Effect of Cichorium intybus L leaves and Spirulina plantensis Algae powder against Osteoporosise in Female Rats induced by Dexamethasone

Safaa T. Gohari

Nutrition and Food Science, Home Economic Dept., Faculty of Specific Education, Ain Shams Univ., Cairo, Egypt- email: drsafaa@sedu.asu.edu.eg

Abstract

Osteoporosis is a multifactorial skeletal disease characterised by a loss of bone mass and alteration of bone tissue's microarchitectural integrity. The current study looked at the effects of *Cichorium intybus L* (CH) leaves and *Spirulina plantensis* algae (SP) powder on Dexamethasone-induced osteoporosis in female rats (DEX).Dried SP algae had a larger amount of protein and fat than CH leaves, according to chemical analysis. On the other hand, CH leaves, were distinguished by a higher percentage of carbohydrates, fibre, ash, and moisture than dried SP algae.Dried SP algae and CH leaves powder were also high in calcium and phosphorus (1341.561.29 and 887.002.0mg/100g)and(295.141.10and1871.0mg/100g),respectively .Forty-eight non-pregnant female albino rats were divided into two groups: the first main group (8 rats) was fed a basal diet (B.D.) and served as a negative control (-ve), and the second main group (40 rats) was injected intramuscularly with DEX at a dose (7 mg/kg b.wt.) once weekly for four weeks to cause osteoporosis, then divided into five subgroups of eight rats each:As a positive control (+ve), group (2) was fed a basal diet, group (3) was fed a B.D. containing 4% dried SP algae, group (4) was fed a B.D. containing 6% dried SP algae, group (5) was fed a B.D. containing 5% CH leaves powder, and group (6) was fed a B.D. containing 10% CH leaves powder.When compared to the positive control group, all osteoporosis groups treated with CH leaves powder (5 and 10%) and dried SP algae (4 and 6%) had significant decreases in liver enzymes, kidney function, calcium and phosphorus in bone, ionised calcium, bone mineral density, and bone mineral content assay.The best results were obtained in groups treated with 10% CH leaves powder and 6% dried

SP algae.After two months, x-rays and histopathology of the positive control group revealed bone loss in various parts such as the fibula, tibia, and femur, as well as bone demineralization and osteoporosis.In comparison to the positive control group, these findings revealed that CH leaves powder and dried SP algae treated degrees of osteoporosis.
Keywords: Osteoporosis; Glucocorticoids; chicory; Microalgae; DEXA; x-ray and histopathology

Introduction

Dexamethasone (DEX) is a type of glucocorticoid (GC) that is widely used to treat allergic disorders, ulcerative colitis, arthritis, pulmonary disorders, and organ transplantation due to its potent anti-inflammatory and immunomodulatory effects. **(Vandewalle *et al*., 2018)**. Despite the therapeutic effects of this drug, its frequent use inevitably results in a variety of health problems.Among these is a severe form of secondary osteoporosis that affects 30-50 percent of GCs therapy patients. **(Nuti *et al*., 2019).** The pathological mechanisms of GCs-induced bone formation include decreased differentiation and maturation of osteoblasts and increased life span of osteoclasts. **(Li *et al*., 2015).** Osteocytes are also affected, with decreased cell function and increased apoptosis resulting in impairment of their ability to detect and repair bone micro damage **(Fraser and Adachi, 2009)**. The combination of increased bone resorption and decreased bone formation may explain the early and rapid loss of bone mineral density (BMD) and bone strength/quality in GCs therapy patients. **(Dobrowolski *et al*., 2017)**. Currently, the use of medicinal plants has emerged as one of the most popular and preferred complementary and alternative medicine modalities. Certain vegetables and fruits have been shown to be essential for maintaining bone mass and preventing osteoporosis. **(Rajput et al., 2018).**

Chicory (*Cichorium intybus*) is a member of the Asteraceae family, and its leaves are commonly used in salads and as cooked vegetables in Mediterranean cuisines. **(Jancic *et al*., 2017)**. Previous research suggested that chicory has antitoxic, hypoglycemic, and a variety of other pharmacological effects, establishing chicory as a functional food. **(Azzini *et al*., 2018).** Chicory was planted by the ancient Egyptians to be used as a medicinal plant.It was used due to its numerous beneficial effects such as hepatoprotective, neuroprotective, hypolipidemic, antioxidant, and anti-inflammatory properties. **(Keshk *et al*., 2019)**. Chemical analysis of fresh chicory revealed that it is primarily composed of inulin and sucrose (68 and 14 percent, respectively), with minor amounts of protein, cellulose, and ash. **(Hassan and Yousef, 2010).** Chicory contains alkaloids, inulin, sesquiterpene lactones, coumarins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins, and tannins in all parts of the plant. **(Abbas *et al*., 2015).**

Many microalgae have been studied, but spirulina is thought to be the most unique due to its high protein content (65 to 70%) and high percentage of amino acids. (**Liestianty *et al*., 2019)**. It's a good source of micro and macronutrients like minerals, vitamins, gamma-linolenic acid, phycocyanin, and sulfated polysaccharides. **(Ljubic *et al.,* 2018).** So it is consumed as a functional food and safe nutritional supplement in the right amount. It is worth noting that the term functional refers to foods that have been shown to help specific functions in the body, leading to enhance health and / or reducing the risk of disease (**Rodriguez *et al*., 2018).**

Spirulina has gained popularity due to its ability to stimulate mineral absorption through its effect on intestinal microflora. **(Siddiqui, 2017).** This evidence suggests that spirulina can influence calcium and bone metabolism, though no studies have been conducted to assess spirulina's activity on bone metabolism in vivo.The green alga has also been investigated as a potential source of macronutrients as well as a viable source of oxygen in the space environment. **(Vandewalle *et al*., 2018).** Plants, seaweeds, microalgae and food by-products are the most important sources of functional compounds such as dietary fiber, phenolic compounds, flavonoids, oils, plant sterols, proteins, prebiotics, probiotics, anthocyanins, carotenoids and many others **(Da Silva *et al*., 2016).** The aim of this research was to investigate the effect of *Cichorium intybus L* leaves and *Spirulina plantensis* Algae powder against Osteoporosise in female Rats induced by Dexamethasone

2. MATERIALS AND METHODS

2.1. MATERIALS

Cichorium intybus L leaves and *Spirulina plantensis* algae were obtained from El-nada farm 59. Egypt, Alex. Desert road. Abo-ghaleb and were identified by El-nada farm laboratory for analyses. While, Commercial kits were obtained from Biodiagnostic Co. Dokki, Egypt. DEX sodium phosphate was obtained from El-Gomhoreya Company for Tarading Drugs, Chemical and Medical Instruments

2.2. Methods

2.2.1. Proximate chemical composition of *Cichorium intybus L* leaves and *Spirulina plantensis* algae

Proteins, fat, ash, fiber and moisture were determined according to **AOAC, (2015).** Carbohydrate contents were estimated by the difference**.**

2.3. Biological experiment

Forty-eight non-pregnant female albino rats of Sprague Dawley weighing 150 ± 10 g were obtained from Agricultural Research Center, Giza, Egypt. For 8 weeks, the animal groups were kept in an environment of filtered, pathogen-free air, water, and a temperature of 20-25oC, with a 12 hour light/dark cycle and a light cycle (8-20 h) and a relative humidity of 50%.All rats were fed a standard diet for one week.The basal diet included 14% casein, 10% sucrose, 4% corn oil, 5% fibre (cellulose), 3.5 percent mineral mixture, 1% vitamin mixture, 0.25 percent choline chloride, 0.3 percent D-L methionine, and 61.95 percent corn starch. **(Reeves *et al.*, 1993).** Before beginning the experiment, allow for acclimatisation.All experimental procedures were carried out in accordance with international guidelines for laboratory animal care and use.The experiment was carried out at the Agricultural Research Center in Giza, Egypt.

2.3.1. Experimental design

After one week of adaptation, the rats were divided into two groups: the first main group (8 rats) was fed a basal diet as a control negative (-ve), and the second main group (40 rats) was injected intramuscularly with DEX at a dose (7 mg/kg b.wt.) once weekly for four weeks to cause osteoporosis according to **(Thakur *et al*., 2013)** then divided into five sub-groups of eight rats each:As a positive control (+ve), group (2) was fed a basal diet, while group (3) was fed a basal diet plus 4% dried SP algae.Group (4) was fed a basal diet plus 6% dried SP algae, Group (5) was fed a basal diet plus 5% CH leaves powder, and Group (6) was fed a basal diet plus 10% CH leaves powder.

Animals from each group were sacrificed at the end of the experiment, and the blood was collected in a clean dry centrifuge tube, left at room temperature until the clot formed, completely retracted, and then centrifuged to separate serum, before being stored in a plastic vial (well stoppered) until analysis.

3.4. Biochemical Analysis

3.4.1. Biological Determination

Biological evaluation of the different tested diets was carried by determination of food intake (FI), body weight gain% (BWG %) and organs weight / body weight% 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

3.4.2. Biochemical Analysis

Serum uric acid was determined by **Barham and Trinder, (1972)** using a spectrophotometer (model DU 4700) adjusted at 510 nm. Serum urea was determined according to the method described by **Fawcett and Soctt (1960)** using a spectrophotometer (model DU 4700) adjusted nm 550 nm. Serum creatinine was determined by (**Tietz, 1986)** using a spectrophotometer (model DU 4700) adjusted at 510 nm. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically using spectrophotometer (model DU 4700) at 505 nm according to the method of (**Reitman and Frankel, 1957**).Alkaline phosphatase (ALP) activity was determined calorimetrically using a spectrophotometer (model DU 4700) at 510 nm according to the method of **(Belfield and Goldberg 1971)**. Calcium O-cpcactivities were determined calorimetrically using a spectrophotometer at 340 nm according to the method of **(Young, 1990).**

2.5. Bone mineral content (calcium and phosphors)

The bone from the left femur was dried overnight at 100°C.To obtain the ash, the femur was incinerated for 12 hours at 1000°C in the Muffle apparatus.The ash was weighed, solubilized in 0.1 Mol/L HCL **(Yang *et al.*, 2008)** and quantitatively transferred into a volumetric flask, which was then filled to 100 ml with 0.1 Mol/L HCL.Using an atomic absorption spectrophotometer, the solutions were used to determine the calcium content of bone ash.A spectrophotometer was used to determine the phosphorus content of bone ash.

3.5. Bone mineral density and bone mineral content assay

Each animal's right femur was dissected and carefully cleaned before measuring 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 Center, Dokki, Egypt to measure the bone mineral content (BMC, g/cm2) and bone area (BA, cm2). The BMD was calculated as BMC/BA **(Bagi *et al*., 2011).**

3.7. Histopathology Investigation

Autopsy samples were taken from the bones and joints of rats in different groups and fixed in 10% formal saline for 24 hours before being decalcified with formic acid.Washing with tap water was followed by dehydration with serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) .Specimens were cleared in xylene and embedded in paraffin for 24 hours at 56 degrees in a hot air oven. Slidge microtome was used to prepare paraffin bees wax tissue blocks for sectioning at 4 microns thickness. Tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin for examination under a light electric microscope. **(Banchroft *et al*., 1996)**

3.8. Scanning X-ray

Radiographic views were undertaken for each rat including lateral, ventrodorsal by using Fischer x-ray apparatus (40 Kilovolt, 100 mA and 100cm FFD) **(Farrow, 2003).**

3.9. Statistical Analysis

The statistical analysis was carried out by using SPSS, PC statistical software (version 25.0; SPSS Inc., Chicago. USA). The results were expressed as mean ± SD. Data was analyzed by one-way analysis of variance (ANOVA). The Differences between means were tested for significance using Least Significant Difference (LSD) test at $p \leq 0.05$ **(Snedecor and Cochran, 1980).**

3. RESULTS AND DISCUSSION

3.1. Proximate chemical composition of *Cichorium intybus L* leaves and dried *Spirulina plantensis* algae

Table (1) shows the chemical composition percentages of CH leaves and dried SP algae.The results showed that dried SP algae had a higher percentage of protein and fat (61.19±0.27 and 5.22±0.26%, respectively) than CH leaves (15.34±0.31 and 5.22±0.26%), which is consistent with previous research. **(Zeller *et al*., 2013).** While CH leaves had a higher percentage of carbohydrates, fibre, ash, and moisture (42.07±0.28, 17.36±0.08, 11.65±0.11, and 8.35± 0.05%) than dried SP algae (18.20±0.55, 2.03±0.06, 9.59±0.42, and 2.21±0.25%).According to **Richmond (2004)** Spirulina contains 50-70 percent protein, 20 percent carbohydrate, 5 percent lipid, 7 percent minerals, and 3 to 6 percent moisture.As a result, unlike proteins obtained from meat and dairy products, it is a protein resource that is low in lipids, low in calories, and free of cholesterol.Spirulina is an energy supplement for the elderly.It is consumed by 73% of people over the age of 50 in Japan.Spirulina has only 36 calories per 10 grammes. **(Seshadri and Jeeji 1992).**Also**,** the results of CH leaves presented in this study were found to be in the range of the value reported by **Nwafor *et al*., (2017)** ,**Hamid *et al*., (2021)**and **Perović *et al*., (2021)**

Table (1): Major chemical constituent of *Cichorium intybus L* leaves and dried *Spirulina plantensis* algae *%*

Samples	Major chemical constituent (%)					
	Protein	**Fat**	**Moisture**	**Ash**	**Fibre**	**Carbohydrates**
CH leaves powder	15.34±0.31[b]	5.22±0.26[b]	8.35 ±0.05[a]	11.65 ±0.11[a]	17.36±0.08[a]	42.07±0.28[a]
Dried SP algae	61.19±0.27[a]	6.77±0.09[a]	2.21±0.25[b]	9.59±0.42[b]	2.03±0.06[b]	18.20±0.55[b]

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at P ≤ 0.05.CH: *Cichorium intybus* SP: *Spirulina plantensis*

Figure (1) depicts the content of important minerals in dried SP algae and CH leaves powder, which were rich in calcium and phosphorus(1341.56±1.29and887.00±2.0mg/100g)and (295.14±1.10 and 187±1.00mg/100g), respectively.The calculated Ca: P ratios of dried SP algae and CH leaves powder were 1.51:1 and 1.58:1, respectively, which are within the European Society for Paediatric Gastroenterology Hepatology and Nutrition's recommended range of 1:1–2:1 (weight/weight) to ensure optimal bone health and development. **(Koletzko *et al*., 2005)**.

Also, results showed that dried SP algae contained a higher percentage of Potassium, Magnesium and Iron (1121.88±1.57, 271.00±1.00 and 171.63±.90mg/100g) than CH leaves (187.33±.90, 118.05±.65 and 9.12±.55 mg/100g), **Carcea *et al*., (2015)** showed that spirulina contains many minerals and vitamins. The most common minerals that are found in spirulina are calcium, iron, zinc, potassium, magnesium, selenium and many others.According to **Nasef , (2018)** the calcium, phosphorus, sodium, and magnesium values in chicory leaves were 100, 47.0, 45.0, and 30.0 (g/100g), respectively.The findings of **Hamid *et al*., (2021)** are also consistent with ours..

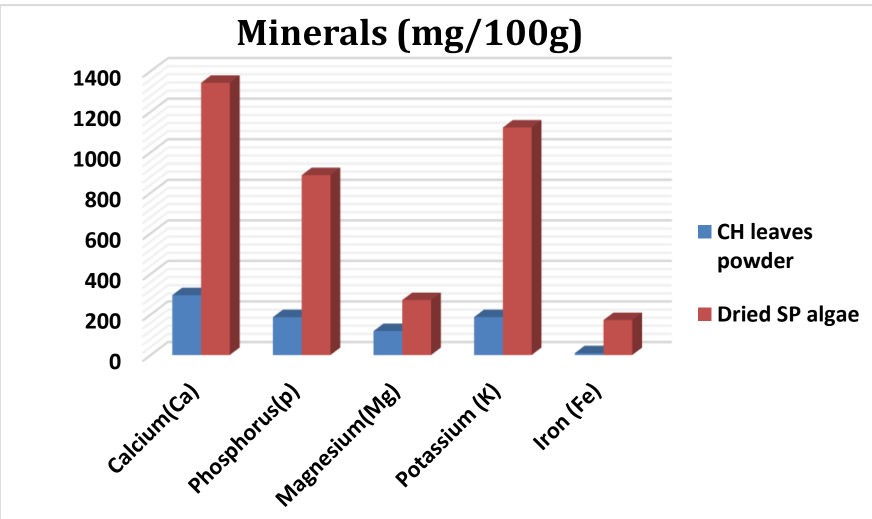


Fig. (1): Minerals of *Cichorium intybus L* leaves and dried *Spirulina plantensis* algae (mg/100g)

3.2. Biological evaluation of experimental rats which treated with two levels of CH leaves and dried SP algae

3.2.1. Body weight gain

The growth curve of experimental rats, final body weights and body weight gain of all rats' groups are given in Fig (2) and Table (2). At the beginning of the investigation, initial body weight showed a non-significant change in all experimental groups. While final body weight of all rats' groups was not significantly different except inducted group (+ve) was significantly (P≤0.05) decreased 194.0±8.27compared with control (-ve) 252.2±6.22 these findings are consistent with those of **Mada *et al*., (2019)**.Dexamethasone (DEX) is a synthetic GC that is widely used as an effective treatment option for a variety of inflammatory diseases **(Vandewalle *et al*., 2018).** However, administration of DEX at moderate to high doses frequently results in a slew of unwanted side effects ranging from minor to severe complications **(Nuti *et al*., 2019).** The majority of attention has been focused on body weight loss as one of the primary indicators of the drug's negative health effects **(Bordag *et al*., 2015).**

On the other hand, (6% dried SP, 10% CH leaves powder, 4% dried SP and 5% CH leaves powder)respectively occurred higher increased in body weight gain (BWG) 43.68±3.87, 42.72±2.24, 41.39±1.24 and 36.75±5.41 respectively compared to inducted groups(+ve) 12.79±1.48. This is consistent with the findings of **Liu, *et al*., (2011)** who discovered that compliance with GC treatment reduced craving and body weight.Various ratios of CH leaves powder and Dried SP algae administration were found to be effective in preventing body weight loss.The improved effect on body weight in dried SP groups could be attributed to Spirulina platensis' unique blend of nutrients, which no single source can provide, such as B-complex vitamins, minerals, proteins, -linolenic acid, and super anti-oxidants such as -carotene, vitamin E **(Kulshreshtha *et al*., 2008 and Holman and Malau-Aduli, 2012).**The oral administration of SP to diabetic rats for 50 days resulted in a significant ($p < 0.001$) increase in BWG, indicating that SP significantly improved their general health status and metabolic mechanisms through effective glycemic control or gluconeogenesis reversal (**Abdel-Daim 2014 and Yusuf *et al*., 2016).** Furthermore, the improvement in body weight gain in rats fed chicory leaves is consistent with the findings of **Urias *et al*.,**

(2007) who revealed that chicory inulin is useful for appetite regulation.

Table (2): Mean body weight gain (g) of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Body weight (g)	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder		Dried SP algae	
			5%	10%	4%	6%
IBW	167.80±7.36a	172.00±6.96[a]	171.60±5.31[a]	170.60±5.68[a]	170.60±4.44[a]	164.0±4.00[a]
FBW	252.2±6.22[a]	194.0±8.27[c]	234.6±9.86[b]	243.4±5.31[ab]	241.2±5.44[b]	235.6±6.65[b]
BWG/ wk %	50.47±6.11[a]	12.79±1.48[d]	36.75±5.41[c]	42.72±2.24[b]	41.39±1.24[bc]	43.68±3.87[b]

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at P ≤ 0.05.IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; WK: Week;

CH: *Cichorium intybus* SP: *Spirulina plantensis*

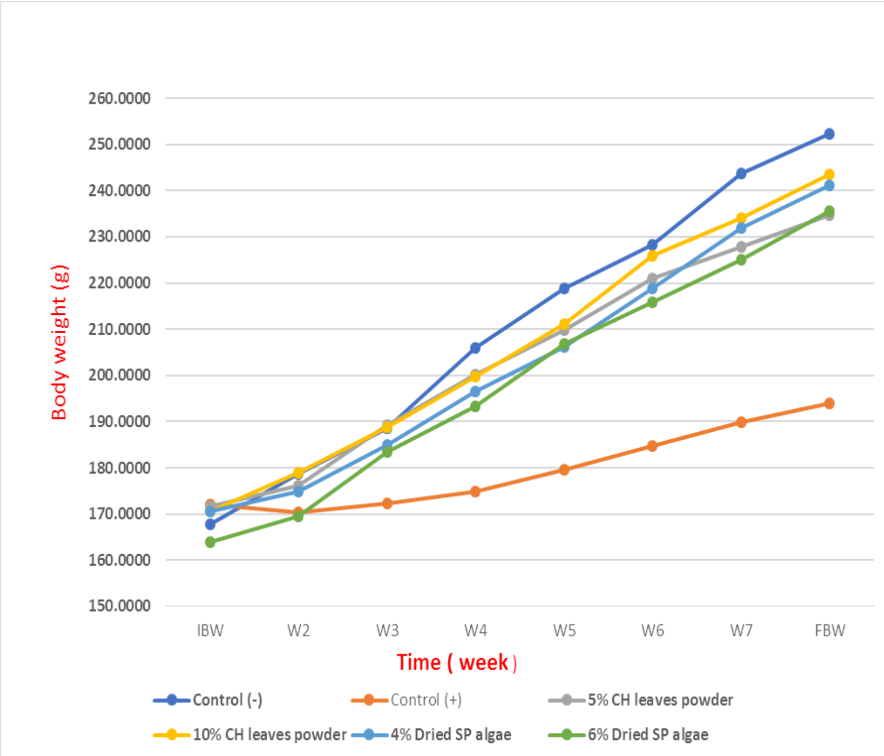


Fig. (2): Mean body weight gain (g) of experimental rats which treated with two levels of CH leaves powder and dried SP algae

3.2.2. Organs weight / body weight %

Osteoporosis is characterized by bone deterioration leading to the development of irregularities as a result of loss of osteoblasts **(Baofeng *et al*., 2010)**. Studies have revealed marked degradation in the femur bone of experimental animals treated with glucocorticoids **(Reid *et al*., 1992)**

Results in Table (3) indicated the effect of two levels of CH leaves powder and dried SP algae on the relative organs weight of rats suffering from osteoporosis. Femur bone weight/body weight % of the (+ve) control group showed a significant decrease $P \leq 0.05$ than the (-ve) control group (0.76±.17and 1.24±.25

respectively).Glucocorticoids have a negative impact on bone formation, turnover, and dependability. Cortisol's action began with osteoblasts, reducing replication and impairing differentiation and maturation, resulting in decreased bone formation **(Ito *et al*., 2007).** It also includes an increased bone resorption component.In the early stages of glucocorticoid treatment, decreased bone formation combined with increased resorption causes a rapid loss of bone integrity and may increase the risk of fracture **(Canalis *et al*., 2004)**

Significant difference $P \leq 0.05$ in Femur bone weight/body weight % among the (+ve) control group and the groups treated with two levels of CH leaves powder and dried SP algae. The best result was recorded for groups treated with 10% CH leaves powder and 6% dried SP algae (1.43±.21 and 1.29±.24, respectively). **Poormoosavi *et al*., (2019)** showed that no significant differences were observed regarding the body and liver weight between the groups receiving S. platensis treatment compared to the controls ($P \leq 0.05$). Furthermore, the experiments had no significant effect on the weight of the kidneys.

As shown in the table (3) It could be noticed that, liver and kidney weight/body weight % of the (+ve) control group (4.26±.34and .99±.09, respectively) showed a significant increase $P \leq 0.05$ than the (-ve) control group (2.93±.26 and .54±.14, respectively) these results agree with **Mada *et al*., (2019)**. Also, Significant difference P>0.05 in liver and kidney weight/body weight % among the (+ve) control group and the groups treated with two levels of CH leaves powder and dried SP algae. The best result was recorded for groups treated by 10% CH leaves powder and 6% dried SP algae (2.91±.41 and.48±.04) and (2.93±.50 and 54±.15) respectively. These results may be due to the plant (chicory) having soluble fiber, it has a hypolipidemic effect and is not hydrolyzed by the digestive enzymes. These results agree with **Alwan (2009)** who reported that adding 1% or 5% chicory water extract to the diet of hypercholesterolemic rats reduced liver weight and found no significant difference in liver weight in the treated group when compared to the normal control group.

Concerning the mean value of heart weight/body weight %, it could be noticed that the (+ve) control group showed a significant increase P≤0.05 than the (-ve) control group (1.45±.19and.45±.06, respectively). All treated groups showed a significant difference P ≤ 0.05 in heart weight/body weight % as compared to the (+ ve) control group. The best result was recorded for groups treated with 10% CH leaves powder and 6% dried SP algae (0.36±.06 and 0.34±.02, respectively).

Table (3): Mean organ weight/body weight (%) of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Organ's weight (%)	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder		Dried SP algae	
			5%	10%	4%	6%
Femur bone	1.24±.25a	0.76±.17 b	1.29±.08 a	1.43±.21 a	1.19±.16 a	1.29±.24 a
Liver	2.93±.26b	4.26±.34 a	3.16±.32 b	2.91±.41 b	3.13±.42 b	2.93±.50 b
Kidney	.54±.14bc	.99±.09a	.60±.11b c	.48±.04b c	.65±.07b	54±.15b c
Heart	.45±.06b	1.45±.19 a	40±.08b	0.34±.02 b	.39±.03b	0.36±.06 b

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at P ≤ 0.05.CH: *Cichorium intybus* SP: *Spirulina plantensis*

3.2.3. Biochemical analysis

The main components of the hydroxyapatite crystal, which is the basic building block of bone minerals, are calcium and phosphate. The mineral hydroxyapatite is important for bone's compressive strength, but the collagen framework within which the hydroxyapatite crystals lie dictates bone size and shape **(Grandfield *et al*., 2018)**.

A significant decrease P≤ 0.05 was observed in the table (4) of serum ionized calcium and femur bone mineral content (calcium and phosphors) mean values in the control group (+ve) **(**1.20±.1, 7.75±.05and 4.53±0.2) respectively when compared with the healthy group (control -ve) (1.75±.18, 8.45±.13, and 5.47±.15) respectively. Glucocorticoids impair bone metabolism by suppressing calcium absorption through the digestive tract as well as calcium reabsorption through the kidney, which can result in hypocalcaemia and, in turn, hyperparathyroidism later on **(Weinstein, 2011)**. Glucocorticoids influence bone mineralization by suppressing Tran's repression of matrix proteins collagen I and osteocalcin.

Furthermore, GCs have a negative impact on muscle mass and strength, resulting in muscle atrophy **(Canalis *et al*., 2007)**. Signs of osteoporosis were similarly observed in the present study as manifested by reduced serum and bone minerals (Ca, P) coupled with the decline of BMD in DEX-treated rats. This could be a consequence of alterations in Ca and P renal excretion **(Hozayen *et al*., 2016)** which may derive bone tissue toward enhanced resorptive activity. In this concern, several studies have been carried out to verify the benefits of natural vegetables and fruits for preventing bone loss **(Stacewicz-Sapuntzakis, 2013).**

The present research showed that treated with two levels of CH leaves powder and dried SP algae had a significant effect on serum ionized calcium and femur bone mineral content (calcium and phosphors). The best result was recorded for groups treated by 10% CH leaves powder and 6% dried SP algae (1.8±0.1, 8.49±.06, and 5.73±.05) and (1.63±.37, 8.30±.1, and 5.67±.25) respectively. Chicory administration significantly increases Ca absorption and results in a significant increase in bone mineral content, which leads to parathormone (PTH) suppression. **(Holloway *et al*., 2007).** This could be because chicory contains inulin, which improves Ca absorption in two ways.The first method involves increased calcium solubility in the colon as a result of decreased pH caused by inulin fermentation.Second, osmotic effects increase fluid transfer in the colonic lumen, increasing permeability between intracellular enterocyte junctions and thus facilitating diffusion.These findings are consistent with those of **Roberfroid *et al*., (2002)** which demonstrated that chicory inulin increases the absorption of calcium and increase the mineral parameters in the bones of the body. In addition, treatment with non-digestible fructans successfully increases Ca absorption and results in a corresponding increase in bone minerals which is followed by a suppression of PTH **(Holloway *et al*., 2007).**

Spirulina has been dubbed a powerful food because it is high in proteins, carbohydrates, polyunsaturated fatty acids, sterols, and other essential nutrients such as calcium, chromium, iron, zinc, magnesium, manganese, and selenium.It contains vitamin B12, vitamin E, ascorbic acid, tocopherols, and a wide range of natural mixed carotene and xanthophyll phytopigments (**Ojiakoi and Nwanjo, 2005).**According to **Ekantari *et al*., (2017)**, rats fed a vitamin D deficient diet and supplemented with spirulina (13 percent spirulina per weight of the diet for 8 weeks) had higher calcium, magnesium, phosphorus, and alkaline phosphatase levels in their serum than the untreated group.Because of the higher mineral absorption coefficient, the treated rats' femur length and diameter, as well as bone mineral density, were also increased compared to the untreated rats.Spirulina is known to contain phycocyanin and kaempferol, which can reduce inflammation, as well as chitosan, which can increase bone mineralization by regulating the associated genes in the mineralization process and calcium-binding proteins during the osteoblast cell differentiation process in Metabolic syndrome MSCs. (**Syarina *et al*., 2015)**

Table (4): Serum ionized calcium and femur bone mineral content (calcium and phosphors) of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Parameters	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder 5%	CH leaves powder 10%	Dried SP algae 4%	Dried SP algae 6%
Ca++(mg/dl)	1.75±.18[a]	1.20±.1[b]	1.5±0.1[ab]	1.8±0.1[a]	1.46±.25[ab]	1.63±.37[a]
	Femur bone mineral content					
Calcium (mg/g ash)	8.45±.13[a]	7.75±.05[d]	7.93±.04[c]	8.49±.06[a]	7.8±.05[cd]	8.30±.1[b]
phosphors (mg/g ash)	5.47±.15[a]	4.53±0.2[c]	5.1±.17[b]	5.73±.05[a]	4.87±.15[b]	5.67±.25[a]

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at $P \leq 0.05$.CH: *Cichorium intybus* SP: *Spirulina plantensis*

The activity of AST and ALT are indicators of hepatotoxicity **(Azab *et al*., 2013)**.Increased activity of serum liver enzymes (AST, ALT) may be due to the deterioration of the functional integrity of the liver cell membranes and the infiltration of these enzymes outside the cell **(Ibrahim *et al*., 2011)**. Alkaline phosphatase (ALP) is a biochemical indicator for the osteoid formation and mineralization **(Chapurlat and Confavreux, 2016).**

Results of liver enzymes for all groups are presented in Table (5). Osteoporotic animals plus fed on standard diet (control +ve) showed severe a significant ($P \leq 0.05$) elevation of serum AST, ALT and ALP levels till the end of the experiment (149.97±0.50, 70.10±0.53 and 180.83±0.60, respectively) compared with a healthy group (control -ve) (65.57±0.56، 32.88±0.92 and 84.14±0.99, respectively).This result agrees with the studies of **Tanyara *et al*., (2019) and Kamel *et al*., (2016).** Also, the present research, are in line with **Xu *et al*., (2012)**, they revealed that sedate initiated liver damage is for the most part brought about by the utilization of antibacterial and glucocorticoids and comprises around one-fifth of hospitalized patients with ALT **Drake *et al*., (2010)** It was also discovered that prenatal glucocorticoid overexposure in rodents increases hepatic lipid aggregation with steatosis.According to research, elevated levels of alkaline and acid phosphatases play a role in the pathogenesis of several bone diseases **(Millán and Whyte, 2016).** Elevated ALP levels may contribute to a faster bone turnover rate, which is characterised by an increase in bone formation and resorption, but bone resorption is more prevalent **(Feng *et al*., 2014).**

The present research showed that significant decrease in indices of Liver enzymes such as AST, ALT and ALP, (10% CH leaves powder, 6% dried SP algae, 5% CH leaves powder and 4% dried SP algae groups respectively when compared with the (control +ve) group. At the final, it could be observed that the best result for 10% CH leaves powder and 6% dried SP algae **(**65.72±0.46, 32.16±0.17 and 83.35±0.25) and (64.86±0.23, 32.25±0.45 and 82.95±0.18) respectively.

The discovery that Spirulina platensis reduced serum AST, ALT, and ALP activity is consistent with the findings of **Bhattacharyya and Mehta, (2012) Gargouri *et al*., (2016a, b)** and **Hozayen *et al*., (2016)**. This could imply that Spirulina protects against liver dysfunction and promotes tissue regeneration (**Gargouri *et al*., 2016b)**. According to **Gargouri *et al*., (2016b)** the liver protective effect of Spirulina may be related to the preservation of the cell's structural integrity, such as the prevention of oxidative DNA damage through the stabilisation of the plasma membrane and thus the repair of hepatic tissue damage.According to **Bhat and Madyastha (2001),** phycocyanin, a blue pigment found in the structure of Spirulina, plays a special protective role against DNA damage. According to **Nasef (2018)** Chicory leaves powder at concentrations of 2.5, 5, and 10% decreased serum AST, ALT, and ALP significantly (p0.05) when compared to the +ve group.Our findings are also consistent with those of **Balasubramanian *et al*., (2013), Helal *et al*., (2011) and Ahmed *et al*., (2009)** who found that chicory treatment reduced ALT and AST activities in rats.

This decrease could be attributed to the presence of isoflavones, polyphenols, and other antioxidants, which aided in the reduction of liver injury.

Table (5): Liver enzymes of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Parameters	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder		Dried SP algae	
			5%	10%	4%	6%
AST (U/I)	65.57± 0.56 d	149.97± 0.50a	92.44±0. 11b	65.72±0. 46d	83.14±0. 13c	64.86±0.2 3e
ALT (U/I)	32.88± 0.92d	70.10±0 .53a	44.55±0. 44b	32.16±0. 17d	39.48±0. 45c	32.25±0.4 5d
ALP(U/I)	84.14± 0.99de	180.83± 0.60a	95.55±0. 51b	83.35±0. 25d	94.52±0. 31c	82.95±0.1 8e

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at P ≤ 0.05. CH: *Cichorium intybus* SP: *Spirulina plantensis* AST: aspartate amino transferase ALT: alanine amino transfers ALP: alkaline phosphatase

Renal insufficiency or age-related loss of renal function is common in patients with osteoporosis **(Cesini *et al.*, 2012).** Patients with chronic kidney disease (CKD) generally have low BMD due to osteoporosis, which puts them at an increased risk of fracture **(Lydiaa *et al.*, 2015).** Results of kidney function for all groups are presented in Table (6). Osteoporotic animals plus fed on standard diet (control +ve) showed significant (P≤ 0.05) increase of serum urea, uric acid and creatinine levels till the end of the experiment (67.00±1.00, 1.89±0.33and 1.06±0.19) respectively compared with a healthy group (control -ve) (37.66±2.05, 0.99±0.22 and 0.48±0.03) respectively**.** The kidney plays an important role in GC metabolism and is the primary source of cortisol production in humans.Reduced 11b-HSD2 activity contributes to the pathophysiology of salt and water retention, as well as hypertension in renal failure.Glucocorticoids have a significant impact on renal function, including an increase in glomerular filtration rate **(Mangos *et al.*, 2003).**

Our results showed that supplemented diet by two levels of CH leaves powder and dried SP algae had a significant effect on serum urea, uric acid and creatinine, (10% CH leaves powder, 6% dried SP algae, 5% CH leaves powder and 4% dried SP algae groups respectively when compared with the (control +ve) group. At the final, it could be observed that the best result for 10% CH leaves powder and 6% dried SP algae (37.33±2.50, 1.03±0.31and 0.46±0.05) and (37.66±2.00, 1.10±0.08 and 0.45±0.3) respectively. Chicory, according to **Wang *et al*., (2019)**, slows renal reabsorption by controlling the expression of urate transporters in fructose-induced hyperuricemia.These findings are supported by **Jin *et al*., (2018)** who investigated the effects of chicory on serum kidney functions and in vitro cell verification and found that chicory extract reduces urea, uric acid, and creatinine elevations in hyperuricaemic rats with kidney damage. Also, **Lin *et al*., (2014)** reported that *Cichorium intybus* could reduce serum uric acid levels and inhibit liver xanthine dehydrogenase and xanthine oxidase.

Furthermore, in a study conducted by **Martin and Sabina (2016)**, spirulina reduced the effect of anti-tuberculosis drugs (isoniazid and rifampicin) on changes in the kidneys of Wister rats.Spirulina (400 and 800 mg/kg/day) treatment reversed kidney injury by lowering serum creatinine, urea, uric acid, and acid phosphatase levels.In addition, **Balasubramanian *et al*., (2013)** discovered that spirulina significantly reduced elevated creatinine and urea levels.It's possible that spirulina, which has antioxidant properties, improved renal function by slowing the oxidative stress-induced decline in kidney function.

Table (6): Kidney function (mg/dl) of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Parameters	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder		Dried SP algae	
			5%	10%	4%	6%
Urea(mg/dl)	37.66±2.05 b	67.00±1.00a	41.66±2.04b	37.33±2.50b	40.00±2.00b	37.66±2.00b
Uric Acid (mg/dl)	0.99±0.22a	1.89±0.33b	1.14±0.07a	1.03±0.31a	1.19±0.18a	1.10±0.08a
Creatinine(mg/dl)	0.48±0.03b	1.06±0.19a	0.59±0.02b	0.46±0.05b	0.52±0.02b	0.45±0.3b

Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at $P \leq 0.05$.CH: *Cichorium intybus* SP: *Spirulina plantensis*

3.3. Bone mineral density and bone mineral content assay

BMD is an indicator for the detection of osteoporosis; this parameter gives knowledge concerning the quantity of minerals in bone, which is only one component of bone strength **(Friedman, 2006).** In the present study, the BMD and BMC were significantly decreased in osteoporotic animals plus fed on standard diet (control +ve) (0.04±0.01 and 0.079±0.04, respectively) compared with the healthy group (control -ve) (0.153±.003 and 0.159±0.04, respectively) as showed in Table (7). Research has shown that calcium failure can reduce BMD and weaken bone strength and long-term calcium deficiency can lead to osteoporosis **(Engstrom *et al*., 2012).**

Our results showed that supplemented diet by two levels of CH leaves powder and dried SP algae had a significant increase in BMD and BMC , (10% CH leaves powder, 6% dried SP algae, 5% CH leaves powder and 4% dried SP algae groups respectively when compared with the (control +ve) group. At the final, it could be observed that the best result for 10% CH leaves powder and 6% dried SP algae (0.152±0.03and 0.145±0.02) and (0.146±0.01and

0.135±0.03) respectively.BMD of the distal femur and proximal tibia in rats, which are rich in trabecular bone, was in fact increased by the spirulina treatment. One of the reasons for this appears to be spirulina's high content of minerals. **Ishiml *et al*., (2006)** proposed that spirulina consumption reduced BMD in rodent trabecular bone under estrogen-deficient conditions.

Chicory leaves are high in anthocyanins, vitamins A and C, as well as calcium, phosphorus, and inulin-type fructans, which improve bone health by increasing bone mineral content (BMC) and bone mineral density (BMD) (**Weisstaub *et al*., 2013).** Previous research found that chicory supplementation improves bone mineral content (BMC), increases Ca absorption, and alleviates the reduction in bone mineral density (BMD) and mineral content that occurs after ovariectomy or gastrectomy in rats **(Hozayen *et al*., 2016)**

Table (7): femur mineral density (BMD) and bone mineral content (BMC) assay of experimental rats which treated with two levels of CH leaves powder and dried SP algae

Parameters	Groups					
	Control (-ve)	Control (+ve)	CH leaves powder		Dried SP algae	
			5%	10%	4%	6%
BMD (g/cm^2)	0.153±.003a	0.04±0.01c	0.0867±0.005b	0.152±0.03a	0.084±0.005b	0.146±0.01a
BMC (g)	0.159±0.04a	0.079±0.04e	0.127±0.02c	0.145±0.02ab	0.114±0.02d	0.135±0.03b

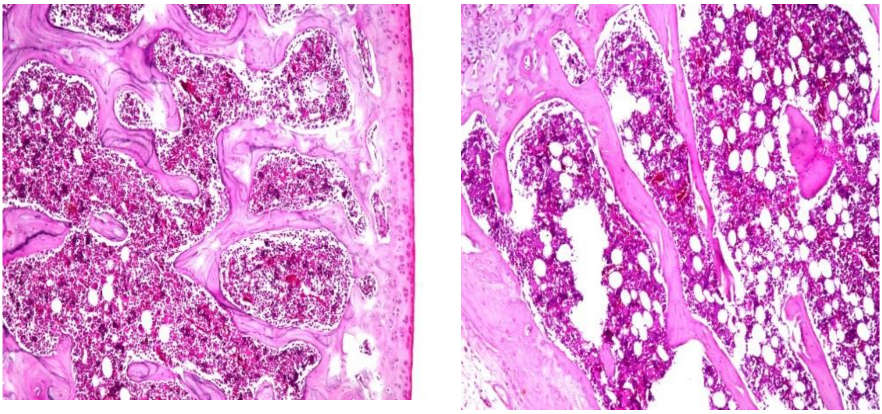
Each value represents the mean value± SD. Mean's value in the same column sharing the different superscript letters are significant different at P ≤ 0.05. CH: *Cichorium intybus* SP: *Spirulina plantensis* BMD: bone mineral density, BMC: bone mineral concentration.

3.3. Histopathological examination and X-ray

Femur bone was examined by X-ray, a histological approach and the photomicrographs of hematolxylin – eosin-stained femur is illustrated in Figures **(3 to 6)**.

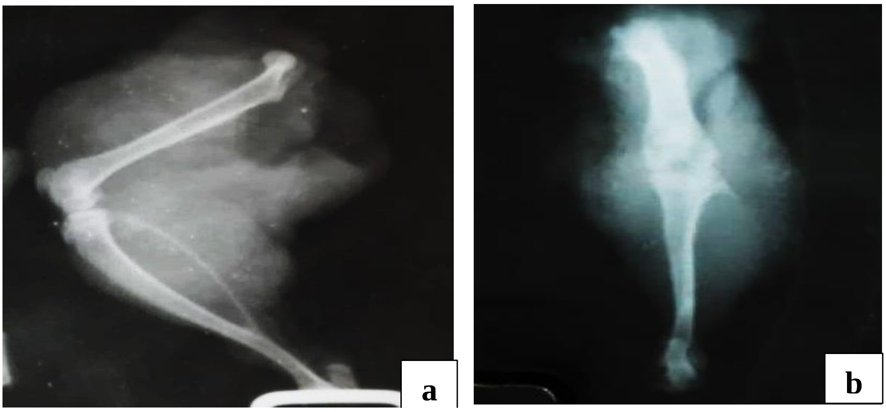
Femur bone

Microscopically, the left femur of rat from (control –ve) group showed the normal histological structure of the thickness and length of the trabecular with normal wide bone marrow spaces in between were recorded in (Fig.3). Contrary, the osteoporotic group (control +ve) severe osteoporosis was detected in the trabeculae as diminish in the thickness and length with the wide area of bone marrow spaces in between were recorded in (Fig.3). Trabecular bone constitutes a major portion of the proximal femur, and it is highly sensitive to glucocorticoids **(Laan *et al*., 1993).**Our radiographic results confirmed these histopathological results as, lateral radiographic view of tibia, fibula and the distal extremity of femur showing normal radiographic findings and architecture (a) and anterior-posterior view of the tibia and distal extremity of the femur showing normal radiographic findings and architecture (b) in (control –ve) group were recorded in (Fig.4).Furthermore**,**lateral radiographic view showing demineralization and thinning of fibula and bony loss of tibia (arrow) and radiolucent area of femur (arrow) (a) and anterior posterior view showing demineralization and thinning of femur, tibia and fibula (b) in (control +ve) group (Fig.4). glucocorticoids (GC) administration for three weeks revealed bone loss of different part such as fibula, tibia and femur in addition to bone demineralization and thinning of femoral cortex in rats, disappear of cortex of tibia in others and fibula and these agree with the observations of **Hallberg *et al.*, (2009)and Sipos *et al*., (2015).** Bone health assessment, especially at the femur, is important for decision making in osteoarthritic patients. Osteoarthritis (OA) which affects the hips is the most common form of arthritis (Breedveld, 2004).

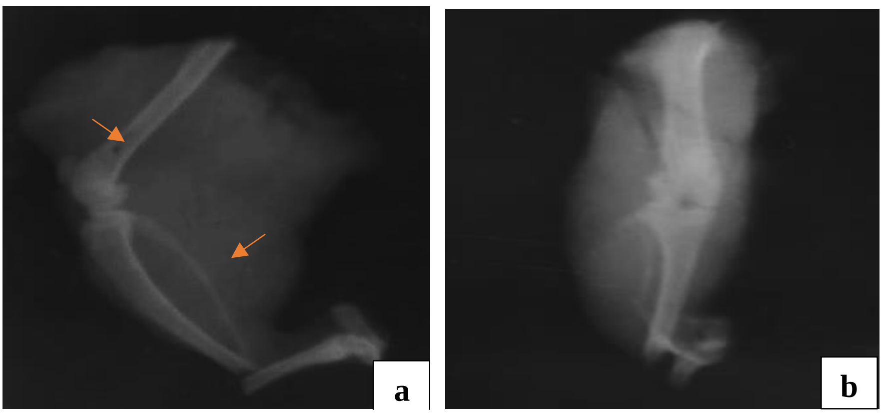


Control (-ve) Control (+ve)

Fig. 3. Photomicrograph of femur sections in (control -ve) and control +ve) groups (H&E), (control –ve) group revealed the normal histological structure of the thickness and length of the trabecular, the osteoporotic group (control +ve) Sever osteoporosis in the trabeculae as diminish in the thickness and length with a wide area of bone marrow spaces in between



Control -ve

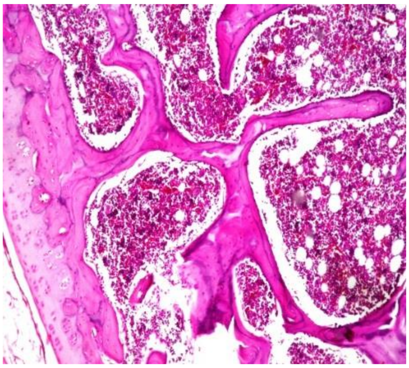


Control +ve

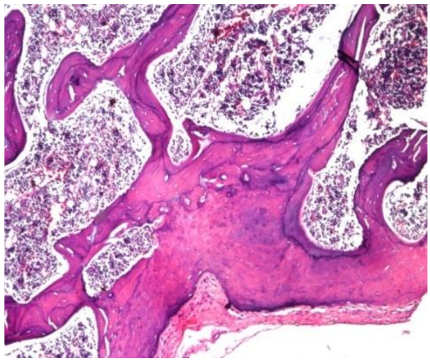
Fig. 4. Radiographic of lateral and anterior-posterior in (control -ve) and control +ve) groups

Femur bone of animals affected by DEX and fed on (5% CH leaves powder) mild form osteoporosis was observed in the bone trabeculae as recorded in (Fig.5). In contrast, no histopathological alteration in femur bone was observed in the animals affected by DEX and fed on (10% CH leaves powder) in (Fig. 5).Our radiographic results confirmed these histopathological results as, lateral radiographic view showing moderate demineralization and thinning of fibula and bony loss of tibia (arrow) (a) and anterior posterior view showing demineralization and thinning of femur, tibia and fibula (b) in (5% CH leaves powder) group were recorded in (Fig.6). Furthermore**,** lateral radiographic view showing normal femur, tibia and fibula (a) and anterior-posterior view showing normal femur, tibia and fibula (b) in (10% CH leaves powder) group (Fig.6). Researchers have shown that the phenolic compounds in various plant species can modulate the functions of the osteoblastic cells, including their maturation and proliferative capacity, by increasing the activity of alkaline phosphatase and calcium deposition in the extracellular matrix **(Hagiwara *et al*., 2011)** 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 **(Rasheed *et al*., 2009).**

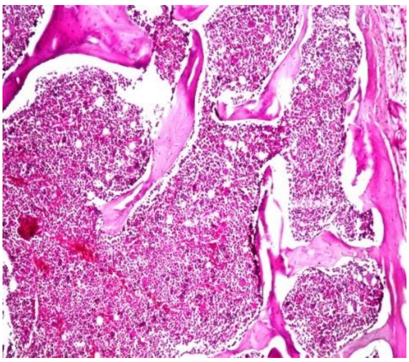
Femur bone of animals affected by DEX and fed on (4% dried SP algae) showed diminished in the thickness and length of the bone trabeculae with wide areas of bone marrow spaces in between as recorded in (Fig.5). In contrast, no histopathological alteration in femur bone was observed in the animals affected by DEX and fed on (6% dried SP algae) in (Fig. 5). Our radiographic results confirmed these histopathological results as, lateral radiographic view showing demineralization and thinning of fibula and bony loss and irregular surface of tibia (arrow) (a) and anterior-posterior view showing demineralization and thinning of femur, tibia and fibula (b) in (4% dried SP algae) group were recorded in (Fig.6). Furthermore**,** lateral radiographic view showing normal femur, tibia and fibula (a) and anterior posterior view showing normal femur, tibia and fibula (b) in (6% dried SP algae) group (Fig.4). Osteoporosis is one of the most common disorders associated with aging and results in bone fracture, this being a substantial public health problem. Specific marine plants have recently attracted attention for their ability to improve bone metabolism, since they are rich in minerals and growth factors. **(Hidaka *et al*., 2004)**. An epidemiological study in Taiwan has shown a seaweed diet to be a significant variable in a multiple linear regression analysis of BMD **(Shaw, 1993)**. **Devesh *et al*., (2012)** and **Gupta *et al*., (2010)** reported an increase in trabecular bone thickness as well as a reduced number of resorptive pits in the femur of diabetic animals treated with spirulina (500 mg/kg/day for 21 days).



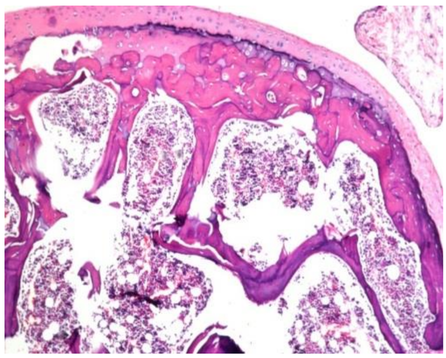
5% CH leaves powder group showed mild form osteoporosis



10% CH leaves powder group showed no histopathological alteration in femur bone



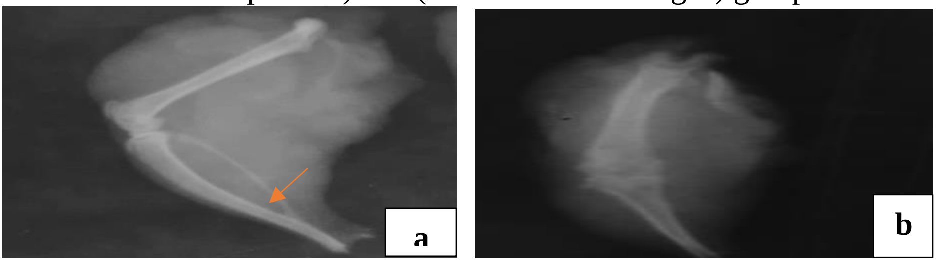
4% dried SP algae group showed diminished in the thickness and length of



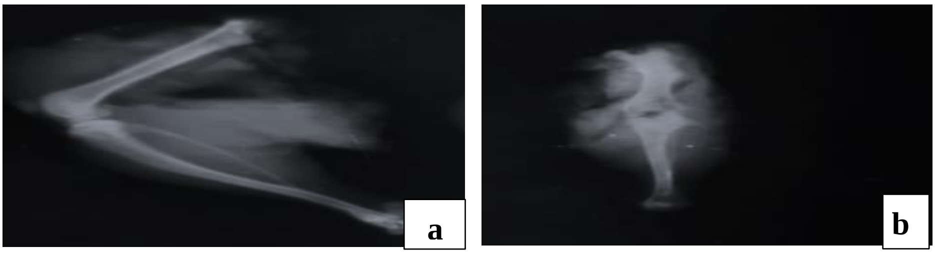
6% dried SP algae group showed no histopathological alteration in femur bone

Fig. 5. Photomicrograph of Femur bone in (5 &10% CH leaves powder) and (4 & 6% dried SP algae) groups (H&E).

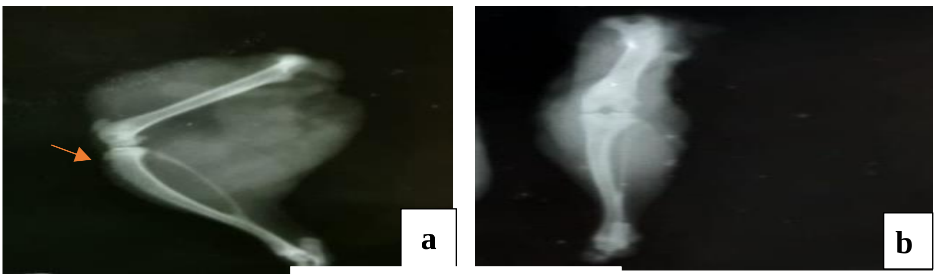
Fig. 6. Radiographic of lateral and anterior posterior in (5 & 10% CH leaves powder) and (4 & 6% dried SP algae) groups



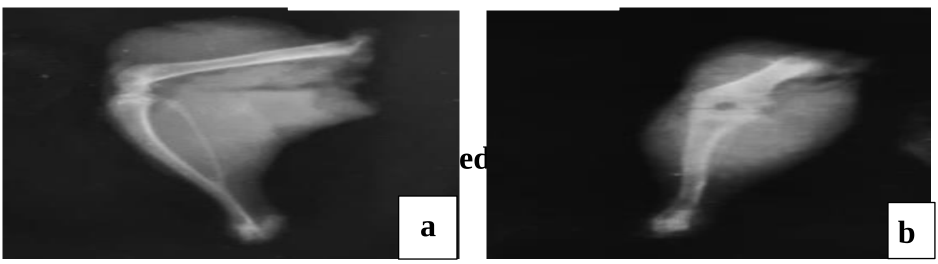
5% CH leaves



10% CH leaves



4% dried SP algae



6% dried SP algae

CONCLUSION

The current study found that supplementing the diet with two levels of CH leaves powder and dried SP algae increases bone mineral density (BMD and BMC), serum and bone minerals (Ca and p), and improves bone histological architecture.This may occur via similar intracellular pathways involving the suppression of oxidative stress and the enhancement of antioxidant defence mechanisms, implying that chicory and Spirulina represent a promising therapeutic option for the prevention of Glucocorticoid (dexamethasone)-induced osteoporosis, and it is consumed as a functional food and safe nutritional supplement.

Referances

Abbas ZK, Saggu S, Sakeran MI, Zidan N, Rehman H, Ansari AA. (2015).Phytochemical, antioxidant and mineral composition of hydroalcoholic extract of chicory (*Cichorium intybus L.*) leaves. Saudi J Biol Sci.; 22:3:322–6.

Abdel-Daim MM. (2014). Pharmacodynamic interaction of *Spirulina platensis* with erythromycin in Egyptian Baladi bucks (*Capra hircus*).Small RuminRes. 120:234–241.

Ahmed L, Ramadan R and Mohamed R (2009). Biochemical and histopathological studies on the water extracts of marjoram and chicory herbs and their mixture in obese rats. Pakistan Journal of Nutrition, 8 (10): 1581-1587

Alwan N A., (2009). Effect of *chicorium Intybus* Extract on Histological changes in Liver and Kidney of Hypercholestrolemic Rabbits. Bas.J.Vet.Res.Vol.8, No.1.

AOAC (2015): Official Methods of Analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5.

Azab, S., Abdel-Daim, M., & Eldahshan, O., (2013). Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of *Delonix regia* leaves extract. Med Chem Res, 22, 4269-4277. http://dx.doi.org/10.1007/s00044-012-0420-4

Azzini, E., Maiani, G., Garaguso, I., Polito, A., Foddai, M. S., & Venneria, E. (2018). The potential health benefits of polyphenol-rich extracts from *Cichorium intybus* L. Studied on Caco-2 cells model. Oxidative Medicine and Cellular Longevity, (9). doi:10.1155/2016/1594616

Bagi, C.M.; Berryman, E. and Moalli, M.R. (2011). Comparative bone anatomy of commonly used laboratory animals: implications for drug discovery. Comp Med 61:76–85

Balasubramanian, B. Swetha, M D.Gayasuddin, Sumayya Samreen. (2013). Antibody activity of *Spirulina Platensis* in high fat diet induced rats .Vol 3; Issue (1)1-5.

Banchroft, J.D.; Sevens, A. and Turner, D.R. (1996): Theory and practice of histological techniques. Fourth ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.

Baofeng L, Zhi Y, Bei C, Guolin M, Qingshui Y, Jian L.,(2010).Characterization of a rabbit osteoporosis model induced by ovariectomy and glucocorticoid. Acta Orthop; 81: 396-401.

Barham, D. and Trinder, P. (1972). An improved col-our reagent for the determination of blood glucose by the oxidase system. Analyst, 97, 142-145.

Belfield A. and Goldberg D.M. (1971), Enzyme .12, 561.
Bhat. VB. & Madyastha. KM. (2001). Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. Biochem Biophys Res Commun, 285(2) (2001(262-266, https://doi.org/10.1006/bbrc.2001.5195
Bhattacharyya, S., & Mehta, P. (2012). The hepatoprotective potential of Spirulina and vitamin C supplementation in cisplatin toxicity. Food Funct, 3, 164-169. http://dx.doi.org/10.1039/c1fo10172b
Bordag N, Klie S, Jürchott K, Vierheller J, Schiewe H, Albrecht V, Tonn J.C, Schwartz C, Schichor C and Selbig J. (2015). Glucocorticoid (dexamethasone)-induced metabolome changes in healthy males suggest prediction of response and side effects. Scientific reports; 5: 15954-15966
Canalis, E.; Bilezikian, J.P.; Angeli, A. and Giustina, A. (2004).Perspectives on glucocorticoid-induced osteoporosis. Bone, 34: 593-598
Canalis, E.; Mazziotti, G.; Giustina, A. and Bilezikian, J.P. (2007). Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int. 2007; 18 (10): 1319–1328.
Carcea, M.; Sorto, M.; Batello, C.; Narducci, V.; Aguzzi, A.; Azzini, E. and Turfani, V. (2015). Nutritional characterization of traditional and improved dihé, alimentary blue-green algae from the Lake Chad region in Africa. LWT-Food Science and Technology, 62(1), 753-763.
Cesini, J.; Cheriet, S.; Breuil, V. and Lafage-Proust, M.H. (2012). Osteoporosis: chronic kidney disease in rheumatology practice. Joint Bone Spine 79: S104–S109.
Chapman, D.G.; Gastilla, R. and Campbell, J.A. (1959). Evaluation of protein in food. I.A. Method for the determination of protein efficiency ratio. Can J Biochem Physiol., 37:679-686.
Chapurlat RD and Confavreux CB. (2016). Novel biological markers of bone: from bone metabolism to bone physiology. Rheumatology 2016; 55: 1714-1725.
Da Silva, V. B., Barreira, J. C. M., & Oliveira, M. B. P. P. (2016). Natural phytochemicals and probiotics as786 bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery787 technologies. Trends in Food Science & Technology, 50, 144–158.788 https://doi.org/10.1016/j.tifs.2015.12.00
Devesh, C., Kritika, M., Anroop, N., Kumar, S.P. & Sumeet, G. (2012). Spirulina reverses histomorphological changes in

diabetic osteoporosis in pioglitazone treated rats. Journal of Diabetes Metabolism 1: 1-7.
Dobrowolski P, Tomaszewska E, Muszyński S, Blicharski T and Pierzynowski SG., (2017). Dietary 2-oxoglutarate prevents bone loss caused by neonatal treatment with maximal dexamethasone dose.Experimental Biology and Medicine; 242: 671-682.
Drake, A.J., P.J. Raubenheimer, D. Kerrigan, K.J. McInnes, J.R. Seckl and B.R. Walker, (2010). Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet. Endocrinology, 151(4): 1581-7.
Ekantari, N., Harmayani, E., Pranoto, Y. & Marsano, Y. (2017). Calcium of Spirulina paltensis has higher bioavailability than those of calcium carbonate and high-calcium milk in Sprague Dawley rats fed with vitamin D-deficient diet. Pakistan Journal Nutrition 16: 179-186.
Engstrom A, Michaelsson K, Vahter M, Julin B, Wolk A, and Akesson A, (2012). Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women," Bone, 50(6): 1372–1378.
Fawcett, J.K and Soctt, J.E., (1960).J, Clin. Path. 13: 156 – 159
Feng M, Zhang R, Gong F, Yang P, Fan L, Ni J, Bi W, Zhang Y, Wang C, Wang K. (2014).Protective effects of necrostatin-1 on glucocorticoid-induced osteoporosis in rats. J Steroid Biochem. Mol., Biol; 144B: 455-462.
Farrow CS. (2003). Veterinary diagnostic: imaging the dog and cat, vol. 1. Mosby Inc.; an Affiliate of Elsevier Science
Fraser L-A and Adachi JD., (2009).Glucocorticoid-induced osteoporosis: treatment update and review. Therapeutic advance sinmusculo skeletal disease; 1: 71-85
Friedman AW (2006). Important determinants of bone strength: beyond bone mineral density. J Clin Rheumatol 12: 70-7.
Gargouri. M., Ben Saad. H., Ben Amara. I., Magné.C. & El Feki. A. (2016b). Spirulina exhibits hepatoprotective effects against lead induced oxidative injury in newborn rats. Cell Mol Biol, 62(10).85-93,https://doi.org/10.14715/cmb/2016.62.10.14
Gargouri. M., Magné. C. & El Feki. A,. (2016a). Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation. Nutr Res, 36(11), 1255-1268, https://doi.org/10.1016/j.nutres.2016.09.011
Grandfield, K.; Vuong, V. and Schwarcz, H. (2018): Ultrastructure of bone: hierarchical features from nanometer to micrometer

scale revealed in focused ion beam sections in the TEM. Calcif Tissue Int 103:606–616.

Gupta, S., Hrishikeshvan, H.J. & Sehajpal, P.P. (2010). Spirulina protects against Rosiglitazone induced osteoporosis in insulin resistance rats. Diabetes Research and Clinical Practice 87(1): 38-43

Hagiwara K., Goto T., Araki M., Miyazaki H., and Hagiwara H., (2011). Olive polyphenol hydroxytyrosol prevents bone loss. European Journal of Pharmacology, 662:78–84. doi:10.1016/j.ejphar.04.023.

Hamid M. Ziena, Soad T. Kheir and Basma R. Ghazie, (2021). Chicory Leave Powder as a Functional Ingredient: Chemical Composition, Bioactive Compounds and Antioxidant Activity.Alexandria Science Exchange Journal, Vol.42, No.4.936-940. Doi: 10.21608/asejaiqjsae.

Hallberg, I.; Bachrach-Lindström, M.; Hammerby, S.; Toss, G. and Ek, A.C. (2009). Health-related quality of life after vertebral or hip fracture: a seven-year follow-up study. BMC Musculoskelet. Disord. 10: 135.

Hassan, H. A., & Yousef, M. I. (2010). Ameliorating effect of chicory (*Cichorium intybus L*.) supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats. Food and Chemical Toxicology, 48(8-9), 2163-2169. doi.org/10.1016/j.fct.2010.05.023

Helal, G.E.; Samia M. Abd El-Wahab, Atef M.Moussa Sharaf and Ghada A. Zedan. (2011). Effect of *Cichorium intybus L*. on fatty liver induced by oxytetracycline in albino rats. The Egyptian Journal of Hospital Medicine (Oct. 2011) Vol., 45: 522 – 535

Hidaka, S., Okamoto, Y., and Arita, M., A. (2004). Hot water extract of *Chlorella pyrenoidosa* reduces body weight and serum lipids in ovariectomized rats. Phytother. Res., 18, 164–168 (2004).

Holloway L., Moynihan S., Abrams S.A., Kent K., Hsu A.R., Friedlander A.L., (2007). Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. British Journal of Nutrition, 97 Suppl 02:365–72.

Holman BW, Malau-Aduli AE (2012). Spirulina as a livestock supplement and animal feed. J. Anim. Physiol. Anim. Nutr. 97:615-623https://doi.org/10.1111/j.14390396.2012.01328.

Hozayen W. G., El-Desouky M. A., Soliman H.A., Ahmed R. R. and Amal K. Khaliefa A. K, (2016).Antiosteoporotic effect of *Petroselinum crispum*, *Ocimum basilicum*, and *Cichorium* *intybus L*. in glucocorticoid-induced osteoporosis in rats. BMC Complementary and Alternative Medicine, 16:165

Ibrahim. M., Khaja. ZU. & Narasu. ML. (2011). Hepatoprotective activity of *Boswellia serrate* extracts: In vitro and in vivo studies. Int J Pharm Appl, 2(1), 89-98

Ishimi, Y. Fumie S, Junko E, Maiko F, and Jian WU. (2006). Effects of Spirulina, a Blue-Green Alga, on Bone Metabolism in Ovariectomized Rats and Hindlimb-Unloaded Mice. Biosci. Biotechnol. Biochem. 70 (2), 363–368. https://doi.org/10.1271/bbb.70.363

Ito, S.; Suzuki, N.; Kato, S.; Takahashi, T. and Takagi, M. (2007). Glucocorticoids induce the differentiation of a mesenchymal progenitor cell line, ROB-C26 into adipocytes and osteoblasts, but fail to induce terminal osteoblast differentiation. Bone, 40: 84-92.

Jancic, D., Todorovic, V., Sircelj, H., Dodevska, M., Beljkas, B., Znidarcic, D., & Sobajic, S. (2017). Biologically active compounds and antioxidant capacity of *Cichorium intybus L*. Leaves from.Montenegro. Italian Journal of Food Science, 29(4). Doi: 10.14674/IJFS-789

Jin YN, Lin ZJ, Zhang B and Bai YF (2018). Effects of chicory on serum uric acid, renal function, and glut9expression in hyperuricaemic rats with renal injury and in vitro verification with cells. Evidence-Based Complemen.Alternative Med.

Kamel AM, Tawfeeq RK, Husin SY, Ahmed SJ, Abdal Al-Kareem M.M. (2016). Lipid profile of Hyperlipidemic mice induced by dexamethasone treated with 105 herbals oil mixture. Diyala Journal of Medicine. 11(1): 24-31.

Keshk, W. A., Soliman, N. A., Ali, D. A., & Elseady, W. S.(2019).Mechanistic evaluation of AMPK/SIRT1/FXR signaling axis, inflammation and redox status in thioacetamide-induced liver cirrhosis: The role of *Cichorium intybus linn* (chicory)-supplemented diet. Journal of Food Biochemistry, 43(8), e12938. https://pubmed.ncbi.nlm.nih.gov/31368578/

Koletzko, B.; Baker, S.; Cleghorn, G.; Neto, U. F.; Gopalan, S. and Hernall, O., (2005): Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. Journal of Paediatric Gastroenterology and Nutrition, 41, 584–599.

Kulshreshtha A, Zacharia AJ, Jarouliya U, Bhadauriya P, Prasad GB, Bisen PS (2008). Spirulina in health care management. Curr. Pharmaceut. Biotechnol. 9:400-405. https://doi.org/10.2174/138920108785915111

Laan RF, Buijs WC, van Erning LJ, Lemmens JA, Corstens FH, Ruijs SH, van de Putte LB, van Riel PL(1993).Differential effects of glucocorticoids on cortical appendicular and cortical vertebral bone mineral content. Calcif Tissue Int; 52: 5-9.

Li G, Bu J, Zhu Y, Xiao X, Liang Z and Zhang R., (2015).Curcumin improves bone microarchitecture in glucocorticoid-induced secondary osteoporosis mice through the activation of microRNA-365 via regulating MMP-9. International journal of clinical and experimental pathology; 8:15684-15695.

Liestianty, D.; Rodianawati, I.; Arfah, R. A. and Assa, A. (2019): Nutritional analysis of *spirulina sp* to promote as superfood candidate. In IOP Conference Series: Materials Science and Engineering, 509, (1), 012031). IOP Publishing

Lin Z, Zhang B, Liu X, Jin R, Zhu W. (2014). The high and low dosage of chicory inulin also decreased serum UA levels on days7, 14, and28.JournalofMedicinal Food .17(11):1214-21

Liu, X.Y.; Shi, J.H.; Du, W.H.; Fan, Y.P.; Hu, X.L.; Zhang, C.C. and Xiang, P. (2011): Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats. Exp. Ther. Med., 2(5): 977-84.

Ljubic, A.; Safafar, H.; Holdt, S. L. and Jacobsen, C. (2018): Biomass composition of *Arthrospira platensis* during cultivation on industrial process water and harvesting. Journal of Applied Phycology. 30(2), 943–954.

Lydiaa, M.; Kumar. S.; Selvakumara, I. and Kumar, E. (2015): Wind resource estimation using wind speed and power curve models. Renewable Energy. Volume 83, Pages 425-434.

Mada SB, Abarshi MM, Garba A, Sharehu KL, Elaigwu OP, Umar MJ,, (2019).Hypolipidemic effect of N-acetylcysteine against dexamethasone-induced hyperlipidemia in rats. Calabar J HealthSci.; 3(2):59-67.DOI10.25259/CJHS_13_2019

Mangos, G.J, Judith A W, Paula M W and John J K., (2003): Glucocorticoids and the kidney. Nehrology 2003; 8, 267–273.

Martin, S.J. & Sabina, E.P. (2016). Amelioration of antituberculosis drug induced oxidative stress in kidneys by *Spirulina fusiformis* in a rat model. Renal Failure 38(7): 1115-1121

Millán JL and Whyte MP. (2016). Alkaline phosphatase and hypophosphatasia.
Calcified tissue international; 98: 398-416.

Nasef, A. N. (2018). Study The Effect Of Chicory (*Cichorium Intybus, L.*) Leaves Powder on Some Biochemical and Biological Parameters of Hepatotoxicity Induced By Ccl4 in

Rats. Journal of Home Economics, Volume 28, December (4) ISSN 1110-2578

Nuti R, Brandi ML, Checchia G, Di Munno O, Dominguez L, FalaschiP,Fiore CE, Iolascon G, Maggi S and Michieli R: (2019).Guidelines for the management of osteoporosis and fragilityfractures.Internal and emergencymedicine; 14:85-102.

Nwafor, I.Ch. K. Shale and M.Ch. Achilonu. (2017). Chemical Composition and Nutritive Benefits of Chicory) *Cichorium intybus*) as an Ideal Complementary and/or Alternative Livestock Feed Supplement. Hindawi: 1-12

Ojiakoi OA, Nwanjo HU., (2005). Effects of pioglitazone on atherogenic risk predictor indices of alloxan-induced diabetic rabbits. Biokemistri 17: 179-184.

Perović, J., V.T. Šaponjac, J. Kojić, J. Krulj, D.A. Moreno, C. García-Viguera, M. Bodroža-Solarov and N.Ilić.(2021). Chicory (*Cichorium intybus L*.) as a food ingredient – Nutritional composition, bioactivity, safety, and health claims: A review. Food Chemistry.336:1-15

Poormoosavi S.; M., Behmanesh M.A., and Najafzadehvarzi H., (2019). Effects of Spirulina platensis on the Improvement of Hepatorenal Toxicity Induced by Iron Oxide in Wistar Rats Using Biochemical and Histological Methods. Shiraz E-Med J. 20(8):e85382. Doi: 10.5812/semj.85382.

Rajput R, Wairkar S and Gaud R., (2018). Nutraceuticals for better management of osteoporosis: An overview. Journal of functional foods; 47: 480-490.

Rasheed W.I., Oraby F.S., Hussein J.S., (2009). Therapeutic efficacy of garlic oil with 1, 25 dihydroxy Vit D and calcium in osteoporotic ovariectomized rats. Australian Journal of Basic and Applied Sciences, 3 Suppl 2:977–81

Reeves, P.G.; Nielsen, F.H. and Fahey, G.C. (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 123(11):1939-51.

Reid IR, Evans MC, Wattie DJ, Ames R, Cundy TF. (1992).Bone mineral density of the proximal femur and lumbar spine in glucocorticoid-treated asthmatic patients. OsteoporosInt; 2: 103-105.

Reitman, S. and Frankel, S. (1975). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Path, 28:65.

Richmond A., (2004).Biological Principles of Mass Cultivation. (A. Richmon editor.(Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Blackwell Science Ltd. Oxford/UK, (2004) 125-177

Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, (2010).Prebiotic effects: metabolic and health benefits. Br J Nutr. 104(Suppl S2):S1–63

Roberfroid M. B., Cumps J., and Devogelaer J. P,. (2002). Dietary chicory inulin increases whole-body bone mineral density in growing male rats. Journal of Nutrition, 132: 3599–3602.

Rodriguez-Concepcion, M.; Avalos, J.; Bonet, M. L.; Boronat, A.; Gomez-Gomez, L.; Hornero-Mendez, D. and Zhu, C. (2018).A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Progress in LipidResearch, 70: 62–93.

Seshadri, C.V. and Jeeji Bai, N. (1992). Spirulina National Symposium. Shri Amm., Murugappa Chettiar Research Center (MDRC), Madras, INDIA (1992).

Sipos, R.S.; Fechete, R.; Moldovan, D.; Sus, I.; Szasz, S. and Pávai, Z. (2015). Assessment of femoral bone osteoclastogenesis by inhibition of the signalosome-associated I kappaB kinase. J. Cancer Res. Clin. Oncol. 135 (2): 173-9.

Shaw, C. K., (1993). An epidemiologic study of osteoporosis in Taiwan. Ann. Epidemiol., 3, 264–271.

Siddiqui RA., (2017). Plums as Potential Dietary Agents to Prevent Obesity and Obesity-Related Disorders. Journal of Obesity and Therapeutics; 1:1

Snedecor, G.W., Cochran, W.G., (1980).Statistical Methods, 7 Th. IBIT Public. Co, Oxford

Stacewicz-Sapuntzakis M., (2013). Dried plums and their products: composition and health effects–an updated review. Critical reviews in food science and nutrition 2013; 53: 1277-1302

Syarina PN, Karthivashan G, Abas F, Arulselvan P, Fakurazi S. (2015). Wound healing potential of *Spirulina platensis* extracts on human dermal fibroblast cells. EXCLI J; 14:385 93.

Tanyara BP, Teixeira CJ, Sato FT, Murata GM, Zonta GA, Sodré FS, Campos CV, Mesquita FN, Anhê GF, Bordin S. (2019).In Utero Dexamethasone Exposure Exacerbates Hepatic Steatosis in Rats That Consume Fructose During Adulthood. Nutrients; 11, 2114; doi: 10.3390/nu11092114.

Thakur RS, Toppo F, Singour P, Chaurasiya P and Pawar RS. (2013). Preclinical studies of various extracts of Polyalthia longifolia for the management of dexamethasone induced osteoporosis in rats. International Journal of Pharmacy and Pharmaceutical Sciences; 5: 267-270.

Tietz, N.W. (1986). Textbook of clinical chemistry WB. Saunders, Philadelphia, pp 1271-1281.

Urias-Silvas, J.E., Cani, P.D., Delmee, E., Neyrinck, A., Lopez, M.G. and Delzenne, M.N, (2007). Physiological effects of dietary fructans extracted from Agaves tequilana and Dasylirion spp. Br. J. Nutr., 99, 254-261.

Vandewalle J, Luypaert A, De Bosscher K and Libert C., (2018). Therapeutic
mechanisms of glucocorticoids. Trends in Endocrinology & Metabolism; 29: 42-54.

Wang Y, Lin Z, Zhang B, Wang X and Chu M (2019). Chicory (Cichorium intybus L.) inhibits renal reabsorption by regulating expression of urate transporters in fructose-induced hyperuricemia. J.Traditional Chinese Med. Sci., 6(1): 84-94.

Weinstein, R.S. (2011). Clinical practice. Glucocorticoid-induced bone disease. N Engl J Med. 2011; 365 (1): 62–70.

Weisstaub, A.R., Abdala, V. and Gonzales, C.M., (2013). Polydextrose Enhances Calcium Absorption and Bone Retention in Ovariectomized Rats. Int J Food Sci 8.

Xu, H.M.; Chen, Y.; Xu, J. and Zhou, Q. (2012). Drug-induced liver injury in hospitalized patients with notably elevated alanine aminotransferase. World J. Gastroenterol. WJG. 18(41): 5972- 5994

Yang, L.; Wu, J.; Ho, L.; Yang, G. S.; Hung, Y. and Lin, W. (2008). Effect of poly-(α glutamic acid on calcium absorption in rats. Biosci. Biotechonol. Biochem. 72:3084-3090.

Young, D.S. (1990).Effects of drugs on clinical laboratory tests. Third edition 1990:3:6-12.

Zeller, M. A., Hunt, R., Jones, A., and Sharma, S. 2013. “Bioplastic and Their Thermoplastic Blends from Spirulina and Chlorella Microalgae.” Journal of Applied Polymer Science 130 (5): 3264-75

تأثيرمسحوق اوراق الهندباء البرية وطحلب الاسبرولينا المضاد لهشاشة العظام علي إناث الفئران البيضاء الناجم عن الديكساميثازون

صفاء طلعت جوهري

قســـم الأقتصــــاد المنزلي – تغذية وعلوم أطعمة - كلية التربية النوعية – جامعة عين شمس – القاهرة – مصر

هشاشة العظام هو مرض متعدد العوامل يصيب الهيكل العظمي، ويظهر انخفاضًا في كتلة العظام واضطرابًا في البنية الدقيقة للأنسجة العظمية. ويدرس البحث الحالي التأثير المضاد لهشاشة العظام لمسحوق أوراق الهندباء البرية وطحلب الاسبيرولينا الناجم عن الديكساميثازون. أظهر التركيب الكيميائي أن طحالب الاسبيرولينا المجففة تحتوي على نسبة أعلى من البروتين والدهون مقارنة بأوراق الهندباءالتي تتميز باحتوائها على نسبة عالية من الكربوهيدرات والألياف والرماد والرطوبة أكثر من الطحالب المجففة ، كما ان الاسبرولينا واوراق الهندباء غنية بالكالسيوم والفوسفور (١٣٤١,٥٦ ± ١,٢٩و ٨٨٧ ± ٢,٠٠مجم / ١٠٠ جم) و (٢٩٥,١٤ ± ١,١٠و ١٨٧ ± ١,٠٠مجم / ١٠٠ جم) على التوالي. تم تقسيم ٤٨ من الفئران البيضاء إلى مجموعتين ، المجموعة الرئيسية الأولى: (٨ فئران) تمت تغذيتها الاساسية (مجموعة ضابطة سالبة) ، المجموعة الرئيسية الثانية (٤٠ فأر) حقنت عضليًا بالديكساميثازون (DEX)في جرعة (٧ مجم / كجم من وزن الجسم) مرة واحدة أسبوعياً لمدة تصل إلى أربعة أسابيع للإصابة بهشاشة العظام ، ثم تم تقسيمها إلى ٥ مجموعات فرعية كل منها ثمانية فئران ، على النحو التالي: المجموعة (٢) التي تتغذى على الوجبة الاساسية (مجموعة ضابطة موجبة) ، المجموعة (٣) تتغذى على علي الوجبة الاساسية مضاف اليها ٤٪ طحلب الاسبرولينا المجفف، المجموعة (٤) تتغذى على علي الوجبة الاساسية مضاف اليها ٦٪ طحلب الاسبرولينا المجفف، المجموعة (٥) تتغذى على علي الوجبة الاساسية مضاف اليها ٥٪ مسحوق أوراق الهندباء والمجموعة (٦) تتغذى على علي الوجبة الاساسية مضاف اليها ١٠٪ مسحوق أوراق الهندباء. أظهرت النتائج أن جميع مجموعات هشاشة العظام التي عولجت بنسب مختلفة من مسحوق أوراق الهندباء (٥و١٠٪)والاسبرولينا المجففة (٤و ٦٪) أدت إلى انخفاض معنوي في إنزيمات الكبد ووظائف الكلى والكالسيوم والفوسفور في العظام والكالسيوم المتأين ومعادن العظام. وكذلك فحص الكثافة والمحتوى المعدني للعظام مقارنة بالمجموعة الضابطة الموجبة. واظهرت أفضل نتيجة للمجموعات المعالجة بـ ١٠٪ مسحوق أوراق الهندباء و ٦٪ الاسبرولينا المجففة. من ناحية أخرى ، كشفت الأشعة السينية والتشريح المرضي للمجموعة الضابطة الموجبة بعد شهرين عن فقدان العظام لأجزاء مختلفة مثل بالإضافة إلى نزع المعادن من العظام وهشاشة العظام. أظهرت هذه النتائج أن مسحوق أوراق الهندباء البرية وطحالب الاسبرولينا المجففة عالجت درجات من هشاشة العظام مقارنة بالمجموعة الموجبة.

الكلمات المفتاحية: هشاشة العظام؛ جلايكورتيكويد ؛ الهندباء ؛الطحالب ؛ ديكسا؛ الأشعة السينية والتشريح المرضي