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ORIGINAL ARTICLE

Antiosteoporotic Effect of Fisetin in an Estrogen Deficient Model of Osteoporosis

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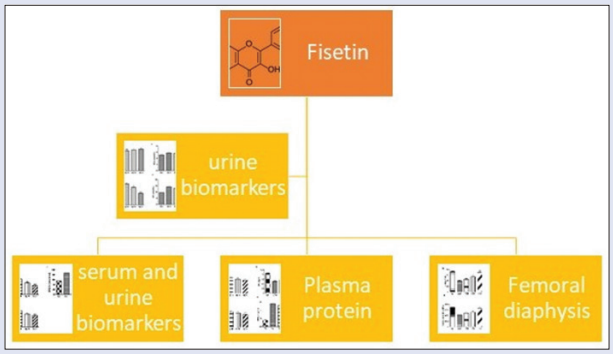
ABSTRACT

Background: Osteoporosis is a serious health problem, especially in the geriatric populations. World widely, it exaggerated the 8.9 million people every year roughly. In the current analysis, we assessed the antiosteoporotic effect of fisetin on the osteoporosis model ovariectomized (OVX) rat. **Materials and Methods:** Fisetin was orally administrated at dose of 5, 10, and 20 mg/kg to OVX rats for 16 weeks. Different biochemical parameters such as alkaline phosphatase (ALP), osteocalcin, phosphorus, calcium, and urinary deoxypyridinoline were also projected. 3-point bending test, bone mineral density (BMD), and histomorphometric feature of the femoral bone were also examined. **Results:** Fisetin significantly decreased the body weight and increased the uterine weight. A significant decrease detected in the level of ALP, serum calcium, while the level of the serum phosphorus, OC augmented after fisetin administration. Fisetin significantly ($P < 0.001$) reduced the homocysteine, C-terminal crosslinked telopeptides of collagen type I, interferon gamma, and increased the level of OC. Fisetin also augmented the level of BMD. Fisetin considerably increased the energy, maximum load, maximum stress, young modulus, and stiffness. The level of cytokines such as tumor necrosis factor-α, interleukin (IL)-6, and IL-1 β also diminished pointedly after fisetin treatment. Fisetin significantly boosted the estrogen (E_2) level and reduced the level of follicle-stimulating hormone and luteinizing hormone. **Conclusion:** Communally, we can accomplish that fisetin exhibited the better protection against osteoporosis through augmenting the bone density and bone mineral content in addition to biomechanical parameters.

Key words: Biochemical parameter, bone loss, fisetin, inflammation, osteoporosis, ovariectomy

SUMMARY

- The current study exhibited the osteoporosis effect of fisetin against the osteoporosis. Fisetin significantly altered the antioxidant, hormone level, biochemical, pro-inflammatory and bone turnover marker parameters. Fisetin significantly boost the level of endogenous antioxidant parameters. Fisetin considerably reduced the level of pro-inflammatory cytokines and showed the anti-inflammatory effect



Abbreviations used: OVX: Ovariectomised; ALP: Alkaline phosphatase; SCa: Serum calcium; SP: Serum phosphorus; OC: Osteocalcin; CTX: C-terminal telopeptide of type 1 collagen; IFN-ϒ: Interferon-gamma; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; IL-1β: Interleukin-1β; E2: Estrogen; FSH: Follicle-Stimulating Hormone; LH: luteinizing hormone; HRT: Hormone replacement therapy; SERMs: Selective estrogen receptor modulators; BMD: Bone Mineral Density; UCr: Creatinine; BUN: Blood urea nitrogen DPD: Urinary deoxypyridinoline; MDA: Malonaldehyde; T-AOC: Total antioxidant activities; SOD: Superoxide mutase

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INTRODUCTION

Bone is a connective tissue encompassing cells, fibers, and ground substance. There are numerous functions such as body formation, movement, development of new blood cells, and to hold the body weight.[1,2] Hence, most of the bone-related glitches such as rheumatoid arthritis, osteomalacia, and osteoporosis are the significant region to emphasis in the development of new drugs.[3] Osteoporosis, characterized by low content of mass in bone and bone tissue structural degradation, is one of the chief health concern topic, predominantly in women with postmenopausal, in spite of differences of racial or ethnic.[4,5] Approximately 200 million osteoporosis-affected populations were anticipated globally. At present, mainly two medical approaches for the prevention and management of postmenopausal osteoporosis

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are hormone replacement therapy (HRT) and bisphosphonate administration.[6] Although, it has been stated that long-term HRT can induce the cardiovascular disease, biliary disease, breast and endometrial cancers, and bisphosphonates which further cause atraumatic fracture in the bones.[6]

Since, the symptoms of osteoporosis are not clear and pathogenesis take longer time to display hence tough to access at early stage.[7] Osteoporosis advanced a significant risk to the health of human and eventually leads to an abridged physical function, decreased quality of life and higher rate of mortality.[8,9] Osteoporosis usually stated in older people worldwide along with the aging of people. After menopause, women are more defenseless in developing osteoporosis due to rapid bone regeneration, which is secondary to deficiency of estrogen, succeeding in higher threat of fractures.[10,11] Yet, the pathogenesis of osteoporosis is not entirely explicated by the scientist.

Currently, the therapeutic substances used in the treatment of osteoporosis act through lessening bone resorption rates, thus dipping bone loss rates, or promoting the formation of bone.[6] Various synthesize agents such as calciton in hormones, calcium, bisphosphonates, and selective estrogen receptor modulators such as droloxifene and raloxifene are employed for the management of osteoporosis[6,7,11] yet all these unified with some side effects, for example, hypercalciuria, hypercalcemia, enhanced risk of endometrial high risk, breast cancer, menstruation, breast tenderness, vaginal bleeds, and thromboembolic event.[6-8]

In many countries, several traditional and medicinal plants have been employed to protect and manage osteoporosis from many years.[12] These plant-derived natural medicines have less side effects and are preferably matched for long-term use oversynthesized medicines.[6,7] Such plant medicinal products that comprise various bioactive constituents typically presented their pharmacological effects through multiple pathways and have multitargets, which similar to the multiple factors of pathogenesis of osteoporosis.[6,13]

Preclinical works recognized the latent advantages of edible diet particularly flavonoid rich in the treatment of osteoporosis.[14] The bioactive flavonoid compound, Fisetin (3,3',4',7-tetrahydroxyflavone) [Figure 1] is polyphenolic in nature[15] which usually stated in fruits and vegetables such as apple, strawberry, onions, and cucumber. Literature familiar the potent antioxidant flavonoid nature of fisetin among various flavonoids. It covers both directintrinsic and indirect antioxidant property through elevating the glutathione level in cells of neurone.[15,16] It possess many therapeutic properties involving antioxidant, anticancer, anti-inflammatory, rheumatoid arthritis, cardioprotective, antiviral, etc.[15-18] Recently, research exposed the allergic respiratory inflammation inhibition, prevention of differentiation of adipocyte, and amelioration of diabetes complication and thwarts bone diseases. The current exploration was planned to systematically inspect the remedial role of fisetin against the ovariectomized (OVX)-induced osteoporosis in estrogen deficient model of animal.

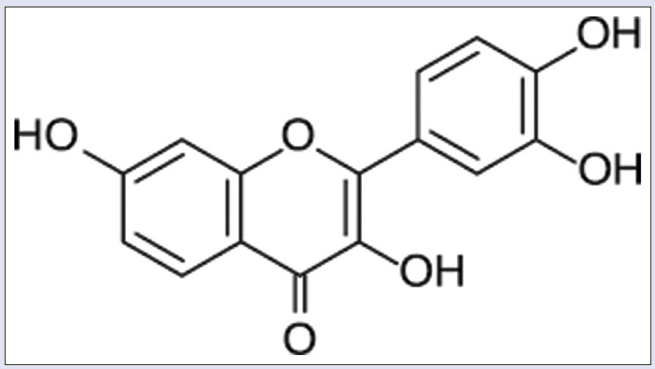

Figure 1: Structure of fiestin

MATERIALS AND METHODS

Animals

Sprague–Dawley rats, aged 20–12 weeks; weight 200 ± 20 g, sex-female were employed for the present study. The rats were obtained from the institute's animal house and housed under normal laboratory conditions. The rats were adapted in the standard laboratory condition for 7 days, before the experimental study. The whole experimental study was carried out through following the animal handling guidelines.

Experimental treatment

After acclimation, the rats were endured for the surgical procedures under the anesthesia condition through using the thiopental sodium (50 mg/kg). After successful surgery (4 weeks), the rats were alienated into five groups and each group comprises eight rats. The groups are – Sham control group – Group I; OVX control group – Group II; OVX control received fisetin (5 mg/kg) – Group-III; OVX control received fisetin (10 mg/kg) – Group IV, and OVX control received fisetin (20 mg/kg) – Group-V, respectively. Sham and OVX control group rats were received the same volume of vehicle during the entire period of experiment. All the group rats received the oral administration of treatment in the morning from the 4th week after the surgery to end of the experimental study (till 16 weeks).[19,20] During the whole experimental study, the rats received the macronutrients (0.9% calcium and 0.7% phosphate) rich diet. During the entire experimental study protocol, the body weight of the all groups rats was assessed at regular time interval. The rats were reserved in the metabolic cage and collected the urine (24 h). After completing the experimental, the rats were anesthetized through using the diethyl ether and collected the blood sample through puncturing the retro orbital plexus and centrifuged to separate the plasma.[21] Serum and urine sample were stored at – 20°C for more biochemical approximation. The organ separate out after sacrifice and weighted immediately. The uterine index was projected as uterine weight/body weights before the sacrifice the rats.[6]

Bone mineral density

For the assessment of bone mineral density (BMD), all group rats were perused before the surgery (baseline) and end of the experiment study. Dual energy-X-ray absorptiometry employed for the estimation of baseline. For the estimation of femur bone, 0.5 mm × 0.5 mm resolution and 1 mm/s speed were used. For the repositioning errors, three repeated measurements achieved, and BMD was estimated as milligrams per square centimeter (mg/cm^2).[6]

Biochemical parameters

The biochemical parameters were used for the estimation of bone turnover comprising bone-specific alkaline phosphatase (ALP), serum phosphorus (SP), and serum calcium (SCa) were valued through using the commercially kits (Boehringer Mannheim GmbH, Mannheim, Germany). Serum osteocalcin (OC) was planned through using the ELISA kit (Streptavidin technology, Boehringer Mannheim GmbH).

Urine parameters

Urine parameters such as creatinine, uric acid, blood urea nitrogen, and total protein were dogged through using the commercially kits (Boehringer Mannheim GmbH). The urinary

deoxypyridinoline (DPD) level was assessed through using the manufacture instruction (Quidel, Mountain View, CA, USA).

Plasma proteins estimations

Osteocalcin ELISA kit was employed for the assessment of OC content following the manufacture instruction (Xinqidi Bio-technology, Inc., China). Enzymatic fluorescence polarization immunoassay was employed for the estimation of homocysteine (HCY) (Abbott, Wiesbaden, Germany). C-terminal cross-linked telopeptides of collagen type I (CTX) level were valued using the ELISA kit through following the manufacture instruction (Sunbio, Inc., China).

Hormone estimation

For the measurement of hormonal level, the serum was detached from the blood through centrifugation for 10 min at 1000 ×*g* and serum samples were successfully separated out and keep in the −80°C for the approximation of hormone level. Hormone such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and E2 were valued using the radioimmunoassay (Sunbio, Inc., China).

Antioxidant enzymes

Malonaldehyde (MDA), total antioxidant activities, and superoxide dismutase (SOD) level were projected using the ELISA kits following the manufacture protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Pro-inflammatory cytokines

Serum pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1 β were assessed using the standard quantitative ELISA kits following the manufacture instruction (MultiSciences Biotech Co., Ltd., Hangzhou, Zhejiang, China).

Biomechanical testing

Bone samples were situated in a similar orientation on two support points, 19 mm apart, and the force was pragmatic by a crosshead to the femoral midshaft moving at a steady speed of 1 mm/min. Central load and displacement until fracture happened were recorded. The maximum load expressed in Newtons (N), stiffness (N/mm), energy absorption (N. mm), maximum load (megapascals or MPa), and Young's module (MPa) were assessed from the load deflection curve defined by Turner and Burr earlier.[22]

Statistical analysis

The present data were seen as mean ± standard mean error. Statistical analyses were conducted using the Student's *t*-test or a single-way variance analysis attended by the *post hoc* Dunnett test. Consider the *P* value to be the applicable.

RESULTS

Body and uterus weights

In the existing study, all rat groups presented the same mean basal body weight. The body weight of control OVX rats was significantly greater than the sham group after 4 weeks of surgery ($P < 0.001$) and remains persistent throughout the study [Figure 2]. The weights of uterus of the group treated with OVX were remarkably observed to be lower as those of the sham group ($P < 0.001$), signifying the effectiveness of the test. Fisetin did not display any major effects on the body or uterine weights of OVX-treated group rats in a dose-dependent manner.

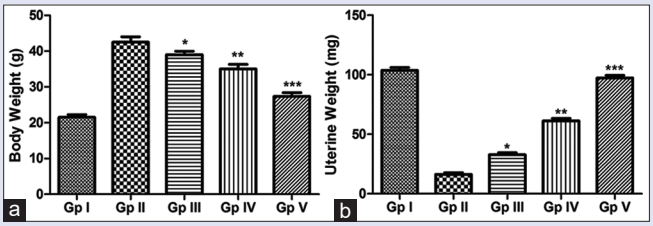

Figure 2: The effect of fisetin on the body weight and uterine weight in ovariectomised rats. (a) Body weight and (b) uterine weight. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to the ovariectomized group

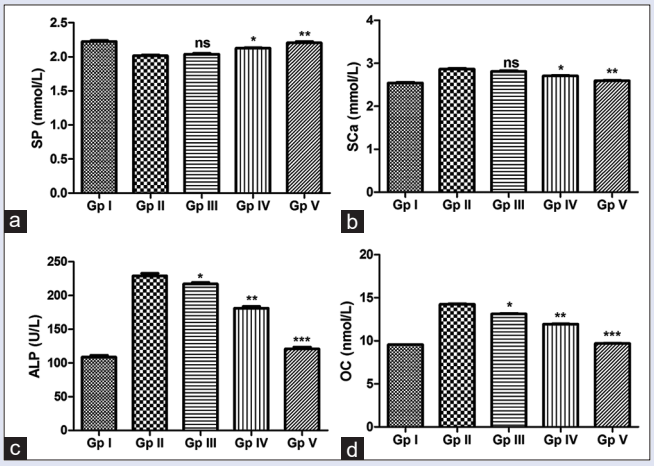

Figure 3: The effect of fisetin on the serum and urine biomarkers of ovariectomised rats. (a) Serum phosphorus, (b) serum phosphorus, (c) alkaline phosphatise, and (d) osteocalcin. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

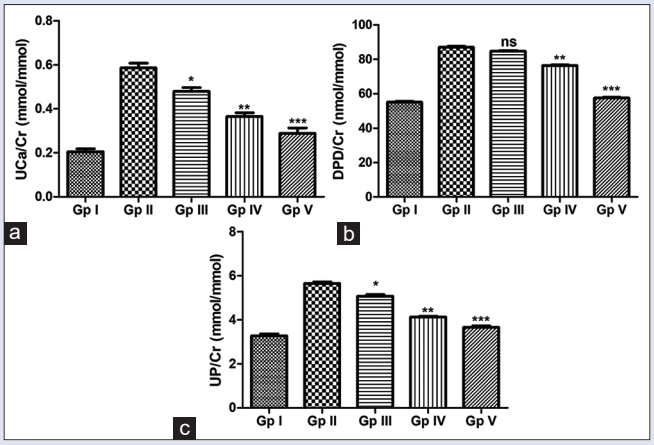

Figure 4: The effect of fisetin on the serum and urine biomarkers of ovariectomised rats. (a) Urine calcium/creatinine, (b) deoxypyridinoline/creatinine, and (c) urine phosphorus/creatinine. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

Biochemical parameters

After the treatment with fisetin and OVX, a noteworthy modification detected during the approximation of SCa and SP, whereas momentous

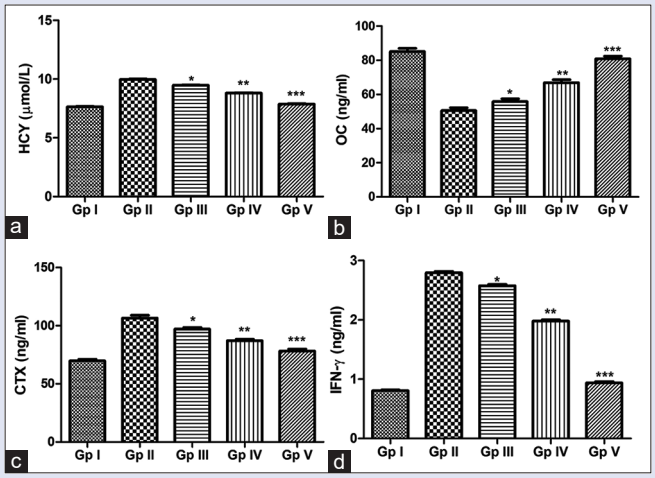

Figure 5: The effect of fisetin on the plasma protein of ovariectomised rats. (a) Homocysteine, (b) osteocalcin, (c) C-terminal crosslinked telopeptides of collagen type I, and (d) interferon-gamma. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

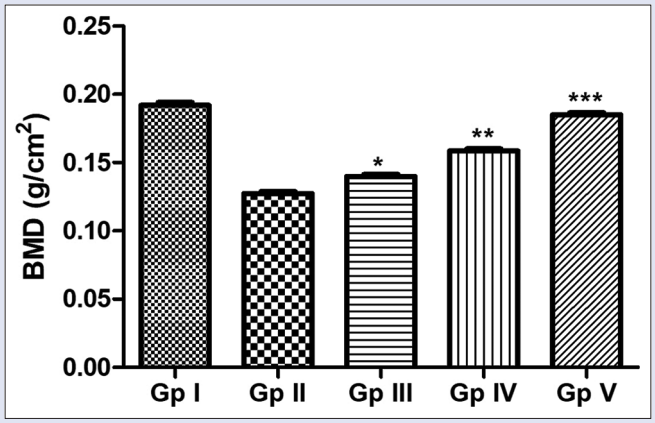

Figure 6: The effect of fisetin on the bone density of ovariectomised rats. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

elevation was found in the excretion of UCa and UP in the group treated with OVX [Figures 3 and 4]. In the OVX sample, bone-specific ALP and serum OC, two putative bone formation markers, and the ratio of DPD/Cr bone resorption markers were suggestively ($P < 0.001$) higher compared to the sham-regulated sample. UCa, UP levels, urinary DPD/Cr, OC, and serum ALP caused by OVX were significantly ($P < 0.001$) abridged by fisetin at a dose level of 20 mg/kg b.w. [Figure 3]. In a same way, lower dose of fisetin also downregulated the bone biochemical parameters but comparatively low fisetin (20 mg/kg b.w.).

Estimation of plasma proteins

The influence of fisetin treatment on level of OC was accessible in Figure 5. All dose-dependent treatment of fisetin elevated the level of OC. There were no major disparities in the content of elevated HCY in fisetin groups as compared with OVX-induced group. For the OVX group, the serum CTX levels were meaningfully ($P < 0.001$) higher than in the other groups, in turn the level was lessened by the fisetin in dose-dependent manner. In the OVX group, the level of interferon gamma (IFN-γ) was significant more than relative to the sham group. However, treatment with fisetin to OVX rats significantly ($P < 0.001$) weakened the level of IFN-γ.

Determination of bone density

In the present research, we employed the OVX rats as an experimental model of rodents with estrogen-induced osteoporosis to assess whether administration with fisetin was operative in preventing estrogen-deficient osteoporosis. Figure 6 proves the BMD, which seems to be significantly ($P < 0.001$) lower in the OVX treated rat left femoral bone as compared to the sham group. Supplementation of fisetin in OVX rats in dose-dependent manner suggestively ($P < 0.001$) enhanced the BMD level in relative to the groups OVX rats [Figure 6].

Figure 7 revealed that the deficiency in estrogen level resulted in a noteworthy suppression in the biomechanical indices of the left femoral bone of OVX rats as a contrast to the sham group. Maximum load, energy absorption, bending stiffness, maximum tension, and the Young's modulus were ominously ($P < 0.001$) abridged in OVX-treated group. Treatments with fisetin 20 mg/kg b.w. for 16 weeks have considerably re-established the OVX-convinced destruction in all the above mention indices. Fisetin at a dose level 10 mg/kg/day significantly ($P < 0.001$) enhanced the maximum load, energy to failure, and the Young's module, while fisetin (5 mg/kg) did not show any substantial progress in either of these biomechanical indicators.

Cytokines

ELISA was employed to measure the inflammatory cytokines in the serum of all groups' rats. Results of pro-inflammatory cytokines were showed in Figure 7. Levels of TNF-α, IL-1 β, and IL-6 in serum were upregulated in OVX- treated rats as compared to the sham group, whereas all these parameters were suggestively ($P < 0.001$) amended after the supplementation of the drug fisetin in a dose-dependent manner [Figure 8].

Oxidative stress status in serum and bone tissue

Figure 9 exhibited the level of ROS in serum and antioxidant profile of all group rats. The amount of MDA displayed a substantial surge in the OVX-treated rats as compared to the SHAM group. In comparison, the overall SOD activity downregulated significantly ($P < 0.001$) in OVX rats for 16 weeks in compared with SHAM group. Administration of fisetin in OVX-treated group in dose-dependent manner meaningfully ($P < 0.001$) downregulated the level of MDA in serum and induced the SOD activity in the serum.

Urine parameter

Figure 10 revealed the data and the impacts of fisetin on the urine parameters in OVX-treated rats. In the OVX group, the rates of U-Ca/Cr, U-P/Cr were noticeably higher relative to the sham group, respectively. These three doses of fisetin substantially ($P < 0.001$) obstructed dose-dependent rises in U-Ca/Cr in OVX-treated rats. Higher dose fisetin therapy (10 or 20 mg/kg/day) significantly ($P < 0.001$) impeded U-P/Cr ascend as relative to the sham group.

Serum hormone measurement

Figure 11 exemplified the level of serum hormone (E2, FSH, and LH). In OVX-treated group, the levels of E2 were downregulated relative to the sham group. On the other hand, there was noteworthy upregulation was detected in the level of serum FSH and LH in the OVX group as

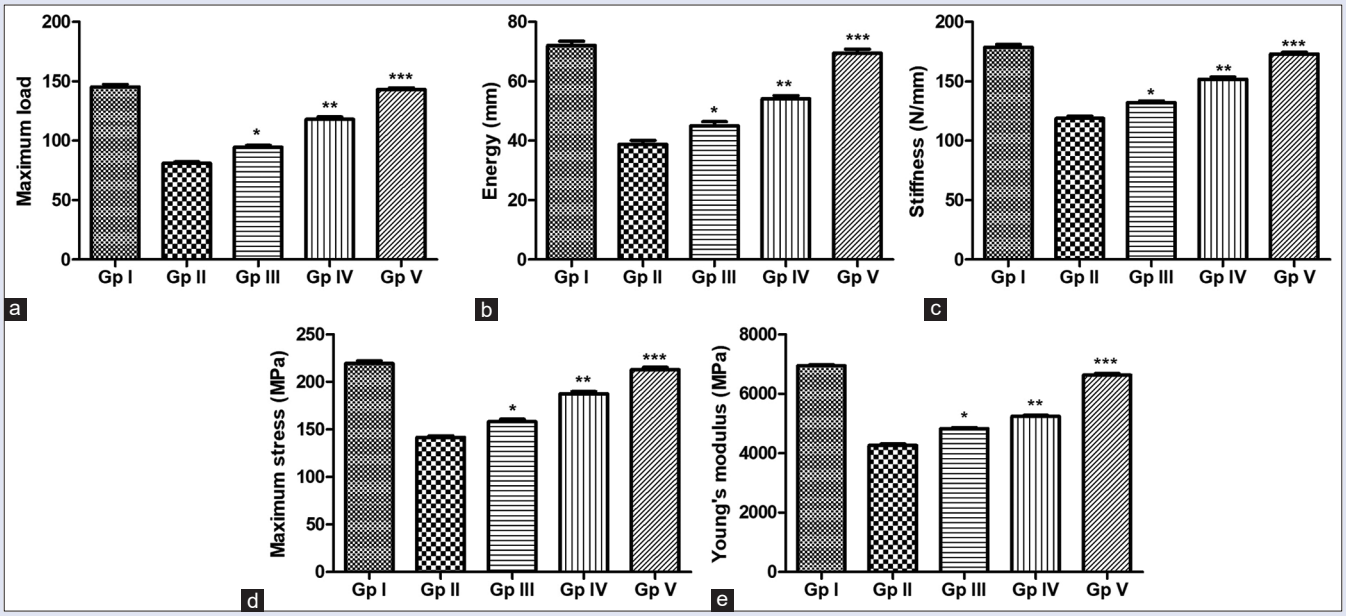

Figure 7: The effect of fisetin on the femoral diaphysis of ovariectomised rats. (a) maximum load, (b) energy, (c) stiffness, (d) maximum stresses, and (e) Young's modulus. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

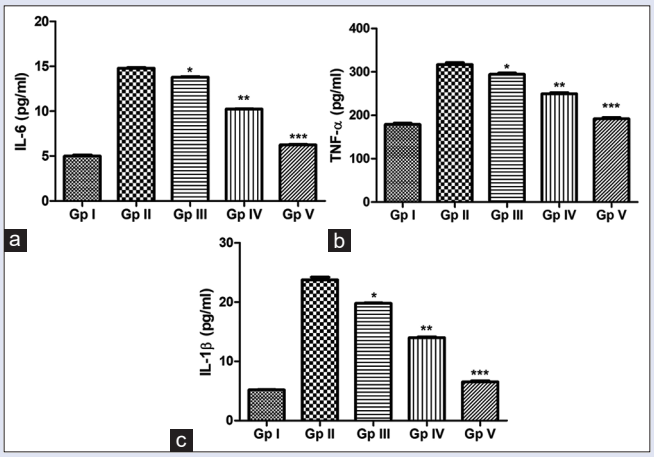

Figure 8: The effect of fisetin on the pro-inflammatory cytokines of ovariectomised rats. (a) Interleukin-6, (b) tumor necrosis factor-α, and (c) interleukin-1β. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomised group

compared to the sham group. Supplementation of higher dose of fisetin significantly ($P < 0.001$) restored the levels of serum E2, FSH, and LH almost to the levels of sham group.

DISCUSSION

Osteoporosis is impartially mutual complaint defined after postmenopausal related with severe morbidity, mortality, worsening in life quality, and economic factor.[23] The rodent OVX model is a mature animal experimental model that is usually employed in the postmenopausal osteoporosis research study.[24,25] In our present work, we engrossed on the OVX model in ratsto determine effects of Fisetin on the osteoporosis caused by estrogen deficiency. We favored the OVX-induced osteoporosis rat model due to alike pathological features of rats postmenopausal osteoporosis to humans.[24] The natural drug fisetin designated due to its antioxidant property which given by oral route in the rat model for the antiosteoporotic activity.

Osteoporosis is the well-known bone metabolic complaint that is categorized by resorption and unregulated bone formation that rapidly augmented bone erosion.[26] The present study is the first achieved in OVX rat model osteoporosis to state a protective influence of fisetin in dose-dependent manner against deteriorating bone build and strength. This dose-dependent impact of fisetin was showed by evaluating different parameters such as determination of bone density, measuring urine, serum, bone markers, and mechanical testing, pro-inflammatory cytokines, as well as hormonal assay.

Previous studies exposed that OVX-induced estrogen deficiency considerably the weight of the body and decay the weight of uterus in rats.[27,28] The fallouts that were totally defended after the administration of E_2. The mechanisms by which E2 reinstate body and uterine weights in OVX rats were also explicated.[27,29] In the present research, any of the doses of fisetin restored the body weight of OVX rats or apply uterotrophic concern that recommended fisetin does not control the growth of body weight and/or uterine tissue; further, earlier studies have shown in concord with this inspection that fisetin do not influenced any phytoestrogenic compounds.[30,31] To comprehend the mechanism through which fisetin exerted its antioestroprosis activity, the serum osteocalcium and level of ALP, markers existing in bone formation markers, and DPD in urine, bone resorption indicator was evaluated.[6] In this study, as showed by reduce bone-specific ALP, serum OC levels, and the urinary DPD/Cr ratio, fisetin lessened dose dependently bone turnover markers induced by OVX. Moreover, fisetin protected the loss of P and Ca in urine in dose-dependent manner, signifying that fisetin downregulated the bone resorption rate. The results seen in this research are reliable with those found in the literature, resulting in a substantial lessening in BMD and a deterioration in bone mass, resulting in diminished bone strength and augmented susceptibility to fracture.[32,33] In the present study, administration of fisetin at doses

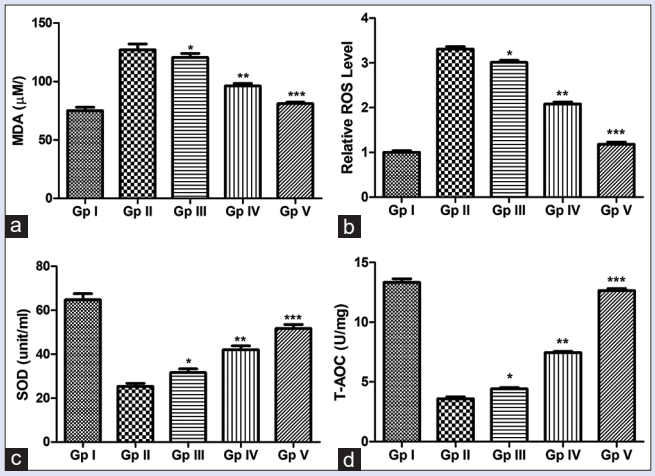

Figure 9: The effect of fisetin on the antioxidant parameters of ovariectomised rats. (a) Malonaldehyde, (b) relative reactive oxygen species level, (c) superoxide dismutase and (d) total antioxidant capacity. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to the ovariectomized group

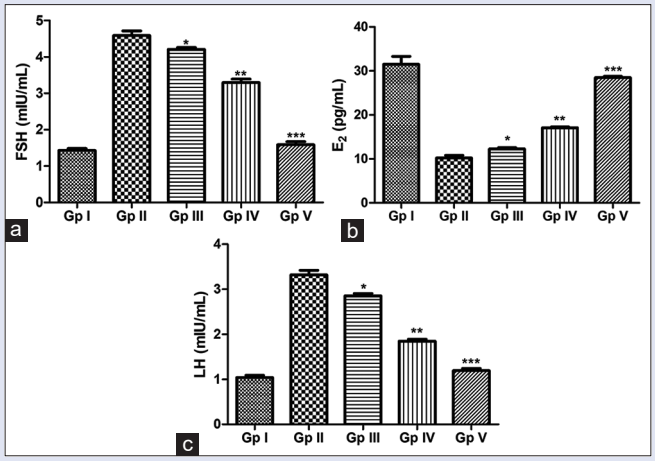

Figure 11: Demonstrate the effect of fisetin on the serum hormonal of ovariectomised rats. (a) follicle stimulating hormone, (b) E2, and (c) luteinizing hormone. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomized group

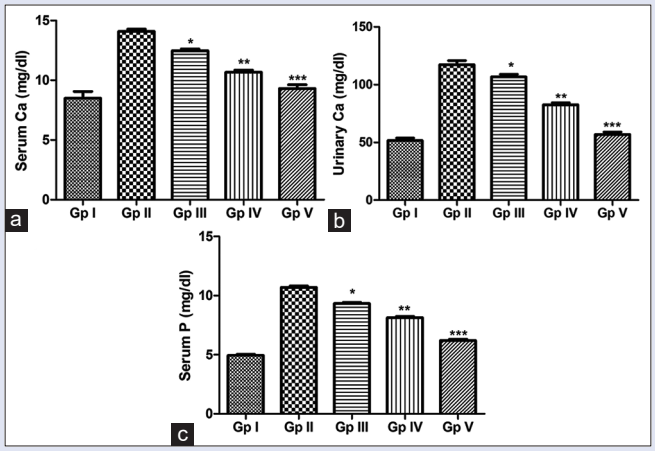

Figure 10: Demonstrate the effect of fisetin on the urinary parameter of ovariectomized rats. (a) Serum calcium, (b) urinary calcium, and (c) serum phosphorus. Values are mean ± standard error of mean. $n = 10$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the ovariectomized group

of 10 and 20 mg/kg b. w. significantly restored the BMD for 16 weeks and prevented diminutions in femoral bone mechanical indices such as maximum stress, failure strength, and module of young in OVX-induced rats as revealed by the 3-point bending test.

ALP is an osteoblast-secreted, noncollagenous protein that is critical for bone mineralization.[34] Augmented serum ALP levels have been existing in various delinquent comprising rapid loss of bone[35] and risk of fracture.[36] Osteocalcin (OC) is categorized as osteoblast-reflecting serum markers, involving formation of bone and turnover.[37] Fisetin therapy enhanced the level of OC. Results designated that oral administration of fisetin would induce the OC secretion. Bone is made up of calcified organic matrix containing of collagen type I of 90%.[37,38]

For osteoporosis, greater CTX are related with lower BMD values.[39,40] In the fisetin-treated rats, the serum CTX levels were considerably weakened as compared to the other rats. Obtained data exposed that IFN-ÿ has been revealed to boost the generation of osteoclast in peripheral blood cultures from osteopetrotic patients meaningfully.[41] The finding was reliable with our analysis. Nonetheless, fisetin treatment in OVX rats noticeably diminished the amount of IFN-Ɣ. These findings showed that the effect of fisetin on osteoporosis was due to a reduction in levels of CTX and IFN-Ɣ as well as a surge in levels of OVX plasma OC.

Phytoestrogens have been stated to have the valued effect of re-establish the parameters of sex hormones such as E2, FSH, and LH in females. FSH and LH are vital stimulators for growing follicles.[42] The FSH and LH preovulation secretion is reaction negatively controlled through E2 circulating through the hypothalamic–pituitary ovarian axis feedback control system.[43,44] In the contemporary research, fisetin strikingly boosted the levels of serum E2 and alleviated the enhanced levels of LH and FSH in serum revealing the exclusion of E2 in OVX-induced rats, signifying that fisetin played a helpful role in controlling hypothalamic–pituitary activity. Besides, measuring the bone markers played a role in the diagnosis and treatment of osteoporosis.[19,20] Loss of Bone mass, as established by augmented U-Ca/Cr, and U-P/Cr levels, proposed bone turnover upregulation with OVX.[6] The above bone turnover markers were dose-dependent inverted by fisetin, suggesting a diminution in bone turnover rate following fisetin treatment. It should also be renowned that fisetin indicated the effectiveness and safety for the treatment of osteoporosis in menopausal women without any sign of toxicity in the present model of experiment.

Estrogen deficiency is one of the decisive causes in induction of osteoporosis condition in women along with whereas epidemiological evidence. The current rodent mechanistic studies also recommended that aging and the connected increase in reactive oxygen species are the other contiguous offender to induce osteoporosis condition.[6,7] In 2010, Manolagas assessed the budding proof which delivers an archetype change from the "estrogen-centric" account of involutionary pathogenesis of osteoporosis to one, in which the protagonists are oxidative stress-related mechanisms intrinsic to bone.[45] More latest courtesy has been devoted to fisetin antioxidant activity. In this study, fisetin reversed the elevated ROS (MDA) production and restored the function of the antioxidant enzyme SOD function, thereby validating fisetin antioxidant position in osteoporosis.

CONCLUSION

These outcomes recommended that fisetin exhibited antiosteoporosis effect through modification of hormone level, bone turnover marker, biochemical parameters, antioxidant, and pro-inflammatory cytokines level. Fisetin expressively sustained the Ca and *P* homeostasis by boosting the level of endogenous antioxidant enzymes. Fisetin also increase the BMD, bone marker formation and suppressed the bone resorption. Fisetin significantly ($P < 0.001$) abridged the level of pro-inflammatory cytokines and presented the anti-inflammatory effect. At the clinical and mechanistic stage, attention wants to be given to the economic factor in the management of osteoporosis care in elderly people and postmenopausal women at high risk of fracture. We reflect fisetin as a natural remedy for postmenopausal osteoporosis prevention and have the capability to growth further.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ballane G, Cauley JA, Luckey MM, El-Hajj Fuleihan G. Worldwide prevalence and incidence of osteoporotic vertebral fractures. Osteoporos Int 2017;28:1531-42.
2. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006;17:1726-33.
3. Ström O, Borgström F, Kanis JA, Compston J, Cooper C, McCloskey EV, *et al.* Osteoporosis: Burden, health care provision and opportunities in the EU: A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos 2011;6:59-155.
4. Biver E, Ferrari S. Osteoporosis. Rev Med Suisse 2020;16:78-80.
5. Sambrook P, Cooper C. Osteoporosis. Lancet 2006;367:2010-8.
6. Fouda AM, Youssef AR. Antiosteoporotic activity of *Salvadora persica* sticks extract in an estrogen deficient model of osteoporosis. Osteoporos Sarcopenia 2017;3:132-7.
7. Li F, Yang X, Yang Y, Guo C, Zhang C, Yang Z, *et al.* Antiosteoporotic activity of echinacoside in ovariectomized rats. Phytomedicine 2013;20:549-57.
8. Nian H, Ma MH, Nian SS, Xu LL. Antiosteoporotic activity of icariin in ovariectomized rats. Phytomedicine 2009;16:320-6.
9. Nian H, Qin LP, Zhang QY, Zheng HC, Yu Y, Huang BK. Antiosteoporotic activity of Er-Xian Decoction, a traditional Chinese herbal formula, in ovariectomized rats. J Ethnopharmacol 2006;108:96-102.
10. Satpathy S, Patra A, Ahirwar B. Experimental techniques for screening of antiosteoporotic activity in postmenopausal osteoporosis. J Complement Integr Med 2015;12:251-66.
11. Trivedi A, Katti HR, Shalavadi MH, Ramkishan A, Chandrashekhar VM. Anti-osteoporotic activity of ethanol extract of *Terminalia Arjuna* (Roxb.) Wight & Arn. on ovariectomized rats. Indian J Nat Prod Resour 2015;6:98-105.
12. Rangrez AY, Balakrishnan S, Parikh PH. Osteoprotective effect three anti inflammatory plants in ovariectomized wistar rats. Pharmacologyonline 2011;1:675-84.
13. Ma XQ, Han T, Zhang X, Wu JZ, Rahman K, Qin LP, *et al.* Kaempferitrin prevents bone lost in ovariectomized rats. Phytomedicine 2015;22:1159-62.
14. Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, *et al.* Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine 2018;36:18-28.
15. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: A dietary antioxidant for health promotion. Antioxid Redox Signal 2013;19:151-62.
16. Adhami VM, Syed DN, Khan N, Mukhtar H. Dietary flavonoid fisetin: A novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management. Biochem Pharmacol 2012;84:1277-81.
17. Maher P, Dargusch R, Ehren JL, Okada S, Sharma K, Schubert D. Fisetin lowers methylglyoxal dependent protein glycation and limits the complications of diabetes. PLoS One 2011;6:e21226.
18. Khan N, Afaq F, Syed DN, Mukhtar H. Fisetin, a novel dietary flavonoid, causes apoptosis and cell cycle arrest in human prostate cancer LNCaP cells. Carcinogenesis 2008;29:1049-56.
19. Geng Q, Gao H, Yang R, Guo K, Miao D. Pyrroloquinoline quinone prevents estrogen deficiency-induced osteoporosis by inhibiting oxidative stress and osteocyte senescence. Int J Biol Sci 2019;15:58-68.
20. Li J, Karim MA, Che H, Geng Q, Miao D. Deletion of p16 prevents estrogen deficiency-induced osteoporosis by inhibiting oxidative stress and osteocyte senescence. Am J Transl Res 2020;12:672-83.
21. Zhang DW, Wang ZL, Qi W, Zhao GY. The effects of *Cordyceps sinensis* phytoestrogen on estrogen deficiency-induced osteoporosis in ovariectomized rats. BMC Complement Altern Med 2014;14:484.
22. Turner CH, Burr DB. Basic biomechanical measurements of bone: A tutorial. Bone 1993;14:595-608.
23. Compston JE, McClung MR, Leslie WD. Osteoporosis. Lancet 2019;393:364-76.
24. Bonucci E, Ballanti P. Osteoporosis-bone remodeling and animal models. Toxicol Pathol 2014;42:957-69.
25. Komori T. Animal models for osteoporosis. Eur J Pharmacol 2015;759:287-94.
26. Bouxsein ML, Myers KS, Shultz KL, Donahue LR, Rosen CJ, Beamer WG. Ovariectomy-induced bone loss varies among inbred strains of mice. J Bone Miner Res 2005;20:1085-92.
27. Zhao X, Wu ZX, Zhang Y, Yan YB, He Q, Cao PC, *et al.* Anti-osteoporosis activity of *Cibotium barometz* extract on ovariectomy-induced bone loss in rats. J Ethnopharmacol 2011;137:1083-8.
28. Wang YF, Liu WT, Chen CY, Ke HP, Jiang HL, Chen XL, *et al.* Anti-osteoporosis activity of red yeast rice extract on ovariectomy-induced bone loss in rats. Genet Mol Res 2015;14:8137-46.
29. Dontas I, Halabalaki M, Moutsatsou P, Mitakou S, Papoutsi Z, Khaldi L, *et al.* Protective effect of plant extract from Onobrychis ebenoides on ovariectomy-induced bone loss in rats. Maturitas 2006;53:234-42.
30. Saeed IA, Ali L, Jabeen A, Khasawneh M, Rizvi TA, Ashraf SS. Estrogenic activities of ten medicinal herbs from the Middle East. J Chromatogr Sci 2013;51:33-9.
31. Enyew A, Asfaw Z, Kelbessa E, Nagappan R. Ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. Curr Res J Biol Sci 2014;6:154-67.
32. Eastell R. Role of oestrogen in the regulation of bone turnover at the menarche. J Endocrinol 2005;185:223-34.
33. Krassas GE, Papadopoulou P. Oestrogen action on bone cells. Musculoskelet Neuronal Interact 2001;2:143-51.
34. Miao D, Bai X, Panda D, McKee M, Karaplis A, Goltzman D. Osteomalacia in Hyp mice is associated with abnormal Phex expression and with altered bone matrix protein expression and deposition. Endocrinology 2001;142:926-39.
35. Miao D, Scutt A. Recruitment, augmentation and apoptosis of rat osteoclasts in 1,25-(OH) 2D3 response to short-term treatment with 1,25-dihydroxyvitamin D3 *in vivo*. BMC Musculoskelet Disord 2002;3:16.
36. Zhang Y, Chen G, Gu Z, Sun H, Karaplis A, Goltzman D, *et al.* DNA damage checkpoint pathway modulates the regulation of skeletal growth and osteoblastic bone formation by parathyroid hormone-related peptide. Int J Biol Sci 2018;14:508-17.
37. Bilezikian JP. New and emerging therapies for osteoporosis. Bone 2010;47:S356-7.
38. Miao D, He B, Jiang Y, Kobayashi T, Sorocéanu MA, Zhao J, *et al.* Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1-34. J Clin Invest 2005;115:2402-11.
39. Khosla S, Oursler MJ, Monroe DG. Estrogen and the skeleton. Trends Endocrinol Metab 2012;23:576-81.
40. Levin ER. Plasma membrane estrogen receptors. Trends Endocrinol Metab 2009;20:477-82.
41. Manolagas SC, Parfitt AM. What old means to bone. Trends Endocrinol Metab 2010;21:369-74.
42. Occhiuto F, Pasquale RD, Guglielmo G, Palumbo DR, Zangla G, Samperi S, *et al.* Effects of phytoestrogenic isoflavones from Red clover (*Trifolium pratense* L.) on experimental osteoporosis. Phytother Res 2007;21:130-4.
43. Kolios L, Daub F, Sehmisch S, Frosch KH, Tezval M, Stuermer KM, *et al.* Absence of positive effect of Black cohosh (*Cimicifuga racemosa*) on fracture healing in osteopenic rodent model. Phytother Res 2010;24:1796-806.
44. Kolios L, Schumann J, Sehmisch S, Rack T, Tezval M, Seidlova-Wuttke D, *et al.* Effects of Black cohosh (*Cimicifuga racemosa*) and estrogen on metaphyseal fracture healing in the early stage of osteoporosis in ovariectomized rats. Planta Med 2010;76:850-7.
45. Manolagas SC. From estrogen-centric to aging and oxidative stress: A revised perspective of the pathogenesis of osteoporosis. Endocr Rev 2010;31:266-300.