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Evaluating the Role of Nano-Bioisoflavonoid, Bioisoflavoinoid and Vitamin D on Immunological Parameters in Glucocorticoid-Induced Osteoporosis Adult Male Rats

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Abstract | This study was designed to investigate the therapeutic effect of nano-isoflavonoids on physiological parameters in male rats with osteoporosis. For this purpose, a total of 60 male rats were divided into two groups where a control group (n=12) whereas the osteoporosis group (n=48) the male rats. After a month, osteoporosis was induced and confirmed by X-rays. The first group was considered a control group. The second group (osteoporosis group), the third group (osteoporosis OP + GbE isoflavonoid extract), fourth group (Osteoporosis OP + GbE isoflavonoid nanoextract) and fifth group (osteoporosis OP + Vitamin D) were treated proportionately. The results obtained showed that osteoporosis induced by glucocorticoids is accompanied by a significant decrease in IL-6 and TNF-a and SOD, and a significant increase in Ca and P and MDA. The results also showed that treatment with nanoisoflavones is better than isoflavones extract followed by vitamin D. These finding highlight the roles of nano-bioisoflavonoid, bioisoflavoinoid and vitamin D in treated model as model to gauge the importance of these treatments in animals.

Keywords | Nanobioisoflavonoid, Gingko Biloba Extract, Glucocorticoid, Vitamin D, (IL-6), (TNF-a)

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INTRODUCTION

Osteoporosis is a major worldwide public health issue that has been growing as populations grow older and live longer, due to the proliferation of low bone mass. However, this condition is prevalent afflicting millions of people post-menopause or aged individuals worsened by prolonged administration of glucocorticoids usually administered for multiple inflammatory and autoimmune diseases. Although it is possible manage those disorders, it is not appropriate to rely mostly on glucocorticoids because they interrupt the osteoblast function and enhance the activity of osteoclast which in turn ending up with bone loss and fragility (Burch *et al*., 2020). Isoflavonoids from the traditional medicinal plant such as Ginkgo biloba rescue bone loss and improve bone health (Li *et al*., 2018). Ginkgo biloba is a well-known herb, possessing various pharmacological properties mostly due to the presence of flavonoids and terpenoids. In this regard, isoflavonoids have attracted much attention due to their potential pharmacological targets on bone metabolism by the regulation of signaling pathways during osteoclastogenesis and/or osteoblastogenesis (Kumar *et al*., 2021). These compounds have the potential to antagonize the

deleterious effects of glucocorticoids, thereby stimulating bone formation and inhibiting bone resorption that could be used as a therapeutic approach for treating osteoporosis (Zhang *et al*., 2020). Nanotechnology also added a new dimension on combining with herbal natural products for therapeutic strategies. Plant extract based green Biosynthesis of nanoparticles have been developed as a novel cost-effective method, not only to improve the bioavailability of drugs but also to combats environmental problems associated with conventional synthesis methods (Thakur *et al*., 2021). Taken together, nanoparticles derived from Ginkgo biloba isoflavonoids provide an improved way to deliver these compounds and helped improve their efficacy during treatment against glucocorticoid-induced bone loss (Yadav *et al*., 2022). Vitamin D is essential for maintaining bone health, as it enhances calcium absorption in the intestines and helps regulate serum calcium levels, crucial for bone mineralization. Insufficient vitamin D levels can lead to impaired calcium absorption, resulting in secondary hyperparathyroidism and increased bone resorption, contributing to the development of osteoporosis (Pilz *et al*., 2021). Recent studies have highlighted the association between adequate vitamin D levels and improved bone density, as well as a decreased risk of fractures in osteoporotic individuals (Liu *et al*., 2022). Additionally, vitamin D supplementation has been shown to complement osteoporosis treatments effectively, emphasizing its role in both the prevention and management of this condition (Sahni *et al*., 2021).

Here we aim to find out the roles of the nanoparticles in treating osteoporosis caused by glucocorticoids. Specifically, to monitor the impact of treatment on physiology, biochemistry, and molecular biology when compared to an isoflavonoid extract of Ginkgo biloba (EGb) and vitamin D.

MATERIALS AND METHODS

This study was carried out at the Faculty of Veterinary Medicine, University of Basra. After the period of acclimation, a total of 60 male rats were divided into five groups with (12) animals in each group. The first group (C) was regarded as control (Negative Control) where each rat in the control group was injected S.C. 1ml of normal saline five days per week for one months. In the second group, the induction of osteoporosis was conducted by glucocorticoid (DXM) injected S.C. 5 days per week for one month (Positive control). The third group (Osteoporosis+ Isoflovonoid Extract GbE) was treated with Isoflovonoid Extract of Ginkgo biloba with a rage of 500mg/Kg B.W dose P.O. daily for one months. The fourth group (Osteoporosis OP+Nano Isoflovonoid Extract GbE) was treated with Nanoparticles Isoflovonoid Extract of Ginkgo biloba with a rage of 500mg/Kg B.W dose P.O. daily for one months as reported before (Lamb *et al*., 2023). The fifth group (Osteoporosis OP+ Vit.D) was treated with Vit. D at the rate of10000 I.U P.O daily for one months (Voulgaridou *et al*., 2023). After the completion of therapy all rates were sacrificed. By sterile syringe, blood samples were taken from the inferior vena cava of the heart in the rat sacrificed with plain tube without anticoagulant. Serum was extracted by centrifuged at 3000 rpm in micro-Eppendorf tubes and stored for 15 minutes at -20 C. These samples were used for laboratory analysis and for immunological testswhereIL-6, TNF-a, MDA, SOD, Ca and P were measured.

RESULTS

Effect of iso bioflavonoid, nano iso bioflavonoid and Vit D on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in osteoporosis adult male rats

As shown in Figure 1, there was a significant (P≤0.05) decrease in serum IL-6 concentration in osteoporosis group (Positive) compared to control group. The treated groups (OP plus iso Bioflavonoid, OP plus Nano iso Bioflavonoid and OP plus Vit D) showed a significant (P≤0.05) increase in the serum IL-6 concentration as compared to the OP Group (Positive). On the other hand, there was no significant difference OP plus nano iso-bioflavonoid and control group. The treated group OP plus iso Bioflavonoid showed a significant (P≤0.05) increase in the serum IL-6 concentration as compared to the OP plus Nano iso Bioflavonoid and OP plus Vit D and control group.

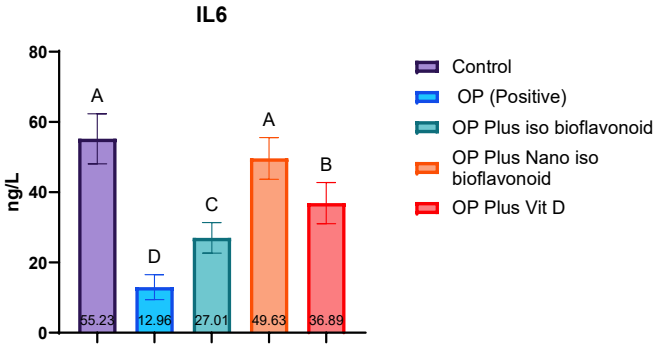

Figure 1: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) in Osteoporosis Adult Male Rats. (Mean ± SD) n=8.

There was a significant (P≤0.05) decrease in serum TNF alpha concentration in osteoporosis group (Positive) compared to control group (Figure 2). The treated groups (OP plus iso Bioflavonoid, OP plus Nano iso Bioflavonoid and OP plus Vit D) showed a significant (P≤0.05) increase in the serum TNF alpha concentration as compared to

the OP Group (Positive). On the other hand, there was no significant difference OP plus Nano iso Bioflavonoid and control group. Also, there was no significant difference OP plus iso Bioflavonoid group and vit D group. Finally, a significant (P≤0.05) decrease in serum TNF alpha concentration in OP plus iso Bioflavonoid group and OP plus Nano iso Bioflavonoid group and control group was observed.

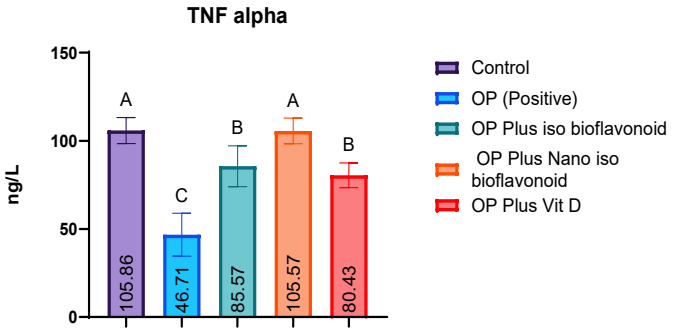

Figure 2: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) in Osteoporosis Adult Male Rats. (Mean ± SD) n=8.

Effect of iso bioflavonoid, nano iso bioflavonoid and Vit D on MDA and SOD in osteoporosis adult male rats

There was a significant (P≤0.05) increase in serum MDA concentration in osteoporosis group (Positive) compared to control group (Figure 3). The treated groups (OP plus iso Bioflavonoid, OP plus Nano iso Bioflavonoid and OP plus Vit D) showed a significant (P≤0.05) decrease in the serum MDA concentration as compared to the OP Group (Positive). On the other hand, there was no significant difference OP plus Nano iso Bioflavonoid and control group. Also, there was no significant difference between OP plus iso Bioflavonoid group and vit D group. Finally, the treated group OP plus Nano iso Bioflavonoid showed a significant (P≤0.05) decrease in the serum MDA concentration as compared to other treated groups. Figure 4 showed that there was a significant (P≤0.05decrease in serum SOD concentration in osteoporosis group (Positive) compared to control group. The treated groups (OP plus iso Bioflavonoid, OP plus Nano iso Bioflavonoid and OP plus Vit D) showed a significant (P≤0.05) increase in the serum SOD concentration as compared to the OP Group (Positive).On the other hand, there was no significant difference in OP plus Nano iso Bioflavonoid and control group. Also, there was no significant difference OP plus iso Bioflavonoid group and vit D group. Finally, the treated group OP plus iso Bioflavonoid showed a significant (P≤0.05) decrease in the serum SOD concentration as compared to OP plus Nano iso Bioflavonoid and control groups.

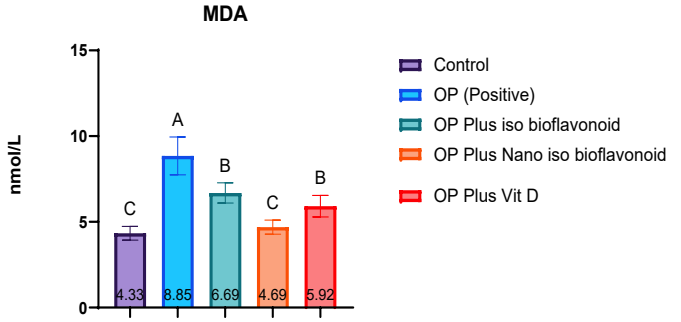

Figure 3: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on MDA and SOD in Osteoporosis Adult Male Rats. (Mean ± SD) n=8.

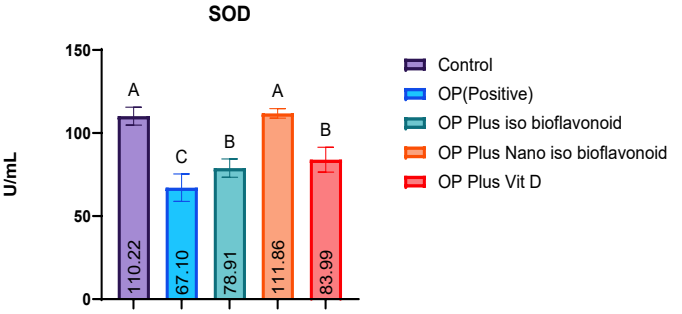

Figure 4: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on MDA and SOD in Osteoporosis Adult Male Rats. (Mean ± SD) n=8.

Effect of iso bioflavonoid, nano iso bioflavonoid and Vit D on Ca and P in osteoporosis adult male rats

As shown in Figure 5, here was a significant (P≤0.05) increase in serum Ca concentration in OP group (Positive) compared to control group. The treated groups (OP plus iso Bioflavonoid, OP plus Nano iso Bioflavonoid and OP plus Vit D) showed a significant (P≤0.05) decrease in the serum Ca concentration as compared to the OP Group (Positive). A significant (P≤0.05) decrease in the serum Ca concentration in OP plus Nano iso Bioflavonoid group was observed compared to all other groups. There was no significant difference between OP Plus Vit D group and Control group.

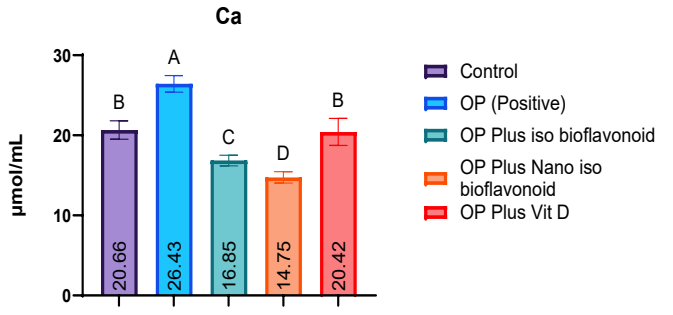

Figure 5: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on Ca and P in Osteoporosis Adult Male Rats. (Mean ± SD) n=8

Data presented in Figure 6 show that there was a significant (P≤0.05) increase in serum P concentration in OP Plus Vit D group compared to all other groups. However, there was no a significant different among all groups excepted OP Plus Vit D.

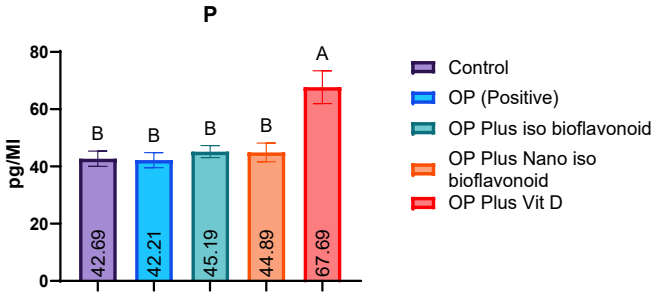

Figure 6: Effect of iso Bioflavonoid, Nano iso Bioflavonoid and Vit D on Ca and P in Osteoporosis Adult Male Rats. (Mean ± SD) n=8

DISCUSSION

This study investigates the effect of isoflavonoid of Ginkgo biloba, nano Ginkgo biloba, and Vitamin D on a broad spectrum of biochemical indicators in male rats that have induced osteoporosis. The results provide valuable insights into therapeutic mechanisms and implications in osteoporosis treatment and management. Effect of isoflavonoid, Nano Gingko biloba, and Vit D were investigated on immunological parameters including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in osteoporosis in adult male rats.

Osteoporosis usually elicits the concentration of inflammatory cytokines (like IL-6 and TNF-α) in serum, which play a role in bone breakdown. We have noticed that the treatment groups had significantly lower serum concentrations of these cytokines than the osteoporosis-positive group (Rao *et al.*, 2018). TNF-α and IL-6 are known to be pro-inflammatory cytokines that contribute significantly to osteoporosis pathogenesis by promoting the osteoclast process, which stimulates bone resorption. The noted decrease in these two cytokines in treated groups indicated a potential ability for treatment to protect against bone loss (Sponholtz *et al.*, 2013).

The Nano Ginkgo biloba group Especially decreased those cytokines to almost match the control negative group, which means that it improved anti-inflammatory action; this may be linked to enhanced cellular uptake and bioavailability, as many research papers find that Ginkgo biloba (our model of iso Bioflavonoid) and other flavonoids and also terpenes blocking the inflammatory signalling pathways such as NF-κB (Noor-E-Tabassum *et al.*, 2022; Salminen *et al.*, 2008).

The optimal effect of nano iso Bioflavonoid in decreasing these two cytokines in relation to iso Bioflavonoid might be because of the better bioavailability and cellular uptake, which are properties of nano-formulation agents that improve and enhance drug delivery through increasing surface area and penetrating into cells (Yusuf *et al.*, 2023; Dwivedi *et al.*, 2023).

The recognizable differences between the effects of iso Bioflavonoid and nano Ginkgo biloba Emphasize how the formulation of the therapeutic agent can alter the influence and the treatment (AbouAitah *et al.*, 2021). While Vitamin D also decreases the inflammatory cytokines, its impact was slight, aligned with vitamin D's known role in regulating the immune system and immune response indirectly through calcium balance (Wobke *et al.*, 2014).

Vitamin D's role focuses primarily on calcium homeostasis, but it also has a role in the immune system, especially as an immunomodulator. This means it can regulate IL-6 and TNF-α concentration in the body by decreasing cytokine production or increasing them also. Even though the effect of Vitamin D in this study was lower than that of iso bioflavonoids, these differences might be because of the vitamin D dosage or the pathway in which it works as an immunomodulator (Wobke *et al.*, 2014).

Oxidative stress, which is detected by increased malondialdehyde (MDA) and reduced superoxide dismutase (SOD), Consistent to be an essential factor in osteoporosis, while all the treatment groups decreased MDA and increased SOD levels, with the nano iso Bioflavonoid group have had the best treatment results among all groups, suggesting that the antioxidant properties of nano iso Bioflavonoid give the best protection to bone against oxidant stress (Marcucci *et al.*, 2023).

MDA is counted to be a marker of lipid peroxidation, which is an essential sign of oxidative stress. While SOD is a little different, SOD is known to be an antioxidant enzyme, and it protects the cells from oxidative damage by catalysing the dismutase of superoxide radicals, which is literally the name of the Enzyme-Super Oxidase Dismutase-. The notable decrease in MDA and increase in SOD concentrations in treated groups indicate that the treatments contribute to decreasing the oxidative stress, which counted as a main factor in osteoporosis progression (Zhao *et al.*, 2021; Sheweita *et al.*, 2014).

Ginkgo biloba (GB) is a bioflavonoid that scavenges free radicals and improves the body's antioxidant mechanisms. The nano iso Bioflavonoid appears to boost those mechanisms by impacting cellular absorption, while Vitamin D's role is significant in Calcium absorption, which affects Bone-Mineral balance (Laird *et al.*, 2010).

As mentioned above, the nanoparticles increase the surface area and allow more penetrating into cells; this may be the reason that the nano iso bioflavonoid performs better than iso bioflavonoid; the nano iso bioflavonoid might facilitate more Potent scavenging to the free radicals and more efficient induction of antioxidants (Barrón *et al*., 2023; Mazzotta *et al*., 2021). Proper calcium and phosphorus regulation is critical for bone health, and imbalances are common in osteoporosis. This study showed that all treatments significantly improved these mineral levels, with Nano Gingko Biloba performing best (Martiniakova *et al*., 2022; Lee and Cho, 2015).

Good calcium and phosphorus regulation is essential for bone health; imbalances in these minerals are common in bone disorders such as osteoporosis. All treatments significantly improved these minerals, with Nano Gingko Biloba having the best therapeutic results.

Calcium and phosphorus play essential roles in bone mineralization; any disturbance in the regulation of these minerals can lead to weakness in the structure of the bones. The strong impact of nano Gingko Biloba on Calcium-phosphorus balance may be linked to the bioavailability and its direct effect on bone cells; by enhancing bone mineralization, nano Gingko Biloba Slove finds a solution to one of the core challenges in the management of osteoporosis. While Vitamin D plays a role in improving calcium absorption from the digestive tract, Nano iso Bioflavonoid provides an extensive approach that affects bone resorption and mineralization (Inpan *et al*., 2023).

CONCLUSIONS AND RECOMMENDATIONS

Osteoporosis has adverse effects on interleukin, antioxidant and endocrine parameters in male rats and is corrected by treatment with vitamin D, Isoflovonoid extract GbE and nano Isoflovonoid extract GbE. Further studies should be conducted to study the effect of osteoporosis in females, avoid exposure to glucocorticoids for a long period because it may have a potential risk to the endocrine axes and other organs, treatment with Ginkgo Biloba extract on cancerous diseases.

ACKNOWLEDGEMENT

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NOVELTY STATEMENT

The present study shows that glucocorticosteroids disrupt the function of osteocyte and enhance the activity of osteoclasts, which in turn leads to bone loss and fragility. The highest efficacy in improving disorders caused by rickets and osteoporosis was observed through treatment with nano-isoflavonoids. This result suggests that nano-isoflavonoids may be useful for treating osteoporosis caused by glucocorticoids in the first place, then isoflavonoid extract of Ginkgo biloba and finally vitamin D.

AUTHOR'S CONTRIBUTION

MHA-S: Conceptualized and designed the experiments.
ARS: Carried out the experimental work and reviewed and approved the final version of the manuscript
ARS and MHA-S: Analysed the data and drafted the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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