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Effect of Hydroalcoholic Extract of Some Medicinal Plants on Obesity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MR, SCA and FS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FS and SSH managed for data collection. Author AZ managed the literature searches. Authors AZ and NH conducted the analyses of the study part with plant extracts. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Obesity is a significant cause of disability and death. This study was conducted to evaluate the efficacy of some medicinal plants in the treatment of obesity and its complications.
Study Design: Male Wistar rats were treated in different groups.
Place and Duration of Study: Department of Physiology, Arak University of Medical Sciences (Iran), December 2015 to July 2017.
Methodology: The groups were defined as the control, the high-fat diet and the high-fat diet with hydroalcoholic extracts of *Camellia sinensis*, *Rosa canina*, *Althaea officinalis*, *Plantago major* and

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Orlistat in their food, for 48 days. In the serum, the profiles of the liver enzymes and the thyroid hormones were measured as parameters of their appetite.
Results: The lowest increase in appetite happened in the *Camellia sinensis* group and the lowest levels of blood glucose and creatinine were in the *Plantago primary* group. The lowest cholesterol and low-density lipoprotein (LDL) were seen in the *Camellia sinensis* group (P<0.05). The amount of alanine aminotransferase (ALP) and the level of aspartate aminotransferase (AST) in the *Plantago major* group were significantly lower than the positive control group (P<0.05). In *Althaea officinalis* group were higher Orlistat and alkaline phosphatase (ALP) than the control group (P<0.05). The level of thyroid-stimulating hormone (TSH) in control group was significantly lower than the positive control, *Camellia sinensis* and *Rosa canina* groups (P<0.05). The level of thyroxin in *Althaea officinalis*, *Rosa canina* and *Camellia sinensis* groups were significantly lower than the positive control group, respectively (P<0.05).
Conclusion: Hydroalcoholic extracts of *Camellia sinensis*, *Rosa canina*, *Althaea officinalis*, and *Plantago major* are effective in reducing the damages caused by high-fat diet through decreasing lipid profiles, liver enzymes, without causing side effects on thyroid and renal functions.

Keywords: Obesity; Camellia sinensis; Althaea officinalis; Rosa canina; Plantago major.

1. INTRODUCTION

Obesity, as a disorder of one's lifestyle, has become a growing concern, due to industrialisation, increased fast food consumption, and reduced physical activity [1]. In addition to increasing risk of diabetes, heart disease, arthritis and some cancers, the psychological effects of obesity, such as social discrimination, depression, and physical inability, isolate the obese person from society [1].

Obesity is the result of an imbalance between receiving and consuming energy [2]. Therefore, one can reduce body weight either by lowering the power taken or by increasing energy consumption. Many studies have been carried out in this field, and it has been reported that the use of herbal compounds containing polyphenols or flavonoids prevents body weight gain [3]. These compounds can well justify the reducing effects of some plants on blood sugar and lipids, as they prevent biochemical changes in blood [4]. Some medicinal herbs that contain such active compounds are: *Camellia sinensis* (Theaceae), which includes a significant amount of polyphenols including catechins, [5,6]; *Rosa canina (*Rosaceae), which contains phenolic acid, proanthocyanidins, polyphenols, unsaturated fatty acids, phospholipids, minerals, galaldipids, carotenoids and vitamin C [7,8]; *Althaea officinalis* (Malvaideae), containing various natural compounds such as peptin, starch, monosaccharides, dicalcium, flavonoids, antioxidants, coumarins, scopolinotine, tannin, asparagine and many amino acids [9,10]; and *Plantago major* (Plantaginacea) which contains alkaloids, caffeic acid derivatives, flavonoids, and iridoid glycosides [11,12]. Orlistat is a drug used for the treatment of obesity [13]. It reduces lipid absorption by inhibiting pancreatic lipase; however, it has some side effects such as increased blood pressure, headache, dry mouth, insomnia and constipation. Orlistat should be taken together with dietary supplements containing fat-soluble vitamins [14,2].

Considering the above, this study aimed to evaluate the effects of the hydroalcoholic extracts of these plants on preventing the possible damages to liver and kidney tissues in a high-fat diet and to compare these extracts with Orlistat as a standard anti-obesity drug. Also, the amount of food intake, weight changes in serum lipid profiles, thyroid hormones and hypoglycemic enzymes were measured in hypercholesterolemic rats.

2. MATERIALS AND METHODS

2.1 Animals

This experimental study (Code: 842-98-03-1393) was performed on 56 Wistar male rats weighing 220-270 g. They were purchased from Laboratory Animal Breeding Center of Baqiyatallah University of Medical Sciences. All animals were kept in compliance with the instructions for treating laboratory animals approved by the Ministry of Health and Medical Education. Animals were kept under the same environmental conditions, i.e., access to water, food, light (12 h) and temperature (21-23°C).

The rats were randomly divided into 7 groups (n= 8) as follows: The control group, which did not

receive any solvent or drug during the experiment with normal diet. The hypercholesterolemia group with high-fat diet which received 0.2 ml of sodium chloride (normal saline) as gavage during the test period, and five experimental groups which daily received their high fat diet together with the hydroalcoholic extract of the following herbs: group 1: *Camellia sinensis* extract (15.73% *w*/*w*) at a dose of 200 mg/kg [15]; group 2: *Rosa canina* extract (15% w/w) at a dose of 500 mg/kg [16]; group 3: *Althaea officinalis* (12.36% w/w) at a dose of 250 mg/kg [10]; group 4: *Plantago major* (18.6% w/w) at a dose of 500 mg/kg daily [12]; and group 5 which received Orlistat at a dose of 10 mg/kg [17].

The treatment period was 48 days. During this period, the extract and solvent were administrated at 9 o'clock in the morning. Daily dietary intake and weighing of rats were done at the end of the test period. In the end, after mild anaesthesia with ether, blood samples were collected from the hearts of all animals and after centrifugation (at 3000 rpm), the plasmas were isolated and sent to the laboratory to measure the needed factors.

2.2 Preparation of High Cholesterol Food

To prepare high cholesterol diet (2%), 20 g of pure cholesterol (Merck, Germany) and 5g of Cholic acid (FlukaChemika) were dissolved in 10 ml of warmed olive oil and mixed with one kilogram of rat food. To prevent spoilage, we tried to prepare the food daily and, in special circumstances, we kept the food in a refrigerator at 4°C for maximum two days [18].

2.3 Extraction Protocol

The taxonomic rectification was carried out at Arak University Medicinal Herbal Farm (Iran). Extraction was done by percolation method. The aerial parts were dried and powdered. Then, methanol was added and the mixture was allowed to sodden for 72 hours. The extract was collected by rotary condensation machine and desiccated as much as possible. Finally, sterile water was used to rehydrate the hydroalcoholic extracts with the different test groups [19].

2.4 Biochemical Measurement

The kits needed to measure blood glucose; triglyceride (TG), cholesterol, high-density lipoprotein (HDL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were supplied by Pars-Azmoon Co. Thyroid hormones test kits were supplied by PishtazTeb Co. An autoanalyzer from Selectric XL, Netherlands, was used to measure different parameters. Data were analyzed by SPSS software version 16 using one-way ANOVA and Tukey's test. The significance level was considered as $p \leq 0.05$.

3. RESULTS

3.1 Weights

Based on the results of the present study, in all groups, the weight of the rats significantly increased during the study ($P<0.001$). Table 1 shows the average weight gain of rats in different groups. Among the groups that received the hydroalcoholic extracts, the average difference in the weight gained was between 63.43 and 48.14. The highest weight gain was in *Plantago major* group (63.43 ± 11.8). *Althaea officinalis* (42.25 ± 13.1) and *Camellia sinensis (*48 ± 14.6) induced the least amount of weight gain in rats, respectively.

3.2 Appetite

The study of the average appetite was determined by the difference in average appetite at the beginning and end of the study. Table 1 shows that range of the average appetite value (0.1 -5.3) between the groups that received the extract. The highest average appetite was observed in *Althaea officinalis* group (5.3 g) and the lowest in *Camellia sinensis* group (0.1 g).

3.3 Biochemical Results

3.3.1 Creatinine

The effects of the hydroalcoholic extracts and orlistat on the serum creatinine level of the rats with a high-fat diet are shown in Fig. 1. The creatinine level was significantly higher in *Rosa canina* group (0.82±0.047) compared to that in the control group (0.62±0.015) ($P<0.05$). Also, creatinine levels were significantly higher in *Camellia sinensis* group (0.79±0.038) and *Rosa canina* group (0.82±0.047) compared to Orlistat group (0.59±0.024) ($P<0.05$).

Table 1. Comparison of the changes in weight gain and appetite in rats

Groups	Dose	Average weight gain (g)	The average increase in rat appetite (g)
Control	—	74.40±10.8	2.4
Positive control	Normal saline	85.17±23.5	3
Camellia sinensis	(200 mg/kg)	48±14.6	0.1
Rosa canina. L	(250 mg/kg)	53±9.9	3.9
Althaea officinalis	(500 mg/kg)	42.25±13.1	5.3
Plantago major. L	(500 mg/kg)	63.43±11.8	3.6
Orlistat	(10 mg/kg)	62.57±20.3	2.2

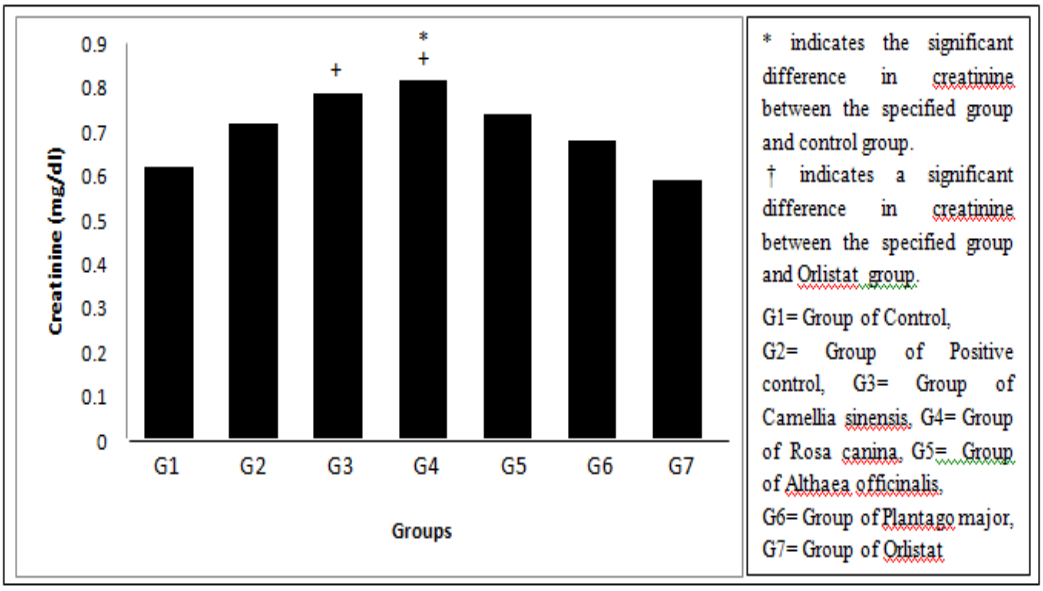

Fig. 1. The average serum creatinine level in different groups

3.4 Lipid Profiles

3.4.1 Cholesterol

Table 2 shows the average serum cholesterol levels in different groups. As paired comparison showed, the cholesterol levels were significantly higher in *Plantago major* (217.28±21.8) and *Althaea officinalis* (178.87±14.3) groups when compared to that in the control group (73±5.7). Also, the level of cholesterol in *Plantago primary* group (217.28±21.8) was significantly higher than that in Orlistat group (92.85±11.1) (P<0.05).

3.4.2 High-density lipoprotein (HDL)

The effects of different treatments on the serum HDL level are presented in Table 2. The highest HDL level belonged to *Althaea officinalis* group (19.5±1.4) and the lowest to *Plantago major L.* (13.86±1.2). According to the results of Kruskal-Wallis test, the tested groups showed significant differences regarding their high-density lipoprotein levels (P <0.05); however, no significant difference was observed in the paired comparison of the groups.

3.4.3 Low-density lipoprotein (LDL)

The effects of *Camellia sinensis, Althaea officinalis, Rosa canina, Plantago major* and orlistat groups on the serum creatinine level were tested for their efficacy against high-fat diet. As shown in Table 2, there were significant differences among different groups in this regard (P<0.001). The comparison of the paired groups showed that the level of low-density lipoprotein was significantly higher in *Plantago major* (125±16.24) and *Althaea officinalis* (101.25±10.17) groups than that in the control group (P<0.05).

Table 2. Serum cholesterol, HDL and LDL level in different groups

Groups	Dose	Cholesterol (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Control	__	73±5.7	18.40±1.5	31.8±2.9
Positive Control	Normal saline	144.5±35.3	16.83±1.8	85±24.28
Camellia sinensis	(200 mg/kg)	166.33±19.1	17±1.3	92.17±16.7
Rosa canina. L	(250 mg/kg)	194.5±46	15±1.2	111±26.7
Althaea officinalis	(500 mg/kg)	178.87±14.3*†	19.5±1.4	101.25±10.17**
Plantago major. L	(500 mg/kg)	217.28±21.8*	13.86±1.2	125±16.24**
Orlistat	(10 mg/kg)	92.85±11.1	14.28±0.9	53.43±6.4

** indicates a significant difference in cholesterol levels between the specified groups and the control group*
† shows a significant difference in the cholesterol level between the specified groups and the Orlistat group.
*** indicates a significant difference in low-density lipoprotein group between the specified groups and the control group*

3.5 Liver Enzymes

3.5.1 Alanine transaminase (ALT)

The paired comparison of groups in Table 3 showed that the amount of ALT in *Plantago major* group was significantly lower than that in the positive control group (P<0.05).

3.5.2 Alkaline phosphatase (ALP)

Table 3 shows serum ALP level in different groups and indicates that the levels of ALP in different groups were significantly varied (P<0.05). The paired comparison of the groups showed that ALP levels in *Althaea officinalis* (90.8±8.9) and positive control (110.97±10.9) groups were significantly higher than that in the control group (44.08±3.9) (P<0.05).

3.5.3 Aspartate aminotransferase (AST)

According to Table 3, the levels of AST in different groups were statistically significant (P<0.01). The paired comparison showed that the levels of AST in the positive control (61.83± 4.4) and Orlistat (75.75±15) groups were significantly higher than that in the control group (29.84± 2.5) (P<0.05).

3.6 Levels of Thyroid Hormones

3.6.1 TSH

Fig. 2 shows the average serum TSH levels of rats in different groups. Based on the results of the present study, the changes were statistically significant (P<0.01). The paired comparison of groups showed that TSH in the control group (0.52±0.07) was significantly lower than those in the positive control (1.17±0.13), *Camellia sinensis* (0.93±0.08) and *Rosa canina* (1.02±0.08) groups (P<0 05).

3.6.2 Thyroxine (T_4)

The effects of different treatments on serum T_4 level and the data obtained are presented in Fig 3. The data showed the levels of T_4 in different groups were statistically significant (P<0.001). The paired comparison of the groups showed that T_4 levels in *Althaea officinalis (64.29±7.7)*, *Rosa canina* (63.84±3.9) groups were significantly lower than that in the positive control group (89.43±2.9), respectively (P<0.05).

Table 3. Serum ALT, ALP and AST levels in different groups

Groups	Dose	Aspartate aminotransferase (U/L)	Alkaline phosphatase (U/L)	Alanine aminotransferase (U/L)
Control	__	29.84± 2.5	44.08±3.9	16.88±1.4
Posirivecontrol	Normal saline	61.83± 4.4*	110.97±10.9**	29.4±1.5
Camellia sinensis	(200 mg/kg)	61.57± 12	89.83±9.3	16.5±3.3
Rosa canina. L	(250 mg/kg)	47.87±7	75.97±8.8	23.77±6.8
Althaea officinalis	(500 mg/kg)	55.82±4.7	90.8±8.9**	17.7±3.1
Plantagomajor. L	(500 mg/kg)	48.83±3.7	85.46±4.2	11.4±1.7†
Orlistat	(10 mg/kg)	75.75±15*	86.54±13.1	24.77±6.1

** indicates a significant difference between the Alanine aminotransferase in the specified group and the control group; ** shows a significant difference in ALP between the specified group and the control group;*
† indicates a significant difference in AST between the specified group and the control group

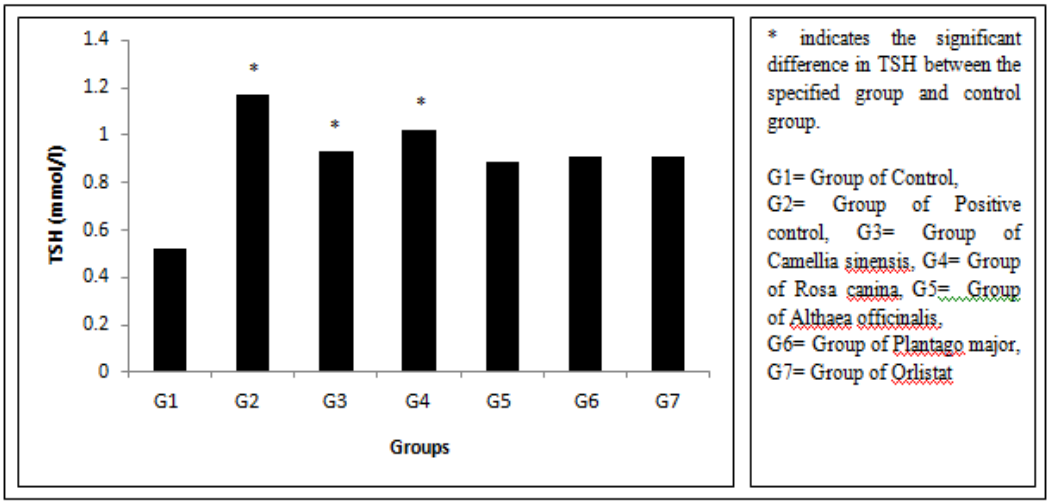

Fig. 2. Comparison of TSH level in different groups

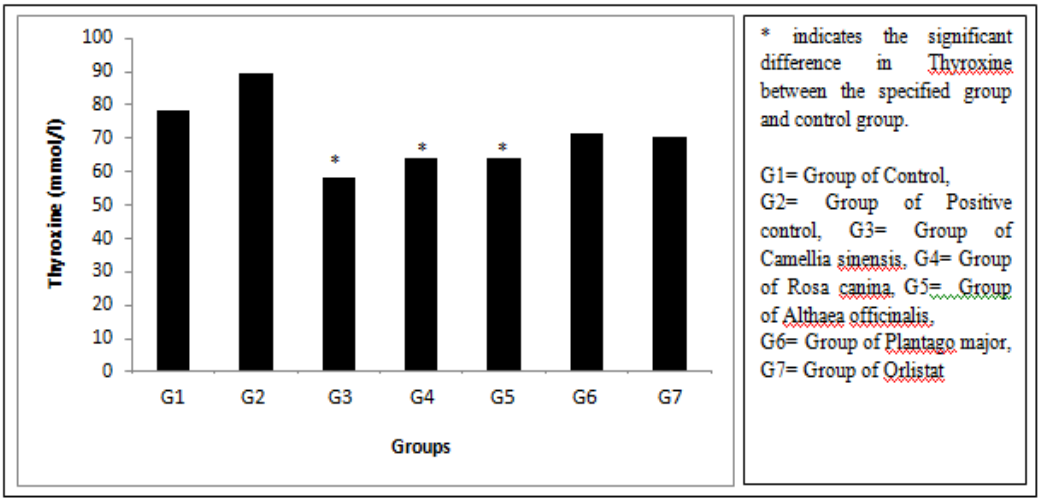

Fig. 3. The average serum thyroxin level in different groups

4. DISCUSSION

Among all the herbs studied in this project, *Camellia sinens* has produced the least appetite increase in rats. Among the groups with plant extracts in their food, the highest creatinine was in *Rosa canina* group and the lowest amount was in *Plantago major* group. Also, the average serum cholesterol and triglyceride levels were respectively seen in *Camellia sinens* and *Althaea officinalis* groups. The highest serum LDL level was in *Plantago major* group and the lowest value was in the control group. Regarding liver enzymes, the lowest reduction in ALT levels was observed in the group with *Plantago major* extract. Similarly, the lowest reduction in ALP happened in *Althaea officinalis* group, AST in *Rosa canina* groups, and T_3 in *Althaea officinalis* group. Among the groups with herbal extracts, on average, the highest T_4 level was in *Plantago major* group and the lowest in *Camellia sinens* group. Besides, TSH was the highest in *Rosa canina* group and the lowest occurred in *Althaea officinalis* group.

Obesity reduces the activity of antioxidant enzymes such as catalase, glutathione peroxidase and glutathione reductase, which results in reduced antioxidant defence, and increased free radicals, followed by increased oxidative stress and decreased the antioxidant capacity of the body [20]. Free radicals destroy

cell membranes, including the hepatocyte cell membrane, and thus result in increased release of ALT, AST and ALP enzymes as the most sensitive indicators of damage to intercellular space of the liver [21,22]. In line with previous studies, body weight gain was seen in hypercholesterolemiac groups, which appears to be normal due to the intake of fatty foods and the high-calorie intake [21]. Previous studies have shown that in a high cholesterol diet, HDL serum levels increase and, as a result, LDL converts to an oxidized form [21,20], which induces cardiovascular disease [23].

In the present study, the serum levels of ALP (110.97±10.9), AST (61.83±4.4) and TSH (1.17±0.13) in rats with high-fat diet significantly increased in comparison with the control group (44.08±3.9, 29.84±2.5, 0.52±0.07, respectively). Since the changes in the serum levels of the above markers in liver steatosis have been already reported, the serum levels of these enzymes were examined in the present study, too [24]. However, anorexia, body weight, regulation of carbohydrate and fatty acids metabolism, and improved fatty liver were observed in the experimental groups receiving the extracts of *Camellia sinensis*, *Althaea officinalis*, *Rosacanina. L* and *Plantago major. L.*

In the present study, the effects of *Camellia sinens* on preventing weight gain, losing appetite, and decreasing ALT (16.5±3.3), AST (61.57±12) and T_4 (58±8.6) were evident in the rats receiving high-fat diet (ALT=29.4±1.5, AST=61.83±4.4, $T_{4=}$89.43±2.9), which is in line with previous studies and probably is caused by the anti-oxidant effects of this plant. EGCG is an active ingredient in *Camellia sinens* which reduces the oxidative stress by decreasing the expression of cytochrome P_{450} and by reducing Malondialdehyde (MDA) production. Also, it increases the antioxidant enzymes of Catalase (CAT) and Glutathione peroxidase (*GPx*). Green tea polyphenols have direct effects on the metabolism of glucose and lipids. They are also able to reduce liver lipid levels through upregulating the pathway for AMP-activated protein kinase (AMPK) [25,5].

Ramadan et al. reported that *Camellia sinens* extract significantly reduced body and liver fats by decreasing hyperglycemia, dyslipidaemia and impairment of liver functions induced by high cholesterol diet [26].

As our data showed, treatment with *Rosa canina* extract in adult rats led to decreased ALT (23.77±6.8), ALP (75.97±8.8), AST (47.87±7), TSH (1.02±0.08) and T_4 (63.84±3.9) compared to the rats receiving high-fat diet (ALT=29.4±1.5, ALP=110.97±10.9, AST=61.83±4.4, TSH=1.17± 0.13, $T_{4=}$89.43±2.9). In line with the present study, Tagighzadeh et al. showed that ALT and AST enzymes reduced in rats treated with *Rosa canina* extract. The changes in the levels of these enzymes can be attributed to the effective compounds of this plant, including phenolic essential oils, parantoicinidine, polyphenols, lipids, carotenoids, and vitamin C and Polyphenols, such as lanolin and tannins, have antioxidant, anti-inflammatory and hypolipidemic effects. Quercetin, as a flavonoid, increases liver glucose and inhibits intestinal glucose absorption. In addition, treatment with quercetin significantly increases antioxidant activity and, as a result, it reduces the oxidative stress [27,7]. On the other hand, Sadigh-Eteghad et al. showed that the antioxidative effects of *Rosa canina* led to a reduction in serum superoxide anion liberation [16].

Our data revealed a decrease in serum level of ALT (17.7±3.1), ALP (90.8±8.9), AST (55.82±4.7), TSH (0.89±0.08) and T_4 (64.29±7.7) following the treatment with *Althaea officinalis* compared to the rats receiving high-fat diet (ALT=29.4±1.5, ALP=110.97±10.9, AST=61.83± 4.4, TSH=1.17±0.13, $T_{4=}$89.43±2.9). The changes in the *Althaea officinalis* group can be attributed to the high concentrations of the plant's chemical compounds, including phenolic compounds, glycosides, alkaloids, glycans, triple panes, vitamins, polysaccharides, peptides, amino acids and proteins. These compounds reduce blood glucose levels [27,28]. In addition, anthocyanins are among the compounds present in this plant [29] which delay the conversion of di-saccharide to monosaccharide by inhibiting the alpha-glucosidase enzyme which finally results in reduced blood glucose [30]. In line with the present study, Boney and his colleagues have shown that the extract of the plant reduces plasma triglycerides due to the presence of regulates.

Phytosterols also lower blood cholesterol by disturbing intestinal cholesterol absorption. In addition, the carvacrol, thymol, linalool, beta-bisabolin and trpinenes present in *Althaea officinalis* extract have several therapeutic properties, such as antioxidant activity, which can improve the function of cell membranes in

various tissues. Therefore, it prevents the leakage of intracellular enzymes to the blood; thereby, it reduces AST, ALT, and LDH in plasma [7].

We also demonstrated that there were no significant differences in serum Cholesterol, LDL and HDL levels in the *Althaea officinalis group compared to the control which* was in agreement with the study done by Hage-Sleiman et al. [31]. We showed that *Plantago major* reduced ALT (11.4±1.7), ALP (85.46±4.2), AST (48.83±3.7) and TSH (0.91±0.06) compared to high-fat diet group (ALT=29.4±1.5, ALP=110.97±10.9, AST=61.83±4.4, TSH=1.17±0.13). Hussain et al. have shown that *Plantago major* could inhibit inflammation by reducing cytokines such as IL-1 and TNF-α, and thereby decreasing ALT and AST. It is worth mentioning that *Plantago major* possess numerous bioactive compounds such as flavonoids, terpenoids, pectin, iridoid glycosides, and tannins which express anti-inflammatory and antioxidant activities [30]. Moreover, the extract of this plant reduces glucose uptake and thus it reduces blood glucose levels. On the other hand, the hypocholesterolemic effect of *Plantago major* is attributed to arbioxylans [32]. Tang et al. [33] have reported that arabinoxylans, with the elimination of lipids, also regulated the activity of 3-hydroxy-3-methyl-glyadar coenzyme A (HMG-CoA) reductase and 7-α-hydroxylase cholesterol and increased the fatty acid in the intestine, and reduced cholesterol and LDL.

Regarding the mechanism by which medicinal plants affect obesity, they seem to act by stimulating the thermogenesis, reducing lipogenesis, increasing lipolysis, inhibiting appetite, and reducing the absorption of lipids. These plants are also able to control renal dysfunctions, to improve liver function and to treat fatty blood by reducing liver enzymes and kidney damage.

On the other hand, in this study, no significant statistical differences in urea levels were seen in different groups. This was probably because hyperlipidemia did not have a rapid effect on renal function, though the level of creatinine showed a statistically significant difference among the groups. These results indicate that, in the event of renal impairment, the extracts of these plants are, to some extent, able to correct it.

The effectiveness of medicinal plants with anti-obesity potential in the hypercholesterolemic model and evaluation of several parameters such as appetite, lipid profiles, liver enzymes and thyroid hormones are the advantages of this research. However, we suffered the lack of information related to the analysis of extracts and we were not able to study the coexisting effects of the main ingredient in this model.

5. CONCLUSION

Hypercholesterolemia and overweight are chronic, epidemic and global diseases. The approved synthetic drugs for treating obesity have low efficacy and many side effects. Therefore, it is essential to do research to identify and replace effective medicines.

This study gives insights into the role of some medicinal plants in the treatment of obesity and its complications and concludes that in rats receiving high-fat diet, the hydroalcoholic extract of *Camellia sinensis* group has a significant effect on weight loss and appetite, as well as on LDL and cholesterol reduction. Also, the extract of *Plantago primar*y group is effective in reducing creatinine and LDL. However, further studies are suggested for developing effective complementary medicines for the treatment of obesity.

Phytochemical studies indicated the prevalence of phenolic compounds in the medicinal plants used in the present study. Research has shown that phenolic compounds, like secondary metabolites, are the most effective in the treatment of obesity by medicinal plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), as well as specific national laws were followed where applicable. All experiments have been examined and approved by the appropriate ethics committee”.

The present study was carried out with the approved ethics code of 1842-98-03-1393 (Tehran University of Medical Sciences).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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