of vegetable origin obtained from natural products have the potential to act as supplements, alone or in mixtures. Due to the synergistic effect, many active compounds may have therapeutic potential much higher than the effects they can give alone when given as a herbal preparation.

M. charantia plant is a natural product known to be used for many years in the treatment of type 2 diabetes. There are many scientific studies supporting the anti-inflammatory and antioxidant properties of the plant, and it is a strong candidate that can clearly contribute to reducing the consumption of plant glycemia as well as reducing analgesic drug consumption in infectious diseases. There are also studies showing its effectiveness in the treatment of obesity, wound healing and supporting the immune system. Many in vitro and in vivo studies have been conducted on the investigation of the anticancer activity of *M. charantia*. The ability of *M. charantia* bioactive components to modulate a variety of cell cycle regulating proteins located in different signal paths has been clearly demonstrated in studies showing anticancer activity. The plant is known to play a vital role in cancer development and progression in cell signaling cascades. The *M. charantia* plant remains popular in the discovery of natural products, with its ability to suppress tumors by targeting multiple oncogenic signaling pathways. More studies are needed where *M. charantia* extracts or bioactive chemicals isolated from the plant are combined with chemotherapeutic drugs to see if they increase drug effectiveness or reduce side effects. There is also a gap in the field of clinical testing of all activities. Few clinical studies on *M. charantia* focused only on the effects on type 2 diabetes and were performed on a small number of subjects. Better clinical studies are needed on hyperglycemic efficacy. In addition, plant efficacy should be verified by increasing in vitro, in vivo and well-organized clinical trials in other areas where its use has traditionally been reported. It is known that the use of *M. charantia* herb together with the drugs used in the treatment of diabetes causes rapid decreases in blood sugar. Better safety studies are needed to perform the safety tests of the plant, to clarify the side effects and to adjust the doses in a controlled manner.

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