

The effects of different levels of peppermint alcoholic extract on body-weight gain and blood biochemical parameters of adult male Wistar ratsBehzad Mesbahzadeh¹, Mohsen Akbari², Nasroallah Moradi kor³, Jalal Bayati Zadeh⁴¹ Atherosclerosis and Coronary Artery Research Center, Department of Physiology, Birjand University of Medical Sciences, Birjand, Iran² Department of Animal Sciences, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran³ Research Centre of Physiology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran⁴ Department of Animal Sciences, Faculty of Agriculture, Shahid Bahonar University, Kerman, Iran**Type of article:** Original**Abstract****Introduction:** Peppermint is an efficient medicinal plant for the treatment of diseases, and it also can be used to produce raw materials in the pharmaceutical industry. The purpose of the current study was to evaluate the effects of various levels of peppermint alcoholic extract on body-weight gain and blood biochemical parameters in adult male Wistar rats.**Methods:** This experiment was conducted using a completely randomized design (CRD). Fifty adult, healthy, male Wistar rats (ages of 2.5–3 months; weights of 190–210 g) were allocated randomly into five groups. T1 was the control group in which the rats received 0.3 ml of distilled water). Groups T2, T3, T4, and T5 received 75, 150, 300, and 600 mg/kg of peppermint extract, respectively. The rats received daily pretreatment by oral gavages for 21 days. We recorded body weights at the beginning and at the end of the study to determine the changes in the body weights. Blood samples were collected for the measurement of glucose, cholesterol, triglycerides, HDL, LDL, albumin, globulin, and total protein. Statistical analysis of the data was done by SAS software. The data statistically analyzed using one-way analysis of variance (ANOVA), which was conducted through Dennett's multiple comparison post-test.**Results:** The results indicated that the rats treated with peppermint gained more weight ($p < 0.05$) and also decreased the serum concentrations of triglycerides, total cholesterol, LDL, and glucose in T3, T4 and T5 than the other groups ($p < 0.05$).**Conclusion:** Peppermint extract had a positive effect on body-weight gain and some blood parameters in adult male Wistar rats. The findings showed that peppermint is a crucial substance at high temperature, and future research should be focused on determining the details of the mechanisms involved in producing the observed effects of peppermint extract.**Keywords:** high temperature, lipid profile, peppermint, serum glucose, Wistar rats**1. Introduction**

Stress is described as any combination of environmental conditions (such as temperature, relative humidity, immobility, and solar radiation) that will cause the temperature of the environment to be higher than the temperature range of the animal's temperature zone/thermal neutral zone. Temperatures above the thermal neutral zone initiate physiological, anatomical, and behavioral responses the aim of which are to increase heat loss and decrease heat production in trying to maintain the body's temperature within its normal range. Homeostasis is influenced by intrinsic and extrinsic stressors (1). Stress is a main factor in depression (2). Episodes of restraint stress for two hours caused great loss of willingness in rats to eat (3).

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There is evidence that shows decreased weight gain in broiler chicks that were raised under heat stress conditions (4). Immobility stress has been shown to have negative effects on some blood parameters in mice (5). Peppermint (*Mentha piperita*) is a medicinal plant of the *Labiatae* family, and it possibly originated in Eastern Asia. Peppermint has been used extensively in herbal medicines, and it's believed that peppermint is efficient in the immune system and in fighting secondary infections (6). Peppermint has biological activities, such as anti-bacterial, anti-fungal, and anti-oxidant properties (6). It has been reported that mint genera have adverse effects on induction of oxidative stress (7). The dietary inclusion of peppermint oil had beneficial effects on some blood biochemical parameters of mice under immobility stress (5, 8). Thus, the current study was conducted to determine the effect of different levels of peppermint extract on body weight and the blood's biochemical parameters in adult male Wistar rats.

2. Material and Methods

2.1. Collection and Preparation of Hydroalcoholic Extract

To prepare the peppermint extract, we collected fresh leaves of peppermint in Jiroft City, Kerman, Iran. The plants were kept by the Department of Pharmacy at Shiraz University of Medical Sciences. The peppermint was dried at 45 °C for five days, and, then, it was ground into tiny particles of powder and homogenized in 96% ethanol at a ratio of 1 part peppermint plant to 10 parts of ethanol. The mixture was left to saturate for four days at 25 °C by occasional shaking and stirring. Then, the mixture was filtered through filter paper, and the filtrate was intensified at low pressure and 45 °C to prepare a dark, gummy, green extract. Then, we dissolved the extract in Tween 20 (10%, w/v).

2.2. Location and Animals

This study was performed at Islamic Azad University, Arsanjan Branch, located in Shiraz Province, Following the preparation of the peppermint extract, 50 adult, healthy, male Wistar rats (aged in 2.5–3 months, weighing 190–210 g) were obtained from the Animal House Unit for this experiment. The Wistar rats were raised in wire-bottom cages at 22 ± 2 °C and 55–65% relative humidity. We initiated a 12-hr light-dark cycle at least a week before the study began, and the rats were maintained under standard housing conditions with free access to a standard diet and water ad libitum. We recorded their body weights at the beginning and end of the study to determine the changes in body weight that occurred during the tests. The animals were allocated randomly into five groups: T1 (control group, received 0.3 ml distilled water), T2, T3, T4 and T5 received peppermint extract 75, 150, 300 and 600 mg/kg respectively. Rats received daily pretreatment by oral gavages for 21 days.

2.3. Biochemical analysis

The animals were anesthetized with diethyl ether 24 h after the last treatment. Blood samples were collected and centrifuged (at 2000 g for 10 min); serum was obtained for the measurement of glucose, cholesterol, triglycerides, HDL, LDL, albumin, globulin, and total protein by spectrophotometer (Shimadzu UV-1700) using a Pars Azmoon commercial kit package (Pars Azmoon, Co., Tehran, Iran). We used standard commercial kits for analysis as recommended by the manufacturer of these kits.

2.4. Statistical analysis

Statistical analyses of the data were done with SAS software (version SAS 9.1.3.). The data were statistically analyzed with one-way analysis of variance (ANOVA) done through Dennett's multiple comparison post-test. The five independent groups were compared using the t-test. The results were presented as mean \pm standard error mean. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

Table 1 presents the effects of the peppermint extract on body weight. The results indicated that rats treated with peppermint produced higher body weights ($p < 0.05$) than the control group. There were significant differences among the experimental groups; rats that received 300 and 600 mg/kg of peppermint extract produced greater gains in body weight than the other groups (235.23 g and 230.17 g vs. 226.05 g or 224.35g and 319.81g). However, the rats that were treated with peppermint in groups T2 and T3 had the greatest gains in body weight compared to the control group. Tables 2 and 3 show the effects of the peppermint extract on the blood parameters of Wistar rats during high temperature. The results showed that the different levels of peppermint extract did not have any significant effects on serum HDL, total protein, globulin, or albumin ($p > 0.05$). The serum contents of cholesterol, triglycerides, LDL, and glucose were significantly lower in the rats in groups T3, T4, and T5 than in the control group ($p < 0.05$). The peppermint extract at high levels had increased effects on the blood parameters (T4 and T5 vs. T3).

Table 1. Gains in body weight by the rats in the different groups

Design of treatment	Weight gain (g/21 days)
Control (received 0.3 ml of distilled water/21 days)	219.281 ± 1.83 ^a
T2 (150 mg/kg peppermint extract)	224.35 ± 0.98 ^b
T3 (150 mg/kg PE)	226.05 ± 1.07 ^b
T4 (300 mg/kg PE)	230.17 ± 1.87 ^c
T5 (600 mg/kg PE)	235.23 ± 1.13 ^c

Values with different superscripts in the same column differ significantly (P<0.05). Values are expressed as mean ± SE

Table 2. Effects of peppermint extract on blood parameters (µg/dl) of the rats in different groups

Groups	Triglycerides	Cholesterol	HDL	LDL
T1 (C)	245.17 ± 1.66 ^a	203.65 ± 1.02 ^a	55.03 ± 1.09	78.43 ± 1.02 ^a
T2	240.22 ± 1.05 ^a	195.52 ± 0.91 ^a	49.12 ± 1.23	75.19 ± 0.85 ^a
T3	229.05 ± 1.13 ^b	179.08 ± 1.08 ^b	58.28 ± 0.93	63.38 ± 0.96 ^b
T4	218.41 ± 1.03 ^c	165.37 ± 1.12 ^c	57.67 ± 1.08	54.17 ± 1.15 ^c
T5	198.02 ± 0.86 ^d	149.14 ± 1.22 ^d	54.53 ± 1.02	51.03 ± 1.02 ^c
SEM	0.728	0.638	0.831	0.669
p-values	0.033	0.029	0.148	0.023

Values with different superscripts in the same column differ significantly (P<0.05). Values are expressed as mean ± SE

Table 3. Effects of peppermint extract on other blood parameters (µg/dl) of the rats in different groups

Groups	Glucose	Total Protein	Globulin	Albumin
T1 (C)	289.53 ± 1.23 ^a	23.25 ± 0.89	19.33 ± 1.62	10.92 ± 0.83
T2	285.17 ± 1.45 ^a	24.38 ± 1.32	18.56 ± 1.27	11.01 ± 0.83
T3	293.62 ± 1.29 ^b	24.55 ± 1.09	17.44 ± 1.39	10.22 ± 0.59
T4	299.51 ± 1.28 ^c	25.07 ± 1.48	19.17 ± 1.05	12.17 ± 1.29
T5	307.13 ± 1.36 ^d	24.13 ± 1.28	20.09 ± 1.33	12.03 ± 1.19
SEM	0.728	0.833	0.693	0.754
p-values	0.035	0.359	0.428	0.331

Values with different superscripts in the same column differ significantly (P<0.05). Values are expressed as mean ± SE

4. Discussion

As the results indicated, the peppermint extract increased the body weight gain in Wistar rats during exposure to high temperature. As mentioned before, stress had negative impacts on appetite and reduced appetite may decrease gains in body weight (3). Unfortunately, we could find no study in the literature that showed the effect of peppermint extract on body weight in rats reared under heat stress condition. In similar study, Akbari and Torki showed that adding peppermint oil had no significant effects on body weight gain in broiler chicks reared under heat stress condition (8). Parallel to our findings, they reported that supplementing their diets with peppermint powder at levels of 4 gm/kg increased body weight gain in broiler chicks (9). There is evidence that shows that adding herbal plants stimulates appetite, the secretion of gastrointestinal fluids, and improves digestion and absorption, thereby producing gains in body weight (10). There is evidence that shows that high temperature can break the homeostasis of cecal microflora and cause damage to the intestinal mucosa and immune function in animals; however, extracts also reduce potentially pathogenic bacteria and cause a shift in the composition of the gut's microflora towards more beneficial bacteria, making it possible to increase body weight (11). This increase may be due partly to the menthol activity of peppermint, because menthol is an appetite-enhancing substance.

In this study, peppermint extract did not have a significant impact on the serum content of HDL, total protein, globulin, or albumin. The peppermint supplements had no significant effect on the serum content of HDL in broiler chicks (9). Peppermint essential oil and chromium picolinate improved the serum content of albumin in heat-stressed broiler chicks (8). The absence of concurrence among these studies may be a result of the levels at which the extract was administered. In addition, other variables, such as differences in background of the selected animals,

the types of animals or genera and age, and the severity of the stress may have affected the efficacy of extract usage, and therefore it was difficult to directly assess different studies that used extracts. In this study, administering the peppermint extract at high levels decreased the serum content of LDL, triglycerides, and cholesterol in Wistar rats that were kept at high temperature. It seemed that peppermint had anti-lipidemic benefits, but it cannot show this benefit at low levels. Parallel to our findings, Johari et al. showed that peppermint extract decreased the serum contents of LDL, cholesterol, and triglycerides in Wistar rats compared with the control group (12). In one human study, peppermint decreased the contents of serum LDL, cholesterol, and triglycerides in university students (13). The decrease of total cholesterol, LDL cholesterol, and total triglycerides of the treatment group might have been related to the involvement of the proposed components in the antioxidant and excess cholesterol protection mechanisms. Heat stress can induce the synthesis of free radicals, which can damage cell membranes by lipid peroxidation of polyunsaturated fatty acids in the cell membrane, thereby destroying the integrity of the membrane (14). Seem antioxidants (peppermint) decrease lipid peroxidation in the plasma and tissues. The idea was confirmed by Young et al. who showed that herbs and spices, along with vitamins C and E (anti-oxidant vitamins) were even more effective at preventing lipid peroxidation in the tissues of birds (15). There was a positive correlation between the total phenol concentration of herbal plants and human low-density lipoprotein oxidation in vitro (16). Also, Marjani et al. reported a decrease in the serum content of malondialdehyde (end product of lipid oxidation) in mice reared under immobility stress when fed a diet supplemented by peppermint oil (from 0.90 to 60 mg/kg) (5). We believe that the decrease in the serum content of cholesterol may be a result of inhibition of HMG-COA reductive activity, which is a key regulatory enzyme in cholesterol synthesis.

In this study, rats supplemented with peppermint at high levels produced lower serum concentrations of glucose. Peppermint reduced the serum concentration of glucose in Wistar albino rats compared with control group (17). Peppermint and chromium picolinate decreased serum glucose in heat-stressed broiler chicks (8). The antioxidant roles found in peppermint tea may be responsible for hypoglycemic effects (18). There is a significant correlation between the serum content of glucose and the levels of lipids. We believe peppermint extract may influence the insulin-sensitive cell receptors or binding activity. It is well-known that insulin reduces lipolysis in adipocytes and prevents gluconeogenesis, thereby decreasing the serum contents of triglycerides and glucose. This idea was confirmed by Xie et al. who showed that cinnamaldehyde (the active component of cinnamon essential oil) increased the release of insulin (19).

5. Conclusions

Supplementation with peppermint extract was effective in improving body weight gain compared with the control group. Treatment by peppermint extract did not improve the serum content of HDL, total protein, globulin, or albumin. The results also suggested that supplementation with peppermint extract can reduce the serum concentrations of cholesterol, triglycerides, LDL and glucose triglycerides, and glucose. This study showed, for the first time, that peppermint extract is able to reduce the serum lipids or glucose and increase the body weight of Wistar rats kept at high temperatures, thereby helping to protect the rats against the deleterious consequences of lipoperoxidation and potentially ensuring antioxidant potential.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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