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THE PREVALENCE OF TAS2R38 GENE PHENOTYPES AMONG THE PATIENTS WITH SOME ENDOCRINE SYSTEM DISORDERS

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Abstract: *PTC (phenylthiocarbamide) is one of the focuses of interest from the medical point of view since a number of associations of the taster/nontaster status of PTC sensitivity with various human diseases have been found. It is estimated that ability of sense of PTC bitterness coded by a single gene TAS2R38. The threshold at which people can taste phenylthiocarbamide (PTC) is bimodal, and some people are tasters while, others are nontasters. In recent years, it is actively conducted the studies of genetic markers in various endocrine disorders. Endocrine diseases, particularly thyroid gland prevalence statistics in Georgia is very high. The goal of our research was to study the phenotype of PTC sensitivity among the patients with some endocrine system disorders in the population of Ajara region of Georgia, particularly diabetes type II and different type of goiter (nodular, diffuse etc.) to reveal any correlation between them. A total of 319 individuals including 136 patients with endocrine system and 183 randomly chosen healthy individuals participated in this study. Obtained results shows, that there is an increased incidence of diffuse toxic goiter in individuals with recessive phenotype of TAS2R38 gene and who are unable to taste PTC bitterness. While, PTC sensibility phenotype inclined to develop nodular goiter. Thus PTC-Sensibility phenotype may be considered one of the genetic markers in the forms of Goiter.*

Keywords: *TAS2R38 gene, PTC sensitivity, taster, nontaster, nodular goiter, diffuse goiter*

2. Introduction

The ability of feeling the PTC (phenylthiocarbamide) bitterness represents one of the well-known and convenient genetic marker with regards to the phenotype or genetic structure of human populations and various biomedical studies [1,2,3] . Synthetic compound of phenylthiocarbamide cause the stimulation of bitterness receptors and feeling of bit taste, while in others do not . Accordingly, there are two phenotypes of PTC sensibility: PTC-taster (sensitive) and PTC-nontaster (insensitive). It should be mention, that phenotype of PTC sensibility is variable in different ethnic groups or populations [4,5,6,7]. The prevalence of taste blindness or an inability to taste bitter chemicals ranges from 3% in West Africa to 6–23% in China, 40% in India, and 50% in Australian Aborigens [8].

Mainly, it is estimated that ability of sense of PTC bitterness coded by a single gene TAS2R38 [9]. Geneticists offered different types of inheritance for the PTC sensitivity, including both single-locus and double-locus models [10]. Most family studies indicated the monogenic nature of the sensitivity to PTC. It was considered that the ability to sense the compound was controlled by a dominant allele of the autosomal gene, and the inability by a recessive allele [11].

The gene contains long exon (1002bp). Sensibility of bitterness is due to presence of three basic single-nucleotide polimorphism (SNPs) that encode three different amino acids (C145G/P49A, C785T/A262V and A886G/I296V) [12,13]. Those three polymorphic variations give 8 combinations (haplotypes), but among them the most frequent are two- PAV (taster) და AVI (nontater) haplotypes. AVI/AVI homozygotes mainly are nontasters of bitterness; PAV/PAV homozygotes are tasters, while AVI/PAV heterozygotes have the moderate sensitivity to bitterness. According to some researchers, the variability of PTC sensibility except of SNPs depends on other genetic and environmental factors [9].

The threshold at which people can taste PTC is bimodal, and some people are tasters and others are nontasters. Family and twin studies suggest that this trait is inherited as a Mendelian autosomal recessive, with two alleles typically represented as T – "tasting" allele and t – "nontasting: allele. Estimated that the majority of the world's population (approximately 70%) belongs to the PTC sensitive phenotype, and the rest 30% - to insensitive one [2]. It is interesting to know that according to these markers, different populations are characterized with different phenotype structure.

PTC is one of the focuses of interest from the medical point of view since a number of associations of the taster/nontaster status with various human diseases have been found. According to PTC sensitivity there was possible to reveal predisposition of some inherited or multifactorial diseases [14,15,16]. Moreover, it has been found that smokers and frequent drinkers were more prevalent among nontasters than tasters [17, 18].

Recently, the researches has proved that the carriers of PTC taster phenotype and the correspondent recessive allele of the gene are more inclined to the pathologies of thyroid glands (68%) than the PTC nontaster phenotype (32%), and, moreover, the detection of polymorphism of TAS2R38 gene at an early stage is the risk groups measuring factor, which, in turn will facilitate an implementation of preventive measures [13].

Prevalence of endocrine diseases, particularly thyroid gland pathologies in Georgia is very high. At the same time it is a fact that, the ability of PTC bitterness sensitivity represents one of the ethno-specific genetic markers, the phenotype of the gene is specific for each particular population among them for Georgians as well.

Thus, the goal of our research was to study of PTC - bitterness sensitivity phenotype among the patients with some endocrine system disorders, namely diabetes type II and different type of goiter (nodular, diffuse etc.) in the population of Ajara region of Georgia to reveal any correlation between them.

3. Materials and methods:

According to phenylthiocarbamide (PTC) sensitiveness the study to reveal different phenotype groups was done on the patients with various pathologies of thyroid gland (nodular goiter, diffuse goiter etc.) The main target group was the patients being registered Batumi endocrinology center (Ajara, Georgia). 319 patients were studied in total, where 136 patients (14 males and 122 females with different ages) were with endocrine system pathologies and 183 randomly chosen healthy individuals (115 Females and 68 males) from Batumi Shota Rustaveli State university students and personal without any thyroid gland pathologies as a control group. The research was made under the protection of the ethic principles of Helsinki Declaration *(World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects)*. Every participant of the experiment confirmed their consents in written form.

The research was based on Harris and Kalmus method [19]; we have used standard taster strips which contained 3,4 mg/kg PTC compounds. The participants fixed the taste sensitivity data of the tester in written form. According to the bitterness sensitivity with the data obtained via PTC testing, the participants of the experiment were divided into two groups: PTC sensitive "tastes" and PTC insensitive "nontasters" phenotypes.

The obtained results were mathematically processed applying the statistical method. We calculated the concentration of PTC gene allelic frequency of its propagation applying Hardy-Weinberg equation - $\mathbf{q^2+2q(1-q)+(1-q)}$, which reflects the distribution of genotypes in panmictic population. The authenticity of the obtained results was confirmed with the formula: $\mathbf{M=\pm\sqrt{p(100-p)/n}}$ Where, P denotes the percentage data, n – the number of the researched people.

4. Results and Discussions:

According to the experimental data majority of the patients has been diagnosed with different form of goiter (nodular and/or diffusive goiter, mainly toxic) and only few (16%) were with diabetes type II. Age of the individuals in the research groups and in the controls were ranged from 16 to 80 years old.

According to the experiment, revealed that part of individuals were tasting phenylthiocarbamide taste universal margin dose 3,4 mg/kg as a very bit taste, while other part of individuals were feeling moderate or little bit more bitterness, and rest part of patients has not been feeling PTC bitterness at all. PTC-insensible individuals have been feeling of only paper taste or none of the taste.

Obtained results has shown, that from the patients portion (136 individuals in total) with both of endocrine disorders (goiter and diabetes mellitus type II) 89 individuals were with PTC-taster phenotype, while 47 were PTC-nontaster. Accordingly, the percentage of PTC-taster phenotype carrier individuals amounted 65%±4.0 from whole research group, and individuals with PTC-nontaster phenotype amounted only 35%±4.0. As for the control group (183 individuals in total) 125 individuals were PTC-taster and only 58 PTC-nontaster. Accordingly, percentage of PTC-taster controls was 68% ±3.4 and the PTC-nontaster controls were 32%±3.4 (diagram 1).

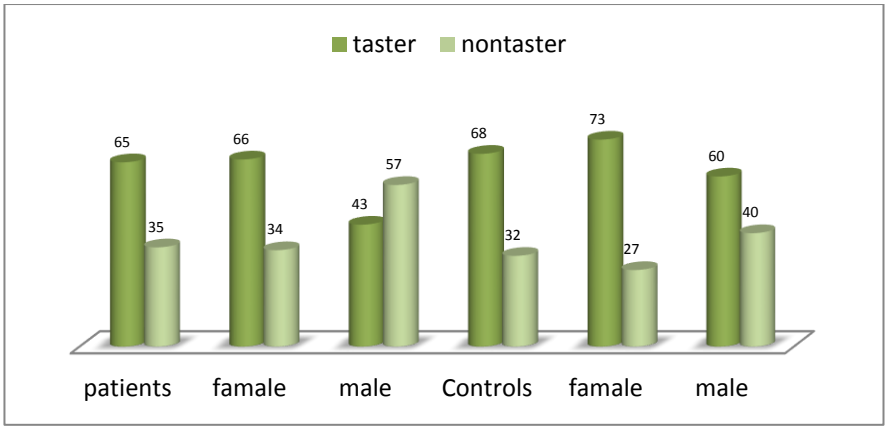

Diagram 1. The distribution frequency (%) of PTC sensitivity among the patients with endocrine system and control group in females and males.

As we can see, the study revealed PTC-taster phenotype in the majority of patients with endocrine disorders (65%±4.0) as in control group (68%±3.4). The phenotype structure of PTC-sensitivity in endocrine system pathologies were analyzed according sex as well, to find out any correlation. In patients, PTC-taster females equaled to 66%±4,3 and PTC-nontasters 34% ±4,3; As for male individuals, actually they were presented less in amount of the patients with endocrine disorders (14 males), where fixed 43% PTC-taster and 57% PTC-nontaster (diagram 1). The data was almost similar in control group number of females in the control group was 115 individuals from where 73%±4,1 of them appeared PTC-taster and 27% ±4,1 - PTC-nontaster. Apparently, the data shows that there is no any correlation between PTC-sensitivity and endocrine system pathologies. According to the obtained results, the distribution of PTC sensitive/taster phenotypes in patients with endocrine system disorders is almost similar to the Georgian population of control group. Accordingly any type of correlation with PTC-sensibility was not revealed in this research group.

The frequency of genotype has shown the following picture: patients – dominant homozygote TT – 0,16, heterozygote Tt – 0,49 and tt 0,35. In Controls TT – 0,27, Tt – 0,5 and tt – 0,23 (diagram 2).

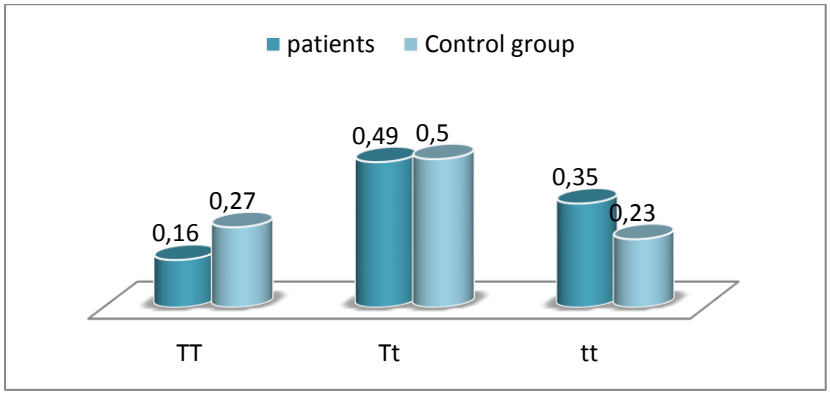

Diagram 2. The distribution frequency of PTC sensitivity genotype in patients and control group

The research of bitterness sensitive genotype frequency in female patients has shown following ratio: TT – 0.18, Tt – 0,48 and tt – 0.34. In male patients the picture was like that TT-0,06, Tt-0,38 and tt-0,56. In the control group males with PTC-taster phenotype equals to 60% of control group while PTC-nontaster is 40%. The genotype frequency in the given population is as follows: TT -0,13; Tt-0,47; tt-0,4.

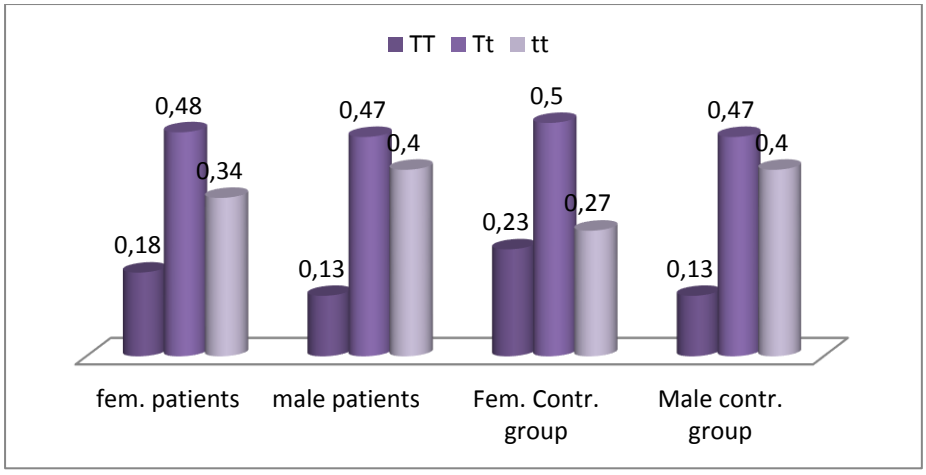

Diagram 3. The distribution frequency of PTC sensitivity genotype in control group and in patients according sex.

According to the obtained results, male patients are much more likely to have PTC-nontaster phenotype, which is proved with 40% in the control group and 57% in the patients with endocrine system disorders. However, due to the small number of male patients, it is difficult to prove that men tasters are more likely to have a tendency to develop endocrine system pathologies regardless there was some sign of inclination.

We tried to clear if there was any inclination according to concrete pathology not like as generalize. there was allocated following group of patients where 60% ±4,2 of patients have been diagnosed with nodular goiter, 16% ±3,1 of patients with diffuse goiter (mainly toxic form), 13%±2.8 of patients have been diagnosed with unidentified etiology and the rest 11%±2.6 with hyperthyroidism, hypothyroidism and an euthyroid. Among them our attention turned out nodular and diffuse goiter.
In the group of patients with nodular goiter the PTC-tasters were 62% ±4,2 of patients and 38%±4,2 PTC-nontesters, respectively. While in the patients with diffusive (toxic) form of goiter the PTC-tasters were pretty low - 36% ±4,2, while most of the patients 64%±4,2 was not able to sense PTC taste (diagram 4). Thus, we can conclude that PTC-taster phenotype is inclined to develop nodular goiter, while diffuse goiter is more likely associated with PTC-nontaster phenotype. This relationship between PTC and thyroid gland activity led by Harris, Kalmus, and Trotter to test the taste response to PTC of groups of patients with thyroid disease. Their data suggested that nontasters of PTC were slightly more susceptible to the development of adenomatous goiter than controls [19]. It had been revealed the high correlation with adenomatous goiter non-tasters in brazil [20]. However, our data contradicts results of the research conducted in endemic area of Brazil, where risk for first-stage diffuse goiter is little affected by taster status but that goiter in a nontaster is more likely to evolve into the nodular form [21].

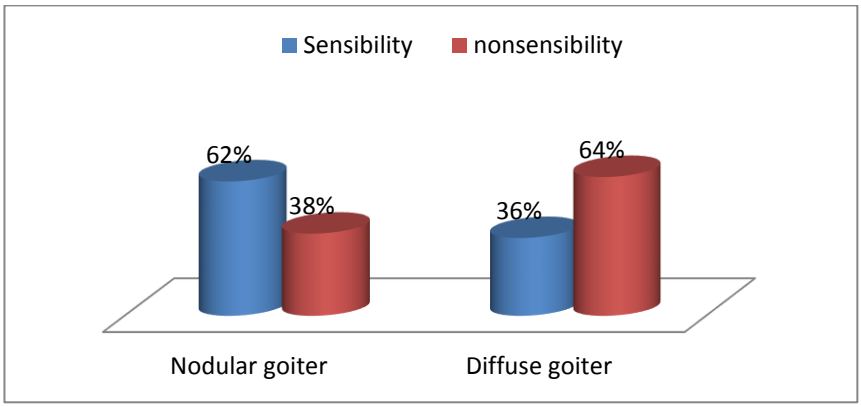

Diagram 4. The distribution frequency of PTC sensitivity phenotype in patients with different form of goiter.

The distribution of genotypes in patients with nodular goiter prove the phenotype results as well which is presented on diagram 5(diagram 5).

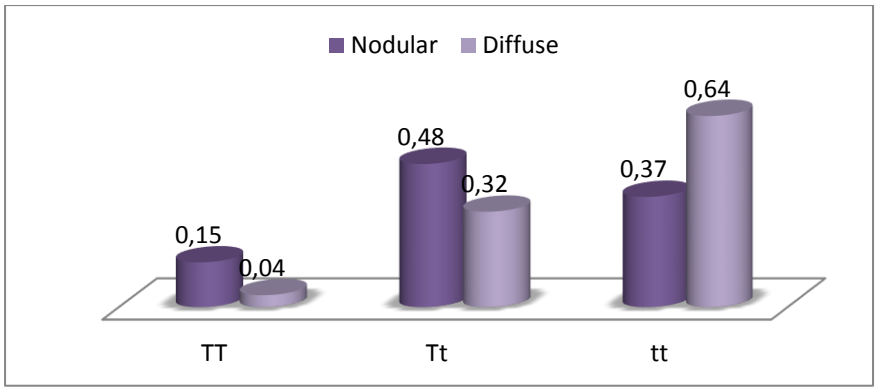

Diagram 5. The distribution frequency of PTC sensitivity genotypes in the patients in nodular and diffuse goiter.

5. Conclusions:

Based on to the results obtained, the analysis of the endocrine system pathologies shows that the phenotypic structure of the PTC sensitivity is slightly different from the general structure of the population, but the explicit correlation between the PTC sensation and the endocrine system different pathologies is not clear. At the same time, the PTC phenotypic structure is radically modified in patients with both of - nodular and diffuse (toxic) forms of goiter. The correlation of nontaster phenotype of PTC gene is quite clear with diffuse goiter (64%±4,2). While, PTC sensibility phenotype inclined to develop nodular goiter. The biochemical base of this pathology may be is an activation of strumogenic/thyreostatic factors supposedly, that leads depression of synthesis and secretion of thyroid hormones and ultimately violates the normal homeostasis of organism. Thus, recessive homozygote (tt) of the gene can be considered as one of the inclined genetic factors for diffuse goiter among various genetic markers associated with the multifactorial diseases, among them TAS2R38 gene recessive may considered as only one of those factors for diffuse (toxic) goiter. As for the results of diabetes melituss type II have not revealed any significant correlation with PTC sensitivity.

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EXPERIMENTAL INVESTIGATION OF THE EFFECTS OF PROBIOTIC SUPPLEMENTATION ON OBESITY AND INFLAMMATION IN OBESE RATS

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Abstract:*TNF-α levels of antiinflammatory and proinflammatory markers were used to investigate effects Obesity is a disease that occurs when body has many endocrine and metabolic functions and fat tissue is higher than normal. There is strong relationship between obesity and inflammation due to production of numerous lipid molecules called adipokines, such as leptin, tumor necrosis factor (TNF-α), interleukins and adiponectin. It was aimed to study obesity and effect on inflammation in experimentally obese animal model study apllied probiotic supplementation. Three different test animal groups were formed. For this purpose; IL-10 and TNF-α on inflammation; leptin levels were used for investigating effect on development of obesity and total cholesterol, HDL and LDL levels from lipid profiles were planned to examine. In obtained serum samples, these parameters were determined by sandwich ELISA technique with commercially available kits. Mean weights at the beginning of study and at the end of 8 weeks were 240,2±9,7 and 283,8±11,0 gr in first, second and third groups; 254,6±16,0 and 307,0±14,5 gr; 330,0±48,6 and 400,5±65,0 gr respectively. At the beginning and end of the probiotic use, weight averages were 288,6±9,9 and 311,8±17,1 gr in first, second and third groups 313.4±17.1 and 339.6±19.7 gr; 412,0±67,7 and 422,0±71,1 gr respectively. When weights were evaluated before and after probiotic use of third group, weight gain was decreased. Although there was no significant difference in HDL, LDL, IL-10 and leptin levels among all group, there was significant difference in total cholesterol and TNF-α values (p<0,05). Compared to first group and second group, there was significant difference in total cholesterol and TNF-α values, in second and third groups there was significant difference in total cholesterol values (p<0,05). As result; probiotics are recognized as living organisms when taken in sufficient quantities, affect health of environment positively, and these beneficial effects are considered to extend to obesity inflammatory diseases. Probiotic supplementation of normal diet may be healthy approach to prevention of various diseases. In order to spread this application to society as whole, it is necessary to increase number of works.*
Key words: obesity, inflammation, probiotic

1. Introduction

Obesity is a disease characterized by excessive fat tissue accumulation in the adipose tissue due to increased consumption of high-energy foods and decreased physical activity,

which has increased in recent years [1]. Adipose tissue is involved in the synthesis of "adipokines", which are defined as metabolically active proteins [2]. While adipokines such as adiponectin and leptin play a role in obesity, adipokines such as adiponectin, visfatin, resistin and leptin are involved in immun system metabolism [3]. The leptin hormone, one of the adipokines, acts on the hypothalamus to increase energy expenditure and to produce a feeling of satiety [4]. It is also similar to helicase cytokines family, structurally containing IL-2 and growth hormone1 and it is thought to have pro-inflammatory activities by stimulating TNF-a and IL-6 [5].

Probiotics are defined as live microorganisms that provide beneficial effects to the health of the environment when taken in sufficient quantities and play a role in the prevention of obesity and inflammation by various mechanisms [6], [7]. Probiotics are effective on the immune system by inducing mucus production by signaling lactobacilli, activating macrophages, increasing secretory IgA and neutrophils, inhibiting the release of inflammatory cytokines, and increasing peripheral Ig levels [8].

When the relationship between obesity and probiotics is examined, some hypotheses have been suggested that intestinal microbiota is effective on diseases with various mechanisms. The first one is the hypothesis of energy extraction. According to this hypothesis, intestinal microbiota accomplishes production of short chain fatty acids (SCFA) from nondigestible polysaccharides and oligosaccharides that escape proximal digestion and absorption and provides additional energy [9], [10].

Another hypothesis that intestinal microbiota affects obesity is based on the relationship between the gut and the brain. The intestinal-brain axis, which exhibits a bidirectional interaction, with the role of blood glucose level, adipocyte function and energy balance modulates the short-term hunger and satiety mechanism by providing the passage and distribution of nutrients through the gastrointestinal tract. In this way, it can lead to changes in eating behavior that are effective on food intake [11].

Based on this information; we aimed to investigate the effects of probiotic supplements on obesity and inflammation in experimental models of obese animals.

2. Material and Methods

The rats to be used in our study were obtained from Ondokuz Mayıs University Experimental Animal Application and Research Center and the steps related to animal research were carried out in this center. In this study; the first group consisted of the control diet fed with the standard diet, the second group was the obese group fed on the high fat diet, and the third group consisted of the obese group with the probiotic addition to the high fat diet. Groups of animals outside the control group were fed a high fat diet for 8 weeks to form an obese rat model. After the 8th week, the third group was given probiotic supplement for 4 weeks. *L. acidophilus, B. lactis, L. paracasei* and *L. rhamnosus* strains were used as probiotic supplement. In addition, rats in each group were weighed weekly and their body weights were measured. For this purpose, IL-10 and TNF-α on inflammation; leptin levels were used for investigating effect on development of obesity and total cholesterol, HDL and LDL levels from lipid profiles were planned to examine. In obtained serum samples, these parameters were determined by

sandwich ELISA technique with commercially available kits (Relassay, Turkey). Statistical SPSS-20 package program was used for analysis of study data. p <0.05 was accepted as statistically significant.

3. Results

According to the results of the study, the following findings were obtained. In Table 1, the weighted average of all groups at baseline, after 8th week, at baseline and after probiotic administration was compared.

TABLE 1. COMPARISON OF THE AVERAGE GROUP WEIGHT

				p			
	Group 1	**Group 2**	**Group 3**	**Group 1- 2**	**Group 1-3**	**Group 2-3**	**All groups**
Baseline (gr)	240,2±9,7	254,6±16,0	330,0±48,6	*0,345*	*0,094*	*0,137*	*0,001**
Week of 8th (gr)	283,8±11,0	307,0±14,5	400,5±65,0	*0,068*	*0,103*	*0,172*	*0,001**
Baseline of probiotic (gr)	288,6±9,9	313,4±17,1	412,0±67,7	*0,083*	*0,100*	*0,166*	*0,001**
After probiotic (gr)	311,8±17,1	339,6±19,7	422,0±71,1	*0,128*	*0,143*	*0,271*	*0,005**

p< 0,05

When examined in Table 1 the mean weights at the beginning of the study and at the end of 8 weeks were 240.2 ± 9.7 and 283.8 ± 11.0 gr, respectively in the first, second and third groups; 254.6 ± 16.0 and 307.0 ± 14.5 gr; 330,0 ± 48,6 and 400,5 ± 65,0 gr, respectively. During the course of probiotic use the mean weights at the beginning and the end were 288,6 ± 9,9 and 311,8 ± 17,1 gr, respectively in the first, second and third groups; 313.4 ± 17.1 and 339.6 ± 19.7 gr; 412,0 ± 67,7 and 422,0 ± 71,1 gr, respectively. Significant differences were found between the groups at baseline, week 8th, according to the weights at the beginning and after probiotic (p <0,05).

In Figure 1, weight averages before and after the use of probiotics were compared and weight gains were evaluated according to groups.

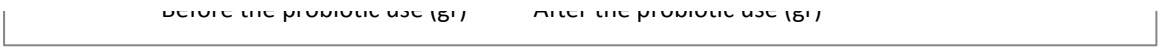

Figure 1. Weight Averages After The Use Of Probiotic

When the effects of probiotic use on weight change were examined, it was determined that group with probiotic use (Group 3) had the least weight gain with 10,0 ± 27,2 gr, group with high fat diet (Group 2) had the highest weight gain with 26,2 ± 7,1 gr. The mean Body Mass Index (BMI) after 8 weeks in all the study groups were 0.49 ± 0,01, 0,53 ± 0,02 and 0,69 ± 0,11 in the first, second and third groups respectively, the mean BMI after probiotic use was determined as 0,54 ± 0,03, 0,59 ± 0,04 and 0,73 ± 0,12 in the first, second and third groups, respectively. When all groups were compared, the change in BMI after probiotic use was statistically significant ($p < 0.05$).

In Table 2, Among the inflammatory markers; TNF-α and IL-10, HDL, LDL and total cholesterol levels from lipid profiles and leptin levels from obesity markers were examined of all groups.

TABLE 2. COMPARISON OF INFLAMMATORY, LIPID AND OBESITY MARKERS

				p			
	Group 1	**Group 2**	**Group 3**	**Group 1-2**	**Group 1-3**	**Group 2-3**	**All Groups**
HDL (µg/ml)	21,7±0,7	21±2,6	18,3±6,3	*0,600*	*0,268*	*0,806*	*0,600*
LDL (µg/ml)	31,5±4,0	43,1±11,8	31,9±2,9	*0,058*	*0,805*	*0,059*	*0,083*
Total kolesterol (mmol/L)	13,7±2,9	22,3±4,0	14,9±3,0	*0,005**	*0,584*	*0,018**	*0,004**
Leptin (ng/L)	2069,3±121,6	2165,3±80,9	2026,7±106,7	*0,193*	*0,530*	*0,058*	*0,149*
TNF-α (ng/L)	1308,6±177,1	1758,7±196	1328,5± 92	*0,005**	*0,921*	*0,067*	*0,034**
IL-10 (pg/ml)	2086,1±400,2	1698,4±50,6	2169,2±402,4	*0,219*	*0,612*	*0,093*	*0,213*

p< 0,05

When the averages of obesity and inflammatory markers were evaluated according to the study groups, HDL averages in the first, second and third groups were 21.7 ± 0.7, 21 ± 2.6 and 18.3 ± 6.3 µg / ml, respectively; LDL averages were 31.5 ± 4, 43.1 ± 11.8 and 31.9 ± 2.9 µg / ml; total cholesterol averages 13.7 ± 2.9, 22.3 ± 4 and 14.9 ± 3 mmol / L; leptin averages were determined as 2069.3 ± 121.6, 2165.3 ± 80.9 and 2026.7 ± 106.7 ng / L, respectively. In the first, second and third groups, the TNF-α averages were 1308,6 ± 177,1, 1758,7 ± 196 and 1328,5 ± 392 ng / L; The mean IL-10 was 2086,1 ± 400,2, 1698,4 ± 50,6 and 2169,2 ± 402,4 pg / ml.

While there was a increase the mean LDL, total cholesterol, leptin and TNF-α levels, there was a decrease in IL-10 level, which a cytokine with anti-inflammatory properties, in the group fed with high fat diet (Group 2) increased compared to the other groups. Although there was no significant difference in HDL, LDL, IL-10 and leptin levels among all groups ($p > 0,05$), there was a statistically significant difference in total cholesterol and TNF-α values ($p < 0,05$). Compared to the first group and the third group, no significant difference was observed in all values, but when comparing the first group and the second group, there was a significant difference in total cholesterol and TNF-α values ($p < 0,05$). When the second and third groups were compared, a significant difference was found in total cholesterol values ($p < 0,05$).

4. Discussion

Literature studies have shown that Lactobacillus and Bifidobacterium spp. Are antiobesity and antiinflammatory effects in many studies. Yoo et al. found an increase in body weight gain and fat accumulation and an increase in cholesterol and proinflammatory cytokines after probiotic supplementation with *Lactobacillus curvatus* and *Lactobacillus plantarum* in obese rats fed with high fat-high cholesterol diet for 9 weeks [12]. In another study conducted during 7 weeks, body weight gain, total cholesterol, HDL, LDL, triglycerides, glucose, and leptin levels were detected a reduction after the addition of probiotic containing *B. longum* to the diet of high fat rats [13]. In another study of probiotic reinforcement containing *Bacteroides uniformis* strain in experimental animals fed on a high fat diet, a reduction was determined in body weight gain, liver lipid content, serum cholesterol, triglyceride, glucose, insulin and leptin levels [14]. In a study using *Lactobacillus curvatus* and *Lactobacillus plantarum* species as probiotic supplement for 2 weeks in mice in which the obese rat model was formed after 8 weeks, a decrease was detected in the proinflammatory genes in the adipose tissue [15].

In our study, similar results were observed to other studies. After probiotic application containing *L. acidophilus, B.lactis, L. paracasei* and *L. rhamnosus* strains for 4 weeks, there was a decrease in body weight gain, total cholesterol, LDL, leptin and TNF-α levels, and an increase in IL-10 which a cytokine with antiinflammatory properties. There was no change in HDL levels.

5. Conclusion

As a result; intestinal microbiology and permeability play a role on inflammatory system and obesity by various mechanisms. Probiotic supplement is thought to be a key strategy that can contribute to the prevention of complications related to obesity and obesity by regulating intestinal permeability and by changing intestinal microbiota to provide immune system activation. It is also thought that it will be useful to clarify issues such as effect mechanism, correct microorganism, correct strains by carrying out more comprehensive studies on the subject.

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INVESTIGATION OF THE EFFECT OF EXERCISE ON IRISIN HORMONE IN EXPERIMENTALLY INDUCED DIABETIC RATS

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Abstract: *Investigating the possible effects of the irisin hormone, which has been discovered in recent years, on energy expenditure and glucose metabolism has been found to have positive effects on end-stage diseases. The irisin secreted by the skeletal muscle in mice and humans; is a hormone thought to improve energy consumption and improve systemic metabolism by making white fat tissue turn into brown fat tissue. Thanks to the knowledge that the structure of the irisin hormone is 100% similar in rats and humans, researches on experimental animals may be helpful for human studies. It has been suggested that irisin, which has been studied in particular for its positive effects on obesity and diabetes, may be a promising alternative treatment for metabolic diseases. In our study that we have planned, we aimed to determine the changes that exercise will bring about at the level of irisin hormone in diabetic animal models that will be created experimentally and to evaluate the effects of these changes on diabetes, insulin resistance, lipid profile, obesity and inflammation from Metabolic Syndrome components. For this purpose, we have 3 groups; control group, diabetic and diabetic exercise group. The effects of exercise on the level of irisin hormone were investigated in the obtained serum samples. In order to observe the obesity process, the rats in each group were weighed weekly and their body weights were measured. When the weights of the groups at the beginning and 4 weeks were observed, a mean increase of 35.8 g ($p < 0.05$) was observed in the control group, 30 g ($p < 0.05$) in the diabetic group and 59 g in the diabetic exercise group ($p > 0.05$). The mean fasting blood glucose value of the diabetic group was 477.17 ± 87.98 mg / dl and the diabetic exercise group had a mean of 485 ± 86.03 mg / dl. When the irisin levels were examined, it was seen that there was a difference between the groups ($p > 0.05$). In the control group, average irisin level was 255.73 ± 374.81 ng/ml, in the diabetic group was 98.09 ± 51.25 ng/ml and in the diabetic exercise group was of 66.43 ± 25.01 ng/ml. As a result, injectable irisin forms are produced because of irisin hormone is an important alternative in the treatment of obesity and diabetes as well as other metabolic diseases; it can be thought that the frequency can be used to control diabetes and obesity which are increasing day by day.*

Keywords: *irisin, diabetes, exercise, obesity.*

6. Introduction

Adipose tissue is an fat tissue composed of lipid-containing cells called adipocytes [1]. Adipocytes are made by lipoblasts that differentiate from mesenchymal cells. Lipoblasts are transformed into two different fatty tissues with different functions and morphology in mammals as white fat tissue (WFT) and brown fat tissue (BFT). Brown fat tissue is a fatty tissue that is specialized in heat generation (thermogenesis) [2]. There is an amount of BFT in the neonatal period, and in adulthood it is quite low [2,3,4]. The functional capacity of WFT is broader and more extensive [2,5]. WFT can represent the largest endocrine tissue of humans. Some of the WFT-secreted adipocytokines; leptin, ghrelin, adiponectin, glucocorticoids, plasminogen activator inhibitor (PAI-1), TNF-α, IL-6, angiotensin, visfatin, resistin, irisin [2].

Irisin are discovered in 2012 by Boström et al. [6], is defined as a hormone secreted by the skeletal muscle linked to the exercise in mice and humans. It is thought that it increases energy consumption and develop systemic metabolism by converting white fat tissue to brown fat tissue to [6,7,8]. Because of these effects, it has gained tremendous popularity as a working topic in many laboratories [9]. Irisin, is a glycoprotein-structured hormone of 12 kDa and composed of 112 amino acids; is a proteolytic product of the fibronectin type III domain 5 (FNDC5) molecule [6,10]. FNDC5, secreted from muscle tissue, is cleaved from the C-terminus and released into the environment. This part which is separated is irisin [11]. As a result of the researches it has been synthesized and released in many tissues and it is stated that the main source is skeletal muscle and fat tissue [8]. While the structure of the irisin hormone is 100% similar in humans and mice, the similarity rate of insulin is 85%, glucagon 90%, leptin 83% [6].

After exercise, increased FNDC5 mRNA has been reported in the skeleton of mice and humans in studies. Although, it is widely known that exercise does not reduce food intake but allows the burning of fat and calories, the molecular mechanism of this phenomenon has not been elucidated until the discovery of irisin [8]. There are studies that suggest that the release of irisin from the skeletal muscle after exercise is promoted, as well as studies that have no meaningful relationship.

Irisin is thought to have many mechanisms of action in the body. Isolation of irisin has been a guide in explaining the metabolic events and especially the fatty tissue metabolism [7,8]. It is thought to be able to prevent the onset of obesity and diabetes thanks to the relationship with glucose/lipid metabolism. A mechanism of increased secretion of irisin in response to reduced glucose/lipid metabolism in non-diabetic obese patients has been reported [12]. The irisin, stimulated by exercise and cold, increases the expression of the UCP-1 pump in white adipose tissue cells. White fat tissue cells which has increased UCP-1 pump in the mitochondria, is called beige fat tissue. These cells work like brown fat tissue cells. Increased UCP-1 expression inhibits ATP synthesis and leads to increased heat production which causes energy consumption in the cell, thermogenesis and glucose homeostasis are achieved [7,13,14,15].

In light of this information, in this study, we aimed to determine the effects of exercise on the level of irisin hormone in experimental diabetic animal models, to evaluate the effects of metabolic syndrome components on diabetes, lipid profile, obesity and inflammation.

7. Methods

In the direction of our purpose, in our study, there are 3 groups; control group, diabetic group and diabetic-exercise group. The rats used in the study were obtained from Ondokuz Mayıs University Experimental Animal Application and Research Center and the steps related to animal research were carried out in this center. A diabetic rat model was developed by applying streptozotocin to rats in the study group [16]. Fasting blood glucose (FBG) levels were measured 72 hours after the administration and rats with 250 mg / dl were accepted as diabetic. While no intervention was carried out in the group in which experimental diabetic rats were placed, diabetic exercise group was applied 3 times a week, 20 m/min walking exercise program. In serum samples taken from the tail veins of all rats in the 4th week, commercial ELISA (Rel Assay Diagnostics) kits were used to determine this parameters: irisin hormone level, FBG, serum total cholesterol, HDL and LDL levels were used to determine lipid profile, leptin level as an indicator of obesity, IL-10 and TNF-α is pre-inflammatory and antiinflammatory markers as an indicator of inflammation. In order to observe the obesity process, the rats in each group were weighed weekly and their body weights were measured.

8. Results

Morphometric measurements of the rats in the study are given in Table I.

TABLE I. MORPHOMETRIC MEASUREMENTS OF GROUPS

	Unit	**Control Mean ± SD**	**Diabetes Mean ± SD**	**Diabetic exercise Mean ± SD**	***p***
Baseline weight	g	289,4±11,9	297,8±22,2	313±7,5	*0,208*
Weight on the 4th week	g	324,4±11,0	267,8±28,7	254±10,6	*0,001**
Length	cm	24,3±1,2	24,4±0,8	24,8±1,1	*0,368*
Body Mass Index	g/cm^2	0,55±0,5	0,45±0,4	0,41±0,3	*0,368*

**p<0,005*

When the weights of the beginning and 4 weeks of the rats examined during the study were evaluated, an average of 35 g ($p < 0.05$) increase was observed in the control group, also 30 g ($p < 0.05$) decrease in the diabetic group and 59 g ($p > 0.05$) in the diabetic exercise group decrease was observed. It has been determined that FBG value of rats administered streptozotocin is over 250 mg / dL. The mean values of the evaluated parameters are summarized in Table II.

TABLE II. AVERAGE VALUES OF PARAMETERS FROM GROUPS

	Unit	**Control Mean ± SD**	**Diabetes Mean ± SD**	**Diabetic exercise Mean ± SD**	***p***
Fasting Blood Glucose	mg/dl	98,06 ± 22,15	477,17 ± 87,98	485,00 ± 86,03	*0,369*
Total Cholesterol	mg/dl	13,74 ± 2,91	15,29 ± 1,44	15,5 ± 3,92	*0,554*
HDL	mg/dl	21,69 ± 0,73	18,87 ± 3,28	20,3 ± 0,25	*0,016**
LDL	mg/dl	21,69 ± 0,73	34,33 ± 1,47	28,77 ± 4,29	*0,000**
IL-10	pg/ml	2086,06 ± 400,22	2046,15 ± 135,87	1812,04 ± 260,69	*0,503*
TNF-α	ng/L	1308,58 ± 177,11	1415,75 ± 110,61	1371,67 ± 84,81	*0,618*
Leptin	ng/L	2069,34 ± 121,61	1911,11 ± 441,63	2008,88 ± 263,09	*0,774*
Irisin	ng/ml	107,73 ± 49,79	98,09 ± 51,25	66,43 ± 25,01	*0,458*

**p<0,005*

As shown in Table II, the mean FBG value of the diabetic group is 477,17 ± 87,98 mg/dl and the diabetic exercise group is 485 ± 86,03 mg/dl. When the lipid profiles were evaluated, HDL level was significantly different between the groups ($p < 0.05$). The HDL level in the diabetic group was lower than in the control group, but it was determined that increased in the HDL level after exercise. Also, there was a statistically significant difference between the groups in terms of LDL levels ($p < 0.05$). It has been found that IL-10 levels which is an inflammatory markers are reduced in diabetic groups because of the fact that diabetes suppresses immunity. TNF-α levels were higher in the diabetic groups than in the control group. When the irisin levels were examined, it was found that there was a difference between the groups ($p > 0.05$). It was determined that the level of irisin in the control group was higher than in diabetic groups. The mean irisin level of the control group was 255.73 ± 374.81 ng/ml, the diabetic group was 98.09 ± 51.25 ng/ml and the diabetic exercise group was 66.43 ± 25.01 ng/ml. The diabetic group has a lower irisin level than the control group, similar to other studies in the literature. Leptin levels were also found that decreased in the diabetic group. In diabetic rats, it was observed that there was a negative correlation between the level of irisin and FBG ($p > 0.05$). It was also determined that there is a strong correlation between leptin and irisin in the negative direction ($p > 0.05$). When the relationship between lipid profile and the level of irisin was examined, it was found

to be positive correlation with total cholesterol and HDL levels and negative correlation with LDL.

9. Discussion

There are a number of studies that have been conducted from the knowledge of increased release of irisin resulting from exercise. Rats with hypothyroid and hyperthyroid were treated with swimming exercise 5 times a week for 1 hour, it was observed that increase in irisin level, decrease in total cholesterol, triglyceride, LDL and HDL levels. [17]. In a study investigating the effects of resistance training on the expression of the irisin in experimental animals and humans, tail-weighted stair climbing exercise was built to the rats 3 days per week during 12 weeks. Also, elastic band exercise program was applied to individuals over 65 years of age 2 days weekly consisting of 1-hour sessions and totally 12-hour. It has been shown in rats that the level of irisin in the serum and muscle tissue is increased and there is an increase in muscle strength, even though there is no change in body composition. Similar results have been observed in humans and it has been found that the level of irisin in circulation rises with exercise, body composition doesn't change and muscle strength increases. These results suggest that resistance training may increase the irisin level and improve muscle function loss that may be seen in later ages [18]. When the effect of 15-20 min walking exercise 5 days a week on the level of irisin was investigated in diabetic rats, serum irisin level was found to be higher in the exercise group than in the control group [19]. It was determined that progression of obesity after 8 weeks of swimming intervention in obese rats induced by high fat diet could be alleviated. Decreases in LDL and total cholesterol levels were observed when increased levels of irisin and HDL were detected [20]. As a result of the study to evaluate the relationship between swimming exercise and serum obesity parameters, it was determined that body fat mass decreased in rats fed a high fat diet after swimming exercise. This condition is thought to result from increased levels of irisin as a result of swimming exercise [21].

10. Conclusion

It has been reported that the irisin is synthesized from many tissues, mainly muscle and fat tissue, as a result of exercise. Irisin is the key hormone that causes UCP-1 release in the lipid tissue, thus causing irreversible thermogenesis and weight loss, and is involved in the regulation of Body Mass Index. As a result of our study, we found that the level of irisin in diabetic rats was lower than in the control group. It was also found that there was a negative correlation between FBG and irisin. These results are similar to those in the literature. Thanks to the knowledge that the structure of the irisin hormone is 100% similar in rats and humans, researches on experimental animals are a guide for human studies.

11. Recommendations

Since the irisin hormone is an important alternative in the treatment of obesity and diabetes as well as other metabolic diseases, injectable irisin forms are produced; it can be considered that it can be used for controlling diabetes and obesity which are increasing day by day. In the light of current information, studies on the relation of irisin hormone with metabolic effects and diseases should be increased and the effects on the treatment of diseases should be emphasized by following developments.

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EXPERIMENTAL INVESTIGATION OF THE EFFECT ON THE CHEMERIN ADIPOKINE AND OBESITY OF PROBIOTIC USE IN OBESE RATS

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Abstract: *Chemerin is a new chemotactic protein that recently joined the adipokines family. It has been shown to play a role in adipogenesis and energy metabolism, including its role on obesity, Type 2 Diabetes Mellitus (T2DM), metabolic syndrome and cardiovascular diseases. Probiotics may play role in the prevention of obesity by various mechanisms and treatment of many diseases such as T2DM. In this study, we aimed to evaluate the effects of probiotic supplementation of chemerin adipokines on serum levels and obesity markers in obese animal models. For this purpose 3 groups of experimental animals were formed. In the obtained serum samples, the effects of probiotic supplementation on chemerin and leptin level which are indicators of obesity will be examined. Weights of all the rats in the groups were weighed each week to monitor the obesity. The weight gain in the group fed with probiotic supplementation was 10,00±27,2 g for 4 weeks and the weight gain for the group fed with high fat diet was 26,200±7,085 g ($p<0.05$). After 8 weeks of feding the changes of BMI values of the rats were found to be statistically significant ($p<0.05$). There was no significant difference between the leptin values of the groups, but the difference between the mean values of the chemerin values after 12 weeks of feeding was found to be statistically significant ($p <0.05$). As a result; this study showed that obese rats reduced the weight gain of probiotic supplementation without calorie restriction, positive effects on BMI and chemerin adipokine serum levels.*

Key words*: chemerin, leptin, obesity*

13. Introduction

Chemerin is a new chemotactic protein that recently joined the adipokines family. In 2007, it was discovered that chemerin and its receptor CMKLR1 are highly exaggerated in human and mouse adipocytes. This suggests that adipose tissue is a source and target for chemerin signaling [1]. Chemerin is a natural ligand for the chemerin receptor (ChemerinR), also known as chemokine receptor 1 [2,3]. Also, chemerin is a chemoattractant composed of 163 amino acids and synthesized as pre-prochemerin once secreted as an inactive precursor, called prochemerin [3,4]. The majority of circulating chemerin is in the form of inactive prochemerin and it has to be converted to bioactive chemerin isoforms (by proteolytic processes) for local biological activities [5]. Chemerin is excreted in the highest levels in the placenta, liver and white adipose tissue while it is excreted less in many tissues such as lung, brown fat tissue, heart, ovary, kidney, skeletal muscle and pancreas [3]. There is growing evidence that this newly discovered adipokine has been shown to play a role in adipogenesis and energy metabolism, including its role on obesity, Type 2 Diabetes Mellitus (T2DM), metabolic syndrome and cardiovascular diseases [3,6,7]. Determining circulating levels and monitoring the levels of chemerin adipokinin is of importance in relation to these diseases [8,9].

It has been suggested that probiotics, which are defined as living microorganisms with beneficial effects on the health of the host cell, may play a role in the prevention of obesity by various mechanisms and treatment of many diseases such as T2DM [10]. Despite the fact that obesity is a multi-factor etiology, changes in intestinal microbiology have attracted attention in recent years [11]. Probiotic reinforcement may be a promising treatment for reversing dysbiosis-related changes in obesity and related diseases [12].

In this study, we aimed to evaluate the effects of probiotic supplementation of chemerin adipokines on serum levels and obesity markers in obese animal models produced by experimental high fat diet.

14. Material and methods

Within the scope of the study, 3 groups of experimental animals were formed. The first group was defined as the control group fed with the standard diet (Group 1), the second group as the group fed with the high fat diet (Group 2) and the third group as the group receive with probiotic capsule supplementation with a high fat diet (Group 3). After feeding the groups for a total of 12 week, in the obtained serum samples were examined with the effects of probiotic supplementation on HDL, LDL and total cholesterol which are indicators of lipid profile, leptin level which are the indicators of obesity, chemerin levels by used commercial ELISA (Rel Assay Diagnostic) kits. In addition, the weights of all rats were determined at the beginning of the study. Weights of all the rats in the groups will be weighed each week to monitor the obesity process and the relationship between the diets in different contents and *Body Mass Index (BMI)* were examined. The rats to be used in the study were obtained from Ondokuz Mayis University

Experimental Animal Application and Research Center and the steps related to animal research are carried out in this center.

15. Results

Initial weights of the rats in the control and study groups, weight changes at the beginning and after the probiotic reinforcement are shown in Table I.

TABLE I. MEAN VALUES OF MORPHOMETRIC PROPERTIES OF RATS

	Group 1	**Group 2**	**Group 3**	***P***	
				Group 1-2-3	**Group 2-3**
Weight (g)					
Baseline	240,2±9,6	254,6±16,0	330,0±48,6	*0,013**	*0,019**
Starter Probiotic	283,8±11,0	307,0±14,5	400,5±65,0	*0,004**	*0,014**
Post-Probiotic	11,8±17,0	339,6±19,6	422,0 ±71,0	*0,009**	*0,027**
Weight gain (g) (9.-12. week)	23,2±7,8	26,2±7,0	10,0±27,2	*0,767*	*0,539*
BMI (g/cm^2)					
Baseline	0,41 ± 0,01	0,44 ± 0,27	0,57±0,08	*0,013**	*0,016**
Starter Probiotic	0,49 ± 0,01	0,53 ± 0,02	0,69±0,11	*0,004**	*0,016**
Post-Probiotic	0.54 ± 0,029	0,54 ± 0,02	0,73±0,12	*0,009**	*0,032**

p<0.05

The mean weights of the rats in Group 1, Group 2 and Group 3 were 240,200±9,67 g; 254,600 g±16,00 and 330,00±48,62 g, respectively. After feeding with 8-week high-fat diet of the rats in group 2 and group 3, the weights were increased to 307,00±14,50 g and 400,50±65,039 g, respectively. After 8 weeks of feding the BMI values of the rats in group 2 were reached from 0,44±0,27 g/cm^2 to 0.53±0. g/cm^2, while the BMI values of the groups in group 3 were reached from 0,57±0,08 g/cm^2 to 0,69±0,11 g/cm^2 ($p<0.05$).

The average weight gains for 0-8th week and 9-12. week are shown in Fig 1.

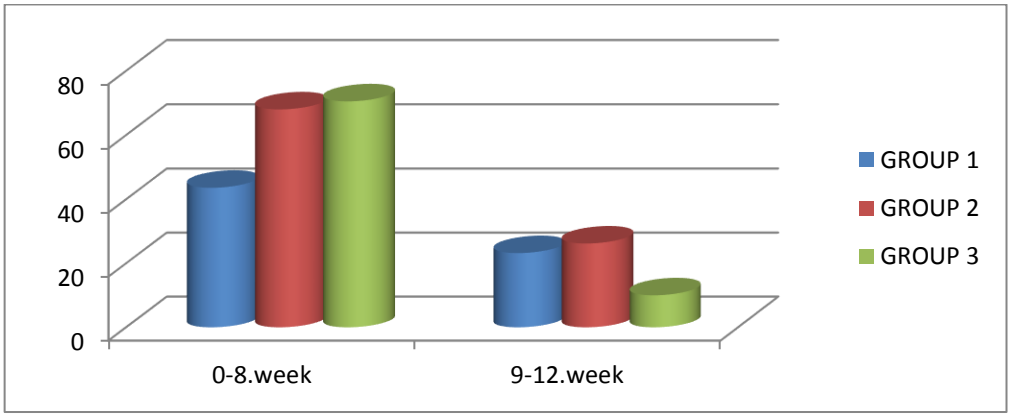

Figure 1. According to Groups Weight Gain from 0-12 Weeks

The weight gain in the group fed with probiotic supplementation was 10,00±27,2 g for 4 weeks and the weight gain for the group treated with high fat diet was 26,200±7,085 g ($p<0.05$).

The BMI changes for the rats are shown in Fig 2.

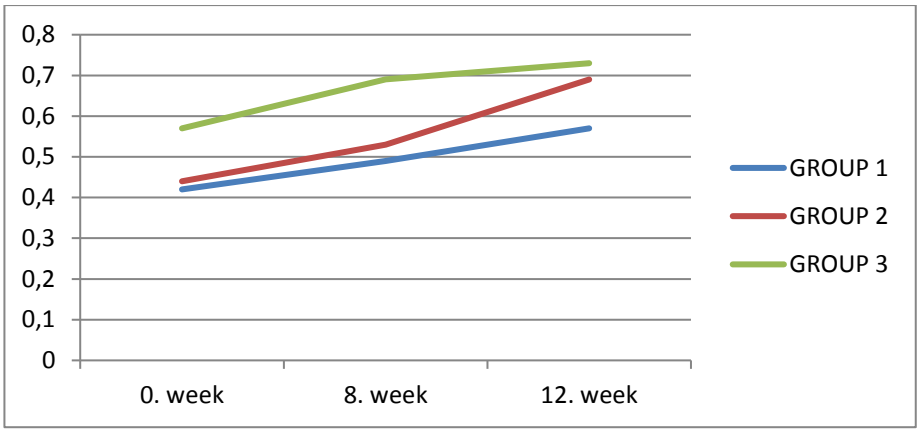

Figure 2. Changes in BMI for Groups from 0-12 Weeks

BMI changes in rats in group 2 are increasing rapidly, while changes in BMI in rats in group 3 tend to increase slowly after the start of probiotic supplementation.

Table II summarizes the laboratory findings of the study rats at baseline and after 12 weeks of feeding.

TABLE II. MEAN VALUES OF SERUM VALUES BY GROUPS

	Group 1	**Group 2**	**Group 3**	***P***	
				Group 1-2-3	**Group 2-3**
HDL (µg/ml)					
Baseline	21,69±0,73	21,69±0,73	21,50±0,68	*0,847*	*0,618*
Final	21,69±0,73	21,00±2,63	23,32±3,76	*0,663*	*0,325*
LDL (µg/ml					
Baseline	31,50±4,02	31,50±4,02	32,15±4,33	*0,965*	*0,907*
Final	31,50±4,02	43,12±11,77	31,87±2,89	*0,083*	*0,059*
Total Cholesterol (mmol/L)	13,74±2,90	13,74±2,90	12,52±1,1	*0,847*	*0,618*
Baseline	13,74±2,90	22,30±3,99	14,87±3,00	*0,004**	*0,447*
Final					
Leptin (ng/L)					
Baseline	2069,34±121,60	2069,34±121,6	2069,34±121,6	*0,977*	*0,827*
Final	2069,34±121,60	0	0	*0,149*	*0,058*
		2165,34±80,88	2026,65±106,7		
			0		
Chemerin (ng/mL)					
Baseline	76,78±33,77	76,78±33,77	70,37±35,32	*0,959*	*0,803*
Final	76,78±33,77	120,42±19,68	116,07±4,25	*0,028**	*0,172*

p<0.05

After 12-week high-fat diets, the rats in Group 2 had HDL levels of 21,00±2,63 µg/mL, LDL levels of 43,12±11,77 µg/mL and total cholesterol levels of 22,31 ± 3,99 mmol/L. The mean HDL, LDL and total cholesterol values of the rats in Group 3 after supplementation with probiotics were 23,32±3,76 µg/ mL, 31,87±2,89 µg/mL and 14,87±3,00 mmol/L, respectively. There was no significant difference between groups in terms of HDL and LDL, but a significant difference was found between total cholesterol values (p <0.05). After 12 weeks of feeding, the mean values of the chemerin values of the groups were 24,26±19,39 ng/mL (Group 1); 71,53±35,23 ng/mL (Group 2); 60,35±9,32 ng/mL (Group 3).There was no significant difference between the initial chemerin values of the groups, but the difference between the mean values of the chemerin values after 12 weeks of feeding was found to be statistically significant (p <0.05). There was no statistically significant difference between leptin values obtained at baseline and after 12 weeks feeding in groups.

The mean leptin and chemerin values of Group 1, Group 2 and Group 3 are shown in Fig 3.

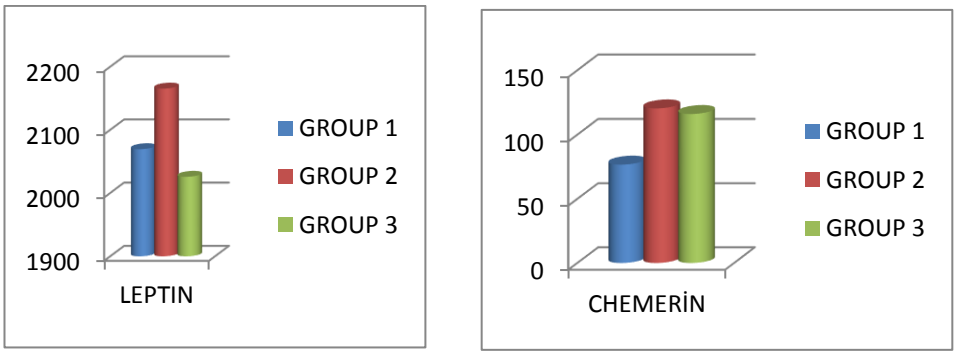

Figure 3. Mean Values of Leptin and Chemerin for The 12th Week According to The Groups

16. Discussion

It has been determined that the probiotic supplementation performed without any weight loss method has significant changes in both weight gain and metabolic parameters. Studies investigated in support of the association between adiposity and serum chemerin levels have observed significant reductions in serum chemerin levels compared to obese individuals who did not enter weight loss interventions (calori restriction or bariatric surgery) in individuals who various weight loss procedures [7,13,14]. Fatima et al. [15] also reported that circulating chemerin levels were significantly higher in obese subjects with BMI > 25 kg/m^2 than subjects with BMI <25 kg/ m^2. In our study, chemerin levels of normal rats were found to be lower than those of obese rats.

In humans, serum chemerin concentration is significantly higher in the BMI and waist circumference than in normal weight patients [16]. In our study, a positive correlation was found between the BMI values and the chemerin levels of the rats.

When examinated of experimental animal studies suggests that probiotic supplementation results in similar results with our study on body weight and metabolic parameters. In a study evaluating changes in high fat diets-related adiposity, intestinal microbiota and serum metabolite levels in rats fed a balanced or high fat diet with and without probiotic for 8 weeks; it has been determined that probiotic supplementation modulates morphometric and metabolic parameters effectively and reduces body weight [11].

Kang et al. [17] investigated the effects of probiotic supplementation on weight loss; rats were fed on a high carbohydrate diet for 12 weeks and received probiotic supplementation twice daily. This study in which changes in body weight and metabolic parameters are observed, shows that probiotics can prevent weight gain, improve metabolic parameters, and be an alternative method of treating obesity. In monosodium glutamate (MSG) -induced obese rats, probiotic supplementation similarly improves body weight and TC, LDL and HDL levels [18].

Studies in the literature regarding the effect of probiotics on the serum level of chemerin adipokine are not sufficient and our results are of importance in terms of the reduced effect on chemerin adipokine levels.

17. Conclusion

As a result; this study showed that obese rats reduced the weight gain of probiotic supplementation without calorie restriction, positive effects on BMI and chemerin adipokine serum levels. The next step of our study is to increase the number of rats in the experimental groups and extend the probiotic supplementation period to achieve more comprehensive results.

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COMPARATIVE ESSENTIAL OIL COMPOSITION OF AERIAL PARTS OF *MICROMERIA PERSICA* POPULATIONS FROM FOUR REGIONS IN FARS PROVINCE, IRAN.

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Abstract*:There are three species of the Micromeria plant in Iran, one of which is Micromeria persica. Different species of Micromeria has been used in traditional medicine. The current study deals with identifying chemical compounds of Micromeria persica populations in four regions of Fars province in Iran. These regions are Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad. First, the aerial parts of Micromeria persica collected as samples were dried. Then, the essence of the dried samples was extracted by water distillation in the Clevenger machine, and identification of compounds was made using the GC/MS machine. In Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad regions, the numbers of recognized compounds were 30, 45, 50 and 25 respectively. The main essence compounds of the four examined populations were Germacrene D, Bicyclogermacrene, spathulenol, and δ-cadinene. Geographical position and ecological parameters of habitat, such as height, annual rainfall, and climate, can change the quality and quantity of the essential oil's compounds in Micromeria persica.*

Key words*:* Micromeria Persica, Phytochemical, essential oil composition GC-MS, Iran

18. Introduction

There are three known species of the *Micromeria* genus in Iran: *Micromeria persica*, *Micromeria hedgei*, and *Micromeria myrtifolia*. The first two species are endemic [1,2]. Some *Micromeria* species are used in folk medicine for different purposes. The aerial flowering parts of the plants are locally used for treatment of cold. Several *Micromeria* species have been reported as antiseptic, abortifacient, antirheumatic, CNS stimulant, and tonic [3]. They are also used for treatment of heart disorders, indigestion, and headaches and as topical anaesthetic for toothache and wounds, inflamed eyes, skin infections and chest pains [4,5]. Some *Micromeria* species have also shown antioxidant and antimicrobial properties [6-8]. *M. biflora* and *M.*

graeca species in Spain are used for treating disorders of the digestive tract and stomach pains, respectively; *M. fruticose* is used in Turkey to relieve headache; in the Canary Islands *M. herpyllomorpha* and *M. varia* are used as a capillary tonic [9].

Sefidkon et al. [10] studied *M. Persica* in Hamadan province and analyzed the essence extracted from aerial parts of the plant before flowering and full flowering stages. They concluded that Thymol, limonene, γ-terpinene, p-cymene and 1,8-cineole are the main constituents of the essence of the plant. Subsequently, essential oil isolated by hydrodistillation from the aerial parts of *M. Persica* Boiss from Persepolis, Province of Fars (Iran) during the flowering stage were analyzed. The main constituents were linalool, a-pinene and (E)-nerolidol [11]. In studies of Kazemi Zadeh et al. [12] on chemical compositions of the essential oil of the two populations of Teucrium hyrcanicum, it was showed that the qualitative and quantitative differences in the essence composition of these two populations may be due to differences in the ecological properties of growth areas such as temperature, humidity, height from sea and other soil and geographical factors.

The current study deals with identifying chemical compounds of *Micromeria persica* populations in four regions of Fars province in Iran. It also investigates the effects of different parameters, such as height, annual rainfall, climate, and location on constituents of the essence.

19. Material and Methods

19.1.1 Plant Material

The aerial parts of *Micromeria persica* were collected in mid-spring (in May and June) from altitude of Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and 14 kilometers after Ghir to Firuzabad in Fars Province in the flowering stage. Geography and climate of the sampling regions are given in Tab. 1. Herbarium plant was identified by the Agriculture and Natural Resources Research Center of Fars province.

Table 1. Environmental Factors of the Sampling Regions.

Region	Location	Height	Climate	Annual Rainfall
Ghir to Firuzabad	southwestern of Shiraz	2500	dry moderate	356
Kuh-e Zireh	80 kilometers south-east of Shiraz	1832	dry moderate	340
Firuzabad	30 kilometers west of Shiraz	2125	dry moderate	315.7
Bezyn defile in Darab	70 kilometers east of Shiraz	1692	dry moderate	237

19.1.2 Isolation Procedure

The air-dried parts of *M. persica* were separately subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The essential oils were obtained in 3.00% (w/w) yield. The oils were dried over sodium sulfate and stored in sealed vials at low temperature before analysis. Identification of the constituents of each of the oils was made using the Gas Chromatography/Mass Spectrometry (GC/MS) machine.

19.1.3 Gas Chromatography

GC analyses were performed using an Agilent 7890A gas chromatograph equipped with a HP-5 column (30 m × 0.32 mm i.d., film thickness 0.25 µm) and connected to flame ionization detector (FID). Nitrogen was selected as the carrier gas with a flow rate of 1 ml/min. The injector temperature was 280°C, and detector temperature was 290°C, while column temperature was linearly programmed from 60 to 210°C (at rate of 3°/min) and then held for 7 min at 210°C.

19.1.4 Gas Chromatography-Mass Spectrum

GC analyses were performed using an Agilent 5975A gas chromatograph equipped with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Nitrogen was selected as the carrier gas with a flow rate of 1 ml/min. column temperature was linearly programmed from 60 °C to 210 °C (at rate of 3°/min) and then 210 °C to 240 °C (at rate of 20 °C/min). MS were taken at 70 eV.

20. Results

20.1. The Composition of the Oils of *M. Persica*

The composition of the oils of the aerial parts of *M. persica* in Kuh-e Zireh, Firuzabad, Bezyn defile in Darab and Ghir to Firuzabad regions are listed in Tables 2, 3, 4 and 5 respectively, in which the percentage and retention indices of components are given.

20.1.1 The Composition of the Oil of M. Persica in Kuh-e Zireh

Thirty compounds were identified in the essential oil of *M. persica* Kuh-e Zireh, representing more than 99.99% of the oil. The major components were found to be Spathulenol (30.25%), Bicyclogermacrene (18.89%), Germacrene D (19.37%). One compound was unknown.

The chemical composition of the essential oil of *M. persica* in Kuh-e Zireh can be seen in Tab. 2.

Table 2. Percentage Composition of the Oils of *Micromeria persica* in Kuh-e Zireh

No	Compound (P920365)	RI	% of compound
1	α-Thujene	925	0.156
2	α-Pinene	932	2.82
3	Sabinene	971	6.228
4	β-Pinene	976	0.959
5	Myrcene	989	1.35
6	p-Cymene	1023	0.259
7	Limonene	1026	1.912
8	1,8-Cineole	1029	0.854
9	(Z)–β-Ocimene	1034	0.949
10	(E)-β-Ocimene	1045	0.493
11	γ-Terpinene	1056	0.442

12	Linalool	1098	0.224
13	Terpinene-4-ol	1175	0.929
14	α-Terpineol	1188	1.115
15	Bornyl acetate	1283	0.274
16	n-Tridecane	1297	1.173
17	δ-Elemene	1334	0.808
18	α-Terpinyl acetate	1347	1.58
19	α-Copaene	1373	0.367
20	β-Bourbonene	1382	1.781
21	β-Elemene	1389	0.401
22	Germacrene D	1478	19.361
23	Bicyclogermacrene	1493	18.882
24	δ-Cadinene	1520	0.534
25	Spathulenol	1574	30.247
26	Caryophyllene oxide	1579	1.085
27	γ-Eudesmol	1634	1.253
28	epi-α-Muurolol	1650	1.317
29	Khusinol	1682	1.681
30	Unknown	1686	0.566

20.1.2 The Composition of the Oil of M. Persica in Firuzabad

There are 45 compounds in *M.Persica* essential oil in Firuzabad region, that is a total of 99.4 percent. The major components of the oil were Germacrene D (35.62%), Bicyclogermacrene (15.72%), Phytol (7%), Spathulenol (6.47%).

The chemical composition of the essential oil of *M. persica* in Firuzabad can be seen in Tab. 3.

Table 3. Percentage Composition of the Oils of *Micromeria persica* in Firuzabad

No	Compound (P920363)	RI	% of compound
1	□-Pinene	932	0.697
2	Sabinene	972	0.186
3	□-Pinene	976	0.719
4	Myrcene	989	0.66
5	□-Phellandrene	1005	0.131
6	□-3-Carene	1010	0.701
7	□-Terpinene	1016	0.052
8	p-Cymene	1023	0.283
9	Limonene	1027	1.273
10	1,8-Cineole	1030	0.163
11	(Z)□□-Ocimene	1035	5.04
12	(E)-□-Ocimene	1045	0.655
13	□-Terpinene	1056	0.519

14	cis-Sabinene hydrate	1064	0.077
15	Terpinolene	1087	0.435
16	Linalool	1098	0.386
17	n-Nonanal	1103	0.127
18	allo-Ocimene	1127	0.275
19	Terpinene-4-ol	1175	0.28
20	□-Terpineol	1188	0.215
21	Carvone	1241	0.466
22	Bornyl acetate	1283	0.102
23	Thymol	1289	0.279
24	Carvacrol	1298	0.201
25	□-Elemene	1335	1.562
26	□-Copaene	1374	4.093
27	□-Bourbonene	1382	0.68
28	□-Elemene	1390	1.351
29	(E)-Caryophyllene	1417	1.983
30	□-Copaene	1426	0.474
31	□-Humulene	1451	0.604
32	allo-Aromadendrene	1458	0.476
33	Germacrene D	1481	35.619
34	Bicyclogermacrene	1496	15.714
35	□-Cadinene	1521	5.221
36	(E)-□-Bisabolene	1529	0.169
37	Spathulenol	1575	6.469
38	Viridiflorol	1588	1.008
39	□-Cadinol	1650	0.513
40	6,10,14-trimethyl-2-pentadecanone	1840	0.32
41	Diisobutyl phthalate	1861	0.364
42	Dibutyl phthalate	1959	0.465
43	epi-13-Manool	2055	1.417
44	Phytol	2116	7.006
45	n-Tricosane	2297	0.57

20.1.3 The Composition of the Oil of M. Persica in Bezyn defile in Darab

50 components in the oil of *M. Persica* in Bezyn defile in Darab, which represented about 100% of the total oil, were identified. The oil of *M. Persica* consisted of GermacreneD (22.1%), Bicyclogermacrene (17.30%), Spathulenol (10.9%), δ -Cadinene (8.7%), α -Copaene (5.9%).

The chemical composition of the essential oil of *M. persica* in Bezyn defile in Darab can be seen in Tab. 4.

Table 4. Percentage Composition of the Oils of *Micromeria persica* in Bezyn Defile in Darab

No	Compound (P920363)	RI	% of compound
1	α-Thujene	925	0.012
2	α-Pinene	932	0.647
3	Camphene	947	0.014
4	Sabinene	971	0.203
5	β-Pinene	975	0.418
6	Myrcene	989	0.341
7	α-Phellandrene	1004	0.056
8	δ-3-Carene	1009	0.051
9	α-Terpinene	1015	0.028
10	p-Cymene	1023	0.125
11	Limonene	1027	1.143
12	1,8-Cineole	1029	0.798
13	(Z)–β-Ocimene	1035	1.668
14	(E)-β-Ocimene	1045	0.287
15	γ-Terpinene	1056	0.239
16	Terpinolene	1086	0.114
17	Linalool	1098	0.775
18	n-Nonanal	1102	0.136
19	cis-p-Menth-2-en-1-ol	1119	0.096
20	trans-p-Menth-2-en-1-ol	1136	0.131
21	Camphor	1142	0.165
22	Borneol	1163	0.1
23	Terpinene-4-ol	1175	1.595
24	α-Terpineol	1189	2.025
25	Carvone	1242	3.136
26	Bornyl acetate	1283	0.14
27	Thymol	1289	0.258
28	Carvacrol	1298	0.958
29	δ-Elemene	1335	1.679
30	α-Terpinyl acetate	1347	0.844
31	α-Copaene	1374	5.879
32	β-Bourbonene	1382	1.292
33	β-Elemene	1390	1.184
34	(E)-Caryophyllene	1416	1.287
35	α-Humulene	1451	0.51
36	allo-Aromadendrene	1458	0.676
37	Germacrene D	1482	22.034
38	Bicyclogermacrene	1497	17.297
39	γ-Cadinene	1512	0.846
40	δ-Cadinene	1522	8.689
41	Spathulenol	1577	10.817
42	Caryophyllene oxide	1581	1.665

43	Salvial-4(14)-en-1-one	1591	0.556
44	epi-α-Cadinol	1639	1.983
45	β-Eudesmol	1647	1.828
46	Khusinol	1683	2.449
47	6,10,14-trimethyl-2-pentadecanone	1840	0.254
48	n-Hexadecanoic acid	1963	1.038
49	Phytol	2114	1.061
50	n-Tricosane	2297	0.473

20.1.4 The Composition of the Oil of M. Persica in Ghir to Firuzabad

In the region of 14 km after Ghir to Firuzabad, there are 25 compounds in the essential of *M. Persica*, which are 100 percent of essential oil. In the oil n-Hexadecanoic acid (16.77), Germacrene D (12.60%), Spathulenol (8.89%), 1-β-ol Eudesma-4 (15) 7-dine- (7.18%), α -Cadinol (5.45%) are major componenets.

The chemical composition of the essential oil of *M. persica* in Ghir to Firuzabad can be seen in Tab. 5.

Table 5. Percentage Composition of the Oils of *Micromeria persica* in Ghir to Firuzabad

No	Compound (P920365)	RI	% of compound
1	Linalyl acetate	1254	0.228
2	□-Bourbonene	1382	0.191
3	□-Elemene	1390	0.549
4	(E)-Caryophyllene	1417	1.617
5	Germacrene D	1479	12.595
6	Bicyclogermacrene	1494	4.294
7	□-Cadinene	1521	0.613
8	Spathulenol	1575	8.898
9	□-Atlantol	1608	2.416
10	epi-□-Cadinol	1639	2.042
11	epoxy-allo-Aromadendrene	1643	1.926
12	□-Cadinol	1650	5.445
13	Eudesma-4(15),7-dien-1-□-ol	1683	7.177
14	6,10,14-trimethyl-2-pentadecanone	1840	3.202
15	Diisobutyl phthalate	1861	1.628
16	Dibutyl phthalate	1959	5.29
17	n-Hexadecanoic acid	1967	16.769
18	Neryl phenylacetate	2018	4.963
19	n-Heneicosane	2101	1.445
20	Phytol	2114	2.186
21	(E,E,E)-Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl	2138	6.932
22	Unknown	2162	4.868
23	n-Docosane	2198	0.589

24	n-Tricosane	2297	2.096
25	n-Pentacosane	2498	2.043

20.2. Comparison of Essential Oil Components of *M. persica* in Four Regions Based on Height

In essence of Micromaria, most frequent compounds are Germacrene D, Bicyclogermacrene, Spathulenol, δ- Cadinene in different regions. Height is one of the important environmental factors which has a significant impact on the amount of active ingredient. Fig. 1 of shows comparing the compounds based on the height of the regions, Germacrene D has the most values at the height of 2125 meters (Firuzabad region), Bicyclogermacrene has the most values at the height of 1832 meters (Kuh-e Zireh), Spathulenol has the most values at the height of 1832 meters (Kuh-e Zireh) and δ - Cadinene has the most values at the height of 1,692 meters (Bezyn defile in Darab), respectively.

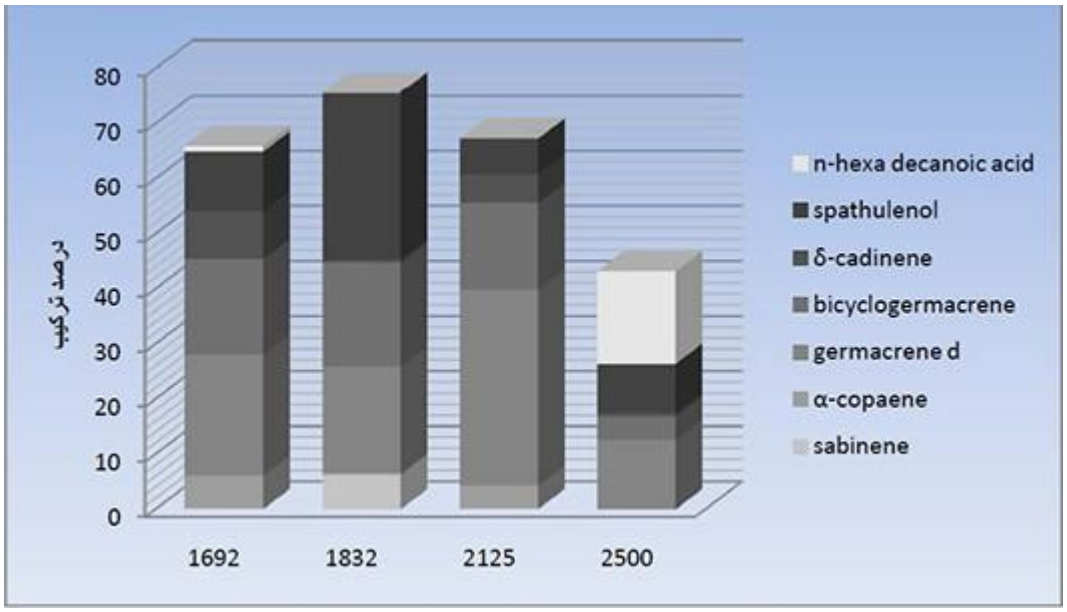

Fig 1. Comparison of Essential Oil Components of *M. persica* in Four Regions Based on Height

21. Conclusion

Changing height can have a direct impact on the temperature and moisture content, so it is an important factor. Increasing the height is along with decreasing temperature, increasing light intensity and increasing wind intensity. These changes along with decreasing temperature affect the moisture content. In addition to changes in climatic factors, height also changes the light quality. Increasing UV created at high altitudes interferes with plant growth. The direction of slope of earth is one of the factors that affect significantly on the amount of light received by ecosystem. This effect is especially evident in medium and high altitudes that sun angle decreases especially in the winter [13].

The growth location of medicinal plants, in terms of height from sea, slope and latitude and the impact of these factors on temperature, light, and relative humidity is very important in

the medicinal plants' metabolism and synthesis of their active ingredients. Height and climate are two important environmental factors in determining the chemical composition of medicinal plants essence. [14]

The results showed that differences in characteristics of the growth location such as height, slope, and direction of slope, cover and other climate conditions has a considerable impact on essence compounds. Differences in the quality and quantity of essence compounds of four populations of Micromaria in Fars province are due to the differences in ecological characteristics of growth areas such as temperature, humidity, altitude or other soil and geographical factors.

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NICOTINE DEPENDENCE LEVEL OF UNIVERSITY STUDENTS RELATING TO TYPE OF EDUCATION AND GENDER

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Abstract: *Smoking is an increasingly important public health problem because of the health problems it causes. We aimed to investigate whether there is a relationship between nicotine addiction level, education type and gender in vocational school of health services students who smoke in the study.*

This descriptive study was conducted with first and second year university students studying in various associate degree programs in health field. In the study, questionnaire forms were used to determine sociodemographic conditions prepared with scientific support as data collection tool. Nicotine dependence was determined by the Fagerström nicotine dependence test (FNBT). A total of 72 students attending 34 daytime education and 38 nighttime education courses participated in the research. 47 of the participants were female(% 65.3) and 25 (%34.7) male. In our study, 57 of the students are between the ages of 17-20 and the remaining 15 are over 21 years old. 29 of the participants in the study were low-level addicts, 34 were moderately addicts, and 9 were high-level nicotine addicts. When the inter-gender dependency levels are examined, 58.8% of the female students and 41.2% of the male students are moderately addicted. 52.6% of evening education students are moderately dependent, 41.2% of daytime education students are moderately and lowly dependent. There was no statistically significant difference between gender, type of education and nicotine addiction. There was no significant difference between the level of dependence, education type and gender in nicotine dependence level study using FNBT. The addiction rate was also higher for women. Most of the students who had nighttime learning were mostly moderate nicotine addicts.

Key Words*:* Nicotine dependence, gender, type of education, students.

1. INTRODUCTION

Smoking is an important public health problem due to health problems. Cigarette addiction is a complicated process in which environmental and genetic influences play a role together. Psychological factors and habits are like being the main factor in nicotine addiction. Nicotine is an addictive substance derived from the leaves of a tobacco plant [1].

Nicotine acts through specific nicotinic acetylcholine receptors and stimulates dopamine release thought to be responsible for the acute rewarding effect of nicotine. It increases the "reward cycle" effect of dopaminergic neurons in the anterior nicotine and increases the release of neurotransmitters such as acetylcholine, norepinephrine, dopamine and serotonin in the presynaptic region[2]. It is known that while acetylcholine causes an increase in performance and memory capacity, the release of dopamine and norepinephrine decreases pleasure and appetite[1]. Smoking cigarettes have been shown to improve performance in nicotine-free jobs[3].

Nicotine dependence has a similar effect on heroin and cocaine dependence. Nicotine dependence is a major cause of continued cessation behavior and failure of treatment interventions [4]. The Fagerstrom Nicotine Dependence Test (FNBT) is often used in studies that measure the prevalence of nicotine dependence. Studies have shown that FNBT is correlated with biochemical measurements and that scales of scale are effective in assessing cigarette smoking and treatment outcome [5].

The purpose of this study is to examine whether there is a relationship between the level of nicotine addiction and the type of education (day and night) and gender in students studying in associate degree programs.

2. MATERIAL AND METHOD

2.1 Participants

This descriptive study was conducted among first- and second-year students studying at the Health Services Vocational School in 2017. A total of 72 smokers participated in the study. During the data collection phase, students were accepted orally and the questionnaires were only applied to students who agreed to participate. The study started with the approval of the Ethics Committee of Sakarya University Medical Faculty.

2.2 Questionnaire Forms

A questionnaire prepared with scientific resources was used as data collection tool. Attendance, gender, age, education class, type of education, social security, parental status, parental education status, number of siblings, etc. Questions about identifying socio-demographic conditions of participants about the descriptive characteristics were directed. After receiving information about the questionnaire and the FNBT questionnaire, they were asked to fill in by the students who agreed to participate.

2.3 Fagerstrom Nicotine Addiction Test

FBNT was developed by Karl O. Fagerstrom to assess the level of physical dependence on cigarettes [5]. The exam consists of 6 questions. The questions were closed. Addiction increases score scale when smoking increases. Those who score 0-2 on the scale are mild, and those who score 8-10 on a scale of 3-7 are considered heavy nicotine addicts. FBNT is also used in smoking cessation clinics.

2.4 Statistical Analysis

Completed questionnaires were evaluated with SPSS 22 statistical program and analyzed statistically. The data show the arithmetic mean ± standard deviation and the number and percentage values.

3. RESULTS

When the distribution of the students according to sociodemographic characteristics is examined; Thirty-seven (65.3 %) of the participants were female and 25 were male (34.7 %). In our study, 79.2 % of the students were between the ages of 17-20 and the remaining 20.8 % were over 21 years old. 49 (68.1 %) of the students were in the first class, 23 (31.9%) in the second class, 47.2% in the first class and 52.8 in the second class. 59.7% of the students are primary school graduates and 48.6 % are high school graduates. 34.7% had an authoritarian family. Moreover, 48.6 % of the total family income is between 3000-5000 TL (Table 1).

Table 1. Distribution of Students by Socio-Demographic Characteristics

Participants Characteristics	*n=637*	*%*
Gender		
Woman	*47*	*65. 3*
Men	*25*	*34.7*
Class		
1st Class	*49*	*68.1*
2st Class	*23*	*31.9*
Type of Study		
Daytime education	*38*	*47.2*
Nighttime education	*34*	*52.8*
Educational status of mother		
Illiterate	*5*	*6.9*
Literate	*8*	*11.1*
Primary school graduate	*43*	*59.7*
High school graduate	*14*	*19.4*
Graduated from a Universty	*2*	*2.8*
Educational status of father		
Illiterate	*1*	*1.4*
Literate	*2*	*2.8*
Primary school graduate	*26*	*36.1*
High school graduate	*35*	*48.6*
Graduated from a Universty	*8*	*11.1*
Family Attitude		
Authoritarian	*25*	*34.7*
Democratic	*22*	*30.6*
Irrelevant	*2*	*2.8*
Protector	*23*	*31.9*
Friend relationships		
Positive	*54*	*75.0*
Verbal controversial	*14*	*19.4*

Physical conflict	*4*	*5.6*
Living place		
Provincial center	*41*	*23.3*
District	*20*	*11.4*
Village	*11*	*6.3*
Income rate		
1000TL	*4*	*5.6*
1000-3000TL	*35*	*48.6*
3000-5000TL	*27*	*37.5*
5000-10000TL	*6*	*8.3*

In our study of all participants smoking, 29 of the students were low-level dependent, 34 were moderately addicted, and 9 were high-level nicotine addicts. 58.8 % of female students and 41.2 % of male students were moderately addicted; 52.6 % of them were dependent on the moderate level and 41.2 % of the primary education students were moderately and lowly dependent on the type of learning and nicotine dependence (Table 2).

Table 2. Nicotine Dependence Levels of Gender and Learning Attendance by Participants

Nicotine Dependency Level	**Type of Study**		**Gender**		**Total (%)**
	Daytime education	**Nighttime education**	**Woman**	**Man**	
Low	14 (41.2 %)	15 (39.5 %)	20 (69 %)	9 (31%)	29 (100 %)
Medium	14 (41.2 %)	20 (52.6 %)	20 (% 58,8)	14 (41.2 %)	34 (100 %)
High	6 (7.9 %)	3 (7.9 %)	7 (77.8 %)	2 (22.1 %)	9 (100 %)
Total	34 (100 %)	38 (100 %)	47 (65.3 %)	25 (34.7 %)	72 (100 %)

4. DISCUSSION

In recent years, smoking cessation campaigns and published legislation, particularly in developed countries, have shown that smoking rates are significantly reduced. There has been an increase in the number of cessation-quitting treatments in our country, especially after leaving cessation in closed areas [6].

Health professionals should be sampled by the community and they should not smoke because they are health educators at the same time. Health workers' cigarettes receive messages about the health effects of smoking [7]. For this reason, it is important to know the smoking status of this group, the levels of nicotine dependence and the factors affecting it.

Health workers need to be taken as an example by the society and at the same time they should be non-smokers because they are health educators. Health workers' smoking, hurts messages about the health effects of cigarettes[7]. For this reason, it is important to know the smoking status of this group and the levels of nicotine addiction and the factors that affect it.

Nicotine dependence is the most common and most important type of substance abuse because smoking is easy and inexpensive and its use is legal. Studies have defined the 15-24 age group as a risky group in terms of the development of substance dependence [8]. In our study, 79.2 % of the students were between the ages of 17-20. Studies conducted for various university students have found smoking rates between 25 % and 63.2 % [9, 10]. Given this high rate, there must be a social awakening in the fight against smoking and special quarantine campaigns should be prepared especially for young people, certain age groups and occupations.

All of our participants are smoking. When the dependency levels of the participants are examined, 34% are moderate 29% are low level nicotine addicts.

It is inevitable that if the younger generation of twenty-year-olds continue to smoke, they will have to be highly dependent on their progressive ages. When the distribution of nicotine dependence level of participants and gender were evaluated, it was seen that women were more dependent on the subject. In our study, 58.8 % of the females and 41.2 % of the males were moderate nicotine addicts, but our results were statistically significant. In some studies it has been reported that there may be an increase in cigarette use with an increase in the level of education in women. A study conducted by university students found that 41.3 % of female students and 16.9% of male students started cigarette smoking at first and second year of university [11]. In a study of 41.5 of the overall average age at which smoking levels were assessed in women and men, 38.2 % of the women were found to be nicotine addicted at high rates [12]. This result supports our hypothesis for the future.

Due to the current conditions, it is becoming increasingly common for a majority of people to work day and study night. Under this preference, universities open night programs of many undergraduate and associate degree programs. When we compared the type of learning with nicotine addiction in our study, 52.6 % of the students in the nighttime and 41.2% of the daytime students were moderately addicted. Studies on smoking habit and nicotine addiction were generally compared with the sociodemographic characteristics of the students and the relation between the type of education and dependency ratio was not questioned. This result in our study may explain that students smoke more to keep their mental activities active until late at night. This may be related to the fact that nicotine has a stimulating effect at low doses, while at higher doses it may initially correlate with sedative effects after stimulants [13]. Smoking cigarettes have been shown to improve performance in nicotine-free jobs. In a study conducted, it was reported that nicotine patches were adhered to non-smokers to develop attention and alertness [14].

As a result; In our study of nicotine addiction level using FNBT, there was no significant difference in nicotine addiction level, age, gender and education type. Nicotine addiction rates were higher in females. We think that factors affecting nicotine dependence can be elucidated by questioning parameters such as age and gender as well as the presence of an additional illness (especially depression, anxiety), age of smoking initiation, social status, smoking history in family and close friends.

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