Therapeutic effect of chia seeds (Salvia Hispanic L.) on rats with obesity and osteoporosis

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Abstract:

The aim of this study was to investigate the ant-osteoporosis and obesity effects of Chia seeds powder. 25 female rats each weighting170 ±5 g was apportioned to five groups. The first group is the negative control group was fed a standard diet while the other groups were given a high-fat diet and injection by dexamethasone (7mg/kg b. wt) once a week for up to four weeks to induce obesity and osteoporosis. The osteoporosis and obese rats were classified into four groups, the positive control group was kept untreated, while three other groups received chia seed treatments at 3%, 6%, and 9% levels in diet for 60 days. This study resulted showed that all treated groups significantly improved their BWG, BWG%, feed intake, FER, BMI, parathyroid hormone (PTH), calcium and phosphorus serum, CH, TG, LDL, VLDL, bone mineral density (BMD) and bone mineral concentration (BMC). They are also all significantly increased their calcitonin and HDL levels. Our findings show that powdered chia seeds can reduce obesity and osteoporosis risk factors.

Keywords: Chia Seeds, Overweight, Brittle bones, Cortisol drug, female rats, high fat diet and Bone Mineral Density

INTRODUCTION

Obesity is a complex and multifaceted health disease that affects individuals all across the world. It is a chronic metabolic condition caused by a mix of genetic, environmental, dietary, and lifestyle factors. The World Health Organization (WHO) considers a body mass index (BMI) of 25 or above to be overweight, and 30 or higher to be obese. Excess adiposity is a substantial risk factor for morbidity and mortality from type 2 diabetes, cardiovascular disease, osteoporosis, and some cancers. Global trade

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liberalization, rapid urbanization, and economic development have all led to an increase in overweight, obesity, and other chronic illnesses throughout the world (Lan and Sulaiman, 2024).

Osteoporosis is a progressive skeletal disorder that raises the risk of fracture by reducing bone strength, density, and quality. It is linked to decreased bone mineral density as well as the loss of biomechanical and structural characteristics that are essential for preserving bone homeostasis (Hsu et al., 2020).

Dexamethasone (DEX) is a type of glucocorticoid (GCs) extensively used as a treatment of allergic disorders, ulcerative colitis, arthritis, pulmonary disorders, and organ transplantation, owing to its potent anti-inflammatory and immunomodulatory effects (Vandewalle et al., 2018).

Chemicals present in plants and their extracts have been used for ages to treat a variety of illnesses due to their proven medicinal properties. Despite global improvements, numerous pathologies are still treated using plant-based medications, which are often utilized as substitutes for contemporary treatments (Dzobo, 2022).

Chia is commonly known as (Salvia Hispanic L.) an oil seed plant, it has several functional components. The importance of these elements might lower the risk of chronic illnesses, such as disorders of the gastrointestinal system, cardiovascular disease (CVD), osteoporosis, and different forms of cancer. Fiber, protein, phytosterols, omega-3 fatty acids, polyphenols, vitamins, and minerals all lower the risk of heart disease by regulating blood pressure, bad cholesterol, and platelet aggregation. Parts of chia seeds lower blood glucose levels and enhance beta-cell function in the GI tract, which lowers type 2 diabetes. Furthermore, chia seeds can help avoid constipation because of their high fiber content, which gives stool volume. However, antioxidants and phenolic portions of these seeds enhance oxidation and contribute in lowering the risk of many forms of cancer. Components of chia seeds may be added in the future to enhance the nutritional content and shelf stability of a variety of food products, including meat and bakery goods (Khalid et al., 2023)

Because of chia's invaluable nutraceutical benefits, it has come to be known as the "seed for the first 21st century". Chia is known as the "new

golden and super seed," offering a number of health benefits (Orona-Tamayo and Paredes-López 2024)

The objective of this study was to investigate the activity curative role of chia seed powder after using the dexamethasone and high-fat diet to induced osteoporosis and obesity female rats

MATERIALS and METHODS

MATERIALS

Chia Seeds: (Salvia Hispanic L.) was obtained from the Agriculture Research Center, Giza, Egypt.

Dexamethasone (DEX): sodium phosphate was purchased as ampules (8mg/2ml) from Amriya Pharmaceutical Industries Alexandria, Egypt.

Animals

Twenty-five female of albino rats (Sprague Dawley) weighting (170 ±5 g), obtained from Experimental Animal House in Agricultural Research Center, Giza, Egypt. The animals were kept under normal laboratory conditions for seven days before experiment and fed one week on standard diet (14% casein (protein >80%), corn oil 4%, choline chloride 0.25%, vitamin mixture 1%, salt mixture 3.5%, fiber 5% and the remainder corn starch) for adaptation according to (NRC 1995) and water ad libitum.

METHODS

Preparation of chia seeds powder

Seeds of chia were thoroughly checked to remove any impurities and then they were ground into a powder. Whole seed powder was saved in well-closed, opaque glass jars in the refrigerator to prevent lipid oxidation until used in diet preparation Barakat et al., (2022).

Induction of obesity

Obesity was induced in rats by fed on a high-fat diet [casein 14% (protein >80%), Choline chloride 0.25%, vitamin mixture 1.0%, salt mixture 3.5%, fibers 5%, L-Cystine 0.18%, sucrose 10%, 20% fat (19% saturated fat 19 g of butter oil + 1% unsaturated fat 1 g of soybean oil), bile salts (0.25%) and the reminder was corn starch] for 4 weeks according to Liu et al., (2004)

Induction of osteoporosis by dexamethasone

Osteoporosis was induced in rats by intramuscular injection of DEX at a dose (7mg/kg b. wt) once a week for up to four weeks according to Thakur et al., (2013)

Animal groups

Twenty-five female of albino rats were randomly divided into five groups (n=5/group) as follow:

- Group 1: Negative control group (-ve) doesn't receive any treatment.
- Group 2: Positive control group (+ve) induced to obesity and osteoporosis rats' group without treatment.
- Group 3: Treated group with 3% chia seeds powder of basal diet.
- Group 4: Treated group with 6% chia seeds powder of basal diet
- Group 5: Treated group with 9% chia seeds powder of basal diet.

After the end of the 60 days (experiment duration), rats were subjected daily to a physical examination for observation of their general body condition such as external appearance, the color of hair, fatigue and lethargy, abnormal movements, and discomfort walking weekly change in body weight gain (BWG) and feed efficiency ratio (FER) were monitored. Food intake (gm.) was determined weekly according to Chapman et al., (1959). All rats were killed by overdose chloroform, and then blood samples were collected from the eye of the venous plexus using a capillary tube in clean, dry centrifuge tubes. Then separate the serum using a centrifuge at 4000 rpm for 10 min and kept at 18 °C until analysis according to El-Refai et al., (2015). All the biological experimental procedures were applied in accordance with internationally guidelines for the care and use of laboratory animals .Ethical guidelines were maintained during animal handling and permission was obtained from the Home Economics Department, nutrition and food science, Mansoura University, Egypt, under animal protocol code No (R/14).

Minerals of chia seeds:

According to Bettinelli et al., (2000) the minerals concentration was determined using Inductivity Coupled Plasma (iCAP™ 7000 Plus Series ICP-OES, Thermo Scientific™) after acid digestion using HNO3 (69%) and H2O2 (30%) in a microwave digestion apparatus (model Milestone MLS 1200 Mega).

Biological estimations

Determination of (BWG), (BWG%), Food intake, (FER) and body mass index (BMI)

Body weight gain (BWG) and Feed intake (gm.) were monitored weekly. feed efficiency ratio (FER) was determined according to Chapman et al., (1959). The body mass index (BMI) was described by Angelova et al., (2013).

Body weight gain (BWG) and feed efficiency ratio (FER) were calculated:

Body weight gain = $\text{Final weight (g)} - \text{Initial weight (g)}$

Body weight gain $BWG(\%) = \frac{\text{final weight(g)} - \text{intial weight(g)}}{\text{intial weight(g)}} \text{x}100$

Feed efficiency ratio (FER) = Body weight gain (g) / Food intake (g) / Number of days of experiment

$$\text{Body Mass Index (BMI)} = \frac{\text{body weight (g)}}{\text{length (cm)}^2}$$

Determination of parathyroid hormone (PTH), calcitonin, serum Ca and P

Parathyroid hormone was determined by immunoenzymatically assay for the in vitro quantitative measurement of human intact parathyroid hormone (PTH) in serum produced by Biosource Europe SA according to Bouillon et al., (1990). Calcitonin was determined by the ratio immunoassay system described by Hackeng et al., (1970). Calcium and phosphorous were assessed by using a colorimetric method in serum according to Tietz, (1970),

Determination of lipid profile

Serum total cholesterol was determined according to Jain et al., (2017), Serum Triglycerides was determined according to Tietz, (1995), Serum high-density lipoprotein cholesterol (HDLc) was determined according to Lopes et al., (1977), Low-density lipoprotein cholesterol (LDLc) and very low-density lipoprotein cholesterol (VLDLc) were calculated by using the method of Friedewald et al., (1972).

Determination of bone mineral density (BMD) and bone mineral concentration (BMC)

At the end of the experiment, the femurs (left) were separated after scarring. The femurs were preserved in formalin. Bone mineral density (BMD) and bone mineral concentration (BMC) were measured using a GE Lunar bone densitometer (DEXA) provided by GE Healthcare (Chicago, IL, USA) using dual-energy X-ray absorptiometry at the National Research Center - Medical Research Excellence Center (MRCE), Giza, Egypt. They were expressed in g/cm². All samples were measured 3 times, and the mean values were calculated according to Chen et al., (2016).

Statistical analysis:

The collected data were presented as means with standard deviations (means±S.D.), statistical analysis was performed using one-way analysis of variance (ANOVA), and the means between groups were compared using the least significant difference (LSD) statistic test. All tests were completed using the computer program of the statistical analysis program (SPSS, version 24, 2016), according to Armitage and Berry (1987)

RESULT AND DISCUSSION

Minerals of chia seeds

The data in Fig. (1) showed that the minerals of chia seeds contains calcium, potassium, magnesium, phosphorus, aluminum, iron, sodium, and zinc with amounts of 630.08±4.43, 627.55±4.57, 354.08±3.46, 146.80±3.90, 85.51±2.05, 37.19±0.07, 28.51±0.05, 7.72±0.12, mg/100g respectively.

These findings are similar to those reported by Knez Hrnčič et al., (2019), who identified magnesium (335) and calcium (631) in Chia seeds (Cs) on a dry weight basis.

According to Anwar et al., (2024), chia seeds include potassium (659), magnesium (309.6), iron (37.73), and zinc (5.1).

Chia seeds are an excellent source of minerals, which are inorganic nutrients necessary for the upkeep of life's physicochemical processes (Prathyusha et al., 2019).

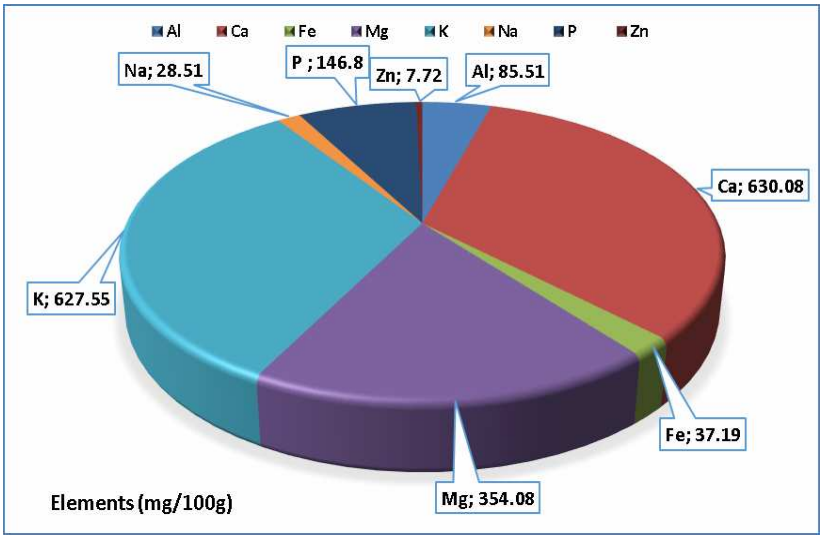

Fig. 1: Minerals of chia seed

Biological estimations

Effects of chia seeds on Body weight gain (BWG), Body weight gain percent (BWG%), Food intake, Feed efficiency ratio (FER) and Body mass index (BMI)

Data in Fig. (2) showed body weight gain, body weight gain percent, food intake, feed efficiency ratio and body mass index

The control positive group (+ve) showed significant increase in BWG, BWG%, food intake, FER, and body mass index (BMI) compared with the control negative group (-ve).

The groups treated with chia seeds powder 3%, 6%, and 9% showed significant decrease in weight gain, weight gain percent and FER.

The groups treated with chia seeds powder 9% showed significant decrease in food intake except the group treated with chia seeds powder 3%

and 6% showed non-significant when compared with the control positive group (+ve).

And the treated groups showed significant decrease in body mass index except the group treated with chia seeds powder 3% showed non-significant when compared with the control positive group (+ve)

The groups treated with chia seeds powder 3%, and 6% showed significant increase in BWG, BWG%, and food intake while showed non-significant in FER. The group treated with chia seeds powder 9% showed non-significant in BWG, BWG% and FER and showed significant increase in feed intake. The groups treated with chia seeds powder 6%, and 9% showed non-significant while the group 3% showed significant increase in BMI when compared with the control negative group (-ve).

The best value of BWG at group treated with chia seeds 9% at (66.00) followed by, 6% at (70.33) followed by, 3% (74.67 g/ml) respectively.

The best value of BWG% at group treated with chia seeds 9% at (38.75%) followed by, 6% at (41.56%) followed by, 3% (43.86%) respectively.

The best value of food intake at group treated with chia seeds 9% at (21.11) followed by, 6% at (21.79) followed by, 3% (22.19g/ml) respectively.

The best value of FER at group treated with chia seeds 9% at (0.052) followed by, 6% at (0.053) followed by, 3% (0.056g/ml) respectively.

The best value of BMI at group treated with chia seeds 6% at (0.554) followed by, 9% at (0.568) followed by, 3% (0.628g/cm2) respectively.

According to Mihafu et al., (2020), giving ground chia seeds to experimental groups resulted in weight reduction; as a result, chia seeds may be a viable option for weight loss. High-viscosity, high-fiber chia seeds have the potential to gel the gastrointestinal system. (Capitani et al., 2012) and, as Vuksan et al., (2010) showed it may function as food additives for satiety.

Conversely, chia seeds' high omega-3 content can reduce obesity by reducing fat deposition, raising energy expenditure and lipid oxidation, and reducing appetite (Buckley and Howe, 2010).

Another study discovered that rats' body weight had decreased somewhat but significantly (Fernández Martínez et al., 2019).

These results are consistent with those of Pai and Prabhu (2019), who found that consuming fiber increases satiety, which in turn decreases excess food consumption and promotes weight reduction. Research by Quaresma et al. (2023) shows that both of the chia groups had significant decreased waist circumference, weight, and body mass index but non-significant between the experiment and control negative group.

According to Roy et al., (2022), chia is generally high source of sterols, fatty acids, phenolic, and dietary fiber, which have proven the potential to optimize metabolism, induce satiety, promote anti-obesity, and utilized as nutritional therapy for several chronic degenerative.

These results agree with those of Pai and Prabhu (2019), who discovered that eating more fiber enhances satiety, which reduces overindulgence in food and encourages weight loss. According to research by Quaresma et al., (2023), there was no significant difference between the experimental and control negative groups, but there were substantial decreases in weight, body mass index, and waist circumference in both of the chia groups.

Roy et al., (2022) state that chia is often high in polyphenols, fatty acids, sterols, and dietary fiber. These nutrients have been shown to have the ability to enhance metabolism, cause satiety, encourage weight loss, and be used as nutritional treatment for a number of chronic degenerative diseases

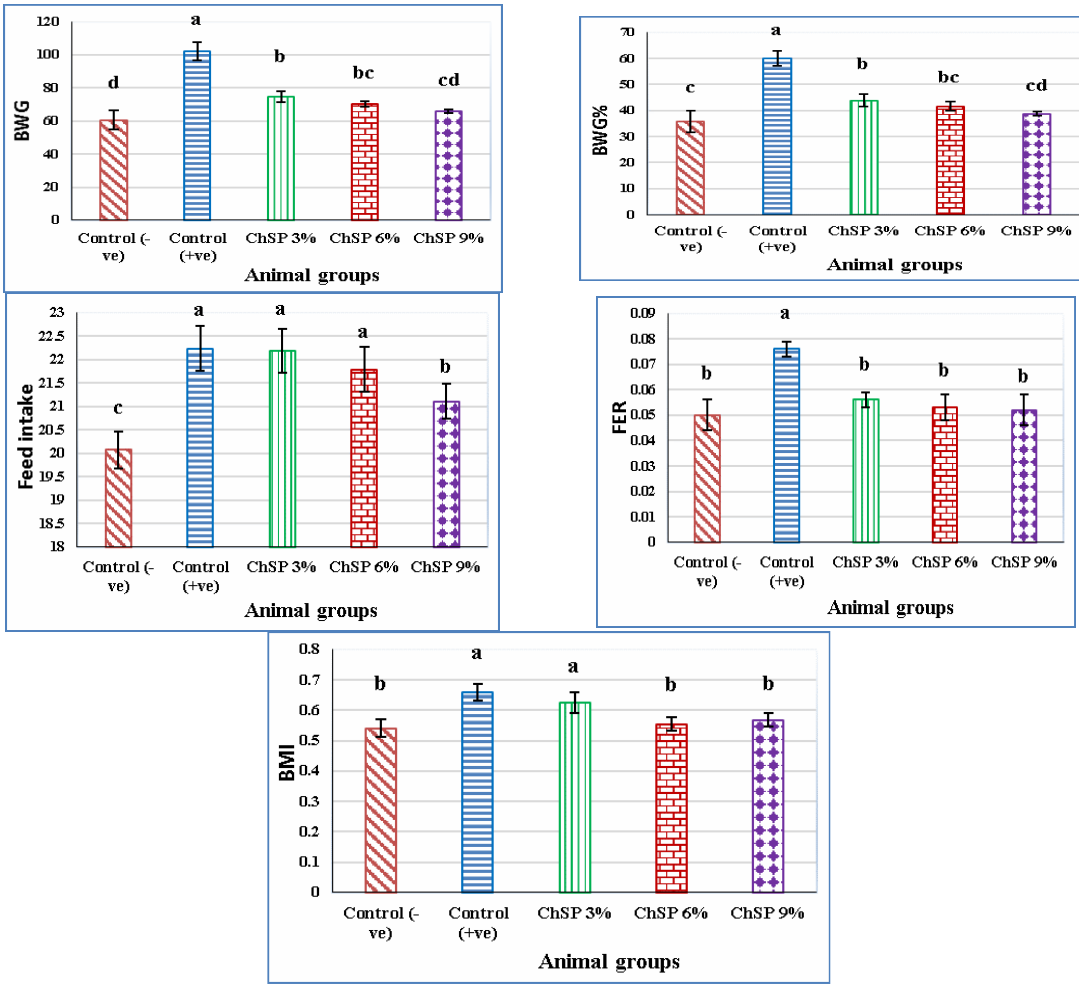

Fig. 2: Effects of chia seeds on (BWG), (BWG%), (FER), Feed intake and BMI

Effects of chia seeds on of parathyroid hormone (PTH), calcitonin, serum Ca and P

Data in Fig. (3) Showed parathyroid hormone (PTH), calcitonin, serum Ca and P

The control positive group (+ve) showed significant increase in (PTH) while showed significant decrease in Ca, P and calcitonin compared with the control negative group (- ve).

The groups treated with chia seeds powder 3%, 6%, and 9% showed significant decrease in Ca, P and Calcitonin while showed significant increase in PTH compared with the control negative group (-ve).

The groups treated with chia seeds powder 3%, 6%, and 9% showed significant decrease in (PTH) at (47.50, 44.80, and 40.73) respectively while showed significant increase in calcitonin at (2.41, 3.07, and 3.82) respectively and Ca at (8.77, 9.12 and 9.71) respectively and P at (5.34, 5.97 and 6.68) respectively when compared with the control positive group (+ve).

This result confirming the studies of (AL-bdeery et al., 2018), who determined that the lowest concentration of serum total calcium in rats as found in the present results in the control negative group (- ve)

Ca and P are extensively utilized as indicators for bone development, as they play an important role in bone mineralization. (Zhang et al., 2021)

The acquired data supported the findings of (Da Silva et al., 2017 and Rizzoli et al., 2018), who found that the diet supplemented with CSP to rats induced an increase in bone mineral content, suggesting that they may be helpful in preventing bone loss. These findings might be related to the high level of calcium (430 mg/100 g) and protein, which maintain healthy bones.

Montes et al., (2018) studied the long-term intake of chia seeds and discovered an increase in bone mineral content in rats, attributing these changes in bone structure to the alpha-linolenic acid (omega 3) content of the seeds.

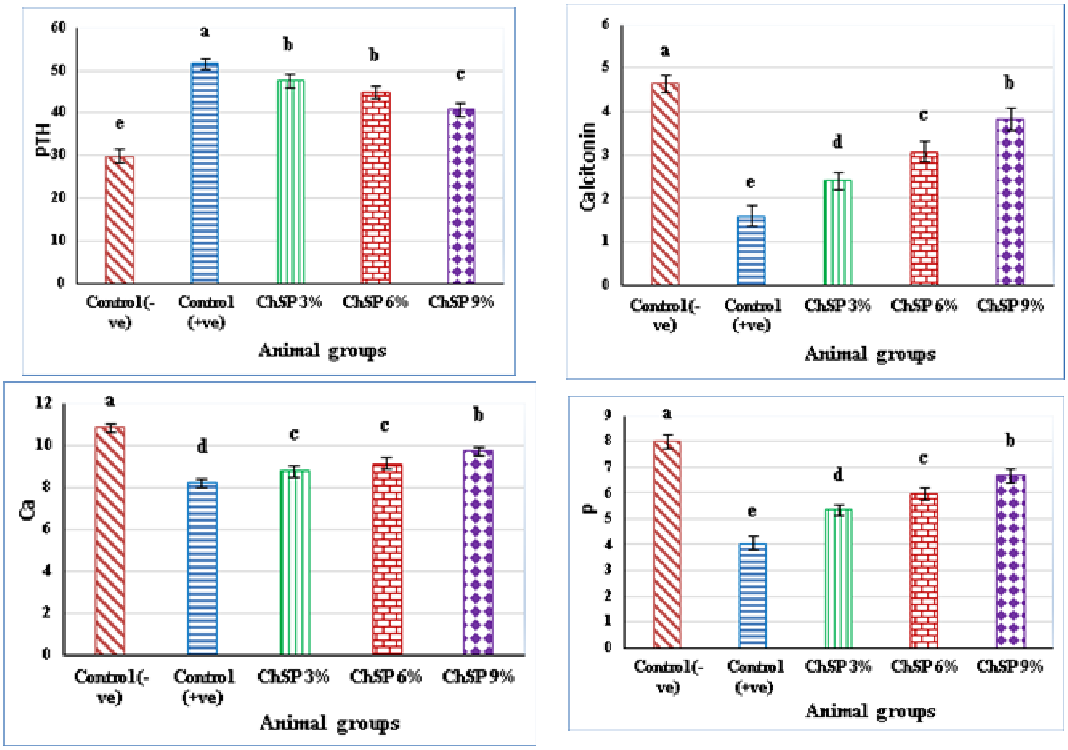

Fig. 3: Effects of chia seeds on PTH, calcitonin, serum Ca and P

Effect of chia seeds on lipid profile in experimental rats

Data in fig. (4) Showed lipid profile CH, TG, HDLc, LDLc and VLDLc.

The control positive group (+ve) showed significant increase in CH, TG, LDLc and VLDLc while showed significant decrease in HDLc compared with the control negative group (-ve).

The groups treated with chia seeds powder 3%, 6%, and 9% showed significant decrease in CH, TG, LDLc and VLDLc while showed significant increase in HDLc compared with the control positive group (+ve).

The groups treated with chia seeds powder 3%, 6%, and 9% showed significant increase in CH, TG, LDLc and VLDLc while the groups showed significant decrease in HDLc except the groups treated with chia seeds powder 6%, and 9% showed non-significant compared with the control negative group (-ve).

The best value of CH at group treated with chia seeds 9% at (96.67) followed by, 6% at (111.33) followed by, 3% (122.77 mg/dl) respectively.

The best value of TG at group treated with chia seeds 9% at (132.33) followed by, 6% at (165.33) followed by, 3% (195.33mg/dl) respectively.

The best value of HDLc at group treated with chia seeds 3% at (42.67) followed by, 6% at (47.00) followed by, 9% (51.67 mg/dl) respectively

The best value of LDLc at group treated with chia seeds 9% at (18.33) followed by, 6% at (31.33) followed by, 3% (41.00mg/dl) respectively.

The best value of VLDLc at group treated with chia seeds 9% at (26.67) followed by, 6% at (36.33) followed by, 3% (39.00mg/dl) respectively.

Chia seeds contain up to 65% of the oil content and are rich in omega-3 fatty acids. Omega-3 fatty acids have been linked to a number of bodily physiological processes. Thanks to their higher quantities of beneficial unsaturated fatty acids, gluten-free protein, vitamins, minerals, and phenolic compounds, chia seeds have the potential to be a strong source of antioxidants. Additionally, dietary fiber—which is fantastic for the digestive tract and for controlling diabetes mellitus and obesity—is another benefit of omega-3 fatty acid. Chia seeds possess antioxidant, anti-lipidemic, and anti-diabetic properties.

These findings are almost in line with those of Alagawany et al., (2019) and Alagawany et al., (2020), who found that consuming chia seed powder decreased the blood triglyceride levels of the treatment group. This decline might be caused by the presence of omega-3 and omega-6 fatty acids as well as the activity of chia's built-in antioxidants.

The cholesterol and LDLc levels were lowered in the groups that received chia seed powder treatment, which is consistent with the findings of (Ahmed 2019 and Alagawany et al., 2020).According to Ayerza and Coates (2005); Mahfouz (2020) and Khafagy and Samir (2022) Triacylglycerol and LDLc cholesterol levels significantly decreased in groups treated with chia seed powder, while HDLc cholesterol levels increased in rat serum. In addition, these research findings imply that Alpha-

linolenic acid in chia seed may be an alternate source of omega-3 for vegetarians and persons allergic to fish and fish products.

Because HDLc and LDLc molecules are the main carriers of cholesterol from its site of synthesis, the liver, to the body tissues, and because they subsequently reduce the amount of cholesterol and triglycerides available for tissue metabolism, lipogenesis in the liver, and fat accumulation in rats, this reduction confirmed the decrease cholesterol levels observed in this study (Alvarenga et al. 2011).

The findings were consistent with (Kulczynski et al., 2019), who established the favorable effects of CS components on lipid profile improvement. This impact might be attributed to its high ALA content, which has been associated to beneficial changes in plasma lipids.

According to Ferreira et al., (2020), chia seeds may have the ability to improve the aberrant lipid metabolism detected in rats' skeletal muscle

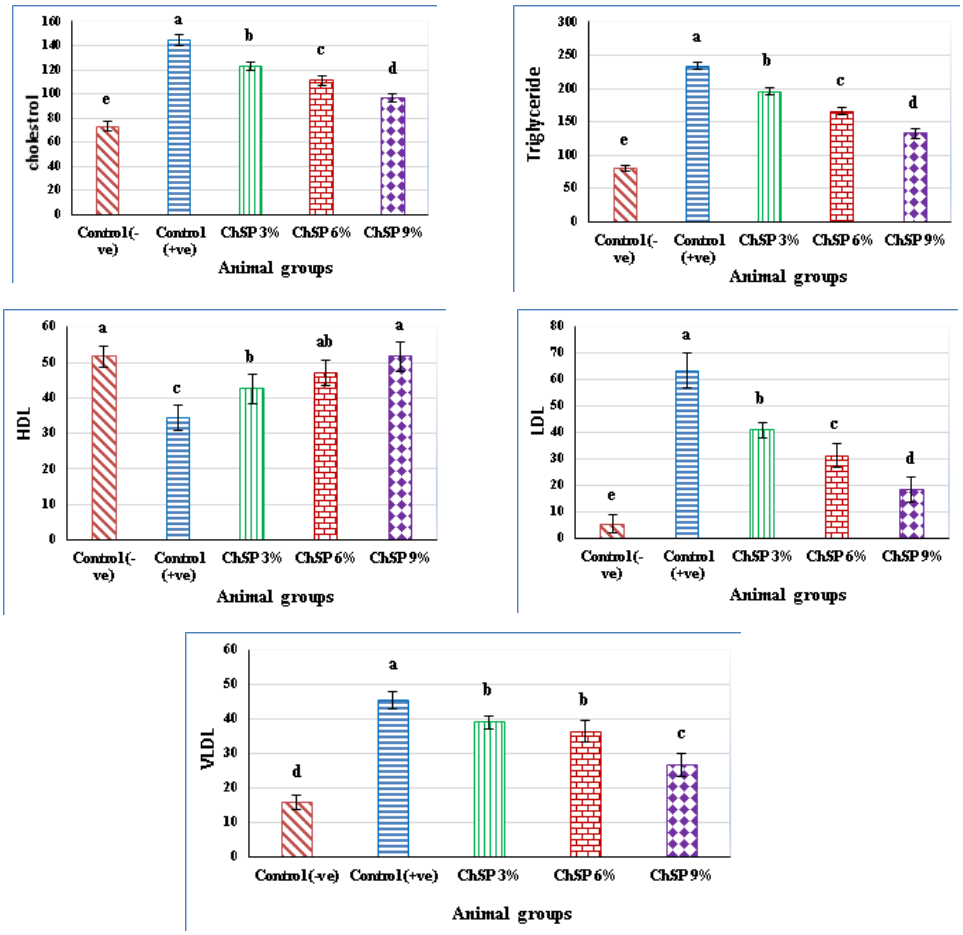

Fig. 4: Effects of chia seeds on lipid profile

Effect of chia seeds on bone mineral density and concentration

Data in figure (5) showed BMD and BMC.

The control positive group (+ve) showed significant decrease in BMD and BMC compared with the control negative group (-ve).

The groups treated with chia seeds powder 3% and 6% showed non-significant in BMD while the groups treated with chia seeds powder 9% showed significant increase compared with the control positive group (+ve).

The groups treated with chia seeds powder 3% and 6% showed significant increase in BMC while the groups treated with chia seeds powder 9% in BMC showed non-significant compared with the control positive group (+ve).

The groups treated with chia seeds powder 3%, 6% and 9% showed significant decrease in BMC while the groups treated with chia seeds powder showed non-significant in BMD except the group treated with chia seeds powder 3% showed significant decrease compared with the control negative group (-ve).

The best value of BMD at group treated with chia seeds 9% (0.243) followed by, 6% at (0.229) followed by, 3% at (0.185) respectively.

The best value of BMC at group treated with chia seeds 6% at (0.049) followed by, 3% at (0.041) followed by, 9% (0.017) respectively.

The results found to agree with Montes et al., (2018) who said that bone structures indicated by the BMC and BMD determinations of the total body skeleton and the total and proximal areas of the left tibia, used as an optimal area for bone measurement in adult and aged rats and chia seeds contain a high percentage of calcium.

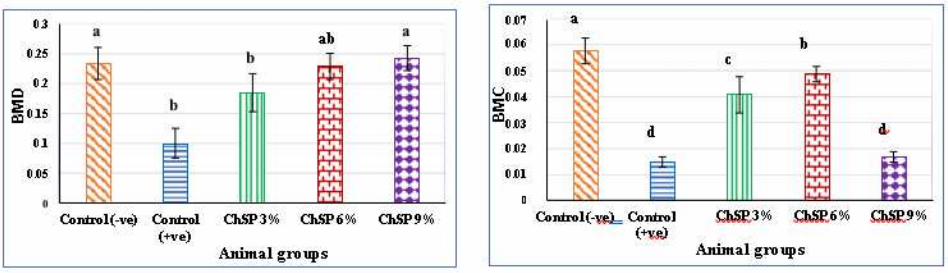

Fig. 5: Effect of chia seeds powder on BMD and BMC

CONCLUSION

Finally, we propose include chia seeds in diets for individuals who are exposed to obesity and osteoporosis since they have the power to reduce weight, fat, and increase minerals such as calcium in the body to avoid osteoporosis

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التأثير العلاجي لبذور الشيا على الفئران المصابة بالسمنة وهشاشة العظام

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الملخص العربي:

السمنة هي تراكم غير طبيعي أو مفرط للدهون التى قد تؤثرعلى الصحة، بينما هشاشة العظام هي حالة تتميز بفقدان الكتلة العظمية، مما يؤثر على العديد من الإناث وتعد بذور الشيا مصدر جيد للمغذيات والمعادن والفيتامينات التي يمكن استخدامها في النظام الغذائي لتجنب أمراض العظام و السمنة ، وقد أجريت الدراسة الحالية على خمسة وعشرين فأر من إناث الفئران وزنها (١٧٠ ± ٥ جم). قسمت الي خمس مجموعات اتخذت المجموعة الأولى مجموعة كنترول سلبية والتي تغذت على الغذاء الأساسي فقط في حين تم تغذية المجموعات من (٢- ٥) بنظام غذائي عالي الدهون و وحقنهم بعقار ديكساميثازون مرة واحدة في الأسبوع بجرعة (٧ ملجم / كجم وزن الجسم / الفئران) لمدة أربعة أسابيع للحث على الاصابة بالسمنة وهشاشة العظام ، اتخذت المجموعة الثانية مجموعة كنترول إيجابية (ve+) بدون علاج بينما تم تغذية المجموعات الثالثة و الرابعة و الخامسة على نظام غذائي أساسي مكمل ببذور الشيا ٣٪ و ٦٪ و ٩٪ من الوجبة لمدة ٦٠ يومًا وفى نهاية التجربة أظهرت النتائج أن جميع المجموعات المعالجة تحسن معنوي في وزن الجسم و نسبة تناول الغذاء و FER ومؤشر كتلة الجسم وهرمون الغده الدرقية (PTH) ونسبة الكالسيوم والفسفور فى السيرم و CH و TG و LDL و VLDL وكثافه المعادن فى العظام BMD وتركيز المعادن فى العظام BMC ، بالإضافة إلى ظهور زيادة ملحوظة فى مستويات calcitonin و HDL مقارنة بمجموعة الكنترول الموجبة . يتضح من النتائج أن مسحوق بذور الشيا له تأثيرات مفيدة على عوامل خطر السمنة وهشاشة العظام. لذلك يوصى بالاستفادة من مسحوق بذور الشيا وإمكانية استعماله في تحضير أغذية اقتصادية خاصة للمساعدة في التغذية العلاجية.

الكلمات المفتاحية: بذور الشيا، السمنة، هشاشة العظام، الديكساميثازون، وكثافة المعادن في العظام

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