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Research Article

EFFECT OF NUTRITIONAL VALUE OF CHESTNUT (CASTANEA SATIVA, MILL) JUICE ON INDUCED OSTEOPOROSIS IN FEMALE RATS

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A R T I C L E I N F O

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A B S T R A C T

The present study aims to study the protective effects of chestnut fruits (*Castanea sativa,*L.) such as rickets and osteoporosis in female rats. The present study carried out on thirty female albino rats (Sprague Dowely) weighing 150 ±5 g. The first group kept as negative control group which fed on standard diet only while the other five groups received injected with by methyl prednisolone acetate (0.2 mg/kg/b.wt./rats) injected subcutaneously to induce osteoporosis. One group served as positive control group while others groups treated with chestnut juice levels at 100, 200 and 300 ml/kg for six weeks. The results revealed that all of the curative groups showed significant increases in feed intake, body weight gain and femur bone/body weight, in addition to the emergence of a significant improvement in BMD, BMC, serum calcuim, ionized Ca, P serum estrogen, osteocalcin and alkaline phosphatase, reduced glutathione, catalase and hydrogen peroxide comparing to positive control group. Also nutritional values and lipid profile have been recorded. Our results demonstrate that chestnut juice have beneficial effects on osteoporosis risk factors that extend beyond the reduction of the symptoms of arthritis of the bones, called osteoarthritis.

INTRODUCTION

Chestnut (*Castanea*) of family (Fagaceae),chestnut fruits considered for many centuries as one of the most important food resources in Europe, but diseases of chestnut tree and rural depopulation caused decreased in its production (Adua, 1999).

Recently, production of chestnut increased as it is a traditional food has nutritional value. Its kernels is used rich in starch, fiber, fat and fatty acids (FA), protein and amino acids, ash, minerals (De Vasconcelos *et al.,* 2007).

Sweet chestnut (*Castanea sativa,* Mill.) is a rich source of phenolic bioactive compounds, especial tannins (Sanz *et al.,* 2010). It has a high content of antioxidants. chestnuts are also rich in carbohydrates and are a good source of essential fatty acids and minerals (Carvalho, 2018). A good source of vitamins and minerals, their high water content means the concentration of these nutrients is less (Vázquez *et al.,* 2017). Furthermore, sweet chestnut extract administration reduced oxidative stress induced by high (n-3 PUFA) and decrease PUFA oxidation; in addition, it prevented DNA damage in blood lymphocytes (Li *et al.,* 2012).

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Osteoporosis is a dangerous health problem because of lossing bone mass, it increased fracture and death-rate and the quality of life in affected women (Ozgocmen *et al*., 2012 and Golob and Laya, 2018).

Methylprednisolone is used to treat various diseases as blood disorders, allergic, cancers, eye, skin, intestinal, kidney, lung diseases, and immune system disorders. It decreases your immune system's response to these conditions and reduces symptoms such as swelling, pain, and swelling that occurs with arthritis and other joint disorders (Strupp *et al*., 2014).

This work was conducted to study the effect of chestnut fruit juice on biochemical analysis of osteoporosis; in addition to, an enhancement in calcium absorption on rats.

MATERIAL AND METHODS

Material

Chestnut was prepared by the from Local market, Kuwait. Methylprednisolone Acetate © (Depo Medrol 40 mg/ml Injection, Pfizer Co., USA).

Rats

Thirty female of albino rats (Sprague Dawley) weighting 150 ±5 g, provided from of National Research Center, Cairo, Egypt.

Methods

Minerals and vitamins composition

Chestnut fresh was analyzed for Ca, P, K, Fe, & Na and vitamins E, K, A & C contents according to (Chapman and Pratt 1978).

The induction of experimental osteoporosis: Methylprednisolone Acetate © (Depo Medrol 0.2 mg/kg/b.wt./rats Injection, Pfizer Co., USA).

Biological experiment

Thirty female albino rats (Sprague Dowely) weighing 150±5 g at the beginning of the study were used. The animals were kept under normal laboratory conditions for five days before experiment and fed one week on standard diet for adaptation according to (NRC 1995) and water ad libitum.

Experimental design

The female rats were fed on the basal diet for 7 days before starting the experiment for adaptation then the rats were classified into five equal groups (n=6 rats). Negative control (-ve) group fed on the basal diet only while the other four animals groups were administered methylprednisolone acetate dose /rate of 0.2 mg/kg/b.wt. injected subcutaneously injection 3times/week according to Rajerdi Kashani, *et al*. (2009), then classified into 4 groups one of them positive control (+ve) "untreated rats" and the other groups fed on diet containing 100, 200 & 300 g chestnut jucie "curative groups". Daily feed intake and weekly (BWG) were calculated. (FER) was determined according to the method of(Chapman *et al*., 1959). For examination of bone metabolic markers, blood was taken by puncture of orbital sinus before and after performing the protocol under diethyl ether anesthesia. The blood samples immediately were centrifuged and serum samples were stored at -70 centigrade degrees until assayed. All rats were killed by overdose chloroform at the end of 7 weeks.

Bone calcium and phosurous

Bone calcium and phosurous were analyzed using fame atomic absorption spectophotometry according to (Fraser, *et al*., 1986).

Bone mineral density (BMD) and bone mineral concentration (BMC):

The bone mineral content of lumbar vertebrae was measured in all groups by Energy X-ray absorptiometry (DEXA) using the Norland, small subject, resolution 0.5 × 0.5 mm, speed 60 mm/s, Host scanner 3.2, 3.2 and 1.1. The bone mineral density was expressed as gram of mineral per unit area of bone (gr/cm2) in Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine University of Cairo.

Biochemical assay

Serum calcium, ionized calcium and phosphatase in serum were determined by spectrophotometer using commercially available test kit (Furuichi *et al*., 2000 and Fishman, 1953). Also, osteocalcin and estrogen and bone alkaline phosphatase (BAP), alkaline phosphatase (AP) and tartarte-resistant acid phosphatase (TRAP) in serum was determined by enzyme immunoassay (Owens and Ashby, 2002 and Shoji *et al*., 2003). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG)), while (LDL-c and VLDL-c) were calculated according to the equation of (Richmod 1973, Lopes *et al*., 1977, Fossati and Prenape 1982, and Foster and Dunn 1973). Fatty acid profiles in serum total lipids were analyzed by the method of (Lepage and Roy 1986). Serum Reduced glutathione (GSH Rd), catalase (CAT), and hydrogen peroxide (HP) according to (Beutler *et al*., 1963, Aebi 1983 and Aebi, 1984) respectively.

Statistical analysis

One way analysis of variance (ANOVA), Duncan and Dunnett tests used to compare the means values between groups. Avalue of $P \leq 0.05$ was considered statistically significant (Snedecor and Cochran, 1967).

Results and Discussion

Table (1), shows that the proximate, mineral and vitamins composition of chestnut freash. The mineral composition of chestnut (Table 1) is good as all the mineral elements Ca, P, K, Fe, and Na (27.48 mg, 94.25mg, 514.05mg, 51.24mg and 3.17 mg) respectively. The vitamins C and K composition of chestnut are 46.82 mg and 194.56. µg/ g) (Table 1). These data are confirmed by (Vázquez *et al.,* 2012 and Carvalho, 2018).

Table 1 Mineral and vitamin composition of chestnut (mg/100gm fresh weight basais)

Element	Composition(mg/100gm)
Ca	27.48
P	94.25
K	514.05
Fe	51.24
Na	3.17
Vit. C	46.82
Vit. K	194.56 µg

Values are the means of 3 independent determinations.

The results in Table (2) show the mean value ± SD of feed intake, body weight gain % and femur bone/body weight of the control group and experimental groups. It could be seen that negative control group (-ve) recorded the highest value of body weight gain %, feed intake and femur bone/body weight, (19.11 ± 1.34 g, 18.54 ±1.12 g and 2.041±0.074 g), respectively. These results are in agreement with Yin *et al.,*(2011), they reported that the 300 mg/kg dosage caused a significant body weight loss in both normal and diabetic rats. There was insignificant increase in the curative groups with level chestnut (100, 200, &300 ml) respectively. While it decreased significantly in the remained of the experimental groups. On the other side, the feed intake recorded the lowest value for positive control group which exposed to medical induction of osteoporosis and without curative. The results reflected that, addition of chestnut may make diet palatable, so it enhanced the animals to eat more was significantly higher compared with the positive control group. There is no doubt that the food intake has an impact on body weight gain. Marked improvement in food intake and body weight gain occurred by groups which curative by chestnut. May be chestnut are able to reduce inflammation and may make diet palatable, so it enhanced the animals to eat more and then increase the weight (Frankic and Salobir, 2011).

These results demonstrated the correlation between feed intake increased and the increment of body weight. The results revealed that femur bone/body weight was affected in induced osteoporosis groups as there was a significant decreasing

compared to negative control which recorded the highest value of femur bone/body weight (2.041±0.074 g), then improvement observed by curative group levels of chestnut to the experimental diet as follow, (1.926±0.075 g, 1.809±0.079g, and 1.689±0.114g) for groups 15%, 10% and 5% respectively. This means that combination of chestnut help to deposition of calcium and phosphors on skeleton. Through the results show clearly that femur bone/body weight was affected by steeps of operation in experimental groups, these results may be due to a lack of calcium and phosphorus absorption rate and then lack of presence on the bone.

Table 2 Effect of levels chestnutof body weight gain, feed intake and femur bone/body weight %in female rats

Groups Variables	body weight gain %	Feed intake g/w	Femur bone/ body weight %
(-ve) control	19.11± 1.34 a	18.54 ±1.12 a	2.041±0.074 a
(+ve) control	10.69 ±1.32 c	12.05 ±1.17 b	0.615±0.118 c
100 ml chestnut	13.02 ±1.61 b	16.38±1.27 a	1.689±0.114 b
200 ml chestnut	13.80 ±1.17 b	17.27±1.21 a	1.809±0.079 b
300 ml chestnut	14.02 ±1.17 b	17.10±1.71 a	1.926±0.075 b

Values with the same letters indicate insignificant difference and vice versa

Table (3) reviews the levels of on bone mineral density (BMD), bone mineral concentration (BMC), serum calcuim, ionized Ca and Pin experimental animals. It can note that, the negative control group reflected the normal result of the aforementioned analyzes (0.163±0.006 g/cm^2, 0.297± 0.023 g/cm^2, 7.52± 2.83mg/dl, 4.22±0.31mg/dl and 5.06±1.57 mg/dl), respectively. Less proportion to the level of calcium showed positive control group which induced osteoporosis without treatment, then gradually improved significantly through groups added level 100, 200, 300 ml curative group levels of chestnut which showed the best results for the level of on bone mineral density (BMD), bone mineral concentration (BMC), serum calcuim, ionized Ca and P, especially curativegroup level of 300 ml and is the closest groups to set negative control group. Many similar studies have shown the role of chestnut in the prevention of bone lose in rats. Compared to the control osteoporosis in rats, have been found to have higher levels of trabecular bone volume, serum estradiol levels, and serum osteocalcin (Akihisa *et al*., 2010) this explains the increased level of calcium in experimental groups which curative group levels of chestnut in our study. Clearly, the role of vitamin D and C in stimulating calcium and phosphorous absorption in rich high sources of chestnut which needed for bone mineral density. These results were agreed withSahni *et al.,* (2016) who suggests that fruits reduce the severity of osteoporosis. Sroga *et al.,* (2012) studied that, have found conclusive evidence that phytoestrogens help to improve bone mass in postmenopausal.

Table 3 Effect of levels chestnut on BMD, BMC, serum calcuim, Ionized Ca and P in female rats

Groups Variables	BMD (g/cm^2)	BMC (g/cm^2)	Ionized Ca (mg/dl)	Ca (mg/dl)	P (mg/dl)
(-ve) control	0.163± 0.006 ^{a}	0.297± 0.023 ^{a}	4.22± 0.31 ^{a}	7.52± 2.83 ^{a}	5.06± 1.57 ^{a}
(+ve) control	0.0087± 0.018 ^{c}	0.153± 0.031 ^{d}	1.64± 0.59 ^{c}	4.39± 0.22 ^{c}	2.98± 0.64 ^{c}
100 ml chestnut	0.155± 0.018 ^{b}	0.286 ± 0.0045 ^{b}	2.65± 0.67 ^{b}	6.18± 0.55 ^{b}	4.33± 0.35 ^{b}
200 ml chestnut	0,159± 0.014 $^{a\,b}$	0.293± 0.0041 ^{a}	3.64± 0.83 ^{a}	6.69± 0.45 ^{a}	4.52± 0.67 ^{b}
300 ml chestnut	0.160± 0.017 ^{a}	0.295± 0.0053 ^{a}	3.94± 0.75 ^{a}	7.31± 0.46 ^{a}	4.92± 0.63 ^{b}

Values with the same letters indicate insignificant difference and vice versa
BMD:Bone Mineral Density.
BMC: Bone Mineral Concentration.

Table (4) reviews the levels of serum estrogen hormone, osteocalcin and alkaline phosphatase in experimental rats. It can note that, the negative control group reflected the normal result of the aforementioned analyzes (17.84± 4.11 mg/dl, 2.98± 0.48 mg/dl, and 93.21±7.31 u/L), respectively. Less proportion to the level of strogen hermon, osteocalcin and alkaline phosphatase showed positive control groups which induced osteoporosis without treatment, then gradually improved significantly through groups added levels of 100, 200 &300ml chestnut the mean values of estrogen hermon, osteocalcin and alkaline phosphatase, of the group treated with chestnut 300ml level recorded the best results (17.11±4.11 mg/dl , 2.82±5.13mg/dl and 92.44±7.15 u/L) and is the closest groups to set negative control group.

Table 4 Effect of levels chestnut on serum estrogen, osteocalcin and alkaline phosphatase in in female rats

Groups Variables	Estrogen (mg/dl)	Osteocalin (mg/dl)	Alkaline Phosphate (U/L)
(-ve) control	17.84±4.11 ^{a}	2.98±0.48 ^{a}	93.21±7.31 ^{a}
(+ve) control	10.19±2.45 ^{c}	1.58±1.82 ^{c}	33.64±5.49 ^{d}
100 ml chestnut	16.46±4.15 ^{b}	2.49±4.12 ^{b}	76.65±6.11 ^{c}
200 ml chestnut	16.99±4.05 ^{b}	2.62±5.01 ^{ab}	87.64±7.13 ^{b}
300 ml chestnut	17.11±4.11 ^{a}	2.82±5.13 ^{a}	92.44±7.15 ^{a}

Values with the same letters indicate insignificant difference and vice versa

Alkaline phosphates (ALP) increases if there is active bone formation occurring, ALP is a byproduct of osteoblast activity (Schiele *et al*., 1998, Qureshi *et al.,* 2010 and Frankic and Salobir, 2011). This explains the obvious change in the level of ALP in the current study, where the ratio increased as a result of induce of methylprednisolone acetate to cause osteoporosis, when chestnutwere added as a curative, clearly decrease in the level of ALP. The decline in estrogen hormone levels associated with menopausal stage led to increase risk for declining bone density and fractures. Ragerdi *et al*., (2009) and Cauley *et al*., (2013) demonstrates that (estrogen and progestin) improve (BMD) and reduces the risk of fracture.

Table (5) reviews the levels of serum total cholesterol (TC) total triglyceride (TG), High density lipoprotein cholesterol (HDL-c), Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c)in experimental rats. It can note that, the (-ve) group reflected the normal result of the aforementioned analyzes. The levels of TC, TG, HDL-c, LDL-c and VLDL-c in the positive control groups increased significantly except HDL-c, as compared to the negative control group. Then gradually improved significantly through groups added levels of 100, 200 &300ml chestnut. The highest level of chestnut showed the best results for the TC, TG, HDL-c, LDL-c and VLDL-c and is the closest groups to set negative control group. Anagnostis *et al*., (2010) suggested that vitamin D plays an important role in decreasing the risk of obesity, chronic diseases, diabetes, metabolic syndrome and cardiovascular disease.

Table 5 Effect of levels chestnut of lipids profile in female rats

Groups Variables	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
(-ve) control	119.22± 9.26 ^{e}	126.74± 11.15 ^{d}	35.45± 3.11 ^{a}	50.78± 5.16 ^{e}	25.17± 3.12 ^{c}
(+ve) control	187.88± 25.98 ^{a}	162.31± 18.19 ^{a}	24.39± ±3.01^{c}	154.01 ±6.21 ^{a}	32.76 ±4.72 ^{a}
100 ml chestnut	129.89± 13.21 ^{b}	135.26± 14.16 ^{b}	32.21± 3.11 ^{b}	86.15± 7.24 ^{b}	27.09± 4.05 ^{b}
200 ml chestnut	125.79± 16.11 ^{bc}	132.38± 15.22 ^{c}	33.66± ±4.01 ^{b}	63.71± 6.71 ^{c}	26.60± 4.07 ^{b}

300 ml chestnut	121.18± 14.29 ^{c}	129.72± 13.22 ^{d}	34.71± 4.33 ^{a}	54.27± 6.22 ^{d}	26.40± 3.01 ^{b}

Values with the same letters indicate insignificant difference and vice versa
TC: total cholesterol TG: total triglyceride HDL-c: High density lipoprotein cholesterol
LDL-c: Low density lipoprotein cholesterol
VLDL-c: very low density lipoprotein cholesterol

The results in table (6) show that the levels of serum on serum reduced glutathione, catalase and hydrogen peroxide in experimental rats. It can note that, the negative control group reflected the normal result of the aforementioned analyzes. Less proportion to the level of on serum glutathione, catalase and hydrogen peroxide showed positive control groups which induced osteoporosis without treatment, then gradually improved significantly through groups added levels of 100, 200 &300 ml chestnut and finally a combination of them and which showed the best results for the level of on serum glutathione, catalase and hydrogen peroxide, especially curative with chestnut 300ml and is the closest groups to set negative control group. Osteoporotic were under oxidative stress as their lipid peroxidation levels were elevated and antioxidant enzymes reduced (Maggio *et al*., 2013). Chestnut is a good source of very important vitamins and minerals such as vit C, vit E, D which they are acting as a free radical scavengers that protect the body from the oxidative stress and free radicals which can cause a wide damage of the organs (Das *et al*., 2018).

Table 6 Effect of levels chestnut on serum reduced glutathione, catalase and hydrogen peroxide in female

Groups Variables	GSH Rd (mg/g)	CAT (U/g)	HP (mm/g)
(-ve) control	1.45±0.06 ^{a}	57.08±4.48 ^{a}	1.21±0.06 ^{a}
(+ve) control	0.49±0.01 ^{c}	22.72±2.32 ^{c}	0.34±0.04 ^{c}
100 ml chestnut	1.26±0.05 ^{b}	49.58±3.07 ^{b}	0.99±0.04 ^{b}
200 ml chestnut	1.30±0.05 ^{b}	51.92±4.01 ^{ab}	1.09±0.03 ^{b}
300 ml chestnut	1.41±4.11 ^{a}	54.85±4.12 ^{a}	1.15±0.05 ^{a}

Values with the same letters indicate insignificant difference and vice versa
GSH Rd: glutathione CAT : catalase HP : hydrogen peroxide

CONCLUSION

It can be concluded that the consumption of level of chestnutjuice as food supplement may be considered a functional food for beneficial to bone health showing an in dependent protective association with a high bone mineral density bone mineral concentration when evaluated.

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