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Impact of Dandelion and Vitamins (C, K) on Osteoporosis Induced by Heparin Drug in Rats

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Abstract: Osteoporosis is a common problem in which the bones become weak and break easily. It is a silent disease that develops slowly over several years. The disease is often diagnosed at a slight fall, or a sudden impact that causes bone fracture as a result of the body losing essential materials that help in nourishing and building bones or lack of nutrients and vitamins the body needs. There are two types of osteoporosis, the first type: Includes osteoporosis in menopause due to Estrogen deficiency, and osteoporosis with aging due to age, the secondary type: Occurs as a result of a specific cause that can be identified, such as osteoporosis resulting from some disease conditions or the use of some drugs. Heparin is an anticoagulant drug by disables thrombin in the blood clotting process. It is widely used in treatments, although it has negative effects such as osteoporosis. Dandelion is a rich source of vitamins, minerals, and other nutrients necessary for the health of the body, as it contains a high percentage of calcium, in addition to antioxidants such as vitamin C and luteolin, and has a critical role in improving bone density, thus reducing the risk of osteoporosis, and helping to maintain bone health and protect it from osteoporosis. The present study was conducted to explore the potential protective effect of the natural plant dandelion in comparison with vitamins C and K on heparin-induced osteoporosis in rats. The study period was 30 days, this study included fifty female Wester albino rats that were divided into five groups: **G1** ate their normal diet for 30 days, **G2** was injected subcutaneously with heparin, **G3** was fed orally with an extract of Dandelion concurrently with being injected with heparin, **G4** was fed with vitamins C and K concurrently with injected heparin and **G5** was fed with dandelion extract and vitamins C and K in the same amount mentioned previously in conjunction with injected with heparin for 30 consecutive days, The results showed that rats treated with dandelion extract responded to the preventive treatment significantly and increased levels of bone minerals (calcium, magnesium, phosphate, vitamin D), also on genetic indicators GAPDH, SOST and OPG unlike rats that were injected only with heparin, the signs of bone resorption and bone formation were increased, but the rates approached in the groups treated with dandelion extract with the control group, which means that it is a protective factor. Also, vitamins C and K, have lesser protective effects but when added to dandelion extract give increased impact results. This study may be preventive against drug-related osteoporosis and preventive care for the general population that may reduce the cost of treating bone diseases.
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Keywords: Dandelion, Osteoporosis, Heparin, Osteoblast, Osteoclast, Vitamin D, Ca, Mg, PO_4.

1. Introduction

Osteoporosis means “POROUS BONES” which is characterized by damage of bone mass over time leading to breakability fractures. This bone disorder can be affected both men and women. Also causes bone fragile and weak due to fragility breaks with small disturbances (Chitra, 2021). Describes osteoporosis by the World Health Organization (WHO) as a “progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture. Treatments aim to slow bone destruction and prevent fractures and there is evidence that bone loss can be restored (Blackie, 2020). Affect many drugs on bone metabolism. Like, heparin can be causes increased resorption of bone by stimulating osteoclasts and suppressing osteoblast function, leading to decreased bone mass (Hamdy, 1999; Hansen and Vondracek, 2004). There is some indication of the possible role of vitamin C in osteoporosis and fracture prevention (Brzezińska *et al*., 2020). Vitamin C (VC) supplementation induced the differentiation of primary bovine osteoblasts and lead to increased synthesis of collagen, osteonectin, and osteocalcin (Urban *et al*., 2012). The same impact, dependent on VC-enhanced collagen synthesis, was detected for human osteoblast-like cells (Takamizawa *et al*., 2004). Vitamins k (VK) have an imperative part in bone strength. Definitely,

VK is required to carry out the process of osteocalcin carboxylation, it appears to help the osteoblast-to-osteocyte conversion and also limit osteoclastogenesis (Atkins *et al*., 2009). Dandelion (*Taraxacum officinale Weber*) is a member of the *Asteraceae Compositae* family (Quer and Davit, 1993). Dandelion contains a wide array of phytochemicals whose biological activities are actively being explored in various areas of human health. Emerging evidence suggests that dandelion and its constituents have antioxidant and anti-inflammatory activities that result in diverse biological effects (González-Castejón *et al*., 2012). Although pharmacological treatment is most often used, for optimal osteoporosis treatment, non-pharmacological management is also important to prevent osteoporosis and limit the risk of fractures. One of the natural ingredients that can be an alternative in preventing osteoporosis. Dandelion is high contents of vitamins (A, C, D, E, and B), inositol, lecithin, and minerals such as iron, magnesium, sodium, calcium, silicon, copper, phosphorus, zinc, and manganese (Ata *et al*., 2011).

2. Materials and methods

Chemicals:

Heparinol, Heparin Sodium 5,000 I.U./ml solution for injection or concentrate for solution for infusion, Ain Medicare Sdn Bhd, Malaysia, Vitamin C (ascorbic acid), manufactured for Doctor's Best, Inc. Tustin, California,92780 USA, Vitamin K (phytonadione) tablets, 500 mcg by Source Naturals and dandelion plant collected in spring from southern Saudi Arabia were used in this study. Kits used for the quantitative determination of different parameters were purchased from MyBioSource (San Diego, United States).

Animals and **experimental:**

Fifty Adult Female Albino Rats (180-200gm) were used in this study. The animals were bought from Faculty of Pharmacy, at King Abdulaziz University. Animals were housed under controlled conditions and provided Diet: Fat content ranges from 4% to 11% Water: Water should be always accessible. Rat handling was performed in accordance with the roles of King Abdulaziz University, Faculty of pharmacy. The animals were left for 7 days for adaptation.

The rats were divided into 5 groups, each of 10 rats

G1: Normal rats that will be treated orally with D.W only for 30 days.

G2: Rats will be treated by subcutaneous injection of unfractionated heparin (0.001 mg/ kg) for 30 days. (Muir *et al*., 1996).

G3: Rats will be co-administered orally with a dandelion water extract (DWE) dose (2.4 mg/ kg) along with heparin injection daily for 30 days. (Cho *et al*., 2002).

G4: Rats will be co-administered orally with vitamin K (50 mg/kg) (Akiyama *et al*., 1999) and vitamin C (200 mg/ kg) with heparin injection for 30 days. (Giordaneo *et al*., 2012).

G5: Rats will be co-administered orally with the combination of dandelion and vitamins C and K with heparin for 30 days.

Blood samples from all groups were collected from eyes after 30 days of treatment with the currently used drugs, rats were fasted overnight (12-14 hours). The final body weight of each animal in all groups was recorded. Blood specimens were gathered into sterilized tubes for clotting and serum separation. The serum samples were separated by centrifugation at 2000g for 15 min and the isolated samples were stored at -20 º until use for biochemical serum analysis. The serum was used to determine the bone mineral test, calcium (Ca) (Watchorn, 1933), Magnesium (Mg^{+2}) (Watchorn, 1933), Phosphate (PO_4) (Weissman and Pileggi, 1974), vitamin D (VD) (Al-Daghri *et al.*, 2017) and genetic markers test Glyceralehyde-3-phosphate dehydrogenase (GAPDH) (Teixeira *et al.*, 2017), sclerostin (SOST) (Li *et al.*, 2009) and Osteoprotegerin (OPG) (Ominsky *et al.*, 2008).

Extraction of Dand**elion leaves**

In the spring season, collected of fresh leaves Dandelion from Kingdom of Saudi Arabia, Southern Region, Faifa, and were frozen at -80 °C for ten hours, then transferred to the lyophilizer (vacuum, freeze at -65 °C), and left for two days until complete drying. The dried plant was smashed in a plastic bag, milled, sieved, and weighed. A weight of 6.37 g of the powdered dry plant was transferred to a round flask 250 ml, mixed with 150 ml methanol HPLC, stopped, shacked for 5 min, left in the ultrasonic bath for 45 min, and filtered through 0.45 Nylon membrane under vacuum. The clear filtrate was transferred to another clean-dry round flask 250 ml, and the solvent was removed using a rotary evaporator at 35 °C. The residue obtained was equal to 0.728 g and kept in the refrigerator at -20 °C until use.

% Yield = (0.728 / 6.37)x100 = 11.43%, g/g% (Cho *et al*., 2002).

Statistical analysis:

Analysis of data by comparing the values for different experimental groups with the values of individual normal ones. Results are expressed as mean ±SD. The significant differences between groups by using analysis of variance (ONE-WAY ANOVA) attached with post-Hoc slightest significance difference (LSD). ANOVA at $p \leq 0.05$

was measured as significant. The statistical analysis was accomplished by statistical package for the social sciences (SPSS) version 23.

3. Results

Effect of dandelion and vitamins (C, K) on osteoporosis indices in heparin-treated female rats

Figures 1, 2, 3 and **4 respectively**, show the serum levels of bone on osteoporosis parameters calcium (Ca), magnesium (Mg), phosphate (PO_4), and vitamin D. The impact of the combination of dandelion, vitamin C, and K with heparin-treated rats was the most potential prophylactic impact on the levels in relation to the treatment with each agent lonely.

As compared to the normal group (G1) after 30 days A very highly significant decrease was found in serum levels of Ca (mg/dl), Mg (mEq/l), PO4 (mg/dl), and vitamin D (ng/ml) in G2 (P≤0.001). Non-significant decrease of Ca (mg/dl), Mg (mEq/l), and PO4 (mg/dl), while a highly significant decrease in vitamin D (ng/ml) in G3 (P ≤0. 01). A very highly significant decrease in Ca (mg/dl), and vitamin D (ng/ml) (P ≤0.001), a Highly significant decrease in mg (mEq/l) (P ≤0.01), a significant decrease in PO4 (mg/dl) in G4 (P <0.05). was found a non-significant increase in serum levels of Ca (mg/dl), PO4 (mg/dl), and vitamin D (ng/ml), and a significant increase in Mg (mEq/l) in G5 (P <0.05).

When compared to heparin treated group (G2) in 30 days, A very highly significant increase was found in serum levels of Ca (mg/dl), Mg (mEq/l), PO4 (mg/dl), and vitamin D (ng/ml) in G 3,4,5 (P≤0.001).

When comparing the group co-administered heparin and **orally with dandelion water extract (DWE) (G3) for 30 days**, A very highly significant increase was found in serum level of Ca (mg/dl) (P≤0.001), but highly significant in PO4 (mg/dl), and vitamin D (ng/ml) (P≤0.01) and significant increase mg (mEq/l) (P<0.05) in G 4. A very highly significant decrease was found in serum levels of vitamin D (ng/ml) (P≤0.001), a significant decrease in Ca (mg/dl), and Mg (mEq/l) (P<0.05), a non-significant decrease in PO4 (mg/dl) in G 5.

When compared to the combination group (G5) co-administered heparin and **orally with dandelion water extract (DWE)** and **vitamin C** and **K for 30 days**, A very highly significant decrease was found in serum levels of Ca (mg/dl), Mg (mEq/l), and vitamin D (ng/ml) (P≤0.001), but significant decrease in PO4 (mg/dl), in G 4 (P<0.05).

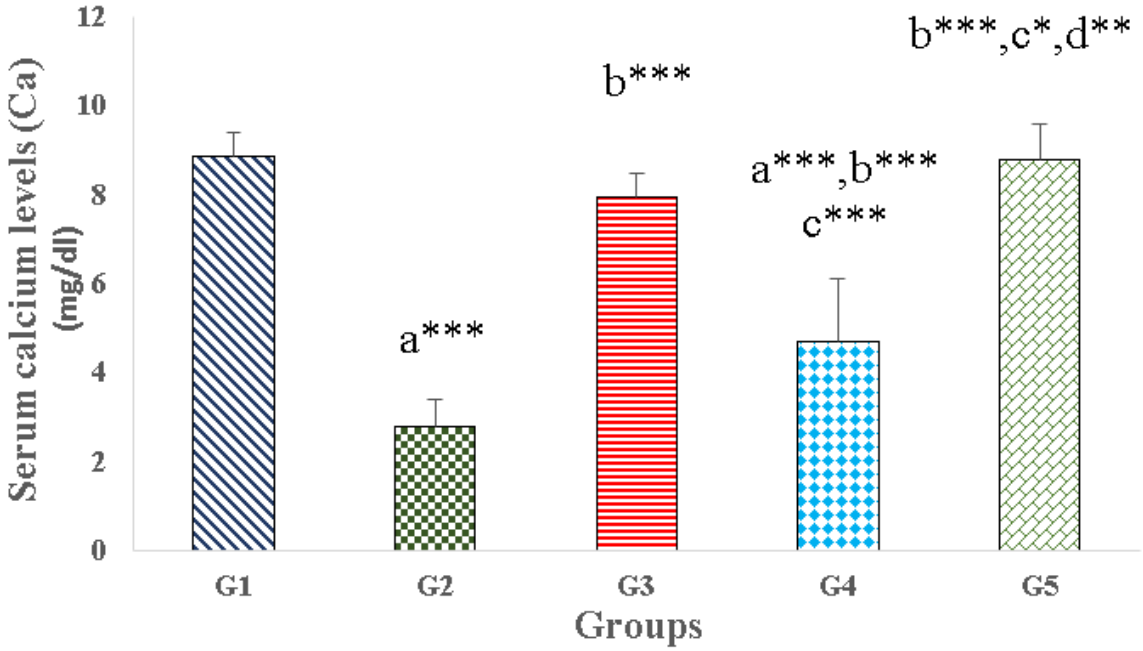

Figure 1. Effect on serum calcium levels (Ca) after 30 days. Mean±SD: stander deviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K) and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.001 very highly significant decrease when compared with G2 and G4. [b]P ≤0.001 very highly decrease significantly when compared with G2. [c]P ≤0.001 very highly significant increase when compared with G4, [c]P<0.05 significant decrease when compared with G5. [d]P ≤0.001 very highly significant decrease when compared with G5.

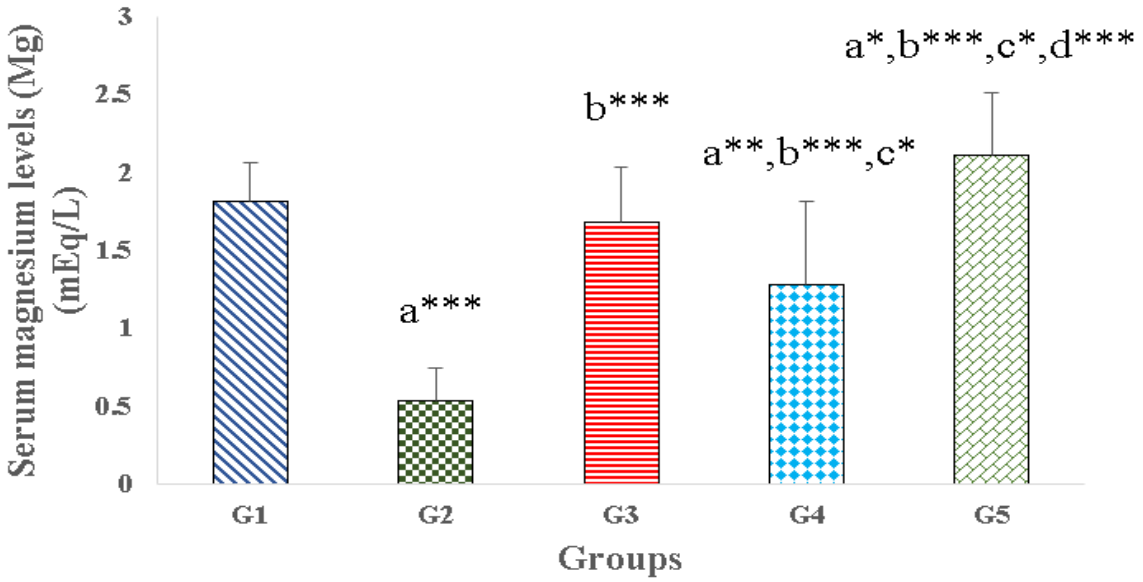

Figure 2. Effect of serum magnesium levels (Mg) after 30 days. Mean±SD: stander deviation G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K) and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.001 very highly significant decrease when compared with G2, [a]P≤0.01 highly significant decrease when compared with G4, [a]P≤0.05 significant increase when compared with G5. [b]P ≤0.001very highly significant increase when compared with G2. [c]P <0.05 significant increase when compared with G4 and significant decrease with G5. [d]P ≤0.001 very highly significant decrease when compared with G5.

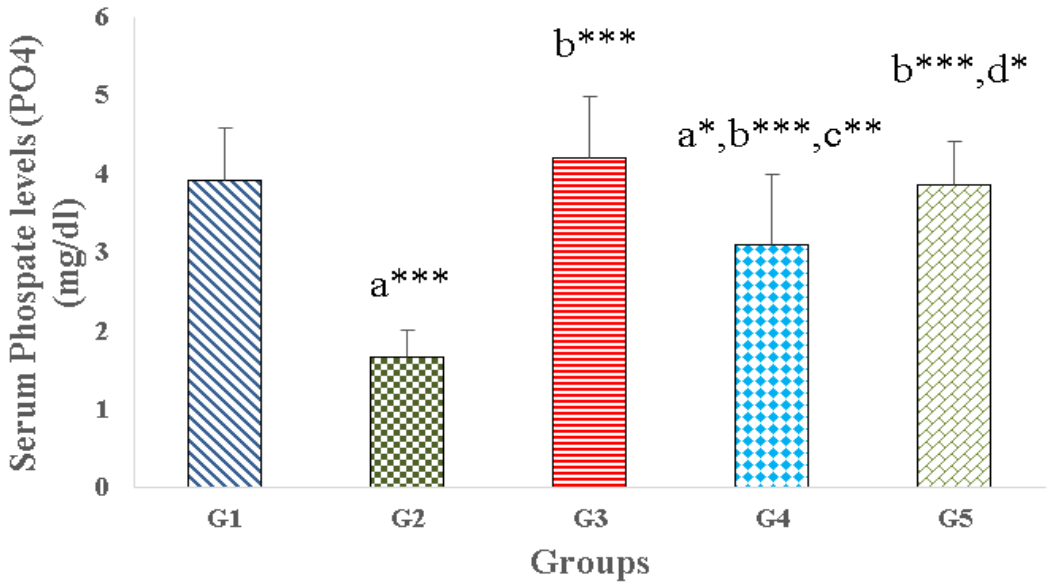

Figure 3. Effect of serum phosphate levels (PO4) after 30 days. Mean±SD: standerdeviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K) and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.001 very highly significant decrease when compared with G2, [a]P<0.05 significant decrease when compared with G4. [b]P ≤0.001very highly increase significant when compared with G2. [c]P≤ 0.001 highly significant increase when compared with G4. [d]P <0.05 significant decrease when compared with G5.

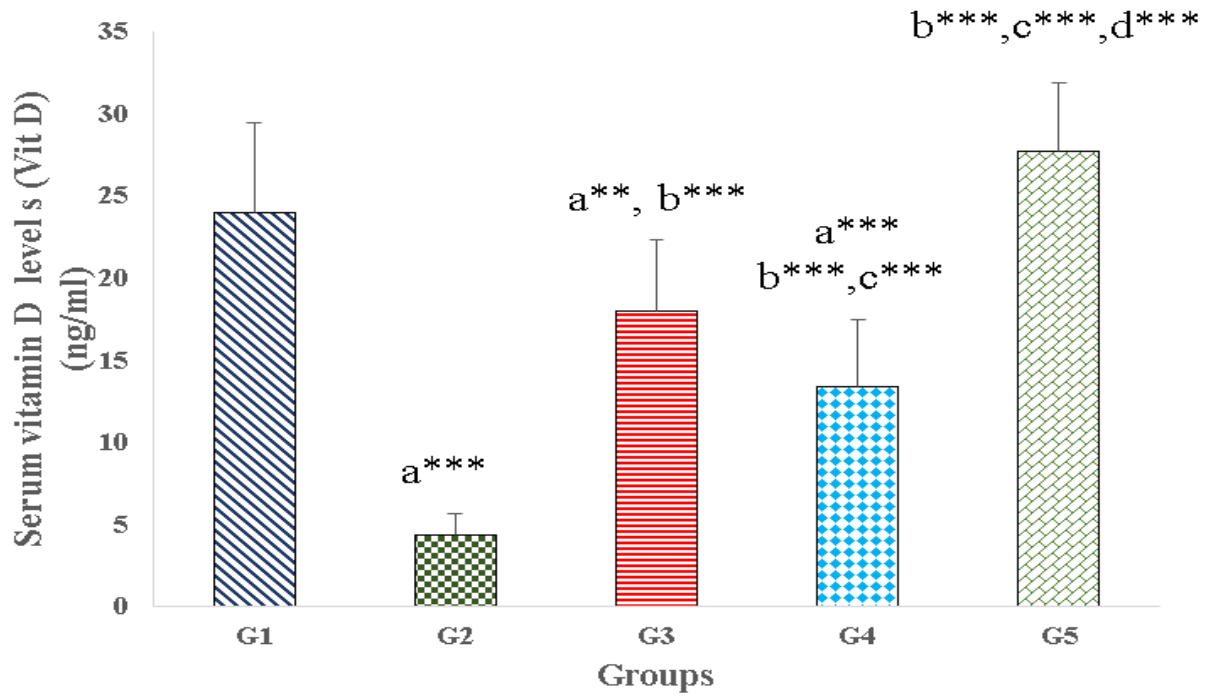

Figure 4. Effect of serum vitamin D (Vit D) levels after 30 days. Mean±SD: stander deviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K) and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.001 very highly significant decrease when compared with G2 and G4, [a]P≤0.01 highly significant decrease when compared with G3. [b]P ≤0.001 very highly significant increase when compared with G2. [c]P ≤0.01 highly significant increase when compared with G4, [c]P ≤0.001 very highly significant decrease when compared with G5. [d]P ≤0.001 very highly significant decrease when compared with G5.

Effect of dand**elion** and **vitamins (C, K) on genetic indicators of osteoporosis in heparin-treated female rats.**

Figures 5, 6 and **7 respectively**, show the serum levels of bone on genetic osteoporosis parameters Sclerostin(SOST), Oteoprotegerin(OPG) and housekeeping gene (GAPDH). The impact of the combination of dandelion, vitamin C, and K with heparin-treated rats was the most potential prophylactic impact on the levels in relation to the treatment with each agent lonely.

As compared to the normal group (G1) after 30 days, a highly significant decrease was found in serum levels of GAPDH in G2 (P≤0.01). A very highly significant decrease was found in serum levels of SOST and OPG in G2 (P≤0.001).

When compared to heparin treated group (G2) in 30 days, A very highly significant increase was found in serum levels of SOST and OPG in G3, G4, and G5 (P≤0.001). Non-significant increase of GAPDH in G3 and G5 while a non-significant decrease in G2 (P ≤0. 01).

When comparing the group co-administered heparin and **orally with d**and**elion water extract (DWE) (G3) for 30 days,** a non-significant decrease was found in serum levels of GAPDH, SOST, and OPG in G3 and G4.

When compared to the combination group (G5) co-administered heparin and **orally with d**and**elion water extract (DWE)** and **vitamin C** and **K for 30 days,** non-significant increase was found in serum level of GAPDH and OPG in G4 while a non-significant i decrease in serum level of SOST G4 (P ≤0. 01).

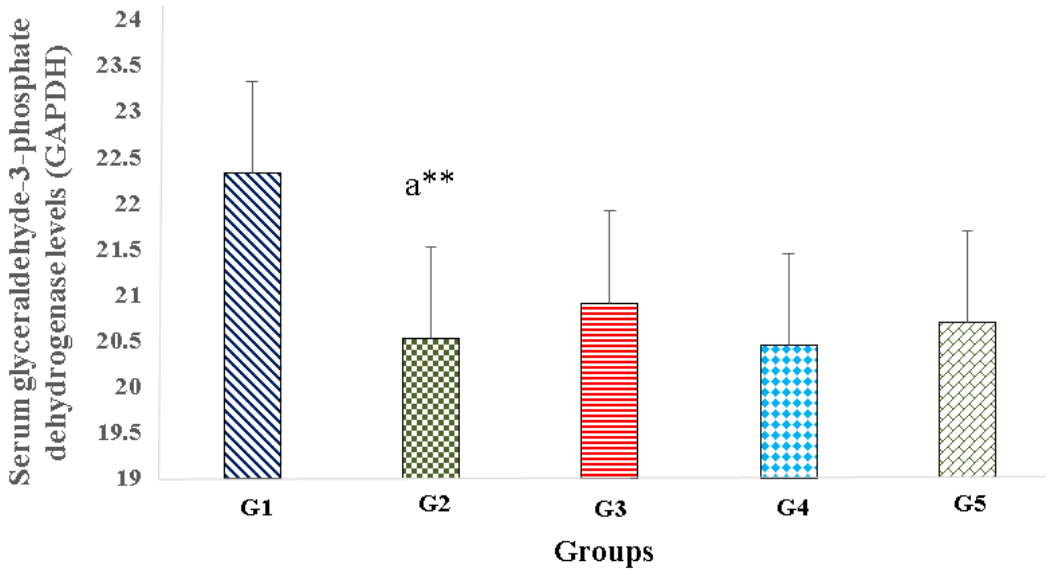

Figure 5. Effect of serum Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after 30 days. Mean±SD: stander deviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K), and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.01 highly significant decrease when compared with G2.

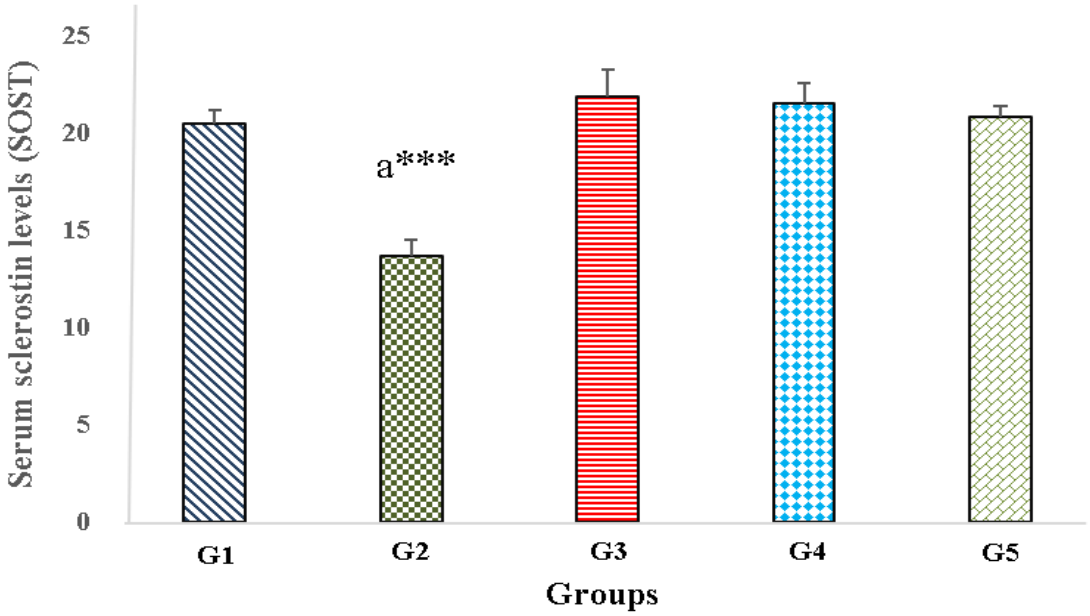

Figure 6. Effect of serum Sclerostin (SOST) after 30 days. Mean±SD: stander deviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K) and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.01 highly significant decrease when compared with G2.

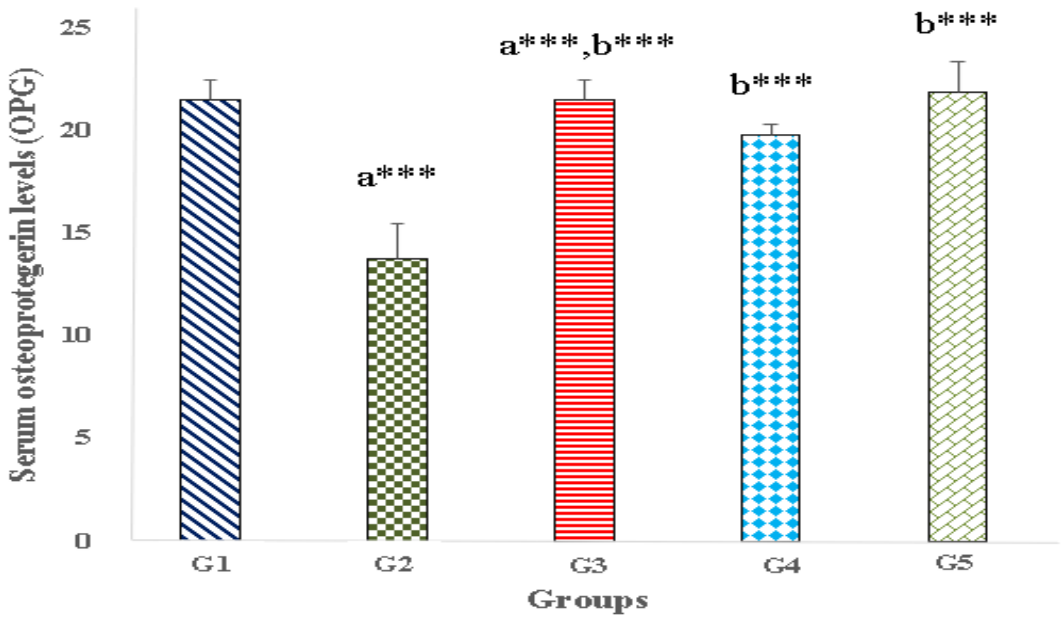

Figure 7. Effect of serum osteoprotegerin (OPG) after 30 days. Mean±SD: stander deviation. G1 (normal group), G2 (Unfractionated heparin), G3 (Unfractionated heparin +Dandelion water extract), G4 (Unfractionated heparin + Vitamin C + Vitamin K), and G5 (Unfractionated heparin + Dandelion water extract + Vitamin C + Vitamin K). [a]P≤0.001 very highly significant decrease when compared with G2 and G4, [a]P<0.05 significant decrease when compared with G3. [b]P ≤0.001 very highly significant increase when compared with G2. [c]P ≤0.001very highly significant increase when compared with G4, [c]P<0.05 significant decrease when compared with G5. [d]P ≤0.001 very highly significant decrease when compared with G5.

4. Discussion

Heparin is broadly used for the prevention and treatment of thrombosis and embolism. At the side required impacts, a pharmaceutical may cause a few undesirable impacts, osteoporosis is an extreme side impact in up to one-third of all patients on long-term treatment (Simann *et al*., 2015), such as bone mass misfortune and auxiliary osteoporosis (Rajgopal *et al*., 2006). Long-term utilization of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) causes bone misfortune and osteoporosis (Yang *et al*., 2020). Clinical trials and creature tests have illustrated that long-term utilization of heparin can actuate bone misfortune and osteoporosis, and a tall total measurement of heparin was related to the movement of bone misfortune (Mätzsch *et al*., 1990; Muir *et al*. 1997; Bick *et al*. 2005; Irie *et al*., 2007) illustrated that heparin improves osteoclastic bone resorption by hindering osteoprotegerin (OPG) action in *vitro*. Heparin ties particularly to OPG and anticipates its interaction with RANKL on the osteoblastic film, so advancing RANK–RANKL interaction and enactment of osteoclasts. Too, Heparin-induced thrombocytopenia (HIT) is ordinarily caused by platelet-activating immunoglobulin G (IgG) antibodies (Abs) against platelet calculate 4 (PF4) complexed with heparin (Vayne *et al*., 2021).

Recently, great interest in folk medicine has been observed, and the discovery of effective prophylactic agents to mitigate heparin-induced bone loss and osteoporosis is very urgent in clinical cases where preventing osteoporosis is more beneficial than treatment in general, because preventing bone loss is easier than restoring lost density. Without effective treatments for osteoporosis in its early stages, it may lead to complications and increase the probability of fractures.

The current study was conducted to investigate the potential prophylactic impact of dandelion and vitamin C and k against the molecular factors involved in osteoporosis and its complications, such as oxidative stress, inflammation, apoptosis, and fibrosis, in female rats treated with heparin.

The current study found that injecting heparin into female rats reduced the levels of biochemical markers of osteoporosis significantly. These findings in electrolytes (Ca, Mg, PO4) are consistent with other studies that found that heparin treatment can cause a decrease in Ca with respect to the effects of heparin, total serum. Ca levels in heparin-treated rats increased significantly after surgery, which was unexpected. This could be because of a decrease in the use of local

calcium as a result of the lower rate of bone formation in heparin-induced secondary osteoporosis (Topal *et al*. 2020).

Furthermore, studies have shown that heparin, due to its high affinity for calcium ions, can inhibit calcification (Nelson, 1997; Hawkins and Evans, 2005). Heparin has been shown in *vitro* to act as a chelating agent to reduce ionized calcium. Reduced ionized calcium levels can stimulate parathyroid hormone, increasing osteoclast activity and bone demineralization. The incidence of symptomatic osteoporosis associated with the use of unfractionated heparin in pregnancy has been reported to be as high as 2-3% of patients on long-term therapy. Although the pathogenesis is unknown, the risk of osteoporosis with long-term heparin use is certain (Le and Rodger, 2008).

The precise cellular mechanism by which heparin contributes to bone loss is unknown. Heparin increases osteoclastic resorption and suppresses osteoblast function, resulting in a decrease in bone mass. Other possible pathways include mast cell loss in the bone marrow and an improvement in the role of parathyroid hormone (PTH), a key calcium regulator in the body. PTH effects increase the release of calcium and phosphorus into the blood, raising serum levels (Taqui *et al*., 2021). Several potential mechanisms have been identified. first, heparin may have an immediate effect on bone cells, either increasing osteoclast activity or decreasing osteoblast activity, or both. Second, due to its high affinity for calcium ions, heparin may inhibit calcification. Finally, as a chelating agent, heparin reduces ionized calcium and stimulates parathyroid hormone, which increases osteoclast activity and bone demineralization (Xia *et al*., 2015). Serum calcium measurement should be one of the first-line investigations in patients with osteoporosis (Rossini *et al*., 2016). A previous study found that serum calcium decreased as the age of osteoporosis in aging women increased. Similar findings have been made by (Khatake *et al*., 2013) who reported that the level of serum calcium in postmenopausal women decreased significantly with age. The same outcome was reported by (Gallagher *et al*., 1979) that postmenopausal women's intestinal calcium absorption declines with age, resulting in lower serum calcium levels These findings are similar to those of Gallagher *et al*., (1979) who observed that serum calcium or active parathyroid hormone levels in osteoporotic women were normal or low when compared to age-matched controls. According to a recent study by Xia *et al*., (2015) revealed that serum phosphorus levels in heparin-treated rats were significantly lower.

Studies on calcitriol levels (1, 25-dihydroxy vitamin D) in women on long-term heparin therapy revealed significantly lower levels than in women not on heparin, indicating that heparin may inhibit the activity of 1hydroxylase in the kidney. Patients taking heparin may require higher doses of vitamin D supplements due to this effect on bone mineralization and resorption (Lycans *et al*., 2016).

Heparin is a drug that affects bone metabolism by interfering with vitamin D absorption, calcium and phosphate metabolism, direct cellular effects on osteoblasts, osteoclasts, and osteoblasts, or interfering with bone quantity or quality (Hofbauer *et al*., 2010).

Furthermore, previous research has shown that magnesium concentrations in those receiving heparin fall below the normal range (Brain *et al*., 2012). Both low and high magnesium levels are detrimental to the bones. Magnesium deficiency contributes to osteoporosis both directly and indirectly by influencing the secretion and activity of parathyroid hormone and by promoting low-grade inflammation (Castiglioni *et al*., 2013).

The results of gene expression studies in osteoclasts and osteoblasts are reported as target genes normalized by the constitutive gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes in gene expression data comparisons (Barber *et al*., 2005).

In *vitro*, heparin increased osteoclastic bone resorption by inhibiting osteoprotegerin (OPG) activity. Heparin binds specifically to OPG and prevents it from interacting with RANKL on the osteoblastic membrane, thereby promoting RANK-RANKL interaction and osteoclast activation. Heparin binds to OPG specifically and prevents OPG-mediated inhibition of osteoclastic bone resorption (Signorelli *et al*., 2019). As a result, despite inhibiting OPG, heparin may not have a significant effect on osteoclast differentiation and may not appear to enhance osteoclastogenesis (Irie *et al*., 2007).

A previous study that examined changes in OPG levels over time observed that heparin-regulated cyclic increases in OPG may play a role in the vascular disease of hemodialysis patients. Some light has been shed in recent years on the relationship between heparin and bone changes, as it has been demonstrated that heparin interferes with osteoprotegerin (OPG) in various ways. OPG acts as a decoy receptor, preventing RANKL from binding to RANK and thus inhibiting all cellular functions associated with this interaction. Several inflammatory cytokines stimulate both the expression and production of OPG and RANKL. Heparin has recently been shown to cause OPG mobilization into circulation via endothelial surface displacement (Cianciolo *et al*., 2011).

In *vivo* studies with intravenous heparin infusions in healthy individuals revealed a 2.2-fold increase in circulating OPG levels within 5 minutes when

compared to preinjection values, but OPG levels were significantly reduced or normalized within 1 hour (Vik *et al*., 2007; Nybo and Rasmussen 2008).

A potential heparin binding site within sclerostin was discovered in a study, which may mediate sclerostin localization on the cell surface of target cells and possibly facilitate the inhibition of Wnt signaling, which is transmitted to osteoblasts to inhibit bone formation (Hens *et al*., 2005). Sclerostin can perform two functions. First, it may keep bone lining cells dormant and thus prevent the initiation of de novo bone formation (Moester *et al*,. 2010).

The authors proposed that the decrease in bone formation was caused by decreased BMP signaling and was independent of sclerostin expression. However, the decrease in bone resorption may be due to increased Wnt signaling caused by the decrease in sclerostin expression. This, in turn, may be due to Wnts upregulating osteoprotegerin in mature osteoblasts, inhibiting RANKL-induced osteoclastogenesis (Goldring and Goldring, 2007).

Our findings show that dandelion and vitamins C and K have a positive effect, and that combining dandelion and vitamins C and K gives more effective results. According to numerous studies, dandelion is a nontoxic herb that can potentially be used for its choleretic, diuretic, antirheumatic, and anti-inflammatory properties. Dandelion has recently received attention for its antioxidant activity and potential benefits against the development of obesity, cancer, and a variety of cardiovascular risk factors. Indeed, dandelion contains a diverse range of phytochemicals, the biological activities of which are being studied in various areas of human health. According to emerging evidence, dandelion and its constituents have antioxidant and anti-inflammatory properties that result in a variety of biological effects (González-Castejón *et al*., 2012). A nutrient analysis of dandelion reveals high amounts of minerals, proteins, fiber, and vitamins, as well as a balanced combination of trace elements, making dandelion an interesting source of micronutrients (Escudero *et al*., 2003 and Shi *et al*., 2008).

According to reports, dandelion leaves are high in fiber, potassium, iron, calcium, magnesium, phosphorus, vitamins A and C, B vitamins thiamine and riboflavin, and protein (Fatima *et al*., 2018). The ca and P ratio in dandelion leaves indicates that they are a good source of minerals required for bone formation. Calcium assimilation by the body is determined not only by the amount of calcium in the product but also by the proportion of calcium to other elements, particularly phosphorus and magnesium. Excess phosphorus consumption, for example, may result in bone mass loss. A high phosphorus intake combined with a low calcium intake can disrupt the hormone that regulates calcium metabolism and vitamin D synthesis. (Biel *et al*., 2017).

Dandelion, on the other hand, contains many bioactive compounds, including phenolic acids, flavonoids, coumarin, sesquiterpene lactones, triterpene, phytosterols, and inulin. Dandelion-derived phytochemicals have well-known health benefits and are used to treat a variety of ailments (Singh *et al*., 2018). Because of their antioxidant and anti-inflammatory properties, bioactive phenolic compounds have long been recognized as health-benefiting components. They can protect bone health by lowering bone loss through antioxidant activity, lowering bone loss through anti-inflammatory action, increasing osteoblastogenesis, and decreasing osteoclastogenesis and osteoimmunological activity. Many flavonoid compounds have been shown to improve bone metabolism (Nicolin *et al*., 2019).

Furthermore, data from a previous study revealed, As a result, coumarin derivatives have the potential to be used as promising natural pharmaceutical agents for the treatment of osteoporosis. After binding to its RANK receptor on osteoclast precursors, RANKL transmits osteoclast differentiation signals via the NF-B pathway and its mediator MAPK proteins, upregulating the expression of the transcription factors c-Fos and NFATc1 to stimulate osteoclast formation and activation (Wada *et al*., 2006 and Kang *et al*., 2016).

Rivera-Huerta *et al*., (2017) have provided tangible evidence that inulin-good health can be especially effective in preventing chronic diseases such as osteoporosis by promoting calcium absorption and uptake. In a study on rat models that evaluated the effects of inulin, rats fed a diet containing a 10% inulin supplement absorbed more calcium, magnesium, zinc, and iron than the other group. Their calcium balance was also improved. Calcium levels in the femur and tibia were found to be significantly higher in the group that received the inulin supplement. Inulin supplementation also increased bone mineral content (calcium and zinc) and bone strength. This study confirms that inulin improves mineral bioavailability (Lobo *et al*., 2009).

Vitamin C is an important antioxidant and cofactor that regulates the development, function, and maintenance of many different cell types in the body. Several epidemiological studies and genetic mouse models show that vitamin C has a positive effect on bone health. Overall, vitamin C promotes trabecular bone formation by affecting the expression of bone matrix genes in osteoblasts (Aghajanian *et al*., 2015).

Several studies have found that nutrients and vitamins, such as vitamin C and, more recently, vitamin K, play an important role in maintaining optimal bone health, particularly in older adults (Fusaro *et al*., 2017).

According to one study, vitamin C may have a protective effect, lowering the risk of osteoporosis in people with low levels of physical activity. Vitamin C is also important in supporting other mechanisms involved in bone health. This vitamin enhances the absorption of calcium, a mineral necessary for the formation of strong bones (Kim and Lee, 2016).

As a cofactor in the hydroxylation of lysine and proline, vitamin C is required for the cross-linking of collagen fibers in bone. Vitamin C also increases the activity of alkaline phosphatase, an osteoblast formation marker (Palacios, 2006). A population-based study found that vitamin C supplementation improved bone mineral density in postmenopausal women (Morton *et al*., 2001). Higher dietary vitamin C intake was linked to lower bone mineral density loss in elderly men participants in the Osteoporosis Study. (Sahni *et al*., 2008).

Vitamin K is required for the synthesis of two bone proteins, osteocalcin and matrix Gla protein, which is controlled by vitamin D. Osteocalcin is a noncollagenous bone protein produced by osteoblasts during the formation of the bone matrix. The hydroxyapatite binding capacity of osteocalcin is provided by vitamin K-dependent y carboxylation of three glutamic acid residues (Joshi and Raghuvanshi, 2020). The matrix Gla protein is found in bones and cartilage, where it binds to both organic and mineral components of bone (Booth and Charette, 2004).

Conclusion:

The current investigation showed that heparin therapy has the potential to cause osteoporosis. Combined treatment with dandelion and vitamin C and K/or a combination of dandelion and vitamin C could result in prophylactic protection of bone tissue from the potential effect of heparin, which was more pronounced in rats treated with the combination of the three agents. The current study may provide a preventive strategy against drug-related osteoporosis that can be considered as enhanced and preventive care for the general population that may reduce the cost of treating bone diseases associated with osteoporosis.

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