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Therapeutic and Prophylactic Efficacy of Garden Cress Seed Oil against Osteoporosis in Rats

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Abstract

The target of this study was to estimate the efficacy of garden cress seed oil in the treatment of osteoporosis in rats. Seventy adult female Sprague-Dawley albino rats were used that were divided into seven groups (n-10/ group). Methotrexate (MTX) was subcutaneously injected (0.65 mg/kg) for two separate 5 days courses (5 days on/9 days off), to induce osteoporotic rat model. Garden cress seed oil was orally gavages with two patterns: the therapeutic pattern in which rats were orally gavages with oil in two doses (200 and 400 mg/ kg b w) after completion of injection of methotrexate. The prophylactic pattern rats were injected with methotrexate in concurrent with the oil at the same doses. The oils injection period for the two treated pattern was four weeks. After the completion of the treatment, animals were anesthetized and blood samples were collected for biochemical analysis. Femoral bones of the treated and control groups were collected to study the expression of genes associated with bone remodeling (Cathapsin K and TNF-αand Osterix). Bones histopathological and morphometric studies were performed. Garden cress oil improved minerals and vitamin D content in osteoporotic animals. The results of the gene expression showed that the oil reduced the gene expression level of Cathapsin K and TNF-α, while increased the level of Ostrix, leading to improving the histological structure and bone thickness of the osteoporotic animals. Garden cress seed oil considerably improved the osteoporosis bone marker through both therapeutic and prophylactic approaches. In addition, data revealed that the therapeutics pattern was more alleviative than prophylactic.

Keywords: Osteoporosis, Minerals, Vitamin D, Bone remodeling genes, Bone morphometric.

1. Introduction

Osteoporosis is a metabolic bone disease resulting in an imbalance of bone remodeling, in which the rate of bone resorption is higher than bone formation (lama *et al*., 2017). In turn, this provides rise to low bone mass, micro-architectural deterioration, and finally an increased risk for fragility fractures (Sucuoglu & Koyuncu, 2017; Poole *et al*., 2017). Methotrexate (MTX) is a folic acid antagonist and it is the most commonly used anti-metabolite agent for different malignancies such as choriocarcinoma and osteogenic sarcoma (Fan *et al*., 2012). MTX is used as first line therapy in treatment of rheumatoid arthritis (RA) and other inflammatory diseases such as psoriasis and dermatomyositis (Minaur *et al*., 2002). MTX was known to cause reduced bone mineral density (BMD), fractures and ingrowths defects (Fischer *et al*., 2005). The current utilize of oral corticosteroids is allied with severe side effects, include osteoporosis (Henneicke *et al*. 2014).

Osteoporosis recent therapies focus on stopping bone resorption and reducing bone remodeling (Wu *et al*., 2017). Parathyroid hormone, and its analogue teriparatide, is the only anabolic therapies obtainable to treat osteoporosis (Reginster *et al*., 2013). The existing drug therapies have been established to improve bone mineral density and moderate fracture risk, but prolonged use has been allied with various side effects (Hough *et al*., 2014). Consequently, the exploration for new drugs is continuing (Fouda *et al*., 2017). The osteoporosis prophylactic agents are restricted to calcium and vitamin D. Epidemiological studies have explored the connotation between fruits and vegetables ingestion and bone health. Most observational studies establish that more intakes of fruits and vegetables are linked with growth in bone mass and reductions in bone loss and fracture risk (Xie *et al*., 2013; Byberg *et al*., 2015; Benetou *et al*., 2016).

Natural products of plant origin as drugs alternative sources are still a main part of traditional medical schemes in developing countries. *L. sativum* (family cruciferae) is cultivated in Egypt by three species: *L. latifolium*, *L. sativum* and *L. aucheri*. The most public one is *L. sativum* and its seeds can be used as functional food (Sakran *et al*., 2014). Garden cress (*Lepidium sativum*) is a high-nutrient plant and has considerable content of vitamins A, C and K and dietary minerals. Garden cress is an excellent source of folic acid, linoleic acid and tocopherols (Datta *et al*., 2011). Seeds, leaves and roots of Garden cress (GC) are commercially vital for their useful pharmaceutical compounds and are utilized in traditional medicine (Pinheiro *et al*., 2011; Mohammad *et al*., 2012; Kashani *et al*., 2013). Garden cress is considered as one of the popular

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medicinal plants used in numerous Arab countries as a good mediator for bone fracture healing (Wadhwa *et al*. 2012). The seeds have carbohydrates, phenolic compounds, flavonoids, proteins, saponins and lipids. Oleic and linolenic acids are the crucial fatty acids in *L. sativum* oil and it has worthy quantity of lignans and antioxidants that can stabilize the n-3 polyunsaturated fatty acids. The plant is recognized to have imidazole, lepidine, semilepidinoside A and B, β-carotenes, ascorbic, palmitic, stearic, sinapic and sinapin acids (Bryan *et al*., 2009).

The objective of the present research was the evaluation the efficacy of Garden cress seed oil as a therapeutic and prophylactic agent in Methotrexate induced osteoporotic rats through biochemical studies to evaluate minerals and vitamin D content and histological as well as morphometric studies. The relationship between the role of plants in improving bone health and expression of genes that regulate bone formation was also studied.

2. Materials and Methods

2.1. Drugs

Methotrexate vial was obtained from EIMC United Pharmaceuticals Company (Cairo-Egypt). Garden cress seed oil (GCSO) was obtained from Haraz Co. Cairo, Egypt.

2.2. Experimental design

Seventy adult female Sprague-Dawley albino rats at the age of 5 weeks, weighing approximately (100-150 g) were obtained from the animal house of National Research Centre, Giza, Egypt. Animals were housed in clear plastic cages with stainless steel wire lids and kept in an animal room with controlled environmental conditions (12-h light/12-h dark cycle, temperature 22 ºC) on closed ventilated shelves. The animals were fed on rat chow pellets and received water *ad libitum.* The Ethics Committee of the National Research Centre (Approval No. 19032) approved the research. Rats were randomly categorized into seven groups of ten animals each. Rats were adapted for one week prior to commencement of the experiment. Irrespective of their allocated treatment groups, all rats received daily subcutaneous injections of MTX as well as oral gavages of GCSO with prophylactic and therapeutic pattern. The MTX group was subcutaneously injected by (0.65 mg/kg/day) for two separate 5 days courses (5 days on/9 days off) according to Fan *et al*. (2012). Garden cress oil groups was administered at 200 and 400 mg/kg once daily according to Yogesh *et al.(*2010) via oral gavages throughout the trial: for 28 days after the final MTX administration (200 and 400 GCSO Ther) and for 28 days concurrently with MTX administration (200 and 400 GCSO Pro). Control groups received oral gavages of saline as negative control and GCSO 400mg/kg.

2.3. Biochemical analyses

At the end of experiment, the rats were fasted overnight, blood samples were collected in tubes, and centrifuged at 3000 rpm under cooling for 15 min to separate the serum that was subjected to different assays. Serum calcium (Ca), phosphorus (P) levels were determined using colorimetric assay kits (BioSystems S.A., Costa Brava, Barcelona, Spain). Serum bone-specific alkaline phosphatase (b-ALP) was estimated by colorimetric assay using specific enzyme kits (Boehringer Mannheim, Germany) according to Nawawi *et al*. (2002). Serum 25(OH) D was analyzed by radioimmunoassays and competitive protein binding assays according to Holick (2005).

2.4. Bone tissue collection

The animals of each treated group were sacrificed by neck vertebra luxation. Femoral bones were isolated and washed with ice-cold saline to carry out the gene expression and histopathology studies. Right femurs were dissected out from all animals and fixed in buffer formol for the histopathological study. The left femur was collected and kept frozen at −80°C for gene expression analysis.

2.5. Real-time quantitative PCR for bone-related gene expression

RNA extraction from the rat right tibias by crushes the bones with liquid nitrogen and homogenized in Easy red total RNA extraction kit (Intronbio, Korea) according to the manufacturer's instructions. The yield and quality of RNA were analyzed using NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA) and gel electrophoresis. RNA (1µg) was treated with RNase-free DNase kit (Promega) to remove any genomic DNA contamination and cDNA was synthesized using HiSenScript ^{TM} cDNA kit (Intronbio, Korea).

2.6. Real-time PCR analysis

Three genes (Cathepsin K, Tumer Necrosis Factor-α (TNFα) and Osterix), related to bone formation and resorption, and glyceraldehyde-3- phosphate dehydrogenase (GAPDH), endogenous control, were used in the present study; see primers properties in Table 2. Real-time polymerase chain reaction (PCR) was performed in Stratagene Mx3005P Real-Time PCR System (Agilent Technologies) in a 20µL reaction. Each 20µL PCR cocktail contained one µL cDNA, 10µl TOPreal^{TM} qPCR 2X PreMIX (SYBR Green with low ROX) (Enzynomics), 0.75µL of forward primer (10 pmol), 0.75µL of reverse primer (10 pmol) and 7.5µL ddH_2O. Amplification conditions included 15 min at 95°C , followed by 40 cycles at 95°C for 15 sec , at 58-63°C for 15sec and 72°C for 30 sec. Melting curve analysis was conducted following each real time PCR. Gene expression data were normalized to GAPDH and analyzed using the $2^{-\Delta\Delta\ Ct}$ method (Livak and Schmittgen, 2001).

Table 1. Primers Property

Gene	Accession no.	Nucleotide sequence 5´–3`	Size of PCR product (bp)
Cathepsin K	NM_007802.4	TGGATGAAATCTCTCGGCGT TCATGTCTCCCAAGTGGTTC	123
TNF	NM_013693.2	CCACCACGCTCTTCTGTCTAC ACCACCAGTTGGTTGTCTTTG	256
Osterix	NM_001348205.1	AGCGACCACTTGAGCAAACAT GCGGCTGATTGGCTTCTTCT	121
GAPDH	NM_001289726.1	AACTTTGGCATTGTGGAAGG ACACATTGGGGGTAGGAACA	223

2.7. Bone histological analysis

Right femurs were dissected out from all animals, fixed in buffer formol for 3 days and decalcified in EDTA solution (10% ethylenediaminetetraacetic acid (in 0.1 M phosphate buffer, pH 7, 8) for approximately 4 to 5 weeks (solution changed once a week). The decalcified specimens were dehydrated processed to form paraffin blocks. Serial sections (5 µm thick) from the femurs were prepared and stained by haematoxylin and eosin (H&E) for microscopic examination (Bancroft, 1994).

2.8. Morphometric study

Morphometric analysis was carried out on routine haematoxylin and eosin stained slides. To measure the mean thickness of the outer cortical bone of the middle shaft of the femur, perpendicular lines were drawn from the periosteum to the endosteum at many sites. The maximum number of osteocytes , mean areas of trabecular concellous bone and the areas of Haversian canal were measured in a ten field/five serial sections for each group at a magnification of ×50 using the image analyzer (a Leica Qwin 500 Image Analyzer (Leica Systems Ltd, Cambridge, UK) in Pathology Department, National Research Center. The results appear automatically on the monitor in the form of the distance measured in µm and area in μm^2 with the mean, SD, the minimum length, and the maximum length and area were measured.

2.9. Statistical Analysis.

Statistical analyses were conducted with SPSS19 software (IBM, New York, NY, USA). Data are expressed as the means ± standard Error (SE). Differences between groups were evaluated by one-way ANOVA followed by Duncan test. A *p*-value ≤ 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Biochemical results

Serum calcium content in osteoporotic animals significantly decreased than in control animals. Therapeutic pattern gavages of GCSO significantly ($p\leq$ 0.05) increased the serum calcium level of osteoporotic animals to the control level. Conversely, in the prophylactic pattern GCSO at 200mg/kg significantly decreased the calcium level in MTX treated animals, while gavages of GCSO at 400mg/kg did not affect the calcium level (Figure 1A). In addition, animals administrated with high dose of GCSO alone exhibited no significant increase in calcium content compared to control.

Phosphorous content of osteoporotic animals was significantly less ($p\leq$ 0.05) than control. Garden Cress seed oil gavages with therapeutic pattern at 200mg/kg none significantly affect the phosphorus content of osteoporotic animals, while, GCSO 400mg/kg significantly ($p\leq$ 0.05) elevated the phosphorous content, while the prophylactic pattern of GCSO gavages at two doses has no change in the phosphorous content of MTX treated animals (Figure 1B). Control animals administrated with 400mg/kg GCSO showed significant decrease ($P\leq$ 0.05) in the phosphorus level than control.

Serum bone alkaline phosphatase (b-ALP) content of osteoporotic animals significantly decreased as compared to control animals,whereas GCSO (200 and 400 mg/kg) gavages with both therapeutic and prophylactic pattern induced significant increase ($p\leq$ 0.05) in the b-ALP content of osteoporotic animals. GCSO 400mg/kg gavage with therapeutic pattern was more efficient. Meanwhile, oil gavages alone significantly increased ($p\leq$ 0.05) the b-ALP content than control (Figure 1C).

Vitamin D analysis result demonstrated that osteoporotic animals showed significant reduction in the concentration of 25 OH Vitamin D as compared to control, while both the therapeutic and prophylactic pattern gavages of GCSO 200mg/kg none significantly increased the Vitamin D level in osteoporotic animals. Moreover, the 400mg/kg GCSO gavages with both two patterns significantly increased ($p\leq$0.05) the Vitamin D level of osteoporotic animals. There was no significant change in vitamin D level between the GCSO treated animals and the control (Figure 1D).

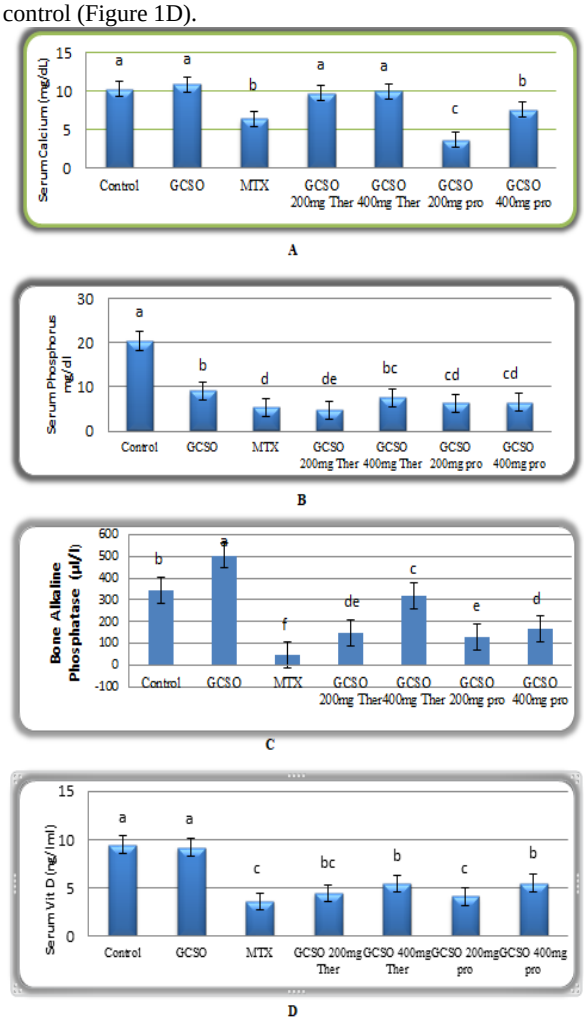

Figure 1. Effects of different doses of garden cress seed oil as therapeutic and protective pattern on serum parameters. A): calcium; B): serum phosphrous; C): bone alkaline phosphatase; D): Vitamin D in MTX osteoporotic rats. Data is presented as mean ± SE (n=5). Mean values with unlike superscript letters were significantly different ($p\leq$ 0.05).

3.2. Gene expression

The gene expression levels of Cat k and TNF-α genes involved in bone resorption and Osterix which involved in bone formation were evaluated to investigate the GCSO therapeutic and prophylactic role against the methotrexate-induced osteoporosis in female rats. Results demonstrate that osteoporotic rats had a significant increase ($p\leq$0.05) in

both Cat k and TNF-α mRNA levels when compared with the control rats. These elevations in gene expressions were significantly ameliorated by either therapeutic or prophylactic treatment with GCSO ($p \leq 0.05$) at 200 or 400 mg/kg b.w. (Figure 2A and B). On the other hand, MTX-induced osteoporotic rats showed significant decrease ($p \leq 0.05$) in Osterix mRNA levels when compared with the control rats. Garden Cress therapeutic (400 mg/kg) treated rats showed a significant mRNA levels increase ($p \leq 0.05$) when compared with the osteoporotic rats, whereas Garden Cress therapeutic (200 mg/kg) and prophylactic treated rats showed non-significant increase in mRNA levels when compared with the MTX-injected rats (figure 2C). In addition, the mRNA expression levels of Cat K, TNF-α and Osterix in rats received only Garden Cress seed oil 400mg/kg was not significantly different from their levels in the control group.

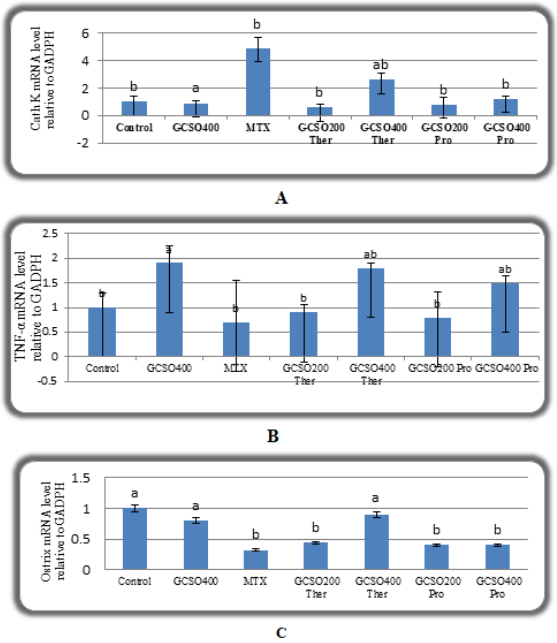

Figure 2. Representative mRNA levels for A: Cathepsin K (Cath K), B:Tumor necrosis factor-alpha (TNF-α) and C:Osterix in rats received normal saline (Control), Garden Cress seed oil at 400 mg/kg (GC400), Methoterxiate (MTX), Garden Cress seed oil as therapeutic pattern at 200 and 400 mg/kg (GCSO 200 and GCSO 400 Ther), Garden Cress seed oil as prophylactic pattern at 200 and 400 mg/kg (GCSO 200 and GCSO400 Pro). Data is presented as mean ± SE (n=5). One−way analysis of variance was used for data analysis (n=5), mean values with unlike superscript letters were significantly different ($p \leq 0.05$).

3.3. Histological and morphometric results

The stained sections of control group showed that the middle shaft of femur bone tissue was of the compact type, covered by two layers, the periosteum located externally, which is a dense connective tissue, and the endosteum, a thin cell-rich connective tissue, lining the internal surface of the bone facing the bone marrow cavity. Histologically, within the bone matrix, osteocytes in their lacunae were detected (Figure 3 A). Compact bone forms a shell around cancellous bone and is the primary component of the long bones. The cancelluos bone trabeculae was formed of a network of bones, consisted of bone lamellae in between which osteocytes stay in their lacunae and bone marrow spaces were present between trabeculae (Figure 3B) .

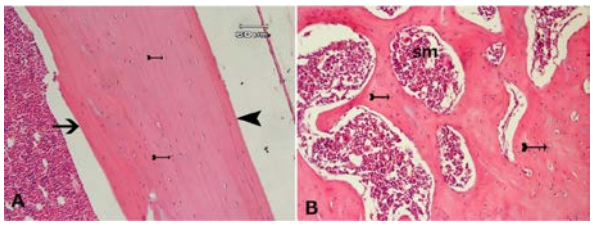

Figure 3. Photograph of femur bone of control rat A) Femur bone shaft showing normal architecture of cortical bone of middle shaft with osteocyte in lacunae (small arrow), surrounded by layer of dense connective tissue periosteum (head arrow) and endosteum an inner layer facing the marrow cavity (arrow); B): Femur head showing normal structure of trabecular concellous bone , osteocyte in their lacunea and bone marrow space (Hx &E x200).

The GCSO 400mg/kg administration revealed no histological alterations in structure of bone tissue, (Fig 4 A). While there was significant increase in shaft of femur cortical thickness as compared to control, no significant difference in Haversian canal areas and the osteocytes number was less than control (195 vs 210) (Table 2). The cancellous bone trabeculae showed no obvious difference in mean areas of trabecular bone compared to control with widening in bone marrow spaces (Figure 4 B & Table 2).

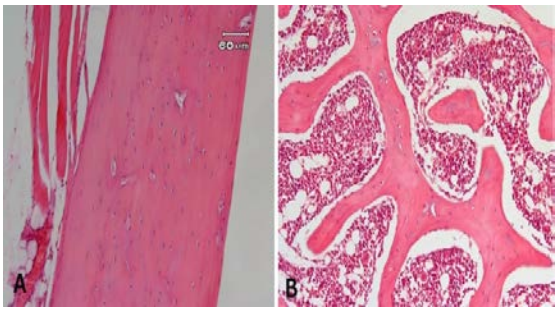

Figure 4: Photograph of bone tissue of rat treated with GCSO 400mg/kg A) The of femur middle shaft with normal structure of bone, significant increase in thickness ,reduction in Haversian canal and osteocytes number ; B) Head femur Trabecular concellous bone showing significant increase in thickness of trabecular bone and widening in bone marrow cavities (H&E x200).

Osteoporotic rat revealed significant decrease in bone thickness of shaft, significant increase in Haversian canal areas and reduction in osteocytes number than control (102 vs 210). Meanwhile, irregularity of the general architecture of bone tissue, gaps, osteoporotic and erosion cavities were observed (Figure 5 A & Table 2). The cancellous bone of osteoporotic animals showed loss of architectures of trabecular bone, with significant reduction of areas of trabecular bone compared to control with widening of bone marrow spaces (Figure 5 B & Table 2).

The microscopic examination of femur shaft of osteoporotic rats treated with GCSO at dose (200 and 400 mg/kg) showed obvious improvement of structure of cortical bone, manifested by significant increase in thickness of compact bone, significant decrease in mean areas of Haversian canal than MTX treated animals (Table 2), and marked increase in osteocyte number (n = 315 and 350, respectively) against MTX group (n=102) indicating recovery of bone tissue. The erosion cavity in endosteal surface of 200mg GCSO treated animals was still found**,** while, no erosion or resorption cavities were noticed in those of 400mg/kg GCSO (Figures 6 A & 7A). Meanwhile, the both two doses of GCSO significantly

increased the mean area of trabecular of cancellous bone than in MTX group (Table 2). The architecture of trabecular bone of 400mg/kg gavages animals appeared nearly normal and in those of both doses narrowing in bone marrow cavities were observed (Figures 6 B & 7 B).

Table 2: Mean area of cortical bone thickness (shaft), mean Haversian canals area, trabecular thickness and number of osteocytes of control and treated groups

Groups	Cortical bone thickness (µm)	Haversian canal area (µm2)	Trabecular mean area (µm2)	Number of Osteocytes
Control	336.62±5.91 [c]	3058.172 ± 535.6 [b]	71109.43 ±10957.0 [a]	210
GCSO (400mg)	372.74± 4.18 [a]	2498.727 ± 434.3 [b]	48873.64 ±7891.7 [a]	195
MTX	90.35 ± 4.45 [e]	45016.49 ± 11453[a]	8669.799 ± 2751.4 [b]	102
GCSO 200mg ther.	272.77 ± 3.75 [d]	5545.00 ±1433.4 [b]	55986.19.±12240.9 [a]	315
GCSO 400mg ther.	365.39 ±2.54 [a]	1557.77± 329.5 [b]	62482.19 ± 14177.7 [a]	350
GCSO 200mg pro	348.82 ± 3.33 [b]	3704.599 ± 786.4 [b]	48815.74 ± 7556.2 [a]	167
GCSO 400mg pro	373.98 ± 3.32 [a]	1087.955 ± 119.5 [b]	71078.5± 11754.4 [a]	189

Note: Values are expressed as the mean ± SEM. Different superscripts within the same column designate significant differences ($p \leq 0.05$)

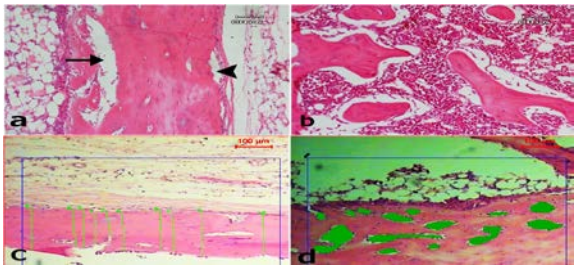

Figure 5. Photograph of MTX treated rat femur bone A) Section of the middle shaft showing significant decrease in bone thickness of cortical bone, irregularity of the general architecture of the tissue, gaps (arrow head),osteoporotic and erosion cavities (arrow) are observed.; A) Section of trabecular of concellous bone showing significant decrease in areas of trabecular bone and widen bone marrow spaces ; C) Section of cortical bone thickness of the same group, the number shown in the figure are the number of measuring lines used by image analyzer system; D) Section of the same group showing sever widen of Haversian canal in cortical bone (H&E x200) (C&d Binary images morphometric measurement).

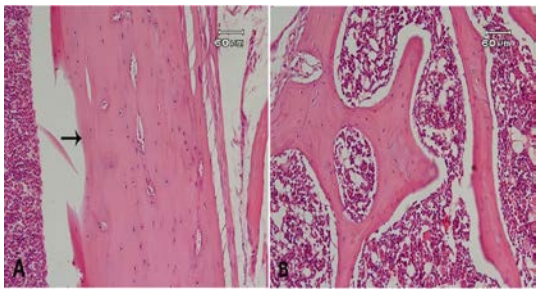

Figure 6. Photograph of femur bone sections of osteoporotic rat treated with 200mg/Kg GCSO A) The middle shaft showing some improvement of structure of cortical bone manifested by significant increase in thickness of shaft reduction in mean areas of Haversian canal and increase in osteocyte count, while, crack (arrow) and the erosion cavity in endosteal surface still found; B) Trabecular bone showing noticeable increase in trabecular area compared with MTX group with increase in the number of osteocytes (H&E x200).

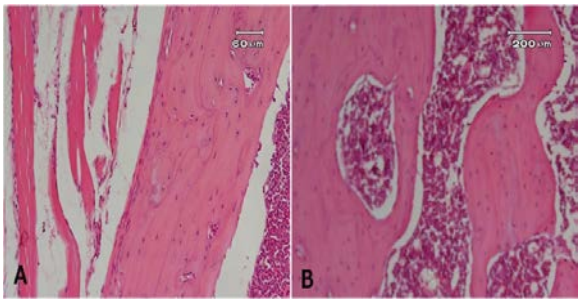

Figure7. Photograph of femur bone sections of osteoporotic rat treated with 400mg/Kg GCSO A) The middle shaft showing highly amelioration in bone tissue represented in significant increase in cortical bone thickness, marked reduction in Haversian canal areas and marked increase in number of osteocytes; B) Trabecular bone showing arked increase in thickness of cortical bone with increase in number of osteocyte and narrowing in bone marrow space (H &E x200).

Examination of shaft cortical bone of rat treated with MTX along with 200 and 400mg/kg GCSO revealed marked protection in architecture of bone tissue represented in significant increase in thickness of cortical bone, significant decrease in Haversian canals compared to MTX treated animal (Figure 8 A & 9 A) and increase in osteocyte number (167 &189 respectively) comparing to MTX (n=102) (Table 2). While, the osteoporotic cavity and erosion in endosteal surface still present in 200mg/kg GCSO treated animals (Figure 8 A). Meanwhile, the trabeculae of cancellous of two dose treated animals showed significant increase in areas of inner cancellous bone trabecular as compared to MTX group (Table 2). Also, calcified cartilage, and dilation of bone marrow space were observed in 200mg/kg treated animals (Figure 8 B). In addition, 400mg/kg GCSO administration revealed normalization of bone trabeculae reach near to control, but increase in osteocytes and narrow in bone marrow spaces (Figure 9 B).

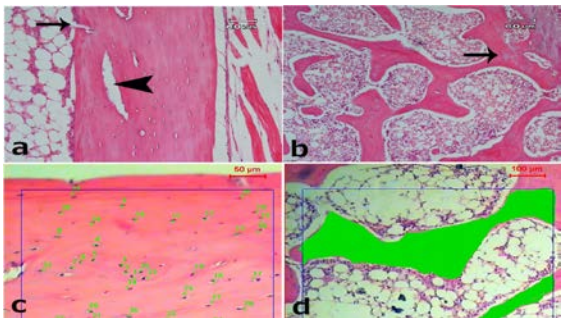

Figure 8: Photograph of femur bone sections of rat treated with MTX along with 200mg/Kg GCSO showing A) Shaft of bone showing significant increase in thickness, marked reduction of Haversian canal area and increase osteocyte count. while osteoporotic (arrow head) and erosion cavity (arrow) in endosteal surface still found; B) Trabecular bone showing significant increase in thickness, calcified cartilage (arrow) and widened in marrow spaces; C): Cortical bone showing count of osteocyte number; D): Concellous bone trabeculae showing area of inner concellous bone (H&E x200) . (C&D: binary of image morphometric measurements).

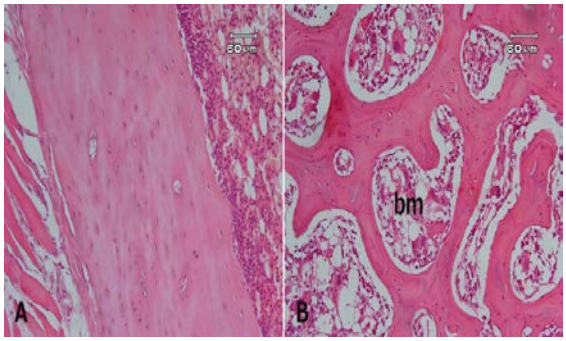

Figure 9. Photograph of femur bone sections of rat treated with MTX along with 400mg/Kg GCSO showing A) Middle shaft bone showing marked protection in structure of bone tissue represented in significant increase in bone thickness, increase in osteocyte number and significant decrease in Haversion canal areas, while some cavity still present.; B) Trabecular of cancelloue bone showing normalization of bone trabeulae, significant increase in thickness of trabecular bone with increase in osteocytes and narrowing of marrow space (bm) (H&E x200).

4. Discussions

In the present study, the methotrexate osteoporotic rat model showed a decline in the levels of serum minerals, including (calcium, phosphorous), bone alkaline phospatase and vitamin D. Corticosteroid-induced osteoporosis was shown to diminish bone mineral density associated with enhanced the fracture risks in animal models (Leonard, 2007). Goralczyk *et al*. (2015) reported that patients treated with MTX verified lower levels of serum 25(OH) D, calcium (Ca), phosphorus (P), and total alkaline phosphatase (ALP). The turn down in the serum levels of Ca and P could be due to enhanced renal excretion and alteration in their transport across the brush border membrane (Banji *et al*., 2014). Extra glucocorticoid declines intestinal calcium absorption and hypercalciuria due to defective vitamin D metabolism. These result in increased bone resorption, declined osteoblast proliferation and biosynthetic activity.

Bone alkaline phosphatase marker (b-ALP) reveals the bone destruction in conditions that affect bone metabolism. ALP, a non-specific bone formation marker, is existing in all tissues of the body, but is particularly intense in liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Iqbal, 2011). The b-ALP values in the current study were significantly reduced in the osteoporosis rats showing an alteration in the bone formation and bone mineralization. Our findings were consistent with Cavalcanti *et al*. (2014); they confirmed that alkaline phosphatase levels decreased after MTX treatment. In addition, several studies indicated a marked decrease in ALP level of glucocorticoid-induced osteoporotic rats, a marker of osteoblast differentiation in primary rat osteoblasts (Elshal *et al*., 2013; Chen *et al*., 2016; Lucinda *et al*., 2017).

Conversely, the administration of GCSO with both therapeutic and prophylactic pattern attenuates the levels of Ca, P, b-ALP and Vitamin D of osteoporotic rats, the therapeutic pattern was efficient more the prophylactic. These findings were in agreement with Elshal *et al*. (2013); they noticed that osteoporotic animals treatment with *Lepidium sativum* recovers the concentration of serum b-ALP to levels higher than that in control. These findings are in harmony with mentioned benefits of *Lepidium sativum* seeds that brought a marked impact on rabbits fracture healing (Juma, 2007). In addition, Gabr *et al*. (2017) reported that *L. sativum* extract is effective in protection against PA-induced osteoporotic hypocalcemia in male and female rats. In this connection, *L. sativum* extract is a worthy source of linolenic acid, which was shown to prevent bone reabsorption, bone remodeling markers and decrease the elimination of Ca.

Cathepsin K is highly expressed in osteoclasts secreted into the osteoclast–bone cell interface leads to efficient degradation of type I collagen. Cathepsin deficiency in humans causes pycnodysostosis that is categorized by enlarged bone mineral density (Drake, 2017). The cytokine tumor necrosis factor α (TNF-α) plays an important role in modulation of bone cell function, regulation and differentiation (Osta *et al*., 2014; Kotrych *et al*., 2016; Mortezazadeh *et al*., 2018). The gene expression data revealed high levels of Cathepsin k and TNF-α mRNA in MTX- induced osteoporotic rats. Song *et al*. (2018) confirmed that the gene expression of Cat K was significantly higher in osteoporotic mice. Various clinical cases revealed TNF-α level up regulation in patients undertaking chemotherapy, recommending its prospective role in chemotherapy-induced osteoclastogenesis. According to the osteoblasts and osteoclasts role in bone regeneration, it is evident that the degree of new osteoblasts and osteoclasts formation has critical impact on bone degradation (Tremollieres and Ribot, 2010). Sex steroids deficiency up-regulates the formation of osteoclasts and osteoblasts by up-regulating the creation of cytokines, including IL-6, TNF, IL-1, which mediate osteoclastogenesis and osteoblastogenesis (Gallagher, 2008).

Epidemiological indication advises that ingesting of vegetables and fruits rich diet that comprises major quantities of bioactive phytochemicals has positive effects for health (Pandey and Rizvi, 2009). In the present study administration of GCSO revealed down regulated the expression of both the Cat K and TNF-α in osteoporotic animals. This finding was in simultaneous with Bu *et al*. (2008) who informed that dried plum polyphenols inhibit the activity of TNF-α and down regulate the transcription factor-T cell nuclear factor (NFATc1) during

osteoclastogenesis. In addition, a study on p-coumaric acid, a polyphenol existing in many vegetables and fruits, established its effective immunosuppressive property for it significantly down regulated the TNF-α expression of in adjuvant arthritic rats (Pragasam *et al*., 2013).

Concerning the osteoblastic differentiation, our data showed a significant down-regulation in the mRNA expression level of osterix which was observed in the MTX-stimulated osteoblasts. Osterix is a major transcription factor that plays a vital character in bone formation and the osteoblast genes expression (Sinha & Zhou, 2013). Lu *et al*. (2006) found that TNF control the Osx expression by suppressing the transcriptional action of its promoter and the inhibition mechanism was mediated *via* a mitogen-activated protein kinase (MAPK) signal. In our results, however, we establish that GCSO promoted osteoblast differentiation and elevated the expression levels of Osterix, the master gene of osteoblast differentiation. This was supported with the study of Choi *et al*. (2016)who found that a novel osteogenic plant showed an capacity to induce osteoblast differentiation. It enhanced osteogenic activity via increased the level of ALP besides the Runx2 transcriptional activity and Osterix.

The ameliorated role of vegetables and fruits against bone osteoporosis or in maintaining bone health turn back to vitamin C, vitamin K, and phytochemicals highly enriched in fruits and vegetables that participate in bone matrix synthesis. Vitamin C has potency to affect bone mass in the hydroxylation of lysine and proline that are required for the construction of stable collagen triple helixes. Vitamin K may show a protecting character against bone loss related age through vitamin K dependent γ-carboxylation of osteocalcin (Ahmadieh and Arabi, 2011). Fruits and vegetables, as a worthy source of alkaline precursors (e.g., K, Ca, Mg), can neutralize the calciuric action of acids diet as confirmed in a modern meta-analysis (Lambert *et al*., 2015).

Regarding the histological examination of our study, osteoporotic rats revealed decrease in bone thickness of shaft, increase in Haversian canal areas, reduction of trabecular bone areas and bone marrow spaces widening and reduction in osteocytes number. The results of the current work are compatible with those of Elsaid and Sadek (2017) who found that MTX caused marked thinning of the periosteum specially the fibrous layer and seeming lessen in the osteocytes number. In addition, the cancellous bone of MTX treated animals revealed thin, commonly detached bone trabeculae with bone marrow spaces widening. Previous animal study revealed that while long term low-dose MTX treatment caused no damage to the growth plate, two cycles of high-dose MTX caused a significant decrease in growth plate height (Fan *et al*., 2009), that was for the diminished of chondrocyte proliferation and collagen-II production, in addition to the stimulation of chondrocyte apoptosis probably through the Fas/FasL death receptor pathway (Xian *et al*., 2007). Due to the growth plate dysfunction, a significant reduction in the thickness of newly formed primary spongiosa bone was originated in the adjacent metaphyseal bone, mirroring the thinning of the growth plate (Xian et al., 2007; 2008). El-Morsi *et al*. (2011) supposed that osteoporosis could be demonstrated as thinning of bone trabeculae or as deletion of some bone trabeculae with remaining trabeculae of normal thickness .

Histological examination showed that administration of GCSO to osteoporotic induced obvious improvement of structure of cortical bone. The architecture of trabecular bone of high dose appeared nearly normal and in those of both two doses narrowing in bone marrow cavities were observed. This data was in line with Elshal *et al*. (2013) who evaluated the role of *Lepidium Sativum* supplementation on histological appearance of tibia trabecular bone in glucocorticoid-induced osteoporosis (GIO) rats, where the trabeculae of inner cancellous bone missing their normal architecture and seemed as irregular bony ossicles disjointed by widened bone marrow spaces. *Lepidium Sativum*-nourished rats revealed marked improvement as compared to those of the GIO-rats, where the cortical bone thickness was exactly like the control and the cancellous bone trabeculae partially recovered near normal structure and looked extra continuous with fewer enlarged bone marrow spaces. Diwakar *et al*. (2008) attributed the constructive effect on bone density *of Lepidium Sativum* to its rich content of calcium, and to its capability to rise serum and liver alpha linolenic acid (ALA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) that have beneficial effects on bone. In addition, numerous epidemiological studies have inspected the link among fruits and vegetables intake and bone health. Most observational studies have reported that more intakes of fruits and vegetables are concomitant with an upturn in bone mass and declines in bone loss and fracture risk (Xie *et al*., 2013; Byberg *et al*., 2015; Benetou *et al*., 2016).

5. Conclusion

We prove that garden cress seed oil oral gavages considerably improved the osteoporosis bone markers through both therapeutic and prophylactic approaches. In addition, data of the tested parameters revealed that the therapeutics pattern was more alleviative than prophylactic. The present article highlights the prospective mechanism of action of garden cress seeds against osteoporosis and recommends further studies for evolving novel therapeutic tools in osteoporosis treatment.

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