Study the effect of different levels of red radish seeds and celery Seeds on rats suffering from osteoporosis

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Abstract:

This study carried out to investigate the effect of different levels of red radish seeds (RRS) and celery seeds (CS) on glucocorticoid induced osteoporosis in rats. Thirty five non -pregnant female albino rats with aged 6-8 weeks, weight 170 to 200g, divided into two main groups, the first group fed on basal diet as negative control and second main group fed on basal diet injected with glucocorticoid (4mg/kg of BW) three times a week for three weeks to induce osteoporosis. Five groups were fed on basal diet containing 10%, 15% of RRS and CS and mixture of 7.5% RRS and CS for sixty days. The results of macronutrients and minerals of RRS and CS were determined; protein, fiber, carbohydrates, calcium, potassium magnesium were high in RRS (29.16, 25.131, 35.589, 495, 396 and 316), while fat, ash, calcium, potassium and magnesium were determined in CS (5.67, 21.13, 115.93, 108.02 and 32.76). The biological results indicated increasing in body weight gain, the best results showed in the mix group (20.07 gm). Vitamin D, Ca and P increased gradually. Estradiol, Parathyroid hormone, AST, ALT, ALP, urea, creatinine, BMD, BMC and bone histological structure showed the best results in mix Histopathological examinations of the liver, kidneys and bones confirmed the results of the biochemical analyses. In conclusion, Red radish and celery seeds have anti osteoporotic activity in glucocorticoid induced osteoporosis, so it was recommended intake of Red radish and celery seeds for patient with osteoporosis.

Keywords: Radish seeds, celery seeds, bone pain, bone stiffness, osteoporosis.

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Introduction

bioactive food components such as polyphenols, carotenoids, phytoestrogens, sterols, stanols, vitamins, minerals, dietary fiber, fatty acids, probiotics, prebiotics, and bioactive peptides in several food and seeds, an increasingly important health-promoting role is ascribed (Kulczy et al., 2016), nutritional or non-nutritional components found in the foods or formed during technological processes of foods may enhance physiological and metabolic functions of bone, homeostasis, formation of bone and normal skeletal result from the osteoclast-mediated bone resorption, so the bone disruption can result in osteoporosis and osteosclerosis (Wang et al., 2017; Huang et al., 2019; Yin et al., 2019), Poor diets resulting in a lower in body reservoirs of iron, calcium, magnesium, potassium, zinc and other essential minerals, it was caused anemia, zinc deficiency, osteoporosis (Montazery et al., 2006). Osteoporosis is a skeletal disease of both genders, specialty female following menopause by the reduced bone mass, bone mineral density (BMD) and microstructural damage, it leads to fracture in the lumbar vertebrae, spine, forearm, shoulder and the hips (Ferdous et al, 2015; Yoo and Park, 2018). Diet rich in plant foods with high antioxidant content with lifestyle changes may improve bone mineral density and reduce the osteoporosis (Jeff et al., 2021). Radish (Raphanus sativus) is one of the most important vegetables in Asia, particularly in China, Japan, and Korea. It has various kinds; their characteristics are due to its phytochemical content, anthocyanin pigments and isothiocyanates responsible for the pungent flavor and distinctive taste (Nishio, 2017), radish is low in calories and a good source of calcium, magnesium, copper, manganese, potassium, vitamin B6, vitamin C, and Folate (Department of Agriculture, 2020). Radish and its extracts is as a source of bioactive compounds with clinical and health impacts in the diseases of hypertension, cardiometabolic disorders and as an antimicrobial and antioxidant agent and used in the pharmaceutical industry (Manivannan et al., 2019). RR is the known old vegetables and consumed of the Brassicaceae family, it has leaves, roots, seasoning vegetables and oilseeds (OECD, 2016), it contains chemical compounds with health benefits (Manivannan et al., 2019) such as,

vitamins and minerals particularly (folic acid, anthocyanin, potassium, calcium, copper, iron, phosphorus, and zinc), fiber and antioxidant (Hossam et al., 2022), these elements appetizing aids in digestion, activates the liver, detoxification and protect from cancer (Jaafar et al., 2020). (Magda et al., **2021**) reported that roots of radish are contain (38.8%) flavonoids, (8.4%) non-flavonoid polyphenols, (8.5%) terpenes and derivatives, (6.4%) fat and fatty related compounds, and (5.6%) glucosinolates. Leaves have high concentrations of macronutrients, calcium, potassium, sodium, fiber, fatty acids and nonflavonoid polyphenols have a positive effect in the prevention and treatment of osteoporosis, while sprouts are a major source of flavonoids (anthocyanin, β -carotene and vitamin C and glucosinolates), glucosinolates also high in seeds, so radish makes on strengthening and improving the density of bones, and improving overall health (Goyeneche et al., 2015; Rosario Goyeneche et al., 2015; Rezk et al., 2019). Various herbal plants have shown pharmacological activity in treating various diseases, because of activity of their various ingredients like antioxidant and organic products which to diminish the osteoclasts (Salihu Shinkafi et al., 2015; Khairullah et al., 2020; Solikhah et al., 2020), such as celery is a popular Chinese vegetable and its seeds consume as a medicinal herbs (Kooti and Daraei, 2017; Sarah and Shajini, 2021), according to Lujuan Jiaa et al., (2020) studied the flavonoids of celery may be used as functional foods, and it has therapeutic impacts on osteoclast-related (Aswin Rafif Khairullah et al., 2021). Also, its roots, stems, leaves and seeds have medicinal uses. Modern pharmacological studies and animal studies have shown the medicinal impacts of celery (C) and celery seeds (CS) in lowering blood pressure and lipid levels because they contain antihypertensive and lipid-lowering ingredients, also it contains vitamins, minerals and nutrients (Moghadam et al., 2013), in addition to it has gastroprotective , cardioprotective, antimicrobial, neuroprotective, hypolipidemic, antioxidant, antifungal and anti-inflammatory (Al-Howiriny et al., 2014; Rumiyati et al., 2016; Genatrika et al., 2019; Jittiwat et al., 2020). The studies have shown that celery powder can be used as an effective nutrient, rich in calcium and increase bone density, it has positive

impact on resist fractures (**Tahuri**, 2011), it also is rich in volatile oils, organic acids, sugars and flavonoids (He et al., 2017; Li et al., 2018; Guijarro Real et al., 2019). Celery contains apigenin and luteolin, effectively inhibit osteoclast differentiation and guard against bone loss to prevent osteoporosis (Lee et al., 2009; Shin, et al., 2012; Kumar et al., 2015). The use of celery seeds have no side effects and have antioxidant properties (Safi Obeais AL-Kafajii, 2013). Celery seeds are also used in the Uyghur medicine of China to treat hypertension, arthritis, rheumatoid arthritis, muscle pain, inflammatory diseases and kidney diseases. Numerous flavones have been isolated from celery seeds, including luteolin, apigenin, corresponding glycosides, graveobioside A, graveobioside B, and apiin (Xu, et al., 2020; Osés et al., 2020; Dias et al., 2021). Flavones of celery seeds, such as luteolin is excellent natural antioxidants that can prevent or treat oxidative stress-related diseases and hyperglycemia (Chao **Zhang et al., 2022)**, so it can be used as an effective alternative to treat diabetic mellitus (Ali et al., 2019). According to (Shaopeng et al., 2019; Anish Desai et al., 2022) reported that celery seed extracts have anti-gout properties, through anti-inflammatory and antioxidative effects. Seyyedeh Maryam Hosseini Shirazi (2015) showed that celery supplements can prevent the development of bone diseases in overweight middle-aged women. The seeds, leaves, and stems of celery can be used to treat gout, rheumatism, arthritis (Kumar et al., 2015), (Kooti and Daraei, 2017) studied the medicinal properties of celery seeds as anti-inflammatory activity because of phytochemical constituents In addition to research studies on the role of celery seeds in treating liver diseases, gout, arthritis, and urinary tract infections (Cooney et al., 2011). Touba and Esmaeil, (2022) showed that eight weeks of Pilates training with celery seed supplement led to decline antinuclear antibody (ANA) in middle-aged women with rheumatoid arthritis. Radish (Raphanus sativus) is a vegetable of the Brassicaceae (cruciferous) family that contains disease-fighting antioxidants and can help reduce blood glucose levels (Manivannan et al., 2019; Banihani, 2017). It has been proposed that oral treatment of radish seeds reduces insulin resistance in Sprague-Dawley rats, mostly via lowering blood viscosity, which enhances the binding affinity between insulin receptors, hence increasing glucose absorption (**Turrini et al.**, **2015**).

Dried radish seeds (RS, Raphani Semen, Lai Fu-zi in Chinese) are classified in the China Pharmacopoeia and have long been used professionally to alleviate food indigestion, upper abdominal distension, constipation, panting, and coughing in China (Jacky, 2023). In India, RS has a therapeutic impact on asthma and other chest disorders (Aruna et al., **2012).** The glucosinolates (GLSs) concentration in radish seeds was 3 to 6fold greater than that in sprouts (Guo and Zhu, 2025). GLSs, which are cruciferous vegetables, peculiar to are precursor chemicals isothiocyanates (ITCs) with specific functional qualities (Alloggia et al., 2023), which is why they are recommended for intake. Treatment of glucocorticoid drugs have many side effects and are needed for treatments of many diseases. So that the purpose of this study was to evaluate the effect of different levels of red radish seeds and celery seeds on glucocorticoid (dexamethasone) induced osteoporosis in female rats.

MATERIALS AND METHODS

Materials

Red radish seeds and celery seeds were obtained from Al Fagr company of agricultural seeds, spices, and medicinal plants, in October, Egypt. The betamethasone was purchased from pharmacy El-Shams-Giza in Egypt, Dexaglobe Ampoules. Kits for serum analysis were purchased from Bio Diagnostic Company, October, Egypt. El Gomhoreya Co., Cairo, Egypt provided casein, sucrose, maize oil, fibre (cellulose), mineral and vitamin mixtures, choline chloride, D-L methionine, and maize starch. Rats were obtained from the Giza Agricultural Research Center

Methods

Experimental procedure

Proximate Composition of red radish seeds and celery seeds

Moisture, crude lipid, ash, crude fibre, and crude proteins, according to the AOAC (2005). The moisture contents were determined by drying the seeds at $105\,^{\circ}$ C until they attained stable weights. The ash concentration was determined by burning of the seeds at $550\,^{\circ}$ C. The nitrogen (N) value was determined using the Kjeldahl procedure. Crude proteins were assessed as N × 6.25. (**Imran et al. 2008**). The lipid content was evaluated using a Soxhlet method. Difference as indicated by calculating total carbohydrate contents (**Muller and Tobin 1980**) by gathering the sample's total fiber, lipids, proteins, moisture, and ash levels and subtracting them from 100. Each therapy was evaluated three times.

Minerals Content in red radish seeds and celery seeds

A burning cup was filled with around 0.5 g of dry samples and 15 mL of pure HNO3. The samples were burnt at 200 °C in a microwave digester (Milstone digester; Ethose—D, GmbH, Leutkirch, Germany), and the solution was diluted with deionised water to the desired amount. The mineral content was analysed as follows: The total nitrogen content was estimated using Kjeldahl's procedures. (Motsara and Roy 2008). Calcium (Ca) and potassium (K) contents in digested dry samples of red radish seeds and celery seeds were measured individually using a Pfp7 flame photometer (Jenway, UK), and the results were represented as mg/kg dry sample.

Biological Experiment

Animal, housing and diets:

Thirty-five non -pregnant female albino rats with aged between (6 to 8 weeks) and about 170 to 200g body weight, divided into two main groups. The first main group (5 rats) and The second main group (30 rats) fed on basal diet according to (Reeves et al.,1993). The second main group were injected with beta methasone at a dose of 4 mg/kg BW three times a week for three weeks to cause osteoporosis. After that one rat was taken from

each group to make sure incidence of osteoporosis, then the second group divided into six groups such as each group consist of (5 rats). The first group fed on basal diet as positive control, but the groups (3, 4, 5 and 6) fed on basal diet containing 10% - 15% red radish seeds (RRS) and 10% - 15% celery seeds (CS) respectively. The last group (7) was a mixture of 7.5% red radish seeds and 7.5% celery seeds for sixty day.

Biochemical Analysis

Biological Determination

Biological evaluation of the various tested diets was carried out by determining the body weight gain percentage (BWG %) and organ weight / body weight percentage according to **Chapman et al.**, (1959).

BWG% = (Final weight - Initial weight) / (Initial weight) X 100.

Organ weight/ body weight % = (Organ weight / Final weight) X 100.

Determination of vitamin D

Serum vitamin D levels were measured using an enzyme linked immunosorbent assay (ELISA) as reported by **Zerwekh (2008)**

Determination of Calcium

Calcium was determined using the technique provided by **Gindler** and King, (1972).

Determination of Phosphorus in serum

Phosphorus was determined using the <u>Daly</u> and <u>Ertingshausen</u> (1972) technique.

Parathyroid hormone (PTH) and Estradiol (E2)

Parathyroid hormone (PTH) was measured using the technique outlined by Bouillon *et al* (1990). Estradiol (E2) was determined using the technique described by **Ratcliffe** *et al.*, (1988).

Liver and kidney function

Blood samples were taken from the orbital plexus veins using thin capillary glass tubes, deposited in centrifuge tubes without anticoagulant, and allowed to clot. Serum samples were analysed using bio diagnostic kits

after being centrifuged at 3000 rpm for 15 minutes. Serum urea was measured using the technique described by **Fawcett and Soctt (1960)** just nm to 550 nm. **Tietz (1986)** determined serum creatinine using a spectrophotometer (model DU 4700) set to 510 nm. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured calorimetrically using a spectrophotometer (model DU 4700) at 505 nm, following the method of **Reitman and Frankel (1957).** The activity of alkaline phosphatase (ALP) was assessed calorimetrically using a spectrophotometer (model DU 4700) at 510 nm, following the method of **Belfield and Goldberg (1971).**

Bone mineral density and content assay

Each animal's right femur was dissected and carefully cleaned before being measured for bone mineral density (BMD) and bone mineral content (BMC) by dual energy x-ray absorptiometry (DEXA) using an XR 46, version 3.9.6/2.3.1 instrument equipped with dedicated software for small animal measurements in the bone mineral density unit, Medical Service Unit, National Research Centre, Doki, Egypt. BMD was estimated using BMC/BA (Bagi et al., 2011).

Histology

Each rat in the seven groups had its liver, kidney, and right femur bone removed and fixed in 10% formal saline for 24 hours. The bone was decalcified using formic acid. Washing was done in tap water, followed by dehydration with serial dilutions of alcohol (methyl, ethyl, and 100% ethyl). Specimens were cleaned with xylene and embedded in paraffin in a hot air oven at 56 degrees for 24 hours. Paraffin bees wax tissue blocks were sectioned at 4 micron thickness using a slide microtome. Tissue slices were collected on glass slides, deparaffinized, and stained with haematoxylin and eosin for routine examination under a light electric microscope (**Banchroft et al., 1996**).

Statistical analysis

The data from the current study was statistically subjected to analysis of variance (ANOVA) according to **Snedecor and Cochran (1980)** using the computerized application SPSS version "20" for Windows. The least significant difference (LSD) value was utilized to determine the difference between means. Data was reported as mean \pm SD. Values were considered significant if P < 0.05, otherwise non-significant.

Results and discussion

Proximate Composition

Table (1): Major chemical constituent of Red Radish seeds and Celery seeds in dry matter

	Constituents					
Materials	Moisture	Protein	Crude Fat	Crude Fiber	Ash	Carbohydrates
	%	%	%	%	%	%
RRS	3.92	29.16	1.08	25.131	5.12	35.589
CS	7.38	19.00	5.67	14.52	21.13	32.3

RRS: Red Radish Seeds *CS: Celery Seeds

As shown in **Table** (1) moisture percentage in celery seeds is higher than the one in red radish seeds (7.38 and 3.92%) respectively. Proteins are complicated molecules that serve a multitude of roles in the body. They can be both good and harmful to bone health, depending on the amount and kind of protein consumed. (**Heaney and Layman, 2008**). Table 1 displays the protein content of RRS and CS, which were 29.16% and 19%, respectively. That indicates RRS has the highest content. However, it is a dietary protein, but that dietary protein is an important nutrient for skeletal health because it effects bone in numerous ways: it forms a substantial component of organic bone matrix, it modulates blood levels of insulin-like growth factor 1 (IGF-1), and it may affect calcium metabolism (**Lorincz et al., 2009**). The results showed that Celery seeds have a higher content of fat (5.67%) Which is estimated at five times the percentage in red radish seeds (1.08%) that makes RRS a good low calorie source with good content. According to

Parry et al. (2005) Lipid profiling of seeds oil revealed beneficial antioxidant phytochemicals as tocopherols, carotenoids, phenolic and polyphenolic components, as well as the unique fatty acid α -linolenic acid. These results were consistent with Köken et al., (2016), which illustrated that Celery oil includes significant active chemicals known as phthalides, which provide protection against cancer, cholesterol, and high blood pressure. The radish seed oil contains two primary fatty acids: linolenic acid and oleic acid (Hou et al. 2011), It has been used in numerous healthrelated applications. This oil has a blend of unsaturated and saturated fatty acids (Silvestre et al. 2017). There is evidence that diets high in omega 3 fatty acids (n-3 FAs) may be advantageous to bone health (Orchard et al., **2012).** On contrast, the carbohydrates content in RRS has a higher (49.03) g/100g) than CS (38.05 g/100g). Results indicate that red radish seeds have high content of crude fiber and Carbohydrates (25.131% and35.589) while in celery seeds is (14.52% and 32.3%) respectively. Giffin et al., (2002) and Abrams et al., (2005) found a robust link between dietary fibre and increased calcium absorption and bone mineralization in children and adolescents.

Table (2): Minerals of of red radish seeds and celery seeds (mg/100g)

Minerals	Minerals (mg /100g)			
Materials	Calcium	Potassium	Magnesium	
RRS	495	396	316	
CS	115.93	108.02	32.76	

As shown in **table** (2) the results demonstrated a high amount of calcium, potassium, and magnesium in RRS (495,396 and316mg/100g) respectively then CS took the second place with its content of minerals (115.93, 108.02 and 32.76 mg/00g). Minerals like Calcium, Phosphor, Potassium and Magnesium have a great importance in management of osteoporosis, According to **Castiglioni**, et al., (2013) who reported that Magnesium shortage leads to osteoporosis directly by acting on crystal formation and bone cells, and indirectly by influencing the secretion and action of parathyroid hormone and encouraging low-grade inflammation,

also Carpenter et al., (2006) explained that Mg supplementation for 12 months improves bone mass accretion in the hip of peripubertal Caucasian girls. Furthermore, it was demonstrated that magnesium consumption is an independent predictor of bone density in young top swimmers. (Matias, et al., 2012). Sakhaee, et al., (2005) Potassium has been linked to improved bone health via a variety of methods. Overall, these data aligned with the finding of Kamchan et al., (2004) which indicated that green leafy vegetables, seeds, and legumes are good alternate sources of calcium, in addition to cow milk and fish with bones, especially for persons who are lactose intolerant or vegans.

Table (3): Mean body weight gain (g) of control and experimental rats given with red radish seeds, celery seeds, or a combination of both

Body weight (g)	IBW	FBW	BWG/ wk %
Groups			
Control (-)	206.00±2.00 ^a	219.33±1.45 ^d	6.48±O.87 ^e
Control (+)	201.66±1.52 ^b	203.33±1.45 ^g	$0.83\pm0.56^{\rm f}$
RRS10%	207.00±1.00 ^a	225.33±1.76 ^b	$8.86\pm0,97^{c}$
RRS15%	199.33±1.52°	222.67±1.45°	11.71±1.05 ^b
CS10%	199.66±01.52 ^c	215.33±1.45 ^f	7.85 ± 1.04^{d}
CS15%	196.33±1.52 ^d	218.67±0.88 ^e	11.38±1.63 ^b
mix (RRS 7.5%+CS 7.5%)	199.33±1.53 ^c	239.33±0.88 ^a	20.07±1.06 ^a

Data were presented as means \pm SDM (n=5). a, b, c and d: Means with different letter among treatments in the same column are significantly different (P \leq 0.05) IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; WK: Week.

As indicated in Table 3, the results showed that the body weight of the experimental rats at the start was not significantly different. Control positive (osteoporotic rats) had a substantial drop (P<0.05) in final body weight (203.33g) and weight increase (0.83g) compared to control negative (healthy rats) (219.33 and 6.48g, respectively). These outcomes are associated with the findings of **Costello et al., (2017) and Arena et al., (2010)** they reported that the amount of weight gain caused by systemic glucocorticoids is less than previously anticipated. Only 10% of the patients

gained more than 10% of their normal weight. Meanwhile, rats fed a food supplemented with mix (RRS 7.5%+CS 7.5%) showed a substantial increase in final body weight and body weight growth (239.33 and 20.07g) as compared to the positive control group.

(**Gouda ,2012**) mentioned that (osteoporotic rats) showed considerable decrease in body weight gain at the end, it could be noted that the best result for BWG% and FER related treatment groups was recorded by group received diet supplemented with combination (RRS 7.5%+CS 7.5%) with (239.33 and 20.07), respectively.

Table (4): Effect of red radish seeds, celery seeds and the mixture of them on weight of organs and weight right femur of osteoporotic female rats and control

Organs /g	Heart	Liver	Kidney	Femur
Groups				
Control (-)	0.81 ± 0.02^{b}	6.95±0.31 ^{bc}	1.73±O.15 ^{ab}	6.62±0.17 ^a
Control (+)	0.93 ± 0.04^{a}	8.83±0.56 ^a	1.83±0.06 ^a	4.85±0.19 ^b
RRS10%	0.77 ± 0.04^{b}	7.01 ± 0.32^{bc}	$1.72\pm0,05^{ab}$	6.48±0.29 ^a
RRS15%	0.63 ± 0.09^{c}	6.18 ± 0.84^{c}	1.56 ± 0.06^{b}	6.40 ± 0.38^{a}
CS10%	0.75 ± 0.07^{b}	6.35 ± 0.58^{c}	$1.69\pm0,14^{ab}$	6.37±0.26 ^a
CS15%	0.82 ± 0.07^{b}	7.32 ± 0.04^{b}	1.74 ± 0.08^{ab}	6.57±0.24 ^a
mix	0.62 ± 0.02^{c}	4.88 ± 0.23^{d}	1.67 ± 0.12^{ab}	6.39±0.14 ^a
(RRS 7.5%+CS 7.5%)				

^{*}Data was represented as Mean \pm SD. Values were considered significant at P \leq 0.05.

Table (4) shows that the positive control group had a significantly higher weight of the heart, liver, and kidney and a lower weight of the femur bone (0.93, 8.83, 1.83, and 4.85, respectively) than the negative control group (0.81, 6.95, 1.73, and 6.62). These findings are consistent with (Yasir, et al., 2023) which demonstrated that corticosteroids have numerous severe side effects due to their multitude of actions affecting almost all organs; one of them is weight gain and significant effects on the liver. Hodges and Joan (1960) found that cortisol administration at 2mg per day

^{*} Means within the same colum with different letters are significantly different $(P \le 0.05)$.

decreased the weight of treated albino rats while increasing the weight of their pituitary glands relative to the control group. Robert et al., (1952) ensured the influence of cortisone on the pace of liver regeneration. The results of adding different levels of red radish seeds and celery seeds (10% and 15%) to the diet of rats suffering from osteoporosis show that there is no significant difference in the weight of the heart, liver, and kidney, but there is a significant difference (an increase) in the weight of bone, and the mixture (7.5% RRS and 7.5% CS) shows a significant difference in all organs compared to the positive control group. These results are consistent with Amar et al (2013) Dexamethasone's influence on body weight was investigated, and it was discovered that both high and low doses of dexamethasone dramatically reduced animal body weight. The findings also revealed that there are no significant differences in heart and kidney weights across the groups.

Table (5): Effect of red radish seeds, celery seeds and the mixture of them on serum vitamin D, Calcium and Phosphorus at the end of study

Micronutrients	Vit. D	Ca	P
Groups	mg/ml	mg/dl	mg/dl
Control (-)	11.97±0.2 ^e	10±0.2 ^b	4.96±0.66 ^a
Control (+)	7.2±0.44 ^g	9.17±0.35°	4.13±0.15 ^e
RRS10%	24.17±0.2°	10.00±0.2ª	4.57±0.15 ^d
RRS15%	29.43±0.47 ^b	10.03±0.1 ^b	4.65±0.18°
CS10%	10.57±0.25 ^f	10.2±0.1 ^a	4.60±0.13°
CS15%	23.6±0.1 ^d	10.13±0.15 ^a	4.75±0.06 ^b
mix (RRS 7.5%+CS 7.5%)	36.23±0.35 ^a	10.17±0.06 ^a	4.85±0.11 ^a

^{*}Data was represented as Mean \pm SD. Values were considered significant at P \leq 0.05.

Table (5) illustrates vitamin D, calcium, and phosphorus concentrations in serum. Serum vitamin D, calcium, and phosphorus

^{*} Means within the same colum with different letters are significantly different $(P \le 0.05)$.

concentrations decreased considerably ($p \le 0.05$) in the positive control group (7.2, 9.17 and 4.13 mg/ml) compared to negative control group (11.97, 10 and 4.96mg/ml). Goodman et al., (2007) explained that calcium deficiency could be caused by cortisone. Glucocorticoids alter mineral homeostasis via lowering calcium absorption. Glucocorticoids directly decrease osteoblastic bone growth, reduce intestinal calcium excretion absorption, and increase renal calcium excretion. Serum vitamin D, calcium, and phosphorus levels have significantly increased ($p \le 0.05$). The seventh group (treated rats with a meal containing 7.5% red radish seeds and 7.5% celery seeds) had the greatest percentage of vitamin D (36.23 mg/ml). There were no significant differences in serum Ca and P levels between the RRS and CS groups. The group fed on a diet supplemented with (15% RRS) had a higher vitamin D content (29.43 mg/dl) than the treatment group with (10%RRS and 10% CS), which came in second place in vitamin D (24.17 and 23.6 mg/dl, respectively). The group fed a mixture of RRS and CS showed a considerable rise in serum Ca and P levels when compared to the other examined groups.

Table (6): Effect of red radish seeds, celery seeds and the mixture of them on some hormone of osteoporotic female rats

Hormones	Estradiol (E2)	Parathyroid hormone (PTH)
Groups	(pg/ml)	(pg/ml)
Control (-)	75.7±0.2 ^a	42.83±0.35 ^a
Control (+)	60.67±0.68 ^e	19.8±0.46 ^f
RRS10%	70.27±0.32°	29.33±0.35 ^d
RRS15%	73.43±0.37 ^b	31.5±0.2 ^b
CS10%	69.73±0.25 ^d	28.47±0.42 ^e
CS15%	70.33±0.45°	30.37±0.45°
mix	75.03±0.15 ^a	42.9±0.2 ^a
(RRS7.5%+CS7.5%)		

^{*}Data was represented as Mean \pm SD. Values were considered significant at P \leq 0.05.

^{*} Means within the same colum with different letters are significantly different ($P \le 0.05$).

Table (6) shows that the positive control group has lower E2 concentrations and higher PTH concentrations (60.67 and 19.8 pg/ml) compared to negative control group (75.7 and 42.83 pg/ml). All treatment groups of rats exhibit a significant rise (p≤0.05) in serum Estradiol (E2) compared to the positive control group. The highest concentration of E2 was found in the serum of rats fed a mix of 7.5% RRS and 7.5%CS, with (75.03pg/ml) in E2 and (42.9pg/ml) in All treatment groups of rats exhibit a significant rise (p≤0.05) in serum Estradiol (E2) compared to the positive control group. The highest concentration of E2 was found in the serum of rats fed a mix of 7.5% RRS and 7.5%CS, with (75.03pg/ml) in E2 and (42.9pg/ml) in PHT. These findings are consistent with (**Micheal et al., 2011**), who reported that increasing PTH recreation plays an important role in increasing bone mineral resorption and osteoporosis.

Table (7): Effect of red radish seeds, celery seeds and the mixture of them on some liver enzymes in experimental rat

Liver enzymes	AST/GOT	ALT/GPT	ALP
Groups	(U/L)	(U/L)	(U/L)
Control (-)	$37.73\pm0.25^{\rm f}$	20.47±0.5 ^e	93.83±0.47 ^f
Control (+)	97.5±0.5 ^a	40.00±1.00 ^a	180.56±0.28 ^a
RR10%	49.23 ± 0.68^{d}	32.53±0.5 ^b	116.43±0.5°
RR15%	44.17±0.76 ^e	26.6 ± 0.52^{d}	95.73±0.64 ^e
CS10%	60.5 ± 0.5^{b}	32.56±0.51 ^b	139.73±0.64 ^b
CS15%	52.57±0.5°	28.43±0.51°	120.5±0.5 ^d
mix (RRS7.5%+CS7.5%)	37.83±0.72 ^f	20.1±1.01 ^e	92.43±0.51 ^g

^{*}Data was represented as Mean \pm SD. Values were considered significant at P \leq 0.05.

Table (7) displays the mean values of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) in rats fed different diets. The control positive group had significantly higher mean values ($P \le 0.05$) for AST, ALT, and ALP (97.5, 40.00, and

^{*} Means within the same Colum with different letters are significantly different $(P \le 0.05)$.

180.56U/L) compared to the control negative group (37.73, 20.47, and 93.83U/L). All liver enzymes decrease as the concentration of RRS and CS increases in all osteoporotic groups as compared to the positive control. According to Xing et al. (2006), Aspartate Aminotransferase and Alanine Aminotransferase were previously known as serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), respectively. AST or ALT levels are also a valuable aid in the diagnosis of liver disease and can be used in combination with other enzymes to monitor the progression of various liver disorders. These results are consistent with(Amira and Wesam 2021). The study found that consuming RRS considerably (P < 0.05) lowered liver enzymes (AST and ALT). RRS contains a large number of phenolic and flavonoid chemicals, making it a valuable source of health benefits. According to **Doha et al.**, (2019) The efficacy of red radish seeds in protecting rats from hepatotoxicity was tested, and serum ALT, AST, and ALP activities in the liver were reduced as compared to a positive control group.

Table (8): Effect of red radish seeds, celery seeds and the mixture of them on some kidney functions

Kidney enzymes Groups	Urea mg/dL	Creatinine mg/dL
Control (-)	33.73±0.66 ^f	0.63±0.01 ^f
Control (+)	51.1±0.2 ^a	0.96±0.02 ^a
RRS10%	40.36±0.91°	0.69 ± 0.01^{d}
RRS15%	36.87±0.32 ^e	0.66 ± 0.03^{e}
CS10%	42.96±0.35 ^b	0.75 ± 0.02^{b}
CS15%	38.27 ± 0.602^{d}	0.70±0.01°
mix (7.5% RRS+7.5%CS)	33.33±0.42 ^f	0.63±0.03 ^f

Data was represented as Mean \pm SD. Values were considered significant at P \leq 0.05.

As shown in table (8) that the positive control group (osteoporotic rats) had the highest urea level compared to the negative control group (healthy rats), and administrated osteoporotic groups. with values of 51.1

^{*} Means within the same Colum with different letters are significantly different (P≤0.05).

and 33.73 mg/dL, respectively. (**Park, et al., 2017**) explained that BMD linked with blood urea nitrogen (BUN) and creatinine levels in both normal and osteoporotic groups. Logistic regression analysis revealed a link between BUN and lumbar and femoral BMD. **Shaohui et al., (2024)** reported that Creatinine has been recommended as a helpful measure of skeletal muscle mass, as well as evidence for future osteoporosis markers.

The results showed that raising the supplementation ratio resulted in a small drop in both the urea and creatinine groups. **Eliza Knez et al.,** (2022) investigated the health benefits associated with the consumption of root vegetables such as celery and radish, including the high nutritional value due to their high content of dietary fibre, vitamins, minerals, and bioactive compounds such as polyphenols, phenols, flavonoids, and vitamin C. These chemicals are responsible for the antioxidant potential. Furthermore, root vegetables have a variety of health benefits, including the management of metabolic parameters such as glucose and blood pressure, antioxidant potential, and prebiotic function. **Shaimaa et al.,** (2022) explained that Supplementing a diet with vegetable seeds such celery, coriander, and purslane, particularly a 20% mixture of seeds, resulted in a significant (p < 0.05) drop in urea and creatinine levels. The combination of celery seeds and red radish seeds resulted in the best serum urea and creatinine levels (33.33 mg/dL and 0.63 μ mol/L, respectively).

Table (9): Effect of red radish seeds and celery seeds on BMD and BMC of osteoporotic female rats and control

Groups	BMD G/cm 2	BMC G/cm 2
Control (-)	0.508 ± 0.01^{b}	0.05 ± 0.01^{a}
Control (+)	$0.180\pm0.008^{\mathrm{f}}$	0.02 ± 0.008^{b}
RRS 10%	0.287 ± 0.007^{d}	0.03±0.007 ^b
RRS 15%	0.316 ± 0.002^{c}	0.03 ± 0.008^{b}
CS 10%	0.231±0.0025 ^e	0.03±0.009 ^b
CS 15%	0.321 ± 0.002^{c}	0.03±0.01 ^b
mix (7.5% RRS+7.5%CS)	0.552±0.01 ^a	0.05±0.01 ^a

^{*}Mean with the same letters in the column are not significantly different at P<0.05.

*BMD: Boon Mineral Density *BMC: Boon Mineral concentration

Table (9) displayed bone mineral density, total BMD, and BMC of the femur bone in each group. It was discovered that the positive control group (osteoporotic group) had a substantial drop in BMD and BMC of the femur bone (0.180 and 0.02 G/cm2). Raczyńska et al. (2003) found that osteoporosis caused a decline in BMD and BMC values. It was discovered that the group that ate diet with sublimation with RRS and CS) had the highest value of BMD and BMC (0.552 and 0.05 G/cm 2), followed by (15%CS and 15% RRS) (0.321 and 0.03 G/cm 2) and (0.316 and 0.03 G/cm). The results showed that administered osteoporotic groups with diets supplemented with RRS and CS had improved BMD and BMC. Perhaps because their mineral content promotes the production of bone osteoblasts and prevents bone resorption. According to Singh et al. (2022), celery leaves, stalks, and seeds are rich in minerals including calcium, iron, copper, potassium, magnesium, phosphorus, manganese, and sodium. (Castiglioni et al., 2013) reported that managing and maintaining magnesium homeostasis is a beneficial intervention for bone integrity. The most easily accepted argument for the impact of dietary potassium on bone health is its effect on acid-base balance, while the involvement of the skeleton in pH regulation is debatable (Hamm et al., 2015). Radish includes a variety of vitamins and minerals, including folic acid, anthocyanin, potassium, calcium, copper, iron, phosphorus, and zinc, as well as fibre and antioxidants. These minerals provide numerous benefits, including increased bone density and improved overall health (Ji g et al., 2014; El Beltagi et al., 2010).

Histopathological Results

pictures (3, 4, 5, 6, and 7) show that rats fed a diet containing 10% RRS, 10% CS, and 15% CS had Kupffer cells activated and few inflammatory cells infiltrated, but rats fed a diet containing 15% RRS had necrosis of sparse hepatocytes, whereas rats fed a 7.5% mix of RRS and CR had slight Kupffer cell activation. **Doha et al.** (2019) found that administration of rats with crude ethanol extract of red radish seeds or roots substantially ($P \le 0.05$) reduced blood levels of ALT, AST, ALP, γ-GT, total and direct bilirubin, liver MDA. Furthermore, the seeds and roots of

red radish exhibit a protective efficacy against paracetamol-mediated. Kyung-A Hwang et al. (2022) also investigated the hepatoprotective benefits of Radish on acetaminophen-induced liver damage by reducing oxidative stress and apoptosis. Radish has anti-inflammatory, anticancer, and antioxidant qualities because it contains useful components such glucosinolates and isothiocyanates. Radish extracts improved histological state of mouse liver tissue, including inflammation and infiltration, dramatically lowering ALT, AST, and malonaldehyde levels while significantly increasing ALP. As a result, radish extracts have been identified as possible natural candidates for the development of hepatoprotective medicines. Sudad et al. (2023) reported that lipid profile were a significantly lowered by the impact of aqueous celery extracts and it has hepatoprotective effects via inhibiting AST and ALT enzymes. Omaima et al., (2016) Celery seed oil is considered to be a rich source of phytochemicals with powerful antioxidant and anti-inflammatory activities. The findings revealed that CSO has an anticancer impact in liver and antiinflammatory.

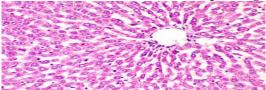
Pictures (12, 13, 11, 10, and 14) show that rats fed a diet containing (10% and 10% CS) have degeneration and cystic dilation in some renal tubules, whereas rats fed a diet containing (15% RRS and 15% CS) have congestion and slight vacuolar degeneration in renal tubules, as opposed to rats fed a diet containing (7.5% RRS +7.5%CS) showing normal renal tissue. Gouda, (2012) described the microscopic inspection of liver tissue, renal tissue, and femur bone. Radish red also protects against kidney disease caused by diabetic and lowers lipid buildup because of its high concentration of natural anthocyanins, which make as antioxidant, anti- and anti-inflammatory properties (Qiang et al., 2024). Irum et al., (2020) discovered the antioxidant ability of red radish seeds in Rat Kidney against CCl4-induced toxicity and it can be used its extracts as a chemopreventive medication. Basma et al., (2019) investigated the preventive effects of celery seed extract on gentamicin (G)-induced renal and liver damage. Celery hexanic extract or celery seed ethanolic extract caused prevention of

hepatic tissue changes, inflammatory of renal cells and fatty degeneration of hepatocyte.

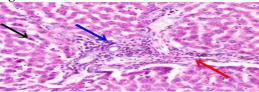
Pictures (19, 20, 21, 22 and 23) show Femur of rats were given a diet containing (10% and 15%RRS) showing congested blood vessels, femur of rats were given a diet containing (10% CS) showing few cracks in the cortex, and bone of rats were given a diet containing diet (15%CS and 7.5% mix of RRS and CS) showing no histopathological lesions and histologically normal bone cortex. The combination level (7.5% RRS and CS) produced the best outcomes in liver, kidney, and bone when compared to the rat group (control negative). According to **Kanti Bhooshan Pandey** and Syed Brahim Izvi (2009), research shows that consumption of riches plant diets in polyphenol improve pain bone and protects osteoporosis. Radish and its seeds considered good sources of bioactive compounds (Manivannan et al., 2019), such as many important vitamins (vitamin C and beta carotene), minerals particularly folic acid, anthocyanin, potassium, calcium, phosphorus, and zinc), fibre, and antioxidants, particularly flavonoids, anthocyanin, and glucosinolates), which are highly concentrated in seeds, these compounds prevent and treat of osteoporosis (Hossam et al. 2022). As a result, radish promotes bone density while also enhancing general health (Rezk et al., 2019). According to Lujuan Jiaa et al. (2020), total flavonoids from celery can be employed as functional dietary components with potential preventative and therapeutic benefits on osteoclast (Aswin Rafif Khairullah et al., 2021). Modern studies on animals have shown the medicinal impacts of celery, its roots, stems, leaves, and seeds; it contains different vitamins, minerals, and nutrients (Moghadam et al., 2013); it is rich in calcium and appears to increase bone density; it also contains volatile oils and flavonoids. (Guijarro Real et al., **2019**) like apigenin and luteolin, which can be used as a treatment bone loss and prevent osteoporosis (Kumar et al., 2015). Celery seeds have effective antioxidant properties to treat arthritis, rheumatoid arthritis, due it contains Numerous flavones, including luteolin, apigenin, corresponding glycosides (Dias et al., 2021), isolated flavone glycosides such as graveobioside A, graveobioside B, and apiin (Xu, et al., 2020; Osés et al., 2020).

Histopathological figures

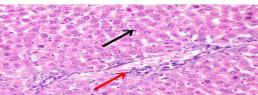
Histopathological figures of liver



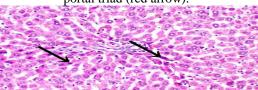
Picture 1: The normal histoarchitecture of rat liver from group1 (negative control).



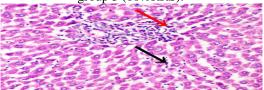
Picture 2: Liver of rat from group 2 (osteoporotic rat) with sporadic hepatocytes (black arrow), portal inflammatory cells infiltration (blue arrow) and fibroplasia in the portal triad (red arrow).



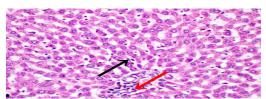
Picture 3: Kupffer cells activation (black arrow) and few inflammatory cells infiltration in the portal triad (red arrow) of liver of rat from group 3 (10%RRS).



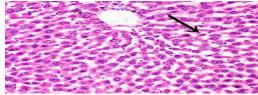
Picture 4: Necrosis of sparse hepatocytes (black arrow) of rat liver from group 4(15% CR).



Picture 5: Kupffer cells activation (black arrow) and portal inflammatory cells infiltration (red arrow) of rat liver from group 5 (10%CS).

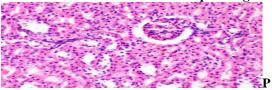


Picture 6: Kupffer cells activation (black arrow) and few portals inflammatory cells infiltration (red arrow) of rat liver from group 6 (15%CS).

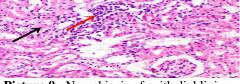


Picture 7: slight Kupffer cells activation (black arrow) of rat liver from group 7(7.5%RRS +7.5%CR)

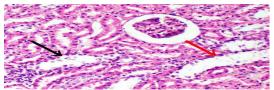
Histopathological figures of kidney



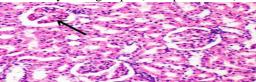
icture 8: Normal histological structure of kidney of rat from group 1(healthy rats).



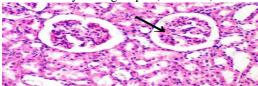
Picture 9: Necrobiosis of epithelial lining renal tubules (black arrow) and interstitial inflammatory cells infiltration (red arrow) of rat kidney from group 2 (osteoporotic rats).



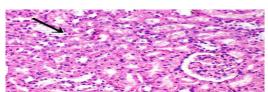
Picture 10: Vacuolar degeneration of epithelial lining some renal tubules (black arrow) and cystic dilation of some renal tubules (red arrow) of rat kidney from group 3 (10%RRS).



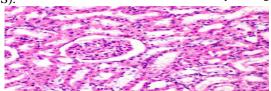
Picture 11: Slight congestion of glomerular tuft (black arrow) of rat kidney from group 4 (15% RRS).



Picture 12: Slight congestion of glomerular tuft (black arrow) of rat kidney from group 5 (10%

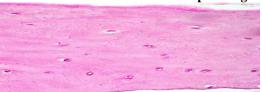


Picture 13: slight vacuolar degeneration of epithelial lining sparse renal tubules of rat kidney from group 6 (15% CS).

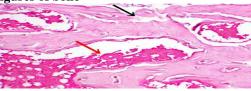


Picture 14: Normal renal tissue of rat kidney from group 7 (7.5% RRS +7.5%CS).

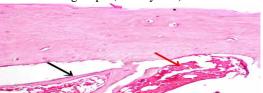
Histopathological figures of bone



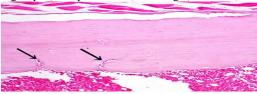
Picture 15: No histopathological alterations with appearance normal bone cortex of rat from group 1(healthy rats).



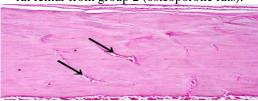
Picture 16: Necrosis of cortical bone with presence of cracks, fissures (black arrow) and dilated bone marrow cavity of Femur of rat from group 2(osteoporotic rats) showing (red arrow).



Picture 17: Thin bone trabeculae (black arrow) and dilated bone marrow cavity (red arrow) of rat femur from group 2 (osteoporotic rats).



Picture 18: Congested blood vessels (black arrow) of rat Femur from group 3(10%RRS).



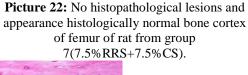
Picture 19: Congested blood vessels (black arrows) of rat femur from group 4 (15% RRS).

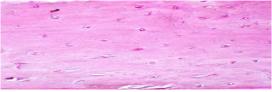


Picture 20: Few cracks in the cortex (black arrow) of rat femur from group 5(10% CS).



Picture 21: No histopathological lesions and appearance histologically normal bone cortex of femur of rat from group 6(15%CS).





Picture 23: No histopathological lesions and appearance histologically normal bone cortex of rat femur from group 7(7.5% RRS +7.5%CS).

Conclusion:

The present study indicated that dietary, red radish seeds and celery seeds could reduce the potential effect of dexamethasone inducing osteoporosis in experimental rats based on phytochemicals, nutrients, and pharmacological activities that the improvement of calcium absorption, regulation of bone metabolism, differentiation of the osteoblast and osteoclast, and decline excretion of calcium and phosphorus. Beside the steroids and flavonoid components which have the potential as natural antioxidants to prevent oxidative deterioration in body, also as an adjuvant in the management of osteoarthritis and the improvement of joint pain and stiffness.it can be recommended that the red radish seeds and celery seeds could be used as a natural dietary approach to avoid the bone loss related with dexamethasone therapy.

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دراسة تأثير نسب مختلفة من بذور الفجل الأحمر وبذور الكرفس على الفئران المصابة بهشاشة العظام

اللخص العربى:

اجريت هذه الدراسة لمعرفة تأثير مستويات مختلفة من بذور الفجل الأحمر (RRS) وبذور الكرفس (CS) على هشاشة العظام التي يسببها الجلوكوكورتيكوييد (ديكساميثازون) في ايناث الفئران. حيث أجريت الدراسة على خمسة وثلاثين أنثى من الفئران البيضاء غير الحوامل التي يتراوح أعمارهن بين ٦- ٨ أسابيع واوزنهم من ١٧٠ إلى ٢٠٠ جرام، حيث قسمت إلى مجموعتين رئيسيتين، المجموعة الأولى تتغذى على نظام غذائي قياسي كمجموعة كنترول سالبةوالمجموعة الرئيسية الثانية تتغذى على نظام غذائي أساسي وتم حقنهم بالجلوكوكورتيكويد (ديكساميثازون) بجرعة ٤ ملغ / كجم من وزن الجسم عن طريق الحقن العضلي ثلاث مرات في الأسبوع لمدة ثلاثة أسابيع لاحداث هشاشة العظام في الفئران. ثم اعادة تقسيهم إلى المجموعة الثانية كمجموعة كنترول موجبة وخمس مجموعات معالجة وتم تغذيتها على نظام غذائي قياسي أساسي يحتوي على ١٠٪ و ٥١٪ من RRS و CS ومزيج من ٥٠٪ RRS و RRS لدة ستون يوماً، و أظهرت النتائج الكيميائية أنه تم تحديد العناصر الغذائية الكبرى والمادن في RRS و CS ؛ كانت البروتينات والألياف والكربوهيدرات والكالسيوم والبوتاسيوم والمغنيسيوم عالية في RRS (29.16 و ٢٥,١٣١ و ٣٥٥٥٨ و ٥٩٥ و ٣٩٦ و ٣١٦) ، بينما تم تحديد الدهون والرماد والكالسيوم والبوتاسيوم والمغنيسيوم في CS (5.67) و ٢١,١٣ و ١١٥,٩٣ و ١٠٨٠٠٢ و ٣٢,٧٦). أشارت النتائج البيولوجية إلى زيادة في زيادة وزن الجسم ، وأظهرت أفضل النتائج في المجموعةالتي تناولت خليط (٢٠,٠٧) جم. كما زاد فيتامين د والكالسيوم والفوسفور تدريجيًا.

الكلمات المفتتاحية: بدور الفجل، بدور الكرفس، آلام العظام، تيبس العظام وهشاشة العظام العظام العظام العظام العظام العظام الفئران.