Protective Role of Chicory Leaves Extract against Gamma Radiation-Induced Bone Loss and Down-expression of Osteocalcin Gene in Albino Female Rats

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ABSTRACT

People all over the world widely use medicinal plants as alternatives to pharmaceutical drugs. Herbal products have fewer side effects in comparison to synthetic drugs. The present study was done to demonstrate the effect of aqueous extract of chicory leaves (*Cichorium intybus L.*) against gamma radiation induced-bone loss in rats' long bones. Twenty-four female albino rats weighing about 130-150 g were divided into 4 groups. Group 1(C): Normal control, in which the rats were neither treated nor irradiated; Group 2 (CH): the rats received orally 100 mg/kg b. wt. of chicory leaves aqueous extract every day for 28 days; Group3 (IR): the rats were exposed to whole-body gamma radiation with a fractionated dose (3 Gy / week up to 9 Gy total doses); and Group 4 (CH + IR): the animals received chicory leaves aqueous extract as group 2 daily for 7 days before exposed to fractionated dose and also within the period of fractionated irradiation (21 days). Gene expression of osteocalcin was measured by real-time PCR in addition to chromosomal analysis and histopathological examination. Also, serum levels of phosphorus, calcium, and calcitonin were measured. Our studies demonstrated that aqueous extract of chicory leaves supplementation was equally effective at preventing the skeletal responses to gamma radiation, through improvement in chromosomal aberrations, bone trabecular architecture, bone minerals elevation and upregulated osteocalcin gene.

Key Words: *Gamma Radiation, Chicory Extract, DNA Damage, Chromosomal Aberrations.*

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INTRODUCTION

The use of natural and herbal products can be useful in protection against damage induced by radiation because they have a few toxicities or even they are non –toxic compared with synthetic products in their optimal protective doses [1]. Chicory (*Cichorium intybus* L.) is a member of the *Asteraceae* family, and a biennial plant, that is one of the important medicinal plants cultivated throughout Egypt [2]. Several authors have reported the medicinal importance of chicory due to the presence of some phytochemicals, such as inulin (starch-like polysaccharide), flavonoids, vitamins, coumarins, tannins, sesquiterpene lactones (lactucin and lactucopicrin), minerals alkaloids, and volatile oils [3, 4]. The secondary metabolites (coumarins, flavonoids, and tannins) of chicory have been proved to have some biological activities such as anticancer, antihepatotoxic, antioxidant, anti-inflammatory, and antiparasitic, which have a positive health effect on humans [5, 6].

On the other hand, ionizing radiation (IR) can cause changes in the chemical balance of cell and affects humans by depositing energy in body tissue [7, 8]. Exposure to ionizing radiation increases the production of reactive oxygen species (ROS) and causes lipid peroxidation in cell membrane and damage to cellular activities leading to dysfunction of cells and tissues [9].

In addition, exposure to ionizing radiation has several challenges to bone health, most notably reducing bone strength that results in a loss of bone mass. Bone damage that occurs after absorption of therapeutic radiation has

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been recorded for decades. Some animal models are showing that the doses and properties of spatial light beams, due to an acute increase in bone resorption and bone formation suppression, represent a health risk to the skeleton [10, 11]. In addition, the increasing use of radiation in agricultural and industrial activities as well as clinical practice, and high levels of radioactivity left by nuclear testing explosions have a significant impact on the potential risk of radiation in humans [12].

Also, ionizing radiation has harmful effects on living cells by damaging the DNA which is the vital cellular target. The lesions generated in DNA by radiation include intra- or inter-strand cross-linking and double and single-strand breaks. The cell responses include cell cycle arrest, progression in the checkpoints of cell cycle and DNA repair induction. Protecting the living system from intensifying ionization radiation is important in radiation biology [13].

Therefore, this study was proposed to assess the protection provided by the aqueous extract of the dried leaf powder of Cichorium intybus in view of its bone protective effects in irradiated rats.

MATERIAL AND METHODS

Experimental Animals

This study was conducted on twenty-four female albino rats weighing 130 ± 150 g. The rats were kept for 7 days, before the start of the experiment under observation in order to adapt the laboratory conditions: temperature, lighting, and ventilation.

Radiation Processing

Gamma – irradiation was conducted at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using a 137Cesium (^{137}Cs) biological irradiator source (γ-cell-40) Atomic Energy of Canada Limited. The rats were exposed to 9 Gy whole body γ-irradiation fractionated over 3 weeks (3 Gy/ week). The dose rate was 0.67 Gy/min.

Plant Material

Chicory leaves (Cichorium intybus L.) were collected from the market of herbal medicine (Cairo, Egypt) and then identified by an ecologist in the plant department, Faculty of women, Ain Shams University.

Preparation of Aqueous Plant Extract

The leaves of the plant were cleaned, shade dried in a dryer oven at 40° C for 3 hours, crushed to a coarse powder and preserved for further processing. Two grams of dried chicory leaves were extracted by adding 100 ml of boiling distilled water then infused for 15 min. The extract (2 % w/v) was filtered, and the filtrate was freshly used [14].

Liquid Chromatographic Analysis of the Plant

The samples were filtered through a 0.45-µm pore size membrane filter before injection. An HPLC system (UltiMate 3000 UHPLC, Thermo Fisher, USA) operated by Windows XP based Chromelion software was used. The HPLC equipment was used with a diode array detector (DAD). The system consisted of a QuadPump, degasser, and an autosampler. The used column was a Hypersil C18 ODS: 4.6 mm × 125 mm, 5 µm. The mobile phase consisted of 2 solvents: Solvent A, water/formic acid (95:5; v/v) and Solvent B, acetonitrile/solvent A (60:40; v/v). The compounds were eluted under the following conditions: 1 mL/min flow rate and temperature at 25 °C, isocratic conditions from 0 to 10 minutes with 0% B, gradient conditions from 0% to 5% B in 30 minutes, from 5% to 15% B in 18 minutes, from 15% to 25% B in 14 minutes, from 25% to 50% B in 31 minutes, from 50% to 100% B in 3 minutes, and then washing and reconditioning the column. The ultraviolet-visible spectra (scanning from 200 to 600 nm) were recorded for all peaks. Triplicate analyses were done for each sample. The identification of compounds were obtained by using authentic standards [15].

Animal Groups

For the achievement of the objectives of this study, twenty-four female albino rats were divided equally and randomly into the following 4 groups (n = 6):

Group 1(C): Normal control, the rats in this group were neither treated nor irradiated.

Group 2 (CH): The rats received 100 mg/kg b. wt. of plant extract daily for four weeks orally [16].

Group3 (IR): Irradiated group that exposed to whole-body gamma radiation with a fractionated dose 3Gy / week up to 9 Gy total doses [11].

Group 4 (CH + IR): The animals received the extract daily for 7 days before exposed to fractionated dose and within the fractionated irradiation period (21 days).

Bone Mineral Density (BMD) Determination

Densitometry was performed by a dual-energy X-ray absorptiometry (DEXA) using high-resolution scans on the whole leg. In brief, the whole leg was placed on the location on the platform of a DEXA and scanned using high-resolution imaging adapted for bone mineral content (BMC, g) and bone area (BA, cm2). The BMD was calculated as BMC/BA. This work has been achieved by bone mineral density unit, National Research Center, Dokki, Egypt.

Quantifying Gene Expression Of Osteocalcin By Real-Time Pcr Analysis

Total mRNA from the frozen femoral bone samples (15 mg) was ground into a powder by using a mortar and pestle containing liquid nitrogen and then isolated using the STRATEC Molecular Kit protocol according to the manufacturer's instructions. The total RNA quality was

analyzed using the RNA 6000 Nano LabChip kit on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

The extracted RNA was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit. 1 µg/µl of total RNA was reverse-transcribed by adding 2 µl Random Hexamer primer, 2 µl Reaction Buffer, 0.8 µl dNTPs, 1 µl RNase Inhibitor, 1 µl Reverse Transcriptase, 10 µl RNA, and 3.2 µl H_2O to a final volume of 20 µl. Then, the reaction was incubated at 37°C for 2 hours to activate the enzyme.

The qPCR reactions were performed in a 20 µl system containing 10 µl SYBR Green Master Mix, 1 µl, each gene-specific primer (sense or antisense), 5 µl Nuclease-Free Water, 3 µl cDNA. Osteocalcin: forward, 5'-AAGCCTTCATGTCCAAGCAG-3'; reverse, 5'-TCGTCACATTGGGGTTGAG-3' (Accession no. X04141). GAPDH: forward, 5'-TGAACGGGAAGCTCACTGG-3'; reverse, 5'-TCCACCACCCTGTTGCAGTA-3' (Accession no. NM_017008). Amplification was performed with 40 cycles with enzyme activation at 95 °C for 10 min, denaturation for 15 sec at 95 °C and annealing/extension for 60 sec at 60 °C.

Direct detection of qPCR products was monitored in real time by measuring the fluorescence increase caused by the binding of SYBR Green I Dye to dsDNA. Subsequently, the threshold cycle (Ct), the number of cycles in which the amount of interested amplified gene was reached a constant threshold, was determined. Relative quantification of mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen [17] after confirming that GAPDH and other cDNAs were amplified with the same efficiency. The relative quantification value of the target, normalized to an endogenous control and relative to a calibrator, was expressed as $2^{-\Delta\Delta Ct}$ (fold), where ΔCt=Ct of the target gene – Ct of the endogenous control gene (GAPDH), and ΔΔCt=ΔCt of the samples for target gene – ΔCt of the negative controls.

Chromosomal Aberration (CA) Assay

To analyze the chromosomal aberrations, the rats were intraperitoneally injected with 0.04% colchicine (0.1 ml/20g body weight), 90 min before sacrifice. The animals were sacrificed by ether anesthesia and bone marrows were harvested according to Preston et al. [18]. In summary, femur bones were excised and then, the bone marrow was extracted in 0.56% KCl. The cells were incubated for 20 min at 37 ^{0}C and centrifuged at 1000 rpm for 10 minutes. Cells were fixed in freshly prepared methanol: glacial acetic acid, 3:1, v/v) and burse opened on clean slides to release chromosome. The slides were stained with Giemsa for 10 minutes. A total of 300 well-spread metaphase plates were scored for CA at a magnification of 1000 for each group. Percentages of different types of CA were scored.

Serum Markers Assays

After 4 weeks that the experiment was over, the rats were anesthetized by diethyl ether and then their blood was collected by cardiac puncture and kept at 25°C for 40–50 minutes in a vertical position for completely clotting, then the serum was separated by centrifugation at 1,000 x g for 10 minutes and stored at -20°C for biochemical markers assays. The level of calcium in serum and inorganic phosphorus were determined colorimetrically using commercial kits supplied by Spinreact Company, Spin according to methods of [19, 20], respectively. The serum levels of calcitonin were also determined as a bone formation biomarker using commercial ELISA kits supplied by Kamiya Biomedical Company, USA according to the methods of Hillyard et al. [21].

Histopathology of Bone and Bone Marrow

Autopsy samples were taken from the femur of rats in different groups and fixed in 10% formaldehyde for 24 hours and decalcified in formic acid. Washing was done in tap water and then dehydration was done by serial alcohol dilutions (methyl, ethyl, and absolute ethyl). The samples were purified in xylene and embedded in paraffin at 56 °C in a hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning with a thickness of 4µm by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin & eosin stain for light electrical microscopy [22].

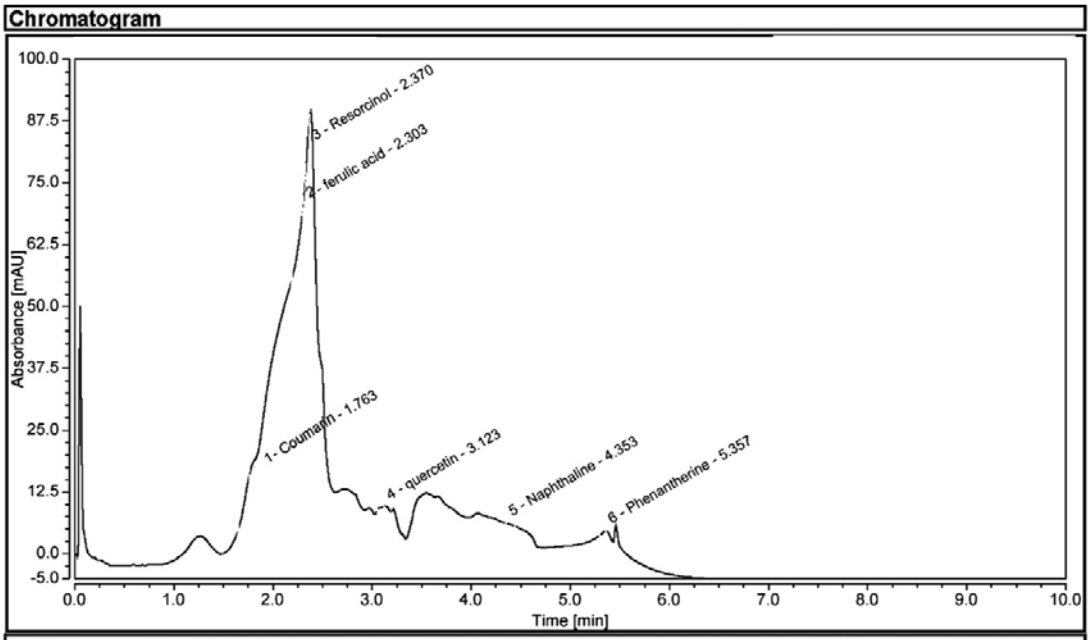
Statistical Analysis

Data was encoded and entered by using SPSS statistical software version 25 and then summarized using the mean and standard deviation of quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test for comparison between each 2 groups [23]. P-values < 0.05 were considered as statistically significant.

RESULTS

Identification of Antioxidants in Extract by HPLC Analysis

The HPLC for the aqueous chicory extract showed the presence of 5 phenolics. The extract from chicory contained coumarin (44.32 ppm), ferulic acid (134.77 ppm), resorcinol (4.03 ppm), quercetin (25.13 ppm), naphthalene (15.19 ppm) with retention times of 1.763 min, 2.303 min, 2.370 min, 3.123 min and 4.353 min, respectively as appeared from the peaks areas (Figure 1).



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount ppm
1	Coumarin	1.763	3.170	33.561	12.14	9.18	44.3241
2	ferulic acid	2.303	8.936	87.330	34.21	23.89	134.7731
3	Resorcinol	2.370	3.965	104.797	15.18	28.67	4.0376
4	quercetin	3.123	1.982	28.689	7.59	7.85	25.1373
n.a.	kaempherol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	Naphthaline	4.353	4.725	54.806	18.09	14.99	15.1907
6	Phenantherine	5.357	3.340	56.408	12.79	15.43	n.a.
Total:			**26.118**	**365.591**	**100.00**	**100.00**	

Figure 1: HPLC chromatogram of the antioxidant compounds of Cichorium intybus L. leaf.

Bone Mineral Density (BMD)

After 21 days of gamma radiation exposure, the BMD of the rats was significantly (P<0.001) decreased (0.0838±0.0002) in comparison to the control group (0.1011±0.0002). After treatment with chicory in continuous with radiation, the BMD of the femurs was increased significantly (P<0.001) (0.0928±0.0002) compared to radiation-exposed group as shown in Figures 2 & 3.

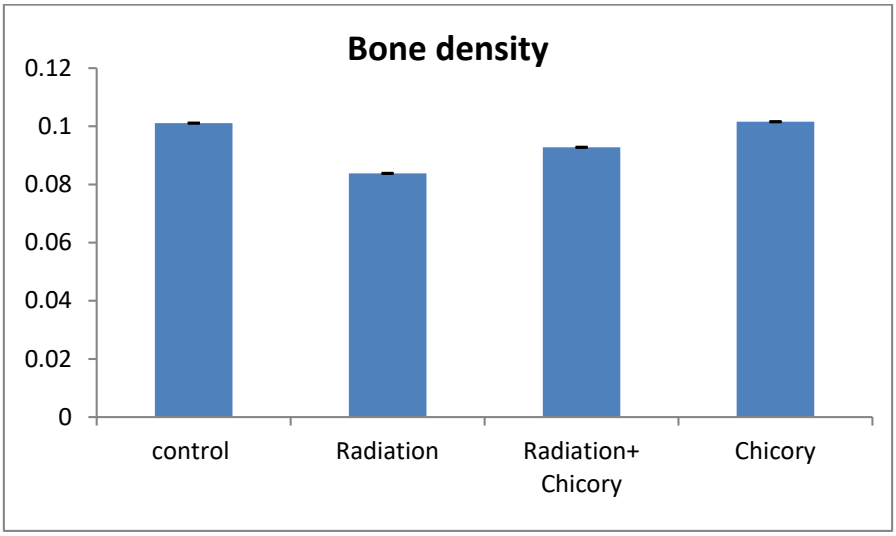


Figure 2: The effect of a fractionated dose (9 Gy) of gamma radiation administration alone or in combination with chicory on bone mineral density. Data were expressed as mean ± SEM (P < 0.05).

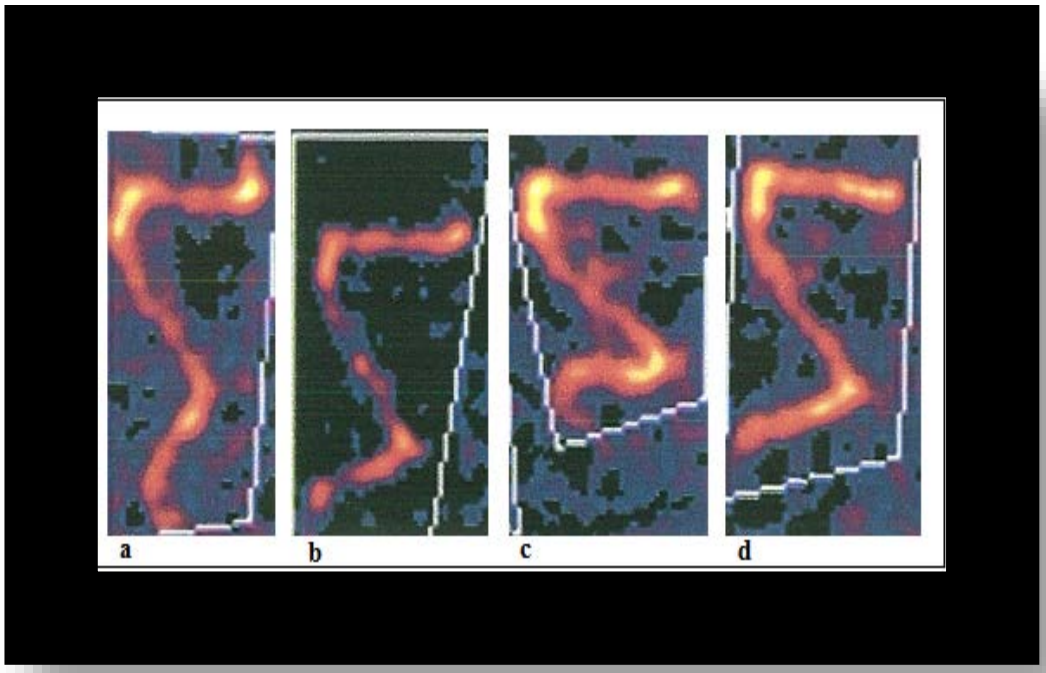


Figure 3: Long bones scan of female rats to detect bone mineral density in a: control group, b: irradiated group, c: chicory extract plus radiation group, d: chicory extract group.

Real-Time PCR

The fold change (FC) is calculated using the Livak and Schmittgen method and the treated samples were compared with control samples (Figure 4). The relative mRNA expression levels of osteocalcin (FC= 0.189465) gene was markedly downregulated in the irradiated group meanwhile, the expression transcript levels of the target gene were upregulated in chicory plus radiation-treated rats (FC= 0.812252).

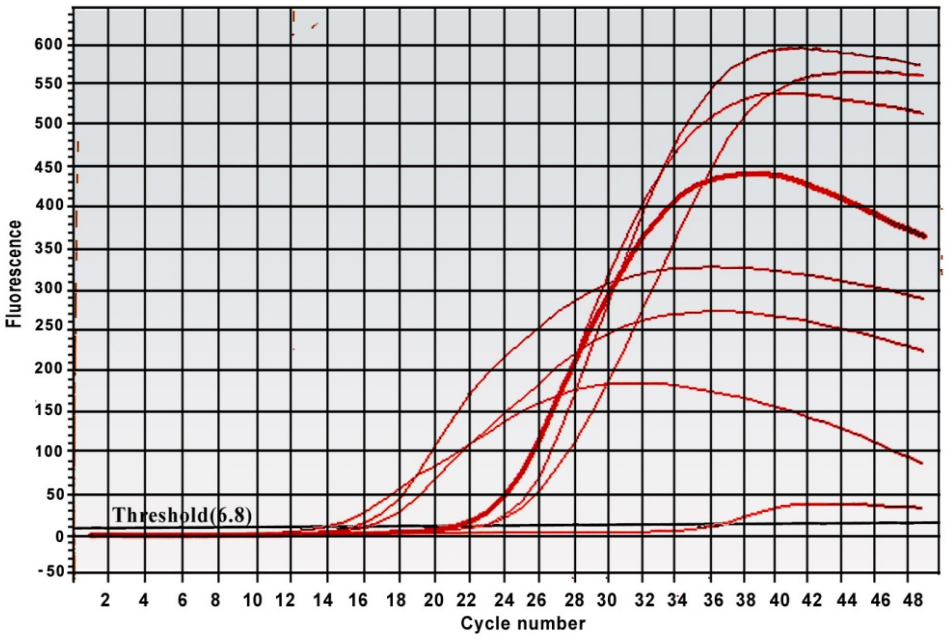


Figure 4: Analysis of mRNA expression of osteocalcin under exposure dose of radiation for 3 weeks

Chromosomal aberration assay

The diploid number of chromosomes of albino rats is n=42. Table (1) shows different types of chromosomal aberrations recorded in the examined groups of rat bone marrow cells. An exposure dose of gamma radiation resulted in a significant increase (P<0.001) in the percentage of aberrant cells (82.7%) in comparison to the control group (1.3%). The higher percentage was 20.7% for pulverization followed by 20% for deletion, 9% for dicentric, 6.3% for end to end association, 5.3% for isochromatid gap, 4.7% for diradial, 4% for centric separation, 3% for hypoploidy, 2.3% for triradial, 1.7% for centric fusion & hyperploidy, 1.3% for break, 1% for gap & polyploidy and 0.7% for ring (Figure 5). On the contrary, the treatment with aqueous extract of chicory besides radiation significantly (p<0.001) reduced the frequency of chromosomal aberrations (48 %) as compared to irradiated rats (82.7%) as shown in (Figure 6).

Table 1: Percentage of metaphases with structural & numerical chromosomal aberrations in rat bone-marrow cells of experimental groups (n=6)

Groups	Structural chromosomal aberration (SCA)/300 metaphase												total SCA %	Numerical chromosomal aberration (NCA)			total NCA %	% of total CA	Mean ±SD
	pulv	tri-rad.	r	g	e to e	isog	dirad	dicen.	br	del	Cf	cs		poly	hyper	hypo			
Control	0	0	0	0	0	1	0	0	0	1	0	1	3	0	0	1	1	4	0.6667±
	-	-	-	-	-	0.3%	-	-	-	0.3%	-	0.3%	1%	-	-	0.3%	0.3	1.3 %	0.5164
Radiation	62	7	2	3	19	16	14	27	4	60	5	12	231	3	5	9	17	248	41.3333±
	20.7%	2.3%	0.7%	1%	6.3%	5.3%	4.7%	9%	1.3%	20%	1.7%	4%	77%	1%	1.7%	3%	5.7%	82.7%	1.9664 *
Chicory+ Radiation	23	2	1	7	12	10	7	11	2	35	6	12	128	1	6	9	16	144	23.8333±
	7.7%	0.7%	0.3%	2.3%	4%	3.3%	2.3%	3.7%	0.7%	11.7%	2%	4%	42.7%	0.3%	2%	3%	5.3%	48 %	1.4720 *#
Chicory	0	0	0	0	0	3	0	3	0	6	3	7	22	0	0	2	2	8 %	4.0000±
	-	-	-	-	-	1%	-	1%	-	2%	1%	2.3%	7.3%	-	-	0.7%	0.7%		0.8944 *#$

Values are expressed as mean±SEM (Standard Error Mean); one-way ANOVA followed by post hoc test; n = Number of rats. * indicates P < 0.05 compared to control, # indicates P < 0.05 compared to radiated group, $ indicates P < 0.05 compared to chicory plus radiation group.

Pulv=pulverization. tri-rad=triradial. r=ring. g=gap. e to e a =end to end association. isog= isochromatid gap. dirad=diradial. dicen=dicentric. br=break. del=deletion. Cf=centric fusion. cs=centric separation. Poly=polyploidy. hyper=hyperploidy. hypo=hypoploidy.

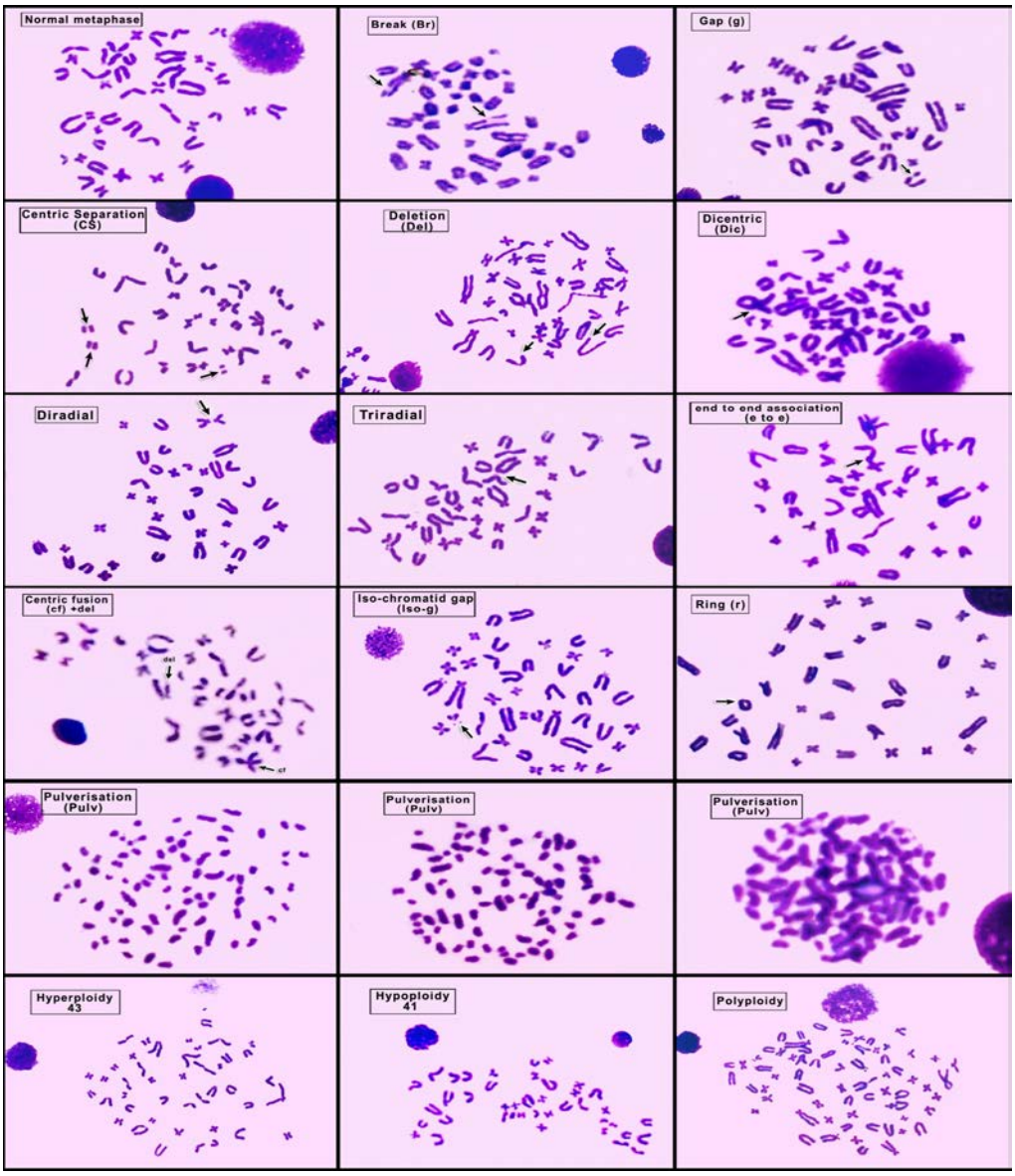


Figure 5: Different Structural chromosomal aberrations in metaphase prepared from bone marrow cells from all groups

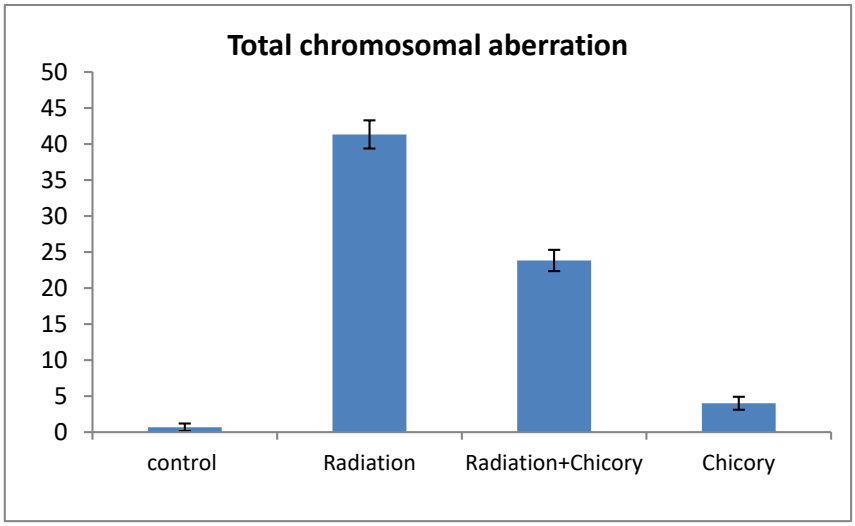
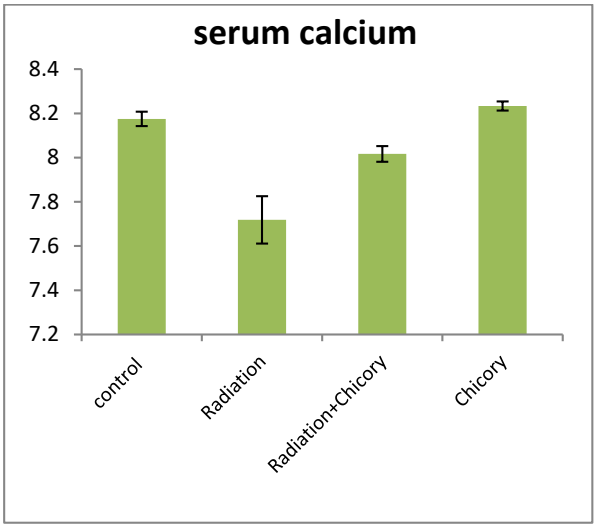
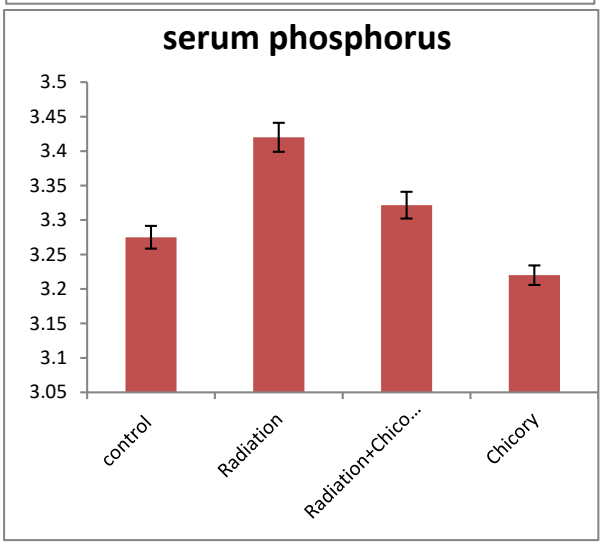


Figure 6: Effect of a fractionated dose (9 Gy) of gamma radiation exposure alone or in combination with chicory on chromosomal aberrations of rat bone-marrow cells. Data were expressed as mean ± SEM (P < 0.05)

Biochemical Analysis of Serum Samples

There was a significant decrease (P<0.001) in serum calcium in the animals exposed to radiation (7.7183±0.1072) compared to the control group (8.1750±0.0327); while, it significantly elevated again (P<0.001) in radiation plus chicory group (8.0167±0.0356) compared to radiation group. Also, there was a significant increase (P<0.001) in serum phosphorus in radiation group (3.4200±0.0210) compared to the control group (3.2750±0.0164) and it was significantly decreased again (P<0.001) in radiation plus chicory group (3.3217±0.0194) compared to the radiation group. While the calcitonin hormone in serum was significantly decreased (P<0.001) in radiation group (2.8233±0.0242) compared to control group (2.9267±0.0437) then significantly elevated again (P=0.009) in radiation plus chicory group (2.8900±0.0141). In addition, there was no significant difference between chicory group and control group for calcium (P=0.637) and calcitonin serum (P=1.000) as shown in (Figure 7).





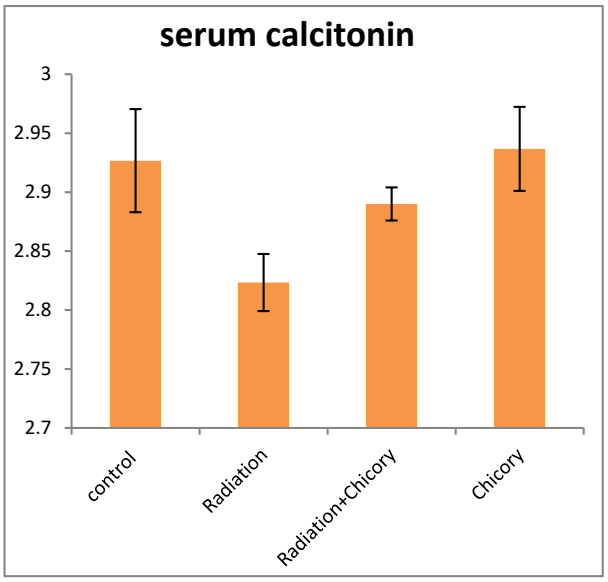


Figure 7: The effect of a fractionated dose (9 Gy) of gamma radiation administration alone or in combination with chicory on serum calcium, phosphorus, and calcitonin. Data were expressed as mean ± SEM (P < 0.05).

Histopathological Findings

The normal histological structure of the bone and cartilages of the epiphysis and metaphysis, as well as the bone trabeculae, were recorded in the control group (Figures 8a & 8b). In the irradiated group, a narrowing and shrinkage of the bone trabeculae were detected in the condyle of the femur epiphysis and metaphysis (Figure 8c). However, the improvement was detected in the trabeculae of the bone and it seems within the normal histological structure in the protected group with chicory then exposed to radiation (Figure 8d). Finally, there was not any histopathological alteration as recorded in the chicory group (Figure 8e). In addition, there were no histopathological changes in the haemopeosis of the bone marrow cells were recorded in all groups (Figure 8f).

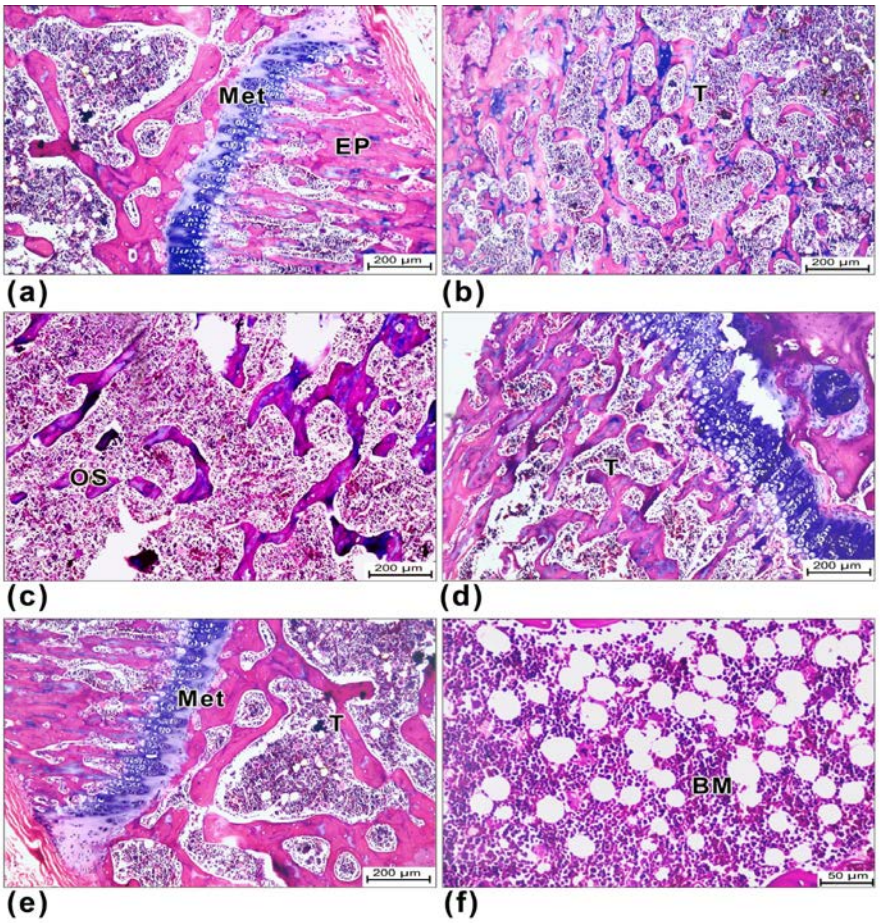


Figure 8: Photomicrographs of femur sections of different experimental groups stained with haematoxylin and eosin. (a & b, x200): negative control group revealed no histopathological alteration in the bone trabeculae (t) of epiphysis (ep) and metaphysis (met). (c, x200): the irradiated group revealed a mild bone resorption in the trabeculae (osteoporosis, OS). (d & e, x200): irradiated rats protected with chicory extract and chicory group respectively showed a normal bone tissue. (f, x400): the irradiated group does not reveal histopathological changes in the bone marrow (BM)

DISCUSSION

Bone health is a major public health issue. Osteoporosis is a disease that affects many millions of people all over the world and will take on increasing significance as people live longer and the number of the population continues to rise [24]. Although nutrition is only one of the various factors affecting bone mass and fragility fractures, this is very important for bone health, because it is modifiable [25]. Therefore, the present study is designed to address the role of chicory extract in the amelioration of bone damage resulting from gamma-radiation exposure.

The γ-irradiation (9 Gy) exposure fractionated over 3 weeks (3 Gy/ week) with a dose rate 0.67 Gy/min for 21 days caused a significant reduction in bone mineral density (BMD) compared to the rats in the control group, which consequently increased the risk of osteoporosis. These results are in agreement with the reports of Kondo et al. who showed that irradiation (2 Gy) declined 20% of tibial cancellous bone volume fraction in three days and declined 43% in ten days, while 1 Gy declined 28%, in 10 days because of the increased spacing and reduced thickness of trabeculae [10]. Radiation also increased acid phosphatase-positive osteoclasts, cancellous surfaces lined with tartrate-resistant, as the index of bone resorption increases. Meanwhile, after a further 4 weeks of treatment with chicory (in continuous with radiation), the BMD of the femurs increased significantly in comparison to the radiation group. Previous investigations found that dietary supplementation with chicory improves bone mineral content (BMC), enhances Ca absorption, and alleviates the reduction of bone mineral density (BMD) and mineral content which follows ovariectomy or gastrectomy in rats [16, 26].

The present study also showed that radiation treatment decreased the osteogenic osteocalcin expression relative to GAPDH mRNAs due to the decreased activity of osteoblasts and osteocytes for producing bone matrix proteins [27]. The obtained results are in agreement with those of Huang et al. who showed that γ-radiation

reduced cell adhesion, spreading, proliferation, and the gene expression of osteogenic osteocalcin gene at days 7 and 14 postirradiation [28]. The ionizing radiation effects on DNA have been studied in details for many years. Irradiation results in single or double-stranded DNA breaks and eventually blocks cell cycle progression [29]**.** Therefore, it is believed that irradiation predominantly induces DNA damage with attendant growth inhibition and that this is responsible for suppressing osteoblast differentiation and mineralization [30].

Besides, the expression of osteocalcin was increased in radiation plus chicory group. This transient increase in the production of osteocalcin is likely associated with the stimulation of osteoblast differentiation by the administration of chicory. Osteoblasts are bone-forming cells derived from undifferentiated pluripotent mesenchymal cells. The bone formation involves complex processes that include the proliferation and differentiation of osteoprogenitor cell and ultimately leads to the formation of a mineralized extracellular matrix. Numerous cytokines, hormones, and growth factors control bone formation by regulating osteoblast cell proliferation and differentiation. Phenolic compounds have been shown to modulate osteoblast cell functions [31, 32]. Flavonoids of phenolic compounds which are abundant in plants, have many beneficial biological effects such as estrogenic, antioxidant, and anti-inflammatory effects [25].

Researchers have shown that the phenolic compounds in various plant species can modulate the osteoblastic cells functions, including their maturation and proliferative capacity, by increasing the activity of alkaline phosphatase and calcium deposition in the extracellular matrix [31, 33]. The proposed mechanisms that make these changes in osteoblastic activity include the improving the function of osteoblast by certain phenolic compounds via the modulation of different transcription factors, e.g., Cbfa1/Runx2, and bone morphogenetic proteins, e.g., osterix and osteocalcin; these are all essential molecules to induce osteoblast differentiation, which in turn may activate genes involved in the bone remodeling process [31, 34, 35].

There is also growing evidence both in vivo and in vitro studies that natural phenolic acids can affect the skeletal system by the stimulation of bone formation and inhibition of bone resorption [36, 37]. In addition, phenolic acid derived from the breakdown of blueberries polyphenols appears in the serum after consumption and stimulates the differentiation of osteoblast by Wnt signaling, indicating their ability to prevent bone loss [38]. It seems that the probable mechanism of phenolic acid on osteoblast is its binding to estrogen receptors in osteoblasts [39]. Phenolic acid, depending on the estrogen receptor, promotes the proliferation of human breast cancer cells in an estrogen receptor and concentration-dependent manner [40]. In addition, quercetin inhibits bone resorption and induces apoptosis in mature osteoclasts [41, 42].

Moreover, A significant increase was existed in the frequency of aberrant bone-marrow cells and in the different types of structural/numerical chromosomal aberration in irradiated rats with gamma radiation in comparison to the control group and these results are in agreement with those of Pillai et al. who demonstrated that radiation caused a significant increase in the percentage of aberrant cells. A corresponding increase in all individual aberrations was found [43]. Meanwhile, Chicory administration (together with radiation) significantly reduced the mean frequencies of aberrant cells, as well as the incidence of structural/numerical chromosomal aberrations in bone-marrow cells, whilst extract itself does not have any marked harmful effect on the bone marrow chromosomes and this result in agreement with that of Thulasi et al. who found that the administration of aqueous *Chicory* leaf extraction to animals showed a significant reduction in chromosomal damage and micronucleus induction [44]. Free radical scavenging may be a likely mechanism of action as the extract was found to possess significant hydroxyl radical scavenging activity [16, 45].

The biochemical results of the present study showed a significant decrease in serum calcitonin and calcium and in contrast, a significant increase in serum phosphorus, and this may be attributed to secondary hyperparathyroidism, which is consistent with the increase in parathormone (PTH) under the effect of radiation [46, 47]. The disorders of PTH insufficiency or excess affect serum levels of phosphorus and calcium and also affect neurologic, skin, cardiac, bone, and other systemic manifestations. PTH is secreted in response to hyperphosphatemia and hypocalcemia and severe hypomagnesemia inhibits it [48].

The present investigation also showed a significant decrease in the serum level of calcitonin in irradiated rats. These results coincided with those of Zaidi et al. who showed that calcium concentration is considered the main stimulus of calcitonin secretion by C-cells of the thyroid gland [49]. The calcitonin secretion stimulus is diminished when blood calcium is lowered, Also, the actions of calcitonin and PTH are antagonistic on bone resorption.

On the contrary, the administration of chicory significantly increases the absorption of Ca and results in a significant increase in the mineral content of bone which leads to PTH suppression [50]. This may be due to chicory contain inulin which enhances Ca absorption in

two ways. First way: increased calcium solubility in the colon due to decreased pH as a result of inulin fermentation. Second way: osmotic effects, increasing fluid transfer in the colonic lumen and thus permeability increase between the junctions of intracellular enterocyte that facilitates diffusion. These results are consistent with those of Roberfroid et al. which demonstrated that chicory inulin increases the absorption of calcium and increases the mineral parameters in the bones of the body [51]. In addition, treatment with non-digestible fructans successfully increases Ca absorption and results in a corresponding increase in bone mineral which is followed by a suppression of PTH [50]. Moreover, non-digestible oligosaccharides have the ability to reduce the rate of bone turnover due to the ability to reduce the osteoclastic activity thus the rate of bone resorption decreases [46].

Also, the chicory extract phytochemical screening confirmed the presence of such bioactive compounds, especially total phenolic, which may help to protect the chicory extract against free radicals [52]. A rat model to mimic menopausal women was established and it was found that the treatment with chicory induced osteogenesis because of osteoporosis preventive properties due to the protective effect of indigestible oligosaccharides in bone and increased absorption of calcium, decreased bone turnover rate, and increased bone mineralization and calcium balance [53, 54].

In the same time, the histological analysis of the irradiated group demonstrated shrinkage of the bone trabeculae which revealed mild bone desorption in the epiphysis and metaphysis of the long bone. These results are consistent with those of Hamilton et al. who showed that a long-term reduction in trabecular bone quality and quantity occurs after a whole body 2 Gy dose γ-rays [55]. Loss of functional bone was detected as early as 3 days after a 2-Gy dose of γ-rays. In addition, Kondo et al. reported that the dose of gamma irradiation caused the loss of cancellous tissue, as well as rapid increases in osteoclast numbers and trabecular surfaces covered by osteoclasts in skeletally mature male mice. These responses were not additive in the short term [56].

However, chicory treatment increased the width of femoral trabeculae and it seems within the normal histological structure in the protected group with chicory in continuous with radiation. This suggests that treatment with chicory may serve as a useful approach to protect bone from the adverse effects of radiation. Chicory increases the production of insulin 1 like growth factor-1 (IG-F) and stimulates osteoblastic activity through an estrogen receptor-mediated action, and thus performs its antiosteoporotic effect, which is known to increase osteoblastic activity [57]. In addition, there was no histopathological alteration and the normal histological structure of the haemopeosis of the cells was recorded in all groups.

CONCLUSION

The present study indicated that the administration of the aqueous extract of chicory leaves before whole body irradiation was able to modulate bone loss in rats, as represented by increasing bone mineral density (BMD) and serum mineral Ca, upregulates osteocacin gene expression, decreases bone marrow aberrant cells, and ameliorates histological architecture of bone. This may occur through similar intracellular pathways, involving the suppression of oxidative stress and enhancement of antioxidant defense mechanisms which suggests that chicory represents a promising therapeutic option for the prevention of gamma (^{137}Cs) radiation-induced osteoporosis, which may be equivalent to the fractions of radiotherapy.

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