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ASSESSING THE EFFECT *SALVIA HISPANICA* AND *AVENA SATIVA* SEEDS ETHANOLIC EXTRACT ON ESTRADIOL LEVELS IN GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN FEMALE RABBITS

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Abstract

In the current study, we investigated the efficacy of the active compounds present in *Salvia hispanica* and *Avena sativa* seeds in the treatment of osteoporosis on methylprednisolone in female rabbits. The study consists of two experiments; the first one is used to estimate the acute toxicity of chia(*Salvia hispanica*) and oat (*Avena sativa*) seeds ethanolic extracts. These results showed the seeds were safe up to 15000 mg/kg B.W dose. In accordance with this doses of 600 mg/kg B.W were selected for experimental study. The second experiment, 80 female rabbits were used, weighted 1500- 2000gm and aged 8 months, were divided into 8 equal groups. Group 1 served as control negative group(G^{-ve}). Group2:Control positive group(G^{+ve}) received S/C methylprednisolone(Meth-Pred.) of 0.2 mg/kg per for 1 month, then received orally 1ml/kg normal saline per day for 2 months. Group3(Meth-Pred+Sh): received S/C Meth-Pred. of 0.2 mg/kg for 1 month then received 600 mg/kg *Salvia hispanica* seed ethanolic extract orally per day for 2 months.Group4(Meth-Pred +As) : received Meth-Pred. for 1 month then received 600 mg/kg *Avena sativa* seed ethanolic extract orally for 2 months.Group5(Meth-Pred+Alend): received Meth-Pred. for 1 month then treated by alendronate 3.6mg/kg.BW weekly orally for 2 months.Group6(Meth-Pred+ Alend + Sh): received Meth-Pred. of for 1 month then treated by alendronate 3.6mg/kg BW orally weekly and 600 mg/kg *Salvia hispanica* seed ethanolic extract orally per day for 2 months.Group7 (Meth-Pred+ Alend + As): received Meth-Pred for 1 month then treated by alendronate 3.6 mg/kg BW orally weekly and 600 mg/kg *Avena sativa* seed ethanolic extract orally per day for 2 months.Group8 (Meth-Pred+ Sh + As): received Meth-Pred. of for 1 month then treated by both *Salvia hispanica* seed ethanolic extract at 300 mg/kg and *Avena sativa* seed ethanolic extract at 300 mg/kg orally per day for 2 months. This study persisted till after 1 week of the medicine's withdrawal, by studying the body weights, BMD, serum ALP, Calcitonin, Estradiol hormone, bone Ca, and phosphorus. The results showed that the body weights, BMD, Calcitonin, estradiol hormone, bone Ca, and phosphorus a significantly declined ($p \leq 0.05$), as well as a significant increase ($P \leq 0.05$) in serum ALP of female rabbits after 2 months of treatment and after 1 week of withdrawal of treatment in control(+ve) and alendronate group compared with control(-ve), but when treated animals with *Salvia hispanica* and *Avena sativa* seeds ethanolic extracts in 3 and 4 group, demonstrated an improvement in the parameters above return to its normal values and produced good results. According to these results, *Salvia hispanica* and *Avena sativa* seeds are effective in preventing bone loss in female rabbits.

Keywords: ***Salvia hispanica* seeds, *Avena sativa* seeds. Ethanolic Extract, Bone, Female rabbits, Osteoporosis**

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Introduction:

Osteoporosis is a chronic skeletal condition characterized by reduced bone mass as a result of microstructural deterioration of bone tissue, with consequent the increasing bone fragility and fracture risk. This loss and degradation of the bone tissue's structure is brought on by a net imbalance in bone remodeling, due either to an increase in the number or activity of osteoclasts or a decrease in the number or activity of osteoblasts(1). Estrogen reduction occurring at menopause plays a main role in the development of osteoporosis in postmenopausal women (2,3). In order to avoid adverse effects of estrogen depletion, estrogen replacement therapy has been proposed and the use of selective estrogen receptor modulators (SERM) has been produced (4,5,6). Therefore, there is a tremendous need to find a natural treatment that has less unwanted side effects and that may decrease the need for now used drugs. Several experimental studies have noted the beneficial effects of a diets rich in phytoestrogens for preventing postmenopausal diseases (7). These compounds, identified in several grains, are recognized for their estrogenic effect thanks to their similarity with 17β-estradiol. They are able to scavenge free radicals and modulate the expression of genes encoding for antioxidant enzymes(8). Phytoestrogens are plant substances having biological action similar to that of estrogen. The main classes of phytoestrogens are isoflavones, flavonoids, and lignans. Particularly after consuming lignans and isoflavones, heterocyclic phenols are produced that, due to their near steriochemical resemblance to estrogen, can bind to estrogen receptors (9,10). In the current study, we aim to investigate the efficacy of the compounds present in the seeds of *Salvia hispanica* and *Avena sativa* in the treatment of osteoporosis on methylprednisolone -induced osteoporosis in female rabbits.

Materials and Methods

Collection of Plant materials seeds, and extraction:

Salvia hispanica L. (chia) and *Avena sativa* L. (oat) seeds were bought from local markets in Basra, Iraq. The seeds were extracted technique according to (11).

Preliminary qualitative chemical analysis of ethanolic extracts of seeds:

Qualitative chemical tests were carried out for the extract of chia and oat seeds as described in table (1):

Table(1): preliminary qualitative chemical analysis extract of ethanolic chia and oat seeds

Phytoconstituent	Reagents	Results
Phenols	1ml extract +1ml FeCl_3	Brown precipitate
Flavonoids	1ml extract+1mlKOH alcoholic	Yellow precipitate
Tannins	1ml extract+1ml lead acetate	Brown to Yellow precipitate
Saponins	1mlextract+1ml HgCl_2	Yellow precipitate
Glycosides	1mlextract+1ml Benedict	Blue coloration
Alkaloids	1ml extract+1ml Mayers	Turbidity of solution
Proteins	1ml extract+1ml Biurete	Green to Yellow coloration
Carbohydrates	2ml extract + 10ml H2O + 2 drops ethanolic α-naphthol (20%) +2ml H2SO4(conc.)	Reddish violet ring

Acute Toxicity Studies:

Acute toxicity study was carried out by using graded doses seeds of *Salvia hispanica* and *Avena sativa* ethanolic extract on female rabbits. The animals were fasted for 12hrs prior to the oral administration of extracts. It is diluted in distilled water, and administered orally in graded doses (5000, 6000,7000, 8000, 9000, 10000, 11000, 12000, 13000,14000,15000 mg/kg B.W), as one animal at one dose of serial extract, and monitoring the animal for (24) hours for

behavioral effects such as nervousness, ataxia, excitement, alertness, dullness, and death, in which the not dosed remaining rabbits let to next step, then used the other diluted extract dose to another rabbit and monitoring to (24) hours.

Experimental animals:

Eight equal groups made up of a total of 80 mature female local rabbits (*Lepus cuniculus*) were formed, each including ten adult female rabbits: **1.** Control negative group(G^{-ve}): received orally1ml/kg normal saline daily for 3 months.**2.** Control positive group(G^{+ve}) (methylprednisolone group) were received S/C methylprednisolone of 0.2 mg/kg per for 1 month (12), then received orally 1ml/kg normal saline per day for 2 months. **3.** Group3(Meth-Pred+Sh): received S/C methylprednisolone of 0.2 mg/kg for 1month then received 600 mg/kg *Salvia hispanica* seed extract orally per day for 1 months.**4.**Group4(Meth-Pred +As) : received S/C methylprednisolone of 0.2 mg/kg per day for 1 month then received 600 mg/kg *Avena sativa* seed extract orally for 2 months. **5.**Group5(Meth-Pred+Alend): received S/C methylprednisolone of 0.2mg/kg for 1 month then treated by alendronate 3.6mg/kg.BW weekly orally for 2 months.**6.** Group6(Meth-Pred+ Alend + Sh): received S/C methylprednisolone of 0.2mg/kg for 1 month then treated by alendronate 3.6mg/kg BW orally weekly and 600 mg/kg *Salvia hispanica* seed extract orally per day for 2 months.**7.**Group7 (Meth-Pred+ Alend + As): received S/C methylprednisolone of 0.2mg/kg for 1 month then treated by alendronate 3.6 mg/kg BW orally weekly and 600 mg/kg *Avena sativa* seed extract orally per day for 2 months.**8.**Group8 (Meth-Pred+ Sh + As): received S/C methylprednisolone of 0.2mg/kg for 1 month then treated by both *Salvia hispanica* seed extract at 300 mg/kg and *Avena sativa* seed extract at 300 mg/kg orally per day for 2 months. Half number of rabbits in each group have been sacrificed after 3 month of therapy, and the other half after 1 week of treatment withdrawal.

Measurement of the body weights

The weight of each animal was recorded in the 0 day, in the 3months and 1 week of withdrawal of treatment by using electronic balance.

Dual- energy X-ray absorptiometry DEXA

Animals were anaesthetized using mixture of ketamine (35mg/kg), xylazine (5mg/kg) intramuscularly. Each scans were performed by using DEXA (Hologic QDR-1000 System, Hologic Inc., Waltham, USA.) after one, two, three months, and one week of withdrawal of treatment. The high resolution scan was performed to estimate the bone mineral density (BMD) at the femur bone of animals.

Blood sample collection.

Blood samples (ten ml) were collected from each rabbit after 3 months of therapy and 1 week of withdrawal of treatment through heart puncture technique, by using 10cc disposable syringe. The blood was placed in a tube without anticoagulant and centrifuged for 10 minutes at a speed of 3000 rpm to get the serum. The serum was then separated in Eppendorf tubes and kept at -20°C until it was needed, which then used to study parameters like Alkaline phosphatase **(**ALP) according to (13), Calcitonin according to(14), and Estradiol hormone was measured by the use of a special kit (Monobind Inc. lake forest CA 92630, USA).

Estimation of Bone Ash

Left femur bones were collected from all groups, are extricated from the flesh and connective tissue, then dried, and burned samples according to ((15), then digestion for determination of bone calcium and phosphorus according to method of (16).

Statistical analysis:

was performed using one-way covariance (ANOVA) test. Through SPSS program V. 24(17).

Results

1-Phytoconstituent of *Salvia hispanica* and *Avena sativa* Seeds Ethanolic Extracts.

The phytoconstituent of the ethanolic extracts of both plants' seeds are listed in table(2). In this study, the active constituents of ethanolic extract of the *Salvia hispanica* were determined using different specific reagents. The results indicated the presence of different active compounds which are (Flavonoids, Phenolic compounds, and Protein). The active constituents of the ethanolic extract of *Avena sativa* were determined using different specific reagents. The results indicated the presence of

variety active compounds which are (Flavonoids, Phenolic compounds, Saponin, Alkaloids, Glycosides, Protein, and Carbohydrate). These results were in accordance with previous studies on the same plants that indicated the presence of these compounds(18,19,20,21).

Table (2): phytoconstituent of *Salvia hispanica* and *Avena sativa* Seeds Ethanolic Extracts

Chemical constituent	*Salvia hispanica*	*Avena sativa*
Flavonoids	+	+
Phenolic compounds	+	+
Tannin	-	-
Saponin	-	+
Alkaloids	-	+
Glycosides	-	+
Protein	+	+
Carbohydrate	-	+

+ Indicates presence - indicates absence

2. Acute Toxicity Study for *Salvia hispanica* L and *Avena sativa* L seeds.

The results clearly indicated there was no mortality observed - along the acute toxicity experiment of both extracts till a dosage rate of 15000 mg/kg B.W. as well as there were no behavioral and any other signs of poisoning observed. Therefore, *Salvia hispanica* and *Avena sativa* seeds were considered practically not toxic . In accordance with this doses of 600 mg/kg B.W were selected for experimental study.

3.Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Body Weight (g) in Female Rabbits.

The body weight showed no significant ($P>0.05$) differences between all experimental groups at 0 day of the experiment. After 2 months of treatment and after 1 week of treatment withdrawal the results indicated a significant ($P\leq0.05$) decrease in final body weight of female rabbits control(+ve), and alendronate group (MP+ Ale) compared with control(-ve), and another treated groups, while showed no significant changes ($P>0.05$) in final body weight of female rabbits treated with *Salvia hispanica* (MP+ Sh), *Avena sativa* (MP+As) and MP+ Sh+ As ethanolic extract groups compared with control(-ve), but the result showed a significant ($P\leq0.05$) rise in MP+ Ale +Sh and MP+ Ale +As groups compared with control(+ve), but it was still significantly less ($p\leq 0.05$) than that of the control (-ve)group (Table 3).

Table 3. Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Body Weight (g) in Female Rabbits

Groups	Body Weight (g)		
	Zero day	Two Month	Withdrawal
C –ve	1797.0± 266.64a	1875.4± 272.86a	1877.4± 272.86a
C +ve	1801.4± 275.14a	1185.0± 259.22c	1174.0± 273.17c
MP+ Sh	1801.6± 273.96a	1955.0± 338.80a	1968.2± 339.80a
MP+As	1802.2± 275.88a	2070.2± 337.46a	2110.2± 337.46a
MP+ Ale	1801.0± 224.29a	1454.0± 353.67c	1452.8± 352.67c
MP+Ale +Sh	1801.8± 273.69a	1570.0± 241.35b	1576.0± 237.66b
MP+Ale +As	1802.4± 274.23a	1574.8± 241.78b	1578.8± 239.24b
MP+ Sh+ As	1800.6± 273.04a	1934.6± 239.28a	1946.6± 237.51a
LSD	689.5	745.2	747.2

4.Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Femur Bone Mineral Density (BMD) in Female Rabbits.

Table (4) shows the effect of ethanolic extracts of *Salvia hispanica* and *Avena sativa* seeds on femur BMD, after one month from treatment, There was a significant decrease ($p \leq 0.05$) in BMD in the control(+ve) group compared with the negative control group, also there was non-significant differences ($p>0.05$) between control (+ve) group and alendronate alone group(MP+ Ale), while there was no significant change ($p>0.05$) was showed in BMD of female rabbits treated with *Salvia hispanica* (MP+ Sh) and *Avena sativa* (MP+As) groups compared with control(-ve), but the results showed a significant increase ($P \leq 0.05$) in BMD of MP+ Sh+ As, MP+ Ale +Sh and MP+ Ale +As groups compared with control(+ve) but it was still significantly less than that of the control (-ve) group. After two month from treatment, a significant decline ($p \leq 0.05$) in BMD in the control(+ve) group compared with the control (-ve) group and other treated groups, and the result of BMD showed a significant increase ($p \leq 0.05$) in the group treated with *Salvia hispanica* (MP+ Sh) compared with control(-ve) and another treated groups, whereas there was no significant change ($p>0.05$) was showed in BMD of female rabbits treated with *Avena sativa* (MP+As) group and control(-ve), so was showed no significant difference ($p>0.05$) in BMD of MP+ Ale +Sh and alendronate alone (MP+ Ale) groups compared with control(+ve), on the other hand, the results showed a significant increase ($P \leq 0.05$) in BMD of MP+ Ale +As and MP+ Sh+ As group compared with the control (+ve) group but remain significantly lower ($p \leq 0.05$) compared with the control (-ve) group. After one week of treatment withdrawal, the results of BMD revealed a significant decrease ($p \leq 0.05$) in the control (+ve) group compared with the control (-ve) group and another groups, whereas a significant increase ($p \leq 0.05$) in BMD of the group treated with *Salvia hispanica* (MP+ Sh) and *Avena sativa* (MP+As) compared with control(-ve), but the results showed a significant decrease ($P \leq 0.05$) in BMD of MP+ Sh+ As, MP+ Ale +Sh and MP+ Ale +As, and alendronate alone (MP+ Ale)group compared with the control (+ve) group.

Table 4. Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Femur BMD (g/cm^3) in Female Rabbits

Groups	BMD (g/cm^3)		
	One Month	Two Month	Withdrawal
C –ve	0.37± 0.01a	0.41± 0.03b	0.41± 0.03c
C +ve	0.22± 0.02e	0.18± 0.01f	0.17± 0.01g
MP+ Sh	0.38± 0.01a	0.46± 0.02a	0.47± 0.01a
MP+As	0.36± 0.01a	0.42± 0.01b	0.44± 0.07b
MP+ Ale	0.24± 0.02e	0.31± 0.02e	0.29± 0.08f
MP+ Ale +Sh	0.28± 0.01c	0.32± 0.01de	0.31± 0.07e
MP+ Ale +As	0.25± 0.02d	0.33± 0.01d	0.32± 0.01e
MP+ Sh+ As	0.34± 0.02b	0.39± 0.01c	0.38± 0.07d
LSD	0.038	0.025	0.033

5.Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on ALP and Calcitonin in Female Rabbits

The results of ALP after 2 months of treatment and after 1 week of treatment withdrawal, showed a significant increase ($p \leq 0.05$) in the control(+ve) group compared with the control(-ve) group, While the results showed non-significant changes ($p>0.05$) in ALP in *Salvia hispanica* (MP+ Sh) group and *Avena sativa* (MP+As) group compared with the control(-ve)group, also there was non-significant changes ($p>0.05$) in ALP in MP +Ale +As and MP + Sh +As group when compared with the control(+ve)group, also there was significant decrease ($p\leq0.05$) in ALP of MP +Ale +Sh and alendronate alone (MP+ Ale) groups when compared to the control(+ve)group and it is still significantly higher than that of the negative control group. The results indicated a significant decrease ($P\leq0.05$) in Calcitonin in female rabbits (control(+ve)) as compared with control(-ve), but no significant changes ($P>0.05$) in serum CT between control (+ve) group and alendronate alone(MP+ Ale) group, also there was no significant ($p>0.05$) different in serum CT between *Salvia hispanica* (MP+ Sh) group and *Avena sativa* (MP+As) group, but there was a significant rise ($p\leq 0.05$) in CT of MP+ Ale +Sh, MP+ Ale +As and MP+ Sh+ As groups compared with the control(+ve) group, but it is significantly lower than that of the control(-ve) group after 2 month, while, after 1 week of treatment withdrawal, there was no significant changes ($P>0.05$) in serum CT between MP+ Ale +Sh, MP +Ale +As group (table 5).

Table 5. Effect of *Salvia hispanica* L and *Avena sativa L* Seeds Ethanolic Extracts on ALP and Calcitonin in Female Rabbits.

Groups	Two Month ALP U/L	Withdrawal ALP U/L	Two Month CT ng/ml	Withdrawal CT ng/ml
C –ve	36.80±3.83e	37.40±0.15e	1.28±0.19b	1.29± 0.30b

C +ve	**121.20±0.45a**	**120.34±0.42a**	**0.38± 0.08f**	**0.34± 0.05e**
MP+ Sh	**36.02±0.70e**	**37.04± 0.72e**	**1.48± 0.15a**	**1.54± 0.11a**
MP+As	**37.90±0.74e**	**37.74± 0 .08e**	**1.42± 0.08a**	**1.48± 0.08a**
MP+ Ale	**70.00±17.32b**	**71.10±13.50b**	**0.40± 0.08f**	**0.38± 0.11e**
MP+Al+Sh	**59.92± 0.78c**	**60.16± 0.89c**	**0.52± 0.05e**	**0.51± 0.05d**
MP+Al+As	**54.40±0.25cd**	**52.76± 0.81d**	**0.70± 0.09d**	**0.63± 0.02d**
MP+ Sh+As	**48.70± 8.34d**	**48.38± 8.13d**	**1.01± 0.08c**	**1.04± 0.08c**
LSD	**17.91**	**14.43**	**0.28**	**0.33**

6.Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Estradiol in Female Rabbits

After 2 months of treatment and after 1 week of treatment withdrawal, The current results listed in Table (6) revealed a significant decrease ($P \leq 0.05$) in estradiol in female rabbits treated with methylprednisolone (control (+ve)) group compared with control (-ve) group, but no significant difference ($P > 0.05$) in serum E_2 between control (+ve) group and alendronate alone(MP+ Ale) group, while the results showed a significant increase ($P \leq 0.05$) of E2 in female rabbits treated with *Avena sativa* (MP+As) group and *Salvia hispanica* (MP+ Sh) group compared with control (-ve) group and another groups, but showed no significant ($p > 0.05$) different in estradiol between MP+ Ale + Sh and MP+ Ale + As groups, but there was a significant increase ($p \leq 0.05$) in E_2 of MP+ Sh+ As group compared with the control(+ve) group and it is still significantly lower than that of the control(-ve) group.

Table 6.Effect of *Salvia hispanica* L and *Avena sativa L* Seeds Ethanolic Extracts on Estradiol in Female Rabbits.

Groups	**Two Month E_2 Pg/ml**	**Withdrawal E_2 Pg/ml**
C –ve	**39.91± 0.07c**	**39.91± 0.17c**
C +ve	**23.00± 2.34f**	**22.84± 2.41f**
MP+ Sh	**45.72± 0.46b**	**45.76± 0.45b**
MP+As	**49.26± 0.42a**	**49.44± 0.49a**
MP+ Ale	**25.00± 4.13f**	**25.02± 3.89f**
MP+ Ale +Sh	**30.36±0. 40e**	**30.06± 0. 08e**
MP+ Ale +As	**31.40± 0.54e**	**30.40± 1.14e**
MP+ Sh+ As	**35.61± 3.06d**	**35.38± 2.94d**
LSD	**5.22**	**5.12**

7. Effect of *Salvia hispanica* L and *Avena sativa* L Seeds Ethanolic Extracts on Bone Ash in Female Rabbits.

After 2 months of treatment, it is clear from the table (7) that methylprednisolone caused a significant decrease ($p \leq 0.05$) in bone calcium of female rabbits in the control(+ve) group, also there was no significant difference ($P>0.05$) was seen in the bone calcium between control (+ve) group, control (-ve) group, alendronate alone (MP+ Ale), MP+ Ale +Sh, MP+ Ale +As and MP+ Sh+ As groups, also it showed no significant changes ($P>0.05$) of bone Ca between *Salvia hispanica* (MP+ Sh) group and *Avena sativa* (MP+As) group compared with control (-ve). After 1 week of treatment withdrawal, the results indicated a significant decrease ($P\leq0.05$) in bone calcium in female rabbits (control(+ve)) as compared with control(-ve), also there was no significant difference ($P>0.05$) was seen in the bone calcium between control (+ve) group, alendronate alone (MP+ Ale), MP+ Ale +Sh, MP+ Ale +As, and MP+ Sh+ As groups, there was no significant change ($p>0.05$) was observed in bone calcium between the control (-ve) group, and *Avena sativa* (MP+As) group, while the results showed a significant increase ($P\leq0.05$) of in bone calcium in female rabbits treated with *Salvia hispanica* group (MP+ Sh) compared with control (-ve) and another treated group. The results of bone phosphorous a significant decline ($P\leq0.05$) in control(+ve) compared with the control (-ve), while it showed no significant differences ($P>0.05$) in bone P between *Avena sativa* (MP+As) group, and with control (-ve), also there was no significant differences ($P>0.05$) of bone P between with *Salvia hispanica* group (MP+ Sh), alendronate alone (MP+ Ale), MP+ Ale +Sh, MP+ Ale +As, and MP+ Sh+ As groups compared with the control (+ve) group. After 1 week of treatment withdrawal, the results indicated a significant decrease ($P\leq0.05$) in bone P in female rabbits (control(+ve)) as compared with control(-ve), also no significant changes ($P>0.05$) between control(+ve), alendronate alone (MP+ Ale), MP+ Ale +Sh, MP+ Ale +As, and MP+ Sh+ As groups compared with the control (-ve), while the results showed a significant increase ($P\leq0.05$) of in bone P in female rabbits treated with *Avena sativa* (MP+As) group compared with control (-ve) and another treated group, whereas it showed no significant differences ($P>0.05$) in bone P between *Salvia hispanica* (MP+ Sh) group and control (-ve).

Table 7. Effect of *Salvia hispanica* L and *Avena sativa L* Seeds Ethanolic Extracts on Bone Ash in Female Rabbits

Groups	Two Month Ca mg/ml	Withdrawal Ca mg/ml	Two Month P mg/ml	Withdrawal P mg/ml
C –ve	36.54± 0.05b	36.62± 0.16b	9.49±0.14a	9.50±0.14ab
C +ve	19.02± 1.02b	19.00± 1.00c	5.77± 0.45c	5.62± 0.50c
MP+ Sh	56.67±12.62a	57.30±12.06a	8.35± 0.44b	8.49±0.08ab
MP+As	50.10±4.41a	51.72±0.46ab	10.46±1.22a	10.67±0.46a
MP+ Ale	26.07± 0.43b	24.99± 0.66c	6.42± 0.01b	6.02± 0.08c
MP+Ale +Sh	29.90±1.24b	30.73± 5.18c	7.42± 1.71b	6.82±1.45bc
MP+Ale +As	31.02±16.46b	31.00±15.41c	7.74± 1.89b	7.03±1.17bc
MP+ Sh+ As	34.20±0.57b	33.92±0.74c	7.79± 1.16b	7.73±1.89bc
LSD	19.39	17.94	2.84	2.50

Discussion

The rabbit model is useful in studying osteoporosis and the methylprednisolone rabbit was determined to be the standard animal for the study of bone loss caused by estrogen deficiency. This effect of Glucocorticoids (GCs) is a significant reduction in body weight is in agreement with (22,23). These findings indicate that GCs have an inhibitory effect on body weight, food intake and appetite peptide expression in the hypothalamus indirectly effect secondary to metabolic changes. GCs treatment causes earlier and more severe glucose and lipid abnormalities, which could lead to a reduction in NPY and AGRP mRNA expression followed by reduced food intake and body weight(24). These results indicate that GCs have an increased risk of osteoporosis, which is characterized by a decline in bone strength and bone weight (25,26), low body weight may result from this(27).While treatment with ethanolic extract of *Salvia hispanica* showed a significant rise in body weight. The rise in body weight could be due to the lesser digestibility of chia protein(28). Furthermore, the high soluble fiber content of chia seeds may have enhanced the period of food digestion in addition to the absorption of nutrients by intestinal cells. The enhancement in body weight when treated with ethanolic extract of *Avena sativa* may be attributed to its content of magnesium, phosphorus, manganese, copper, iron, zinc, proteins, and carbohydrates which are essential for growth, body repair, and maintenance. Interestingly, administering *Avena sativa* seed extract prevented body weight loss effectively (29). BMD measurement is a great endpoint for evaluating treatment effects on the skeleton, was assessed by DEXA, the gold standard technique for evaluating osteoporosis clinically(30). Reduced BMD, osteoblast numbers, and bone formation rates are frequently associated to glucocorticoids (31). It has been shown that administering glucocorticoids increases bone resorption, lowers total body bone mineral content, and lowers bone mineral density as a result (32,33). When *Salvia hispanica* extract was used as therapy proved effective in restoring BMD levels, demonstrating that the extract raised bone mass in those rabbits and possibly by lowering osteoblast apoptosis, chia seeds contain a large amount of -linolenic (ALA, -3 fatty acid), which may have contributed to the rise of weight of the musculoskeletal system, and the alterations seen with the DXA analysis of the bone structures (34). According to the results of the current study, the *Avena sativa* extract may have a positive effect by preventing osteoclast differentiation and may be able to influence osteoclastogenesis by changing the expression of RANKL and OPG. Oat seeds high in β -glucan were able to prevent BMD declines, by promoting osteoblast differentiation and bone formation and inhibiting osteoclast activity and bone resorption, Furthermore, it has the ability to maintain bone strength and mass and increasing the rate formation of bone (35). Hence, indicating that β -glucan may be useful to prevent and treat fractures caused by osteoporosis. (36). Alkaline phosphatase is a phosphomonoesterase that is secreted from osteoblast and is widely distributed in a variety of tissues and organs, particularly liver and bone (37). ALP activity can represent the degree of differentiation in osteoblast, which is used to evaluate bone formation ability, the enhanced bone turnover, and risk of fracture(38). In accordance with previous reports (27,39) we noticed that glucocorticoids increased the activity of ALP, it is an important index in the early stage of osteogenic differentiation, which participates in skeletal calcification by raising the local content of inorganic phosphate. These GCs-induced alterations can lead to an increase in bone resorption and turnover state, which compensatory rises ALP activity to promote bone formation (40). *Salvia hispanica* ethanolic extract administration significantly reduced levels of ALP near to normal control value. These results were in agreement with El-Wakf *et al*.(41) who reported a significant decline in ALP, and showed a positive influence of polyphenols on ALP level, suggesting reduced bone turnover rate with consequent antiosteoporotic effects. In Particular, the osteoporosis preventive properties of chia seeds perhaps in part mediated by its polyphenols, and their effects on osteoclast precursors and osteoblast-mediated signaling for osteoclastogenesis (42). supplementation with *Avena sativa* ethanolic extract seeds decreased serum ALP in the treated group, in the current study resulted in a lowering of serum ALP in the treated group, which received methylprednisolone,

demonstrating the therapeutic effects of this extract on the bone in rabbits exposed to GCs, β-glucan, macronutrient and minerals such as magnesium, iron, and zinc contained in oat seeds may be beneficial for enhancing bone health (43, 44). Additionally, GCs lower serum levels of calcitonin, which is a potent bone forming hormone acts directly to suppress bone resorption by binding to high affinity receptors on osteoclasts (45). The current study showed similar hormonal changes following methylprednisolone treatment which seemed to be successfully attenuated upon *Salvia hispanica* and *Avena sativa* seeds ethanolic extracts administration. *Salvia hispanica* seed extract administration modifies CT levels, These results were in agreement with Varela-López *et al*.; (46), who reported that the protective effect of ω-3 PUFAs on bone loss was due to the modulation of systemic calcitrophic hormones, including CT. Administration of an ethanolic extract of *Avena sativa* seeds to rabbits receiving methylprednisolone significantly raised the level of CT, This may be due to the high levels of polyphenol chemicals in oat seeds, which are known to have beneficial effects on bone health by perhaps enhancing the effect of CT on the calcium metabolism (41). Serum estrogen levels in the GC-treated group significantly decreased. Zhang *et al*.; (47) reported that in rats, treatment of glucocorticoids showed bone resorption due to estrogen deficiency resulting in a marked bone loss, leading to elevated osteoclast formation, and enhanced bone resorption. The flavonoids like quercetin and kaempferol are present in chia seeds, these types of compounds have been demonstrated to be powerful estrogen-like (48). These compounds might be useful in preventing symptoms arising from estrogen deficiency, and could have a positive effect on bone remodeling as well as inhibitory action on GIO in female rabbits (49). When quercetin was given to ovariectomized mice to study the effect on bone loss, the animals' bone mineral density increased significantly (50). Ma *et al*.; (51) and Zhang *et al*.; (47) who found that the flavonoid prevents osteoporosis that was induced by ovariectomy. The possible role of isoflavones like genistein, and daidzein found in chia seeds extract, in this study, is that these compounds act on bone cells through estrogen receptors (ERs) (52, 53). ERs have been present in osteoblasts, bone forming cells, and bone marrow stromal cells. These compounds have a high affinity for ER (54, 55). These compounds demonstrated estrogenic potency and had the ability to reduce calcium excretion, and restore serum calcium levels in rabbits towards normal values. Some studies have been evaluated the estrogenic activity of isoflavones extracted from *Cicer arietinum* in vitro and in vivo(56, 57). As lignans, which are phytoestrogens found in oat seeds, perform their antiosteoporosis effect by stimulating osteoblastic activity by an estrogen receptor mediated action, or through raising the production of insulin 1 like growth factor-1 (IG-F) which is known to promote osteoblastic activity (58). This results was desirable as an agent intended to replace conventional estrogen must mimic the role of estrogen in preventing excessive bone resorption. Phenolic acids which are present in oat seeds were also investigated for their osteoprotective effect with ferulic, coumaric, caffeic and chlorogenic acids to counteract skeletal changes caused by estrogen deficiency in female rabbits, these results were in agreement with other study on Phenolic acids as antiosteoporotic effect (59). Led to raised bone formation and significantly inhibits resorption.

Administration of glucocorticoids may reduce intestinal absorption of calcium and increase its excretion, which may lead in secondary hyperparathyroidism(60), According to the current study, the bone Ca and P levels were significantly lower than in the control group. These results are in agreement with (61) who observed that GCs lowered the levels of bone Ca and P. is marked by decreased intestinal calcium absorption, which may be a contributor in the accompanying bone loss. This could be a consequence of alterations in Ca, and P renal excretion, which may derive bone tissue toward improved resorptive activity. The positive effect on bone density of chia and oat seeds is probably due to its rich content of calcium, and phosphorous, which have been shown to have beneficial effects on bone and restored the lowered levels of bone Ca, and P to normal values (62, 63, 64). To clarify the mechanism at play in the chia and oat seeds' regulatory effects on calcium metabolism, the expression of active calcium transport proteins in the duodenum, and the kidney, the kidney (renal re-absorption) as well as the exchange of calcium to, and from bone (65, 66,67). Other study

shown that Phytoestrogens including daidzein, and genistein found in chia seeds extract were proven to have an anabolic effect on bone metabolism, prevented bone loss, prevent the decline of bone density, and strength, raise the activity of osteoblasts, and improve bone mineralization, In cortical bone culture of female rats, genistein and daidzein induced an rise of Ca and P content, and in bone tissues (68, 69). Additionally, it has been shown that the saponin in oat seeds extract has an anabolic effect on bone metabolism by promoting osteoblast proliferation, The result in concordance with the study of Niu *et al.,* (70) demonstrated that the saponin extracted from Radix Dipsaci, which has been shown to promote the proliferation, differentiation, and mineralization of osteoblastic cells, In order to prevent osteoporosis, saponin may be useful. Beside, chia and oat seeds are specially rich in magnesium which acts as a cofactor of glycosyltransferase enzyme required for production of proteoglycans being essential for bone formation and mineralization (42). Our results clearly demonstrate that *Salvia hispanica* and *Avena sativa* could be considered as a natural alternative to hormone replacement therapy for the preventing of bone loss in postmenopausal women.

Ethics

The experimental design, and procedures used in this study were reviewed and approved in accordance with animal welfare ethical standards by the Scientific Committee of the Department of Physiology, Biochemistry, and pharmacology, College of Veterinary Medicine, University of Baghdad, in its session held on 4/7/ 2021, and the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

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