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Article *in* Methods and Findings in Experimental and Clinical Pharmacology · September 2010

DOI: 10.1358/mf.2010.32.7.1487085 · Source: PubMed

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ORIGINAL ARTICLE

THE EFFECTS OF *CANNABIS SATIVA* L. SEED (HEMPSEED) IN THE OVARIECTOMIZED RAT MODEL OF MENOPAUSE

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SUMMARY

Cannabis sativa L. has been used for the treatment of various gynecological diseases in traditional medicine. The potential of this plant to protect against complications of menopause has been raised but rarely studied. Twenty female rats were divided into five groups: sham-operated (sham), ovariectomized (OVX) and three other ovariectomized groups: HST1%, HST2% and HST10% which received 1%, 2% and 10% hempseed, respectively, in their diet for 3 weeks. The effects of hempseed on plasma lipid and lipoprotein profiles, estradiol and calcium levels were evaluated. Rats were tested for behavioral changes using the forced swimming test. The results showed that ovariectomy, independent of the type of diet, caused elevation of plasma calcium, total cholesterol and HDL-cholesterol levels, while hempseed modified this effect. Plasma estradiol levels were significantly lower in the OVX group compared to other groups. The swimming times for the OVX and sham groups were significantly shorter than that of the HSD10% group. All hempseed-treated groups were less anxious and showed significant declines in fecal boli compared to the sham group. The exploratory diving percent decreased in the HST10% group compared with other groups. These results suggest that hempseed may improve post-ovariectomy complications in rats.

INTRODUCTION

Biological menopause, whether natural or surgically induced, is associated with elevated concentrations of circulating total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), placing postmenopausal women at greater risk for coronary heart disease (CHD) (1, 2). Estrogen replacement therapy (ERT) in postmenopausal women reduces the risk of CHD, in part by modulating plasma cholesterol levels. However, because of the side effects of ERT and cholesterol-lowering pharmacological agents, patients often seek alternative treatments (2-4).

In Iranian traditional medicine, *Cannabis sativa* L. (Shadanaj and/or Shahdaneh in Persian) has been used for various ailments alone or as part of herbal formulations. Herbal formulations utilizing *C. sativa* L. as the main ingredient have been prescribed for headaches, insomnia, depression, agoraphobia, uterine hemorrhage and gastroenteritis. The historical and traditional use of *C. sativa* L. in obstetrics and gynecology has been reviewed (5).

Hempseed is a rich source of phytochemicals, including terpenes, such as cannabinoids, phytoestrogens and n-3- or n-6-polyunsaturated fatty acids. Hempseed oil contains 100-148 g/L of the phytoestrogen β-sitosterol (β-Sit), which has been shown to reduce hypercholesterolemia (6). Since Δ^9-tetrahydrocannabinol (Δ^9-THC) was first identified as marijuana's primary psychoactive ingredient (7) great strides have been made in understanding its actions on human physiology. Δ^9-THC and its congeners, known as cannabinoids, exert their effects by acting at the cannabinoid CB_1 and CB_2 receptors that are distributed unequally in all tissues (8). The concentration of THC in hempseed depends on the type of plant (fiber or drug hemp), as well as the degree of contamination when harvested (9). THC concentrations were found to be < 2 mg/kg in drug hemp (marijuana) and < 0.5 mg/kg in fiber hemp (9, 10). Therefore, the source and purity of the seeds are the most important factors determining the concentration of THC in the seeds.

We previously found that THC-free hempseed reduced plasma cholesterol levels in rats (11). Numerous studies suggest that phytoestrogens increase levels of high-density lipoprotein cholesterol (HDL-C) while lowering levels of LDL-C in a manner similar to estradiol (3). The primary aim of the present study was to investigate whether hempseed prevents ovarian hormone deficiency-induced hypercholesterolemia in ovariectomized rats as a model of menopause (2).

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In women, reduced estrogen levels at menopause are associated with depression, sleep disturbances, irritability, anxiety, panic disorders and cognitive dysfunction (12). Estrogen receptors, which are distributed ubiquitously in many regions of the central nervous system, are implicated in the modulation of neurobiological systems (13). Therefore, all preventive and therapeutic strategies that help premenopausal women to conserve estrogen levels and thus affect estrogen receptor (ER) activity in the physiological range may delay cardiovascular and bone loss problems, as well as improve mood and feelings of general well-being during this climacteric period of life. Accordingly, the second objective of this study was to evaluate the effect of hempseed on complications of estrogen deficiency such as depression, anxiety and possibly impaired cognition in the ovariectomized rat model of menopause.

MATERIALS AND METHODS

Analysis of dietary fatty acids

Lipids were extracted with ethyl ether in a straight-through extractor. The isolated triglycerides were then transesterified to the corresponding methyl esters using potassium hydroxide in methanol. Fatty acid methyl ester composition was then determined using gas chromatography (Hewlett-Packard HP6890) (14).

Animals and diets

The present study was approved by the Laboratory Animal Care Committee of Urmia University, Western Azerbaijan, Iran. Six-month-old breeder female Wistar rats with regular 4-day estrus cycles were used in this experiment. Rats were housed in individual cages with polyethylene bottoms in a room maintained at 23 ± 1 °C with a 12-h light/dark cycle (light, 07:00 to 19:00 h). Rats were acclimatized for 7 days by feeding on a commercial solid diet (Niro–Sahand Diet Co., Tabriz, Iran). After acclimatization, a bilateral ovariectomy was performed under anesthesia by intraperitoneal (i.p.) injection of a ketamine/xylazine cocktail. Rats were fed a commercial solid diet during the 7-day recovery period and were then divided into five dietary groups (n = 5 in each group) on the basis of body weight: sham-operated (sham), ovariectomized (OVX) and three other ovariectomized groups, HST1%, HST2% and HST10%, which received 1%, 2% and 10% hempseed, respectively, in their diet for 3 weeks. The sham (non-ovarectiomized) and OVX groups received a standard commercial diet (Niro–Sahand Diet Co., Tabriz, Iran), while HST1%, HST2%, and HST10% groups received 1%, 2% and 10% whole hempseed plus 99%, 98% and 90% standard commercial diet, respectively. The compositions of the experimental diets are shown in Table I. The fatty acid composition and lipid characteristics of hempseed and commercial food pellets are shown in Table II. Diets were freshly prepared each week and stored at 4 °C until required. All rats were given free access to food and water over 21 days. Body weight was measured on the first and last day of the study.

Forced swimming test (FST)

Rats were initially exposed for 15 min to the swimming apparatus (individual glass cylinders, 20 cm diameter, 46 cm height, 30 cm water depth and 23-25 °C water temperature) 24 h prior to their test session (15). Swimming sessions were conducted 16 days after the dietary change. Each rat was forced to swim for 5 min and then removed from the cylinder, dried with paper towels and placed in heated cages for 30 min, before returning to their home cages. The tests were run between 19:00 and 22:00 h. A single observer who was blind to the treatment conditions did all of the behavioral scoring. The cumulative fecal boli output was recorded during the FST as a measure of stress.

Table I. *Macronutrient content of sources of diet.*

Diet	Energy (Kcal/kg)	Protein (%)	Carbohydrate (%)	Fat (%)	Fiber (%)
Standard chow	2600	23.0	62.0	10.0	5.0
Hempseed	5030	22.5	35.8	22.9	35.0

Table II. *Lipid characteristics of sources of diet.*

Source	Oil (%)	SFAs (%)	UFAs (%)	PUFA (%)	MUFA (%)	n6/n3
Standard chow	10.0	79	21	1.2	11.1	0.00
Hempseed	22.9	13	87	64.2	20.9	2.82

SFAs, saturated fatty acids; UFAs, unsaturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

Behavioral scoring

A time-sampling technique was employed to score three different behaviors during FST. The following behaviors were rated 2 min after the beginning of the test: 1) immobility– floating in the water without struggling, and doing only necessary movements to keep the head above the water; 2) swimming– showing moderately active motions around in the cylinder, more than necessary to merely keep the head above water; and 3) diving– presenting active vigorous exploratory movements with complete head and body dipping, usually directed against the bottom of the cylinders. The exploratory diving percent was considered a cognitive behavior.

Blood collection

On the last day of the experiment (day 21), after a 14- to 16-h fast, anesthesia was administered by an i.p. ketamine/xylazine injection and a blood sample was collected into a collection tube containing heparin via cardiac puncture. Plasma was separated by centrifugation at 1400 × g at 4 °C for 15 min, and stored at –20 °C until analysis. After blood collection, the rats were decapitated and the abdominal fat was removed, washed with cold saline, blotted dry on filter paper and weighed.

Chemical analysis

Plasma concentrations of TC, LDL-C and triglycerides (TGs) were enzymatically determined with commercial diagnostic kits (ELITech Diagnostic, France). Plasma HDL-C levels were determined by an immunoinhibition method (ELITech Diagnostic, France). Calcium

concentrations were measured by atomic absorption spectrophotometry (Spectrophotometer 400; Perkin-Elmer Cetus, Norwalk, USA). Samples were previously diluted with lanthanum oxide solution (1 g/L; Carlo Erba Reagenti, Val de Reuil, France). Plasma 17β-estradiol (E2) was measured by radioimmunoassay using an Immuchen kit (ICN Pharmaceuticals, Inc., Costa Mesa, USA). The assay sensitivity was 10 pg/mL, and the intra-assay coefficient of variation was 12.26%.

Statistics

Comparisons between group means were made using one-way analysis of variance (ANOVA). When an effect was statistically significant ($P < 0.05$), mean comparisons were done by post hoc comparisons with a Tukey HSD multiple comparison test. Results are expressed as mean ± SEM. The analysis was carried out using SPSS version 16 (Chicago, USA).

RESULTS

The extracted hempseed oil (22.94%) contained considerable amounts of linoleic acid (46.99%), oleic acid (19.90%), α-linolenic acid (16.66%) and palmitic acid (8.25%), while stearic acid (4.46%), eicosenoic acid (0.53%) and arachidic acid (0.08%) were low. The distribution of saturated and unsaturated fatty acids was 13% and 87%, respectively. The standard commercial pellet contained substantial amounts of palmitic acid (51.56%) and stearic acid (20.01%) and very low amounts of other fatty acids. The lipid characteristics of the source of diets are shown in Table II.

Throughout the experimental period, the mean body weight of the rats in the HST1% and HST2% groups decreased, while that of rats in the sham and OVX groups increased. The mean body weight of rats in the HTS10% group remained approximately constant (Table III). The abdominal fat mass of the OVX group and rats treated with hempseed was similar to sham rats, but the differences between rats treated with hempseed and the OVX group were significant ($P < 0.05$). Hempseed-treated rats had similar food intake, regardless of the amount of dietary hempseed (data not shown).

To evaluate effects on lipid profile induced by hempseed treatment, plasma concentrations of TGs, TC, HDL-C and LDL-C were determined (Table IV). No significant differences were observed for TGs and LDL-C among experimental groups. Plasma TC levels were significantly lower in the sham and HSD2% groups than in the OVX, HSD1% and HSD10% groups. HDL-C levels were significantly lower in the sham and HSD2% groups when compared to those of the OVX, HSD1% and HSD10% groups. Although the plasma calcium concentrations were significantly higher in the OVX group than those of the sham group, there was no significant difference in calcium concentrations for the other experimental groups (Table IV). Plasma E2 levels of the OVX group decreased to one-third of those of the sham group ($P < 0.05$; Table IV), but plasma levels of E2 in the hempseed-treated groups were restored to similar levels obtained for the sham group ($P > 0.05$; Table IV). The swimming times for the OVX and sham groups were significantly shorter than those animals that had been treated with a hempseed-supplemented diet (Table V). The HST10% group had by far the longest swim times of all the test animals. Fecal boli numbers decreased ($P > 0.05$) in the OVX group but were not significantly different than the sham group, while hempseed-treated groups exhibited lower fecal boli numbers than the OVX group ($P > 0.05$). All hempseed-treated groups showed a significant decline in fecal boli numbers when compared to the sham group. The exploratory diving percent decreased significantly in the HST10% group, but it decreased moderately in the OVX group relative to the sham group ($P > 0.05$). Furthermore, the exploratory

Table III. *Body weight and abdominal fat mass during the experiment.*

Treatment group (n = 5)	Initial body weight (g)	Final body weight (g)	Abdominal fat mass *(g)
Sham	211.80 ± 9.73	215.8 ± 16.05	7.94^{ab} ± 3.46
OVX	256.6 ± 51.07	264.2 ± 49.71	4.51^{b} ± 0.28
HST1%	256.2 ± 19.17	250.4 ± 22.22	6.44^{a} ± 0.09
HST2%	254.0 ± 21.13	242.8 ± 21.37	6.37^{a} ± 1.75
HST10%	265.8 ± 36.41	266.2 ± 32.69	10.25^{a} ± 0.35

Data are mean ± SEM. OVX, ovariectomy; HST, ovariectomized rats treated with 1% (HST1%), 2% (HST2%) and 10% (HST10%) of hempseed, respectively. *Values with various superscripts are significantly ($P < 0.05$) different in the column.

Table IV. *Effect of hempseed on serum parameters of ovariectomized rats on the last day of the experiment.*

	Group (n = 5)				
	Sham	OVX	HST1%	HST2%	HST10%
TGs (mg/dL)	34.6 ± 5.68[a]	30.8 ± 5.89[a]	33.2 ± 6.53[a]	32.2 ± 9.36[a]	31.4 ± 14.58[a]
TC (mg/dL)	72.2 ± 5.40[a]	97.2 ± 7.01[b]	87.0 ± 8.86[cb]	79.8 ± 0.67[eadc]	87.0 ± 9.13[db]
HDL-C (mg/dL)	56.6 ± 8.79[a]	85.6 ± 5.17[b]	74.0 ± 7.44[bc]	62.8 ± 7.46[ac]	76 ± 8.74[bc]
LDL-C (mg/dL)	12.0 ± 4.30[a]	13.6 ± 2.79[a]	11.6 ± 1.67[a]	11.4 ± 3.20[a]	10.6 ± 1.51[a]
Calcium (mg/dL)	9.21 ± 0.50[a]	9.87 ± 0.17[b]	9.61 ± 0.20[ab]	9.75 ± 0.17[ab]	9.36 ± 0.34[ab]
17β-Estradiol (pg/dL)	7.10 ± 0.45[a]	2.13 ± 0.39[b]	6.88 ± 0.37[a]	7.02 ± 0.52[a]	6.67 ± 0.45[a]

Data are mean ± SEM. Values with various superscripts are significantly ($P < 0.05$) different in the row.

Table V. *Effect of hempseed on behavioral categories of ovariectomized rats.*

	Group (n = 5)				
	Sham	OVX	HST1%	HST2%	HST10%
Swimming time (s)	78.8 ± 25.06[a]	63.2 ± 29.57[ac]	92.8 ± 38.09[acb]	93.0 ± 27.59[acb]	137.80 ± 22.70[b]
Fecal boli no.	7.4 ± 3.57[a]	5.8 ± 2.48[ab]	3.8 ± 0.83[b]	3.6 ± 3.28[b]	4.0 ± 3.67[b]
Exploratory diving (%)	0.8 ± 0.27[a]	0.6 ± 0.17[b]	0.8 ± 0.34[a]	0.8 ± 0.40[a]	0.2 ± 0.09[c]

Data are mean ± SEM. Values with various superscripts are significantly ($P < 0.05$) different in the row.

diving percent was not different between the HST1%, HST2% and sham groups (Table V).

DISCUSSION

The effects of hempseed were evaluated in 6-month-old ovariectomized rats fed a hempseed-supplemented diet for 3 weeks after surgery and compared with ovariectomized and sham-operated controls which were fed normal rat chow. The antidepressant activity of hempseed was examined using FST, and plasma lipids, lipoprotein, E2 and total calcium levels were considered as endpoints. The duration of this study was shorter than the majority of similar studies (16, 17) using ovariectomized rats to control for synthesis and release of E2 from extra-ovarian organs (18-20). E2 levels in ovariectomized rats were 75% of normal values 8 weeks after ovariectomy (16). As previously reported (17), both subcutaneous adipose and liver tissues contributed to the extragonadal aromatization to promote circulating E2 levels in rats along with time after ovariectomy; the adrenal compensation might also be naturally activated. The body fat of ovariectomized rats increased significantly (21), possibly due to the activity of adipose mesenchymal cells, which might be closely related to the higher aromatase expression along with time after ovariectomy (17). In this study, the nonsignificant difference of abdominal fat mass in hempseed-treated rats compared with sham animals may be due to the finding that there were no statistical disparities between these groups in E2 levels, while hempseed-treated rats showed significantly higher abdominal fat mass and E2 levels than the OVX group.

Recently, it has been found that the activation of the cannabinoid CB_2 receptor attenuates ovariectomy-induced bone loss in mice (22). Δ^9-THC, which is found in hempseed, is a partial agonist for both cannabinoid CB_1 and CB_2 receptors (8). According to these studies, it is proposed that hempseed may decrease osteoporosis via activation of the CB_2 receptor. Generally, results have shown that ovariectomy, independent of the type of diet, caused elevation of plasma calcium levels, but hempseed nonsignificantly modified this effect. There is certainly a close relationship between the abnormality in calcium homeostasis and the accelerated bone mass loss that occurs in the early stage of menopause (23). As previously reported (23, 24), serum calcium concentrations were significantly greater in postmenopausal women as compared with control premenopausal women.

Among the several hormones and cytokines that control bone metabolism, estrogen, as a reproductive hormone, plays different roles in the maintenance of bone homeostasis (25). Estrogen has a distinct vitamin D-independent effect at the genomic level of active duodenal calcium absorption mechanisms, mainly through a major upregulation of the transient receptor potential channel TRPV6 (26). Estrogen tends to have more of a bone-sparing effect than nutritional intervention (e.g., calcium and vitamin D supplement) in healthy postmenopausal women (25). In this study, supplementation of hempseed prevented the considerable decline of estrogen relative to the sham group. On the other hand, the maintenance of estrogen in the normal range prevented increased bone resorption and the accrual of blood calcium. A high dietary ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) is believed to reduce bone formation capacity and cause greater bone resorption activity through increased endogenous production of prostaglandin E_2 (PGE_2) (27). In contrast, the levels of urinary pyridinium cross-links (biomarkers of bone resorption) were significantly higher in rats fed an n-6 PUFA diet compared with those fed an n-3 PUFA diet (28). Different dietary ratios of n-6/n-3 PUFAs were also tested in piglets for their effects on growth and bone metabolism, revealing that higher n-3 PUFA levels in the blood were associated with lower bone resorption (29). The protective effect of n-3 PUFAs on bone loss was also shown in ovariectomized rodents. For example, an eicosapentaenoic acid-enriched diet was effective in minimizing bone loss in ovariectomized rats (30), and feeding fish oil to ovariectomized mice also attenuated bone loss (31). In this study, the ratios of n-6/n-3 PUFAs in hempseed-enriched diets were 0.03, 0.06 and 0.30, respectively, in the HST1%, HST2% and HST10% groups. Therefore, the normal ranges of blood calcium in hempseed-treated rats may be related to the protective impact of low n-6/n-3 PUFA ratios of the respective diets on bone loss.

As previously reported, ovariectomy resulted in a significant weight gain, most likely brought about by increased food intake. In this study, the first observation was that hempseed could prevent weight gain induced by ovariectomy and reduce the body weight gain below the values observed in the sham group. This implies that any other changes related to hempseed cannot be attributed to a change in body weight. Hempseed nonsignificantly altered the partitioning of fat distribution. The abdominal fat mass increased in hempseed-treated rats compared to the OVX group. These changes are not related to food intake because all groups were not different in terms of food intake (data not shown). Estrogen replacement resulted in a significant reduction in body weight in ovariectomized rats, indicating that the low estrogen level in ovariectomized rats was responsible for the increase in body weight (32). Accordingly, estrogen replacement also resulted in an attenuation of the increased food intake observed in ovariectomized rats via the ERß receptor in the central nervous system (33). In this study, the constancy of estrogen concentration in the hempseed-treated ovariectomized rats may prevent the increase in body weight gain above the values observed

in the sham group. Body weights of male and female rats exposed to the phytoestrogen genistein at any dose level examined were lower than those of controls (34). Pure Δ^9-THC and its metabolites did not interact with the ER, while apigenin, the aglycone of a flavonoid phytoestrogen found in cannabis, displayed high affinity for the ER (35).

The capacity for plant sterols to affect de novo cholesterol synthesis is known to occur in the human disorder sitosterolemia. Sitosterolemic patients experience impaired whole-body de novo cholesterol synthesis by downregulation of key synthetic enzymes (36), and this sensitivity suggests that de novo cholesterol synthesis in other species may also be impaired by phytosterol exposure. Therefore, the level of phytoestrogens present in a diet of hempseed and/or a possible increase in plasma E2 following intake of the hempseed may prevent unwanted dyslipidemia that usually occurs after ovariectomy. Although the amount of β-Sit in the hempseed-supplemented diets in the present study may be negligible, the non-significant difference of LDL-C levels among hempseed-treated and the sham group reflects an improvement of the lipid profile following hempseed intake. Also, the decline of HDL-C in hempseed-treated groups compared to the OVX group further confirms the hypothesis that cholesterol biosynthesis decreased in the presence of hempseed. The present study is in agreement with the observations of Scott et al., who showed that estrogens decrease both LDL-C and HDL-C plasma levels in rats (37). One striking result of our study is the potential of hempseed to prevent dramatic plasma level increases of both LDL-C and HDL-C in rats following ovariectomy. Previously, we demonstrated that hempseed that contained high levels of THC led to hypercholesterolemia in guinea pigs (38), while THC-free hempseed improved the lipid profiles of rats (11). Dietary hempseed prevented ovariectomy-induced hypertriglyceridemia and conserved TG levels close to those of the sham group. The high content of UFAs, especially high PUFA, prevented the elevation of TC and TGs in hempseed-treated groups, most obviously in the rats treated with HST10%.

Anxiety and depression are major symptoms in postmenopausal women. Decreased blood levels of sex hormones are thought to be involved in these disorders, as postmenopausal syndrome is significantly improved by hormone replacement therapy utilizing a combined estrogen–progesterone regimen (40). Systemic administration of sex hormones, for example estrogen and progesterone, modifies the affective behavior of ovariectomized rats (41). Estrogen may have different effects on "anxiety" responses under different conditions. In the elevated plus maze, E2 has shown an anxiolytic-like action in estradiol-treated ovariectomized rats (42). In contrast, E2-treated ovariectomized females interacted less with the partner animal in the social interaction test compared with controls, suggesting anxiogenic-like effects that have not been reversed by administration of progesterone (42). E2 possesses long-lasting antidepressant-like effects in the FST (43). In the present study, exposure to a water-filled cylinder in the FST is considered anxiogenic, and decreased defecation in hempseed-treated rats suggests that those animals were less stress-responsive than the OVX group ($P > 0.05$) and sham rats ($P < 0.05$). Likewise, the smaller number of fecal boli ($P > 0.05$) observed in the OVX group suggests that rats devoid of E2 may be slightly less anxious than sham rats ($P > 0.05$). Increased defecation and urination are considered to reflect stimulation of autonomic responses in stress (44). In this study, the anxiety level of the all the ovariectomized rats independent of the type of diet was lower than that of the sham rats. However, only hempseed-treated groups showed significant differences compared to the sham group ($P < 0.05$). In the FST we observed that all three hempseed-treated groups had similar increases in swim time. The swimming time of the HST10% group was significantly different ($P < 0.05$) than both the sham and OVX groups.

The antidepressant activity of hempseed could be the result of its main phytochemicals, such as phytoestrogens and/or phytocannabinoids. The antidepressant activity of other phytoestrogens, such as quercetin and anthocyanins, was reported (45, 46). The antidepressant-like actions of oral apigenin treatment have been reported in rats and mice (47). However, it is possible that other factors that were not measured may be acting as antidepressants, since in the present study it has been demonstrated that scores for depression were reduced after hempseed treatment without concomitant changes in serum estradiol levels. The antidepressant-like effect of hempseed appears to depend, at least partially, on its ER agonist properties. Furthermore, manipulation of the endocannabinoid system due to the presence of Δ^9-THC or possibly other cannabinoids that are found in hempseed may also be involved. Altered function of the endocannabinoid system is attributed to depressive behaviors and the potential of endocannabinoid metabolism modulators as therapeutics for the treatment of depression is currently the subject of considerable research (48). Bambico et al. (49) have shown that CB_1 agonists possess antidepressant-like properties in rats, while the CB_1 antagonist rimonabant, which is currently used for obesity management, increased the adverse effects of depression and anxiety (50).

The present study assumes that diving by the rats is an active behavior that circumstantially shows an exploratory behavior. Exploratory diving was considerably decreased for the HST10% group, while the HST1% and HST2% groups showed a moderate increase when compared to the OVX group. This means that the high amount of hempseed in the diet may increase fear or decrease exploratory behavior. One criticism of this study was that the frequency of diving was assessed as an exploratory behavior in lieu of the duration of the diving. Struggling, swimming and diving behaviors were all classified as active behaviors (15). Finally, the HST10% group showed overt antidepressant-like activity compared to the sham group, since at this amount (10%) hempseed increased the swimming time, while it decreased fecal boli numbers in the FST paradigm.

In this study, rats were fed amounts of hempseed which mimic the amounts that are generally consumed daily by humans from nuts or maximally as a part of the meal. Dietary exposure to hempseed resulted in desirable and acceptable effects on the prevention of weight gain, dyslipidemia, depression and anxiety that occurred following ovariectomy in a rat model of menopause. Hempseed also showed a putative osteoprotective effect by preventing abrupt changes of blood calcium levels. However, in order to develop a hempseed-based food additive or supplement for the treatment of estrogen deficiency-related complications, more research will be needed to identify the active ingredients in hempseed, as well as the mechanism that mediates the action of hempseed in vivo.

DISCLOSURES

This project was financed by Biotechnology Research Center, Urmia University, Urmia, Iran.

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