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The Effect of ChiaSeeds (Salvia hispanica L.) on Osteoporosis of Ovariectomized Rats

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Abstract

The present work aimed to investigate the anti-osteoporotic effect of chia seeds (*Salvia hispanica* L.) powder (CSP) consumption for ovariectomized (OVX) rats by determination of the phytochemical of CSP and serum biochemical and bone tissue analysis of rats. Bilateral ovariectomy was performed in rats under ethyl ether anesthesia. Thirty-five female rats were randomly divided into 5 equal groups (n=7). Group 1 was Sham-operated (SHAM), while the other 4 groups were OVX-operated. After 3 weeks of the surgical operation when the malondialdehyde (MDA) was highly elevated as a serum biomarker then the experiment was started.Group 2 was kept as OVX control and groups 3, 4 and 5 were fed on a diet containing CSP in doses 2.5, 5 and 10%, respectively for 5 weeks. Rats weighed twice a week and weight gain was calculated. Blood samples were collected for biochemical analysis and femur bones were removed for estimating bone markers. Resultsof chemical composition of CSP showed that each 100 g contained 31.42% fat, 25% fiber, 16.50% protein and 16.08% carbohydrate. The major constituents of CSP were polyunsaturated fatty acids mainly α-linolenic acid in 38.97% and linoleic acid in 21.08%.The phenolic compounds,myricetin 75.19 mg followed by chlorogenic acid 41.9 mg and quercetin 38.06 mg have been identified by HPLC. The antioxidant activity DPPH was 87.24% in 10% of high tested sample. The results of biological study

showed that administration of CSPto OVX ratsdecreased weight gain, serum interleukin-1 beta,MDA,aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphates (ALP). On the other hand,levels of serumglutathione peroxidase, total calcium, ionized calcium and femur bone minerals (calcium and phosphorus) contents were increased by CSP administration.In conclusion, chia seeds exhibit an antioxidantactivity and effective in reducing bone minerals loss in OVX rats. Intake of chia seeds may be beneficial in the elimination of postmenopausal osteoporotic women.

Keywords: Chia seeds, Osteoporosis,Ovariectomized rats.

Introduction

Osteoporosis is a progressive skeletal disorder whereby the bone strength (bone density and quality) is compromised thereby predisposing an individual to an increased risk of fractures ***(Hsu et al., 2020)***. It is associated with low bone mineral density and loss of structural and biomechanical properties that are vital for the maintenance of bone homeostasis ***(Ivanova et al., 2015).*** Osteoporosis is a serious health issue among aging postmenopausal women. The majority of postmenopausal women with osteoporosis have bone loss related to estrogen deficiency ***(Cheng et al., 2022).*** A decline in estrogen hormone levels after menopause leads to an altered balance between bone formation and bone resorption favoring bone resorption ***(Fischer and Haffner-Luntzer, 2022).*** One cause are the direct effects of estrogen on bone cells. Estrogen increases osteogenic differentiation of mesenchymal stem cells and osteoblast maturation, thereby enhancing bone formation. Furthermore, estrogen inhibits osteoclast formation and induces osteoclast apoptosis, which limits bone resorption. When estrogen is insufficient in the female body, these osteo-anabolic and anti-

osteoclastic effects are reduced, leading to ongoing bone destruction ***(Borjesson et al. 2013).***

Herbal derivatives are widely used by middle-aged women to manage menopausal symptoms due to their beneficial effects, availability and low side effects ***(Farshbaf-Khalili et al., 2022).*** Chia(*Salvia hispanica* L.), is an annual herbaceous plant belonging to the family *Lamiaceae* ***(Grancieri et al., 2019).*** Chia seeds contain healthy ω-3 fatty acids, polyunsaturated fatty acids, dietary fiber, proteins, vitamins, and some minerals (such as iron and calcium). Besides this, the seeds are an excellent source of polyphenols and antioxidants such as caffeic acid, rosmarinic acid, myricetin, quercetin and others ***(Silva et al., 2017 and Hrnčič et al., 2020).*** These compounds have beneficial effects on the human body including anti-inflammatory, antioxidant effects and improvement of the blood lipid profile***(Hamedi et al., 2016; Scapin et al., 2016 and Kulczynski et al., 2019).***

Therefore, this study was conducted to investigate the anti-osteoporotic activity of chia seeds powder (CSP) consumption onosteoporosisof ovariectomized rats.

Materials and Methods

Materials

Dried chia seeds were purchased from Egyptian local market. Chemicals, casein, cellulose, choline chloride, D-L methionine, vitamin and mineral constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soy oil, and sucrose were obtained from the Egyptian local market. Thirty-five adult female albino rats (Sprague Dawley strain), weighing about 220-230 g b.wt. were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

Methods

Preparation of Chia Seeds Powder:

The seeds were washed and dried, then grinded using a coffee grinder into a fine powder till used for both chemical analysis and for preparation of supplemented diet.

ChemicalAnalysis of Chia Seeds Powder:

Chemical composition, phenolic compounds and 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical-scavenging activity of CSP were conducted at Food Safety and Quality Control Lab, Faculty of Agriculture, Cairo University, Egypt.

The major of chemical composition such as carbohydrates, protein, fats, fiber, moisture and ash was determined according to ***AOAC, (2012).***

Phenolic compounds were determined by HPLC according to ***Agilent, (2014).***

DPPH radical-scavenging activity was conducted according to ***Brand-Williams et al., (1995).***

Fatty acids content wasdetermined at Food Technology Research Institute, Giza, Egyptby gas chromatography according ***toISO, (2017).***

Surgical Procedure of Ovariectomy:

The bilateral ovariectomy in rats was performedat Department of Surgery, Faculty of Veterinary Medicine, Cairo university, Giza, Egypt. The surgery procedure was described by ***Shalaby, (1977).***

Diet Preparation and Experimental Animal Design:

The basal diet was prepared according to AIN-93M diet ***(Reeves et al., 1993).*** Thirty-five adult female albino ratswere

housed in well conditions and fed on basal diet in Research Labs, Agricultural Research Center, Giza, Egypt. After one week of acclimatization, the rats were randomly divided into five equal groups (7 rats of each) as follow:

Group 1 was sham-operated (SHAM) fed on basal diet as -ve control, and the other four groups were ovariectomized (OVX) and left for 3 weeks post-operation to ensure almost complete clearance of their bodies from any sex hormone residues.

Group 2 was left as OVX control and fed on basal diet.

Groups3 was OVX and fed on basal diet supplemented with 2.5% of CSP.

Group 4 was OVX and fed on basal diet supplemented with 5% of CSP.

Group 5 was OVX and fed on basal diet supplemented with 10% of CSP.

During the experiment period (5 weeks), the quantities of diet, which were consumed and/or waste, were recorded every day. In addition, rat's weight was recorded weekly to determine body weight gain and feed efficiency ratio according to ***Chapman et al., (1959).***

Biochemical Analysis of Serum:

At the end of the experimental period (5 weeks), rats were fasted overnight before sacrificing and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtainserum for biochemical analysis. Serum AST and ALT were determined according to the method described by ***Young, (2001),*** and ALP was determined according to ***Roy, (1970).*** Interleukin-1 beta, malondialdehyde and glutathione peroxidasewere determined according to ***Dinarello, C. (1996); Draper and Hadley, (1990)*** and ***Hissin and Hilf, (1970*)**,** respectively. Serum total calcium and ionized calcium were assessed according to ***Gosling, (1986)*** and***Jafri et al., (2014),*** respectively.

Determination of Phosphorus and Calcium in Bone Tissue:

After sacrificing of rats, both femurs were removed immediately for bone analysis. Thereafter the femurs were dehydrated and defatted in acetone and anhydrous ether, dried for 6 h in a muffle furnace at 700°C to obtain the bone ash for estimation of calcium and phosphorus contents in ash using colorimetric method described by ***El-Merzabani et al., (1977).***

Histopathological Examination of Liver Tissue:

Liver tissue specimens were fixed in 10% saline, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4-6 µm thick. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H&E) for histopathological examination through the electric light microscope ***(Bancroft et al., 1996).*** The hepatic tissue sections were examined under a light microscope (Olympus CX31, Ireland) by an experienced pathologist blinded to the groups, and test materials.

The frequency and severity of lesions in the livers were assessed semi-quantitatively as previously reported by ***Plaa and Charbonneau, (1994)*** using a scale where, grade 0: No apparent injury, grade I: Swelling of hepatocytes, grade II: Ballooning of hepatocytes, grade III: Lipid droplets in hepatocytes and grade IV: Necrosis of hepatocytes.

Statistical Analysis:

Results were expressed as the mean ± standard error (x± SE). Data were statistically analyzed for variance “ANOVA” test at $P \leq (0.05)$ using SPSS statistical software, version 20 according to ***Armitage and Berry, (1987).***

Results and Discussion

Chemical composition of chia seeds powder (CSP) was recorded in **Table 1**. The data indicated that chia seeds contained fat, fiber, protein, carbohydrate, ash and moisture at 31.42, 25, 16.50, 16.08, 5 and 6%, respectively.

Results of chemical composition were nearly similar to those reported by***Segura-Campos et al., (2014); Cotabarren et al., (2019); Coelho et al., (2019) and Lara et al., (2021).*** The previous authors demonstrated thatchia seeds possess excellent nutritional value as they are good source of carbohydrates, fats, proteins, ash and dietary fiberswith contents of 41, 30, 23, 4 and 18-30%, respectively. Chia seeds can play a vital role in eradication and minimization of health disorders such as cardiovascular diseases and diabetes due to its high fiber content ***(Oliva et al., 2021).***Chia seeds possess excellent balance of amino acid containing high concentration of cysteine, lysine and methionine as compared to the primary cereals to help minimize the issues related to protein energy malnutrition ***(Pereiraet al., 2019).*** Another prominent characteristic of chia seeds is the absence of gluten which is helpful to prepare gluten-free products for celiac disease ***(Motta et al., 2019).***

The chemical analysis of fatty acids content in CSP usinggas chromatography was depicted in **Table 2**. Data showed that chia seeds contained polyunsaturated fatty acids (PUSFAs), monounsaturated fatty acids (MUSFAs) and saturated fatty acids (SFAs). The PUSFAs were alpha-linolenic acid (ALA) in 38.97% and linoleic acid (LA) in 21.08%.Percentages of MUSFAs were 14.31% for oleic acid, 0.41% for gondoic acid and 0.17% for palmitoleic acid.The SFAs were palmitic, stearic, arachidic, heptadecenoic, behenic, margaric and myristic acidswith percentages14.61, 8.22, 0.87, 0.24, 0.18, 0.17 and 0.12%, respectively.

Many researches on the phytochemicals have been reported, highlighting that the major constituents of chia seeds are polyunsaturated fatty acids [PUFAs: α-linolenic (ω-3 fatty acid) and linoleic (ω-6 fatty acid) acids]***(Silva et al., 2016).Ciau-Solís et al., (2014); Das, (2018) and Di Marco et al., (2020)*** reported that high content of PUFA from chia seeds with a value of64-68% to ω-3 and a value of 19% to ω-6 fatty acid, these findings were nearly similar to the present study.***Campos et al., (2016) and Kulczynski et al., (2019)*** stated that the chemical composition of each product can vary due to different factors such as year of cultivation, environment of cultivation, and extraction method used.

PUFAs are essential for human health, but cannot be synthesized by the human body itself, and should be received them by the diet***(Villanueva-Bermejo et al., 2019).*** Omega 3 is an important fatty acid of long-chain-PUFA. These fatty acids are increase being used in the prevention and treatment of several cardiovascular risk factors, regulate the nervous system, blood pressure, hematic clotting, glucose tolerance. In addition, their potential anti-inflammatory and antioxidant activity may provide health benefits and performance improvement especially in those who practice physical activity due to their increased reactive oxygen production***(Carrillo et al., 2018 and Gammoneet al., 2019).***

As shown in **Table 3,** HPLC- analysis identifiedthe phenolic acids and flavonoids. Data demonstrated that CSP contained myricetin, chlorogenic acid and quercetin with values of 75.19, 41.90 and 38.06 mg, respectively, followed by vanillic acid 12.12 mg and o-coumaric acid 11.59 mg. Resvertol, kampherol, ellagic, syringic acid, p- hydroxy benzoic acid, caffeic acid and rutin were identified in the polyphenolic fractions of CSP in meagerly amount.Thus, the DPPH radical-scavenging activity was assayed to investigate the antioxidant potential of chia seeds. Data in **Table 4** indicated that CSP recorded

higher DPPH radical scavenging activity with 87.24% in the high tested level 10% of sample compared with 5% and 2% of sample that recorded 61.72% and 46.50% of antioxidant activity, respectively.
The most important and widely investigated chemical property of polyphenolic compounds is their antioxidant activity.***Firtin et al., (2020) andDinet al., (2021)*** reported that myricetin, chlorogenic acid, quercetin, caffeic acid and kaempferol recorded the highest contents which known with their antioxidant and anti-inflammatory properties, these findingswere adapted by the present results.

Results in **Table 5** showed that the mean value of feed intake increased in OVX control rats when compared with the sham control rats. Theovariectomy caused a significant increase (*P<0.05*) in body weight gain (BWG) and feed efficiency ratio (FER) when compared to the sham control group. The mean value of BWG was 50.33 g in OVX control group versus to 30.20 g in sham control group.Supplementation with CSP to OVX rats decreased the feed intake, BWG and FER when compared with the OVX control group.
It is well-known that ovarian hormones such as estrogen are involved in the control of feed intake and body weight that related to increased adiposity which attributable to the modification of central responses to metabolic hormones ***(Sloan et al., 2018 and Costa-Beber et al., 2021).*** Results of FI and BWG confirmed by***Sharma et al., (2017); Xu and López, (2018)andBurch et al., (2022)***who reported that ovariectomy caused an escalation in gain of feed intake and body weight due to loss of estrogen.

Mihafu et al., (2020) reported thatadministration of ground chia seeds decreased weight gain in experimental groups, as found in the present study, so chia seeds can be consideringa good candidate for weight loss. As found in the present study, chia seeds rich in fibers which have high viscosity leading to gel formation in gastrointestinal tract ***(Capitani et al., 2012)***and may act as food additives for feelingsatiety, as demonstrated by ***Vuksan et al.,***

(2010). On the other hand, chia seeds have high content of omega-3 that can help to reduce obesity by suppressing appetite, improving lipid oxidation and energy expenditure and reducing fat deposition***(Buckley and Howe, 2010).***

Table 6 illustrated that ovariectomy caused a significant decrease (*P<0.05*) in serum total calcium and ionized calcium when compared with sham control group. CSP administrated to OVX rats with two levels 5 and 10% significantly increased (*P<0.05*) in total calcium and ionized calcium when compared to sham control group. As recorded in **Table 7**,the bilateral ovariectomy in rats induced significant decrease (*P<0.05*) in phosphorus (P) and calcium (Ca) levels of bone tissue when compared to negative control group (SHAM group). OVX rats that treated with CSP increase in P and Ca levels of femur bone tissue when compared with positive control group.

The lowest concentration of serum total calcium and ionized calcium in OVX rats as found in the present results were confirmed by ***AL-bdeery et al., (2018).*** Estrogen is the most potent inhibitor of osteoclastic bone resorption, so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis ***(Oršolić et al., 2018). de Barboza et al., (2015) and Rathod et al., (2015)*** reported that decrease of estrogen in the blood caused reduction in concentration of calcium and ionized calcium which has an important role in the absorption of calcium from the intestine and absorption at the kidney.

Ca and P are widely used as markers for bone formation as they have a vital role in bone mineralization **(*Zhang et al., 2021).***On the other hand,***Mustafa et al., (2018)*** demonstrated that the bilateral ovariectomy in rats produced decreases in Ca and P levels in the femur bone when compared to the SHAM group, as depicted in the present study. Ovariectomy causes accumulation of ROS with

subsequent oxidative stress and in turn promotes the production of cytokines, as IL-1β which cause osteoclast generation so increasing bone mineral loss ***(Chakuleska et al., 2019).***

The diet supplemented with CSP to OVX rats caused increase in bone mineral content and therefore they may effective in preventing bone loss. These findings could be attributed to high content of calcium (430 mg/100 g) and protein ***(Silva et al., 2017)*** whichmaintain healthy bones ***(Rizzoli et al., 2018).Montes et al., (2018)*** evaluated the long-term ingestion of chia seed and observed an increase in bone mineral content in rats and attributed these changes in bone structure to the alpha-linolenic acid (omega 3) content of chia seeds. Studies have suggested that polyunsaturated fatty acids intake affects bone metabolism, thus plant-sources of omega 3 have been shown to have a protective effect on bone metabolism ***(Vannice and Rasmussen, 2014 and Subramanian and Schilling, 2015). Boeyens et al., (2014)*** showed that omega 3 inhibited the formation of osteoclasts induced by the receptor-activated nuclear kappa B ligand.

Data presented in **Table 8** revealed that serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)significantly increased (*P<0.05*) in OVX control group when compared to negative control group. Chia seedspowder administrated to OVX rats decreased ALT, also this result applied to AST in the two levels 5 and 10% and applied to ALP only in 10% level whereas the two levels 2.5 and 5% showed no significant when compared to positive control group.

Results of liver enzymes were similar to that obtained by ***Grigoryan et al., (2017) and Yousefzadeh et al., (2021)*** who reported thatOVX rats had increased in serum levels of AST, ALT and ALP. On the other hand, the consumption of chia seeds could reduce liver damage by reducing its enzymes as found in the present

study that supported by ***Marineli et al., (2015)***[a]. An improvement in liver function can be attributed to the high level of omega-3 fatty acids in chia seeds ***(Valdivia-López and Tecante, 2015 and Oliveira-Alves et al., 2017).***

Results recorded in **Table 9** showed that the ovariectomy caused a significant reduction (*P<0.05*) in level of glutathione peroxidase (GPx) while caused a significant (*P<0.05*) elevation in seruminterleukin-1 beta (IL-1β) and malondialdehyde (MDA) concentrationswhen compared with the sham control group. It was observed that, there was a significant increase (*P<0.05*) in GPx level and decrease in IL-1β and MDA content and for OVX rats that treated with CSPwhen compared to positive control group.

These results were in agreement with ***Ajibade et al., (2021);Sadeghian et al., (2021) and Alasmari et al., (2022)***who demonstrated that ovariectomy induced elevated in levels of IL-1β and MDA while GPx activity was decreased.The negative effects related to reduced estrogen level may be associated with increased oxidative stressand inflammation ***(Silva et al., 2019).*** Oxidative stress, an imbalance between an excessive generation of ROS and insufficient antioxidant defense mechanisms such as GPx, induces severe damage to DNA, protein and lipids in the cells ***(Machado et al., 2021).*** Malondialdehyde is an oxidative stress marker ***(Sun et al., 2022)*** whileIL-1β inflammatory factor ***(Jenei-Lanzl et al., 2019).***

As observed, chia may reduce inflammation factor (IL-1β) resulting from ovariectomy. This benefit is consistent with antioxidants found in chia seeds, including phenolic compounds, vitamins, minerals, dietary fiber and polyunsaturated fatty acids (PUFA***)(Silva et al., 2017).Marineli et al., (2014) and Martínez-Cruz and Paredes-López, (2014)*** reported that the protective effects against oxidative stress (MDA) caused by ovariectomy might be due

to either natural antioxidants present in chia or to PUFA action, especially ALA(omega 3) acid which presented antioxidant effects by reducing lipid peroxidation formation by scavenging free radicals and restoring antioxidant enzymes such as GPx ***(Pal and Ghosh, 2012).*** ***Marineli et al., (2015)***[b] showed that phenolic compounds and antioxidants in chia seeds caused an increasein GPx activity in OVX rats, as found in the present study. The main phenolic compounds found in chia are rosmarinic acid, quercertin, myricetin, kaempferol, caffeic acid, and galic acid***(Oliveira-Alves et al., 2017 and Pelegrini et al., 2018).*** These compounds provide benefits to the body due to the presence of hydroxyl groups that are readily oxidized to produce the corresponding O-quinones, which are effective scavengers of reactive oxygen species***(Fraga et al., 2010 and Tresserra-Rimbau et al., 2018).*** Phenolic compounds can also alter the recruitment of inflammatory cells, decreasing the production of pro-inflammatory mediators.

Liver tissue section of **control group (1)** showed normal hepatic lobules which are made up of radiating plate's or strands of polygonal cells with prominent round nuclei and eosinophilic cytoplasm vertical to central vein**.** Polygonal cells were joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes. Sinusoids lined by a discontinuous layer of fenestrated endothelial cells with fine arrangement of Kupffer cells. The portal area revealed normal histological structure of bile duct, portal vein and hepatic artery grade (0) **Photo 1a-b-c**.

The hepatic lobules of experimentally OVXrats (positive group) showed disorganization of hepatic cords with marked dilatation of both central and portal veins **Photo 2a-b**. Histopathologically, the changes in hepatic parenchyma were characterized by vacuolation of hepatocytes located near the liver capsule and around the terminal hepatic venules. Pyknosis and lytic

changes of hepatocytes nuclei were also seen. Apoptosis of hepatocytes which appeared as deeply eosinophilic apoptotic bodies contained nuclear fragments. Few numbers of intracellular fat droplets were noticed. Also, massive area of coagulative necrosis was seen. Hyperplasia of Kupffer cells, widening of hepatic sinusoids and few number of mononuclear cells infiltration mainly lymphocytes and macrophages were seen grade (IV) **Photo 3a-b**.

The hepatic lobules of experimentally ovariectomized (OVX) rats and treated by 2.5% chia seeds (group 3) revealed histological picture resembling to group 2. Marked dilatation of both central and portal veins were seen **Photo 4a-b**. Hepatic lobule showed pyknosis and lytic changes of hepatocytes nuclei. Apoptosis of hepatocytes which appeared as deeply eosinophilic apoptotic bodies contained nuclear fragments. Few numbers of intracellular fat droplets were noticed. Hyperplasia of Kupffer cells, dilatation of hepatic sinusoids with few numbers of mononuclear cells infiltration mainly lymphocytes and macrophages were seen grade (IV) **Photo 4-c**.

The hepatic lobules of experimentally OVXrats and treated by 5% chia seeds (group 4) showed moderate improvement as compared with group 3; most of the central veins appeared normal and mild dilatation of portal vein with normal organization of hepatic lobules**Photo 5a-b**. The hepatocytes showed low-grade of the cellular swelling and narrowing of hepatic sinusoids with hyperplasia of Kupffer cells grade (I)**Photo 5c**.

The hepatic lobules of experimentally OVX rats and treated by 10 % chia seeds (group 5). The histological picture of hepatic lobules appeared similar to group that treated by chia seeds 5%. The hepatic parenchyma showed normal appearance central vein and mild dilatation of portal veins **Photo 6a-b**. The hepatocytes showed low-

grade cellular swelling, the necrotic areas and hyperplasia of Kupffer cells disappeared grade (I) **Photo 6c**.

The abnormalities of histology analysis of liver tissue are mainly attributed to the lack of estrogen's hepato-protection. Estrogen has been proven to be very crucial to hepatocyte functionality as it promotes mitochondrial function, cellular immunity, and antioxidant capacity ***(Wang et al., 2015).*** Menopausal changes and estrogen deficiency result in mitochondrial dysfunction. Numerous studies reported that estrogen receptors are found in the hepatic mitochondria. Mitochondrial dysfunction leads to disrupted cell membrane permeability, cellular aging with the profound cessation of cellular growth and eventually cellular death ***(Chen et al., 2005).*** Moreover, an abrupt decline in liver antioxidant capacity occurs in menopause which lead to elevation in lipid peroxidation ***(Moorthy et al., 2005).*** Interestingly, estrogen deficiency has also been linked to the potentiation of inflammatory processes in different bodily organs ***(Davis et al., 2013).***

On the other hand, the obtained results were in the same context of results found by ***(ElYamany, 2020)*** who revealed that consumption of 5% chia seeds by diabetic rats showed a slight hydropic degeneration of hepatocytes. Another study by ***(Silva et al., 2019)*** concluded that concentrations of inflammatory markers in female rats could be decreased by chia seeds consumption.

Conclusion and Recommendations

Chia seeds powder are rich source in beneficial polyunsaturated fatty acids especially omega-3 (alpha-linolenic acid, ALA). HPLC analysis of CSP revealed that it contained many phenolic acid and have potent antioxidant properties. Chia seeds exhibit an antioxidant activity and effective in reducing bone minerals loss in ovariectomized rats. The study recommends that

intake of chia seeds may be beneficial for the treatment of postmenopausal oxidative stress and osteoporosis due to estrogen deficiency.Isolation of bioactive constituents from chia seeds is necessary to search for safe natural antioxidants instead of the least safety chemical antioxidants.

Table (1): Chemical composition of chiaseeds powder

Compounds	g/100g
Fat	31.42
Fiber	25.00
Protein	16.50
Carbohydrate	16.08
Ash	5.00
Moisture	6.00
Total	100

Table (2): Fatty acids content of chiaseeds powder

Fatty acids content	%
Myristic acid (C14:0)	0.12
Palmitic acid (C16:0)	14.61
Palmitoleic acid (C16:1)	0.17
Margaric acid (C17:0)	0.17
Heptadecenoic acid (C17:1)	0.24
Stearic acid (C18:0)	8.22
Oleic acid (C18:1)	14.31
Linoleic acid (C18:2)	21.08
Alpha-linolenic acid (C18:3)	38.97
Arachidic acid (C20:0)	0.87
Gondoic acid (C20:1)	0.41
Behenic acid (C22:0)	0.18
Unknown	0.64
Total	99.99

Table (3): Polyphenolic compounds concentration of chiaseeds powder

Polyphenolic content	mg/kg
p- Hydroxy benzoic acid	2.00303
Chlorogenic acid	41.90659
Vanillic acid	12.12012
Caffeic acid	1.14116
Syringic acid	3.12422
Rutin	0.32529
Ellagic acid	6.32090
o- Coumaric acid	11.59691
Resvertol	9.11955
Quercetin	38.06856
Myricetin	75.19528
Kampherol	8.38951
Total	209.31109

Table (4): The antioxidant activity (DPPH) of chiaseeds powder

Sample	%DPPH Radical-Scavenging Activity
2 %	46.50
5%	61.72
10%	87.24

Table (5): Effect of chia seeds powder on feed intake (FI), body weightgain (BWG) andfeedefficiency ratio(FER) of ovariectomized rats

Parameters / Groups	FI (g/d)	BWG (g)	FER
G1: -ve Control (Sham group)	16.60	30.20±5.32^{d}	0.052±0.005^{c}
G2: +ve Control	19.59	50.33±7.69^{a}	0.073±0.004^{a}
G3: 2.5% CSP	18.50	45.00±2.74^{b}	0.069±0.001^{a}
G4: 5% CSP	18.00	41.86±3.94^{b}	0.066±0.002^{b}
G5: 10% CSP	17.10	35.94±1.62^{c}	0.060±0.008^{b}

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (6): Effect of chia seeds powder on serum total calcium (TCa) and ionized calcium (ICa) of ovariectomized rats

Parameters / Groups	TCa	ICa
	Pg/ml	
G1: -ve Control (Sham group)	53.33±6.98^{a}	44.67±3.76^{a}
G2: +ve Control	10.34±1.45^{d}	07.33±1.20^{d}
G3: 2.5% CSP	13.67±3.38^{d}	09.00±1.06^{d}
G4: 5% CSP	17.67±1.76^{c}	11.67±2.73^{c}
G5: 10% CSP	24.00±1.52^{b}	18.01±1.53^{b}

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (7): Effect of chia seeds powder on bone tissue phosphorus (P) and calcium (Ca) of ovariectomized rats

Parameters / Groups	P	Ca
	Ppm	
G1: -ve Control (Sham group)	8461.54±150.36[a]	1624.29±75.26[a]
G2: +ve Control	8001.57±172.44[c]	1115.33±69.64[c]
G3: 2.5% CSP	8222.29±165.29[b]	1189.65±89.24[b]
G4: 5% CSP	8259.97±201.09[b]	1195.98±74.33[b]
G5: 10% CSP	8278.72±139.98[b]	1212.23±91.08[b]

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (8): Effect of chia seeds powder on serum AST, ALTand ALP of ovariectomized rats

Parameters / Groups	AST	ALT	ALP
	mIU/ml		pg/ml
G1: -ve Control (Sham group)	100.33±14.48[c]	6.00±0.74[c]	550.33±70.67[b]
G2: +ve Control	131.50±01.67[a]	25.63±3.55[a]	577.60±22.46[a]
G3: 2.5% CSP	123.79±19.50[a]	16.11±01.66[b]	570.83±28.37[a]
G4: 5% CSP	118.66±11.14[b]	16.00±02.90[b]	568.80±29.00[a]
G5: 10% CSP	111.17±07.69[b]	14.03±01.04[b]	559.47±11.39[b]

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Table (9): Effect of chia seeds powder on serum GPx, MDA and IL-1β of ovariectomized rats

Parameters / Groups	GPx U/ml	MDA ng/ml	IL-1β pg/ml
G1: -ve Control (Sham group)	136.00±4.58[a]	124.33±11.69[d]	257.37±21.33[d]
G2: +ve Control	81.30±12.70[d]	456.33±16.50[a]	426.33±13.83[a]
G3: 2.5% CSP	94.90±7.64[c]	419.67±10.93[b]	391.40±15.01[b]
G4: 5% CSP	95.23±5.55[c]	416.67±10.49[b]	381.17±17.12[b]
G5: 10% CSP	124.43±18.35[b]	370.67±07.69[c]	363.57±16.03[c]

*Mean values are expressed as means ± SE.

*Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

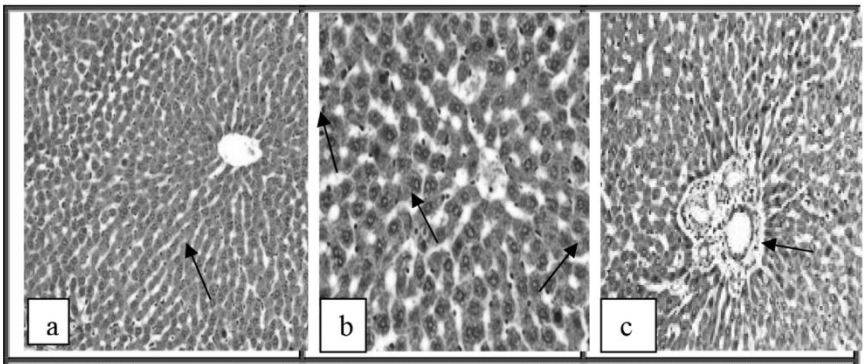

Photo 1 Photomicrograph of Liver tissue from **group (1)** showing **(a)** normal histological structure of hepatic lobule **arrow** (H&E x100) **(b)** polygonal cells with prominent round nuclei and eosinophilic cytoplasm vertical to central vein **arrow** (H&E x200) **(c)** normal histological structure of bile duct, portal vein and hepatic artery **arrow** (H&E x100)

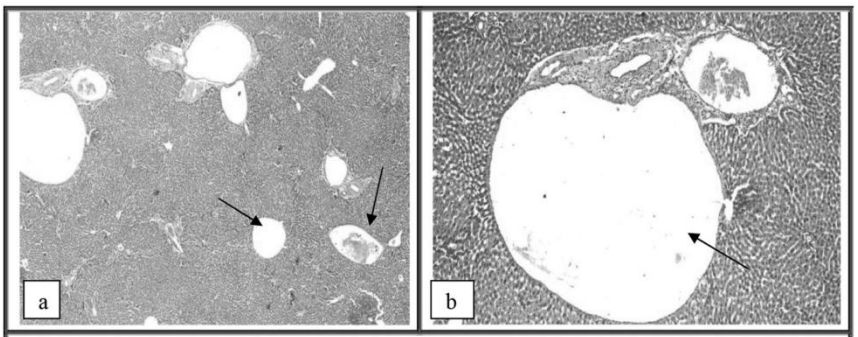

Photo. 2 Photomicrograph of Liver tissue from **group II** showing **(a)**disorganization of hepatic cords with marked dilatation of central vein **arrow** (H&E x40) **(b)**marked dilatation of portal vein **arrow** (H&E x100)

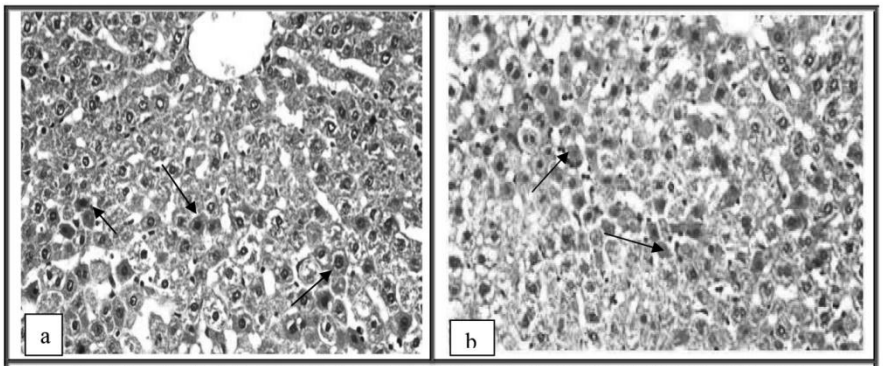

Photo 3 Photomicrograph of Liver tissue from **group (2)** showing **(a)** Pyknosis and lytic changes of hepatocytes nuclei **arrow** (H&E x200) **(b)** Apoptosis of hepatocytes which appeared as deeply eosinophilic bodies contained nuclear fragments and intracellular fat droplets **arrow** (H&E x200)

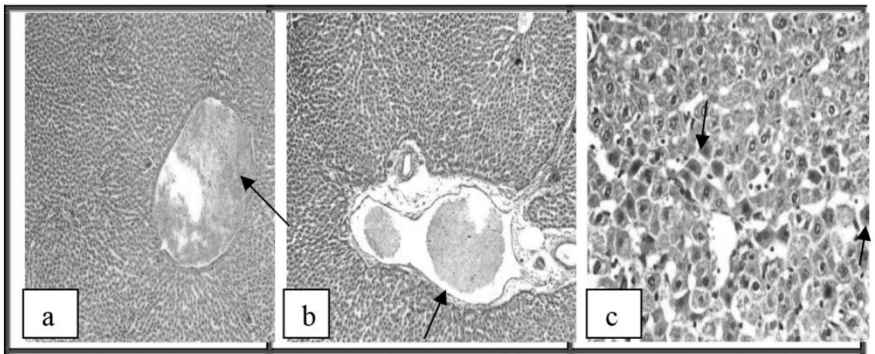

Photo 4 Photomicrograph of Liver tissue from **group (3)** showing **(a)**dilatation of central vein **arrow** (H&E x40) **(b)** dilatation of portal vein **arrow** (H&E x100) **(c)** Apoptosis of hepatocytes and mononuclear cells infiltration **arrow** (H&E x200)

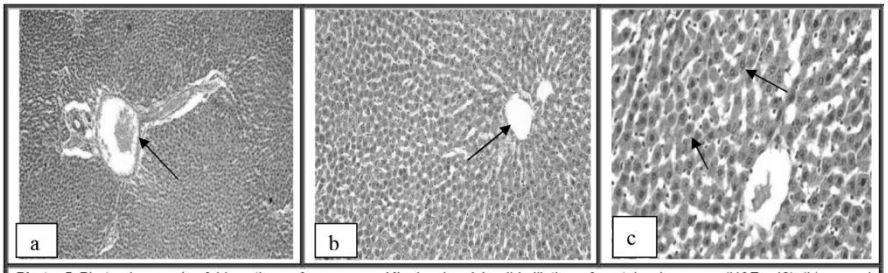

Photo 5 Photomicrograph of Liver tissue from **group (4)** showing **(a)** mild dilation of portal vein **arrow** (H&E x40) (b) normal organization of hepatic lobule with normal appearance of central vein **arrow** (H&E x100) (c) mild cellular swelling and hyperplasia of Kupffer cells **arrow** (H&E x200)

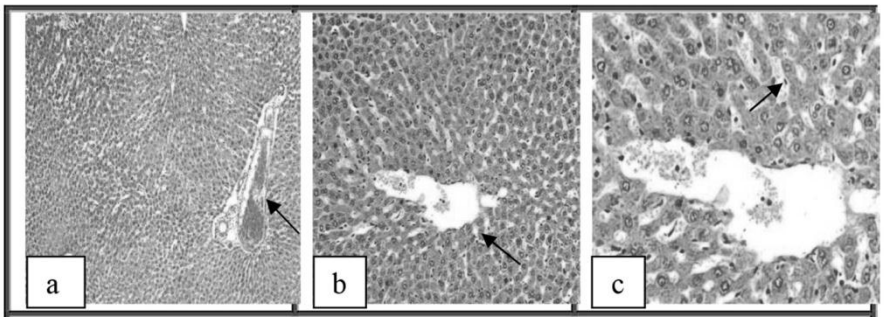

Photo 6 Photomicrograph of Liver tissue from **group (5)** showing **(a)** mild dilation of portal vein **arrow** (H&E x40) (b) normal appearance of central vein **arrow** (H&E x100) (c) mild cellular swelling**arrow** (H&E x200)

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تأثير بذور الشيا على هشاشة العظام فى الفئران مستأصلة المبيض

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قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلى - جامعة حلوان

الملخص العربى

استهدف هذا البحث دراسة التأثير المضاد لهشاشة العظام لمسحوق بذور الشيا في الفئران مستأصلة المبيض باستخدام التحليل الكيميائي لبذور الشيا وتحليل الكيمياء الحيوية في الدم وأنسجة العظام للفئران. وقد تم استئصال المبيضين تحت تأثير مخدر الإيثيلي. وتم توزيع خمسة وثلاثين من إناث الفئران بشكل عشوائي إلى 5 مجموعات متساوية كل منها 7 فئران. وتم إجراء العملية الجراحية فى المجموعة الأولى بدون استئصال المبيضين واستخدمت كمجموعة ضابطة سالبة، وتم استئصال المبيضين فى فئران المجموعات الأربعة الأخرى. وبعد ثلاثة أسابيع من إجراء العملية الجراحية عندما كان تركيزمالوندهيد مرتفعًا كعلامة حيوية في مصل الدم ، بدأت التجربة. استخدمت المجموعة الثانيةكمجموعة ضابطة موجبة (مستأصلة المبيضين) ، وتم تغذية المجموعات الثالثة و الرابعة و الخامسة على مسحوق بذور الشيا بجرعات 2.5 و 5 و 10 ٪ على التوالي لمدة 5 أسابيعبجانب النظام الغذائي الأساسي. وتم وزن الفئران مرتين في الأسبوع وتم حساب زيادة الوزن. جمعت عينات الدم للتحليل الكيميائي الحيوي وأخذ عظام الفخذ لتقدير دلالات أنسجة العظام. أظهرت نتائج التركيب الكيميائي لبذور الشيا أن كل 100 جرام يحتوي على 31.42٪ دهون ، 25٪ ألياف ، 16.50٪ بروتين و 16.08٪ كربوهيدرات. ولقد كانت المكونات الرئيسية للدهون هي الأحماض الدهنية غير المشبعة وبشكل رئيسي حمض ألفا لينولينيك بنسبة 38.97٪ وحمض اللينوليك بنسبة 21.08٪. كما تم تقدير المركبات الفينولية ومنها ميريستين 75.19 مجم يليه حمض الكلوروجينيك 41.9 مجم وكيرسيتين 38.06 مجم بواسطة HPLC. كان النشاط المضاد للأكسدة 87.24 ٪ في عينة 10 ٪المختبرة. أظهرت نتائج الدراسة البيولوجية أن إعطاء مسحوق بذور الشياللفئران مستأصلة المبيض خفض منوزن الجسم المكتسب، ومستويات انترلوكين 1 بيتا في الدم ، مالونديالديهايد ، ومستويات انزيمات الكبد. ومن ناحية أخرى تم زيادة مستويات الجلوتاثيون بيروكسيديز في الدم والكالسيوم الكلي والكالسيوم المتأين ومعادن عظام الفخذ (الكالسيوم والفوسفور). وتدل النتائج أن لبذور الشيا نشاطًا مضادًا للأكسدة وفعالًا في تقليل فقد معادن العظام في الفئران مستأصلة المبيض. وتوصي الدراسة أن تناول بذور الشيا قد يكون مفيدًا في علاج السيدات المصابات بهشاشة العظام بعد انقطاع الطمث.