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***ANTI-OSTEOPOROTIC EFFECT OF DIET FORTIFIED WITH BROCCOLI AND  
FLAXSEED OIL ON RATS***

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**Research Journal Specific Education**  
**Faculty of Specific Education**  
**Mansoura University**

***ISSUE NO. 66, APRIL , 2022***

مجلة بحوث التربية النوعية - جامعة المنصورة  
العدد السادس والستون - أبريل ٢٠٢٢

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## **ANTI-OSTEOPOROTIC EFFECT OF DIET FORTIFIED WITH BROCCOLI AND FLAXSEED OIL ON RATS**

*Nawal Tahoon\**

### **Abstract:**

**Background/aim:** Dexamethasone is one of glucocorticoids and recorded to induce osteoporosis in experimental rats. This study aimed to evaluate the effects of broccoli and flaxseed oil supplementation on nutritional values, bone , serum markers and urinary indicators in osteoporotic rats.

**Materials and methods:** The study was performed on forty two Sprague-Dawley male rats weighted  $130\pm 5$  g, injected with dexamethasone 7 mg/kg body weight once/week for 8 weeks to induce osteoporosis then divided into control positive group , which was fed on basal diet; broccoli powder group, which fed on basal diet contain 10% broccoli powder; broccoli extract group which fed on basal diet and administered 330 mg/kg body weight broccoli extract by stomach tube;, flaxseed oil group, which fed on basal diet contain 7% flaxseed oil; broccoli powder + flaxseed oil group, which fed on basal diet with 10% broccoli powder and 7% flaxseed oil; and broccoli extract + flaxseed oil group, which fed on basal diet contain 7% flaxseed oil plus administered 330 mg/kg body weight broccoli extract for sixty days. Serum, femur bone and urine samples were collected.

**Results:** Osteoporotic rats fed broccoli either powder or extract and or flaxseed oil showed elevation of bone markers {bone mineral density (BMD), bone mineral content (BMC), calcium and phosphorus} and serum calcium but showed decline of serum alkaline phosphatase and osteocalcitonin and urinary markers (calcium, phosphorus, deoxypyridinoline and pyridine) compared with control positive group.

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**Conclusion:** Broccoli and flaxseed oil demonstrating an antiosteoporotic effect on rats exposed to dexamethasone.

**Keywords:** Vegetable-oil- polyunsaturated fatty acids - bone markers, bone resorption - glucocorticoids, fracture risk - rats.

### ***Introduction***

Synthetic glucocorticoids are potent immunosuppressive and anti-inflammatory action so that are common used for the treatment of rheumatic disease of joint and muscle, autoimmune diseases, cancer, gastrointestinal diseases and also in organ transplantation. Dexamethasone is member of glucocorticoids and reported to induce osteoporosis in experimental rats (**Weinstein 2012, Ren *et al.*, 2015 and Vandewalle *et al.*, 2018**).

Sadly, secondary osteoporosis and non-traumatic osteonecrosis are resulted from glucocorticoids administration that elevates the incidence of fractures. There are many risk factors including old age, high daily dose, prolonged duration and cumulative dose (**Steinbuch *et al.*, 2004 and Compston 2018**).

Osteoporosis induced by glucocorticoids is produced mainly from decline of replication and differentiation and maturation of osteoblasts resulting in lowered in bone formation. Also, decline of osteocytes function and elevation of apoptosis could diminish the detection and repairing of bone micro-damage (**Canalis *et al.*, 2007**). At the initial phase of the osteoporosis induced by glucocorticoids, lower of bone formation together with elevation of resorption in addition to increase osteoclast generation and affection of osteoblast signal is resulting in osteoclastogenesis. Glucocorticoids treatment lowers calcium absorption by gastrointestinal tract and increase renal calcium loss beside muscle weakness increase the speedy loss of bone integrity and fracture risk. Suppression of growth hormone and insulin-like growth factor 1 production are complicated in the formation of glucocorticoid-induced osteoporosis (**Sivagurunathan *et al.*, 2005, Canalis *et al.*, 2007 and Caplan *et al.*, 2017**).

Therefore, the needs of suitable nutrition for preventing bone resorption are increased specially for glucocorticoids treated patients.

Recent evidence demonstrates that cruciferous vegetables have many protective ingredients as vitamin C, fibres, tocopherols, folate, carotenoids, and polyphenols and also glucosinolates which are secondary sulphur metabolites (**Lin and Harnly 2009 and Vasanthi et al., 2009**). Broccoli (*Brassica oleracea Italica*) is one of the most common cruciferous vegetable and is interested by biological and chemical studies. It has been reported that broccoli have antifungal, antibacterial, antidiabetic and anti-inflammatory effects in addition to experimentally hepatic and renal protective effects. Also, consumption of broccoli could diminish risks of the gastrointestinal tract cancers and many of metabolic disease (**Jaiswal et al., 2011 and Piruthiviraj et al., 2016**).

One of the most functional oil seed crop and widely grown all over the world is flaxseed (*Linum usitatissimum L.*), attributed to unsaturated fatty acids, particularly  $\alpha$ -linolenic acid content that assists the equilibrium the percentage of n-6 and n-3 fatty acids in the diet. The nutritional value and pharmaceutical efficacy of flaxseed oil were proved in reduction of atherosclerosis, colon tumours and mammary cancer (**Iolascon et al., 2017 and Wang et al., 2020**).

Therefore, the main aim of this study was to evaluate the possible protective effect of broccoli and flaxseed oil consumption in reduction of osteoporosis in dexamethasone induced osteoporosis in experimental rats.

#### **MATERIAL AND METHODS:**

Flaxseed oil was obtained from the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Broccoli was obtained from grocery. The experimental rat basal diet was formulated according to **Reeves et al., (1993)**. It is obtained from Amriya for pharmaceutical industries, Alexandria – Egypt. Forty two Sprague-Dawley male rats weighted  $130 \pm 5$  g were bought from the Research Institute of Ophthalmology, Giza, Egypt, and caged (seven rats per cage) under standard conditions. After acclimatization period (14 days), all rats were intramuscular injected with dexamethasone 7 mg/kg body weight once/week for 8 week to induce osteoporosis (**Wood et al., 2018**).

Broccoli heads were washed, trimmed florets to small parts and dried at hot oven at 60°C then grinded to powdered. The broccoli powder was either added to the basal diet as 10% or extracted by mixing of 500 mg broccoli powder with 10 ml of methanol/water (60:40, v/v) by ultrasonic bath for an hour at room temperature and centrifuged at 2500 rpm for 15 min. The supernatant was taken. The remaining part was dissolved again in 10 ml of methanol/ water followed by the same procedure. All the supernatants were kept at -20 °C. (**Bertelli *et al.*, 1998**)

After two weeks of acclimatization, the rats were classified into 6 groups as follows:

- Control positive group which was fed on basal diet only.
- Broccoli powder group which fed on basal diet contain 10% broccoli powder in substitution with fiber of the basal diet.
- Broccoli extract group which fed on basal diet and administered 330 mg/kg of body weight broccoli extract by stomach tube.
- Flaxseed oil group which fed on basal diet with 7% of flaxseed oil in substitution with sunflower oil of the basal diet.
- Broccoli powder + Flaxseed oil group which fed on the basal diet contain 10% broccoli powder plus 7% flaxseed oil.
- Broccoli extract + flaxseed oil group, which fed on the basal diet contain 7% of flaxseed oil plus administered 330 mg/kg of body weight broccoli extract.

Daily food intake and weekly body weight were recorded during the experimental period (60 days) to calculate feed efficiency ratio. Urine samples were collected via a metabolic cage.

After sacrificing rats, blood samples were collected separately to estimate serum calcium, phosphorus, osteocalcin and alkaline phosphatase values were assessed using colorimetric assay kit according to **Gindler and King (1972)**, **El-Merzabani *et al.*,(1977)**, **Craciun *et al.*,(2000)** and **Roy (1970)**, respectively. The femur bones were collected and all tissues surrounding the bone were removed, and then washed with saline. Bone

mineral density (BMD) and bone mineral content (BMC) were determined in right femur with Dual-Energy X-ray Absorptiometry scanner. (Lochmüller *et al.*, 2001)

Femur ash and bone minerals (calcium and phosphorus in dry weight) were determined by Inductively Coupled emission spectrometer according to previous study of Ren *et al.*, (2015). Urinary calcium, phosphorus, pyridine and deoxypyridin were estimated according to Murray *et al.*, (2000).

### Statistical analysis

The obtained results were expressed as mean  $\pm$ SD using one-way analysis of variance test of the Statistical Package for the Social Sciences for comparisons between means of different groups. The difference was considered significant when *P* value less than or equal to 0.05 Abo-Allam (2003).

### Results

**Table (1):** Nutritional indicators of the experimental rats groups at the end of study.

Groups Variables	BWG	FI	FER
Control positive	45.60 $\pm$ 3.11 <sup>c</sup>	16.31 $\pm$ 1.25 <sup>a</sup>	0.0465 $\pm$ 0.003 <sup>c</sup>
Broccoli Powder	65.75 $\pm$ 4.22 <sup>ab</sup>	16.99 $\pm$ 1.30 <sup>a</sup>	0.0644 $\pm$ 0.02 <sup>d</sup>
Broccoli Extract	71.31 $\pm$ 6.11 <sup>ab</sup>	17.51 $\pm$ 1.16 <sup>a</sup>	0.0678 $\pm$ 0.004 <sup>b</sup>
Flaxseed oil	69.61 $\pm$ 4.96 <sup>ab</sup>	17.45 $\pm$ 1.25 <sup>a</sup>	0.0664 $\pm$ 0.002 <sup>bc</sup>
Broccoli Powder+ Flaxseed oil	72.31 $\pm$ 5.11 <sup>a</sup>	17.81 $\pm$ 1.33 <sup>a</sup>	0.0676 $\pm$ 0.004 <sup>b</sup>
Broccoli Extract+ Flaxseed oil	74.03 $\pm$ 4.31 <sup>a</sup>	17.79 $\pm$ 1.41 <sup>a</sup>	0.0693 $\pm$ 0.005 <sup>a</sup>

Results are expressed as Mean  $\pm$  SD

BWG: Body weight gain FI: Food intake FER: feed efficiency ratio

Mean values in each column having the same letter (a ,b,c, d & e) are non - significant different at  $P \leq 0.05$

The results in table (1) showed significant increases in body weight (BWG) gain and feed efficiency ratio (FER) osteoporotic rat groups fed

flaxseed oil and broccoli either powder or extract and or with flaxseed oil compared with control positive group. Among the experimental rat groups, the highest improvement in nutritional value appeared in the osteoporotic rat group administered broccoli powder plus flaxseed oil in basal diet and rat group administered broccoli extract plus flaxseed oil. There were non-significant differences in body weight gain, and food intake among osteoporotic rat groups fed broccoli either powder or extract and or with flaxseed oil.

**Table (2):** BMC, BA, BMD, ash, calcium and phosphorus of the experimental rats groups

Groups Variables	BMC (g)	BMD (g/cm <sup>2</sup> )	Femur ash (g)	Calcium (mg/g)	Phosphorus (mg/g)
Control positive	0.05±0.001e	0.12± 0.03e	0.55±0.03f	60.41±5.22d	30.22±3.11c
Broccoli Powder	0.08±0.002cd	0.14±0.02cd	0.60±0.02e	75.69±7.11bc	38.61±3.77b
Broccoli Extract	0.09±0.004c	0.16±0.03ab	0.63±0.01d	80.71±8.14ab	41.22±4.22ab
Flaxseed oil	0.082±0.003cd	0.15±0.04c	0.96±0.97a	40.22±3.77e	39.46±0.04b
Broccoli powder+ Flaxseed oil	0.10±0.01ab	0.16±0.05ab	0.68±0.03bc	85.22±8.51a	41.85±3.99a
Broccoli Extract+ Flaxseed oil	0.11±0.03a	0.17±0.06a	0.70±0.02b	88.14±7.99a	45.11±4.35a

Results are expressed as Mean ± SD

BMC: bone mineral concentration

BMD: bone (a ,b,c, d & e) are non -

significant different at  $P \leq 0.05$

The osteoporotic rat groups fed broccoli powder or extract and or with flaxseed oil showed significant increase of femur bone mineral density (BMD), bone mineral concentration (BMC), ash, calcium and phosphorus compared with control positive group. The osteoporotic rat groups fed flaxseed oil, broccoli powder plus flaxseed oil and broccoli extract plus flaxseed oil showed non-significant differences in bone mineral concentration (BMC), femur ash and calcium. There was also non-significant difference in bone mineral density and bone phosphorus in rat

groups fed broccoli powder plus flaxseed oil and broccoli extract plus flaxseed oil, as shown in table 2.

**Table (3):** Serum calcium, phosphorus, OC and ALP of the experimental rats groups

Groups Variables	Serum calcium (mg/dl)	Serum phosphorus (mg/dl)	OC (µg/l)	ALP (U/l)
Control positive	7.88±0.55c	5.91±0.41c	10.55±1.14a	280.77±36.55a
Broccoli Powder	10.51±1.12a	7.31±0.66a	8.01±0.80b	167.81±20.14b
Broccoli Extract	10.14±1.14a	6.31±0.49b	7.91±0.63bc	148.77±14.18c
Flaxseed Oil	9.96±0.97ab	6.11±0.45b	7.88±0.65bc	149.45±15.17c
Broccoli Powder+ Flaxseed Oil	10.55±1.30a	7.21±0.56a	8.76±0.66b	150.41±16.22c
Broccoli Extract+ Flaxseed Oil	10.71±1.25a	6.88±0.47ab	8.22±0.77b	145.55±13.77c

Results are expressed as Mean ± SD

OC: osteocalcin ALP: alkaline phosphatase

Mean values in each column having the same letter (a ,b,c,d & e) are non - significant different at  $P \leq 0.05$

As compared with the osteoporotic positive control rat group, rat groups fed broccoli either powder or extract and/or with flaxseed oil and flaxseed oil showed significant elevation of serum calcium and phosphorus and decline in serum osteocalcin (OC) and alkaline phosphatase(ALP). Serum calcium and osteocalcin were in non -significant difference among rat groups fed broccoli either powder or extract and or with flaxseed oil. Serum phosphorus was in non-significant difference among rat groups fed broccoli extract or flaxseed oil and also non -significant difference among rat groups fed broccoli powder, broccoli powder plus flaxseed oil and broccoli extract plus flaxseed oil. Moreover, rat groups fed broccoli extract, flaxseed oil and broccoli powder plus flaxseed oil and also broccoli extract

plus flaxseed oil showed significant decline in alkaline phosphatase compared with rat group fed broccoli powder as presented in table 3.

**Table (4):** Urinary calcium, phosphorus, pyridine and deoxypyridine of the experimental rats groups

Groups Variables	Calcium (mg/d)	Phosphorus (mg/d)	Pyridine (μmol/mol)	Deoxypyridine (μmol/mol)
Control positive	8.88±0.55a	11.91±1.21a	98.61±9.77a	135.66±12.22a
Broccoli Powder	6.33±0.29b	9.14±0.90b	79.07±7.25b	95.14±8.77b
Broccoli Extract	6.11±0.40b	8.61±0.87bc	76.99±7.14bc	92.77±9.11b
Flaxseed Oil	6.55±0.33b	9.11±0.73b	75.14±8.07bc	90.16±9.20bc
Broccoli Powder+ Flaxseed Oil	5.60±0.29c	8.51±0.63c	73.60±7.11c	86.18±8.31c
Broccoli Extract+ Flaxseed Oil	5.77±0.34c	8.10±0.65c	70.11±6.96cd	85.16±7.94cd

Results are expressed as Mean ± SD

Mean values in each column having the same letter (a ,b,c, d & e) are non - significant different at  $P \leq 0.05$

The osteoporotic rat groups fed broccoli powder or extract and or with flaxseed oil showed significant decline of urinary calcium, phosphorus, pyridine and deoxypyridine compared with control positive group. The osteoporotic rat groups fed broccoli powder, broccoli extract and flaxseed oil showed non-significant differences in the tested urinary biomarkers. There was also non-significant difference among osteoporotic rat groups fed broccoli powder with flaxseed oil and broccoli extract with flaxseed oil but showed the lowest values compared to other experimental rat groups as shown in table 4.

## **DISCUSSION**

It has been reported that dexamethasone was associated with decline of osteogenesis and elevation osteoblast apoptosis that lower bone formation. The decline of bone anabolic state are related to catabolism of the bone proteins, osteopontin and osteocalcin with prolong life span of the mature osteoclasts (**Sivagurunathan et al.,2005, Jia et al., 2011 and Böcker et al.,2014**). Several studies reported that glucocorticoids interrupt

of calcium-phosphorus homeostasis by reducing resorption of calcium in the gastrointestinal tract, blocking intestinal calcium and phosphorus absorption and prevent renal tubular calcium reabsorption resulting in development of hypocalcemia (Suarez-Bregua *et al.*, 2018). Also in our results, higher value of serum alkaline phosphatase, pyridine and deoxypyridine reflect glucocorticoids induced osteoporosis (Lin *et al.*, 2014, Liang *et al.*, 2016 and Saad *et al.*, 2019).

The improvement of nutritional indicators in our study is mainly related to advantage constituents in broccoli and flaxseeds oil. Broccoli is a vital nutrient source, may be due to a greater content of antioxidants, flavonoids, bioactive glucosinolates, minerals and protein than other vegetables. Also, Broccoli was supposed to improve the digestion processes and treat anemia (Lin and Harnly 2009, Avula *et al.*, 2015 and Fahey 2015). However, flaxseed is the richest dietary source of omega-3 fatty acids among plant sources that diets contain flaxseed oil has reflective favourable health effects in different pathologies (Moreno *et al.*, 2006, Podsedek 2007 and Elbahnasawy *et al.*, 2019).

Our data confirm that rats fed broccoli either powder or extract with flaxseed showed significant elevation of serum calcium that reflects the enhancement of intestinal calcium absorption and reduction of urinary calcium excretion and subsequently, the restoration of bone metabolism and prevention of bone loss as identified by the improvement of serum markers of bone resorption as reported by Mühlbauer *et al.*, (2002). Previous researches proved that antioxidants are molecules that inhibit or neutralize cell damage caused by free radicals. This can lead to reduced inflammation and an overall health-protective effect. Presence of a respectable source of vitamin K and calcium in broccoli has vital roles for maintaining strong, healthy bones. Vitamin K-dependent proteins are placed within the bone. Healthy bones need zinc, phosphorus, and vitamins C and A, which found in broccoli (Vasanthi *et al.*, 2009 and Yagishita *et al.*, 2019).

Owing to major  $\omega$ -3 poly unsaturated fatty acids of flaxseed oil which comprise alpha-linolenic acid and its longer chain derivatives as

eicosapentaenoic and docosahexaenoic acid are essential for regulation of osteoblastogenesis and hinder of bone resorption that definitely associated with bone mineral density (Kelly *et al.*, 2013 and Lavado-García *et al.*, 2018).

In our study, feeding rats with broccoli and flaxseed oil could increase bone mineral density in all osteoperotic groups, which is in line with results (El-Wakf *et al.*, 2019). The decline in femur mineral is associated with bone loss and bone resorption reflected of fall in bone mineral density and an increase the risk of bone fractures. Osteocalcin secreted from osteoblasts and found mainly in the bone matrix and a minor portion is secreted into blood circulation that reflects the activity of osteoblasts and osteoclasts (Neve *et al.*, 2013). Several studies reported that poly unsaturated fatty acids of flaxseed oil in osteoclast and osteoblast activity are regulated and controlled the bone metabolism via improving calcium balance and bone turnover resulting in higher bone minerals (Dorni *et al.*, 2018 and Elbahnasawy *et al.*, 2019).

Experimental evidence indicates that organosulphur compounds, such as glucosinolates and sulphoraphane in broccoli have a potent phytochemical for detoxifying ability by regulating cellular antioxidants and reducing of free radicals (Conzatti *et al.*, 2015). It has been reported that broccoli could prevent bone disorders owing to glucoraphanin that metabolized into an effective antioxidant sulforaphane during digestion which inhibiting osteoarthritis. The intake of fruit and vegetables is correlated with better bone mineral density attributed to the extra base of buffering non-carbonic metabolic acids to protect from bone dissolution (Qazi *et al.*, 2010, Tomofuji *et al.*, 2012 and Yanaka 2018).

## **CONCLUSION**

The present study indicated that dietary flaxseed oil and broccoli could reduce the potential effect of dexamethasone inducing osteoporosis in experimental rats based on pharmaceutical components containing omega-3 fatty acids and antioxidants compounds by improvement of calcium

absorption, regulation of bone metabolism, differentiation of the osteoblast and osteoclast, and decline excretion of calcium and phosphorus.

### **RECOMMENDATION**

Thus, broccoli and flaxseed oil could be used as a natural dietary approach to avoid the bone loss related with dexamethasone therapy. There is a continuous search for natural agents' anti-osteoporosis.

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## التأثير المضاد لهشاشة العظام للوجبات المدعمة بالبروكلي

### وزيت بذور الكتان على الفئران

#### الملخص العربي:

الديكساميثازون هو أحد أنواع الجلوكورتيكويدات والذي يؤدي الي الاصابة بهشاشة العظام في فئران التجارب. تهدف هذه الدراسة إلى تقييم تأثير التدعيم بالبروكلي وزيت بذور الكتان على المؤشرات الغذائية ودلائل العظام والسيرم وكذلك المؤشرات البولية في الفئران المصابة التي تعاني من هشاشة العظام. أجريت الدراسة على اثنين وأربعين فأر من ذكور سبراجيو داوولي وزن  $130 \pm 5$  جم تم حقنها بالديكساميثازون 7 ملجم / كجم من وزن الجسم مرة واحدة أسبوعياً لمدة 8 أسابيع لإحداث هشاشة العظام ثم إعادة تقسيمهم لست مجموعات . المجموعة الاولى الضابطة الموجبة التي تم تغذيتها علي الوجبة القياسية فقط والمجموعة الثانية التي تغذت على الوجبة القياسية ومسحوق البروكلي 10٪ والمجموعة الثالثة التي تغذت على الوجبة القياسية ومستخلص البروكلي 330 ملجم / كجم من وزن الجسم من خلال أنبوب المعدة ، والمجموعة الرابعة التي تغذت على الوجبة القياسية وزيت بذور الكتان 7٪ والمجموعة الخامسة التي تغذت علي الوجبة القياسية ومسحوق البروكلي 10 ٪ + زيت بذور الكتان 7٪ والمجموعة السادسة التي تغذت على الوجبة القياسية ومستخلص البروكلي 330 مجم / كجم من وزن الجسم + زيت بذور الكتان 7٪ واستمرت الدراسة لمدة ستون يوماً. تم جمع عينات من السيرم وعظام الفخذ والبول لتحليلها وأظهرت النتائج ان الفئران التي تعاني من هشاشة العظام والتي تم تغذيتها على مسحوق البروكلي أو المستخلص أو زيت بذور الكتان اظهرت ارتفاعاً معنوي في علامات العظام الصحية (كثافة المعادن بالعظام ، BMC ، الكالسيوم والفوسفور) ومستوي كالسيوم السيرم ، لكنها أظهرت انخفاضاً معنوي في انزيم الفوسفاتاز القلوي في الدم وهرمون الأوستيوكالسيونين والعلامات البولية (الكالسيوم والفوسفور وديوكسيبيريدينولين والبيريدين) وذلك بالمقارنة مع المجموعة الضابطة الموجبة.

الخلاصة: البروكلي وزيت بذور الكتان اثبت تاثيرهم كمضاد لهشاشة العظام على الفئران المعرضة لمرض هشاشة العظام بعقار الديكساميثازون.

الكلمات المفتاحية: زيت نباتي- احماض دهنية عديدة عدم التشبع- دلالات تحليل العظام الجلوكوكورتيكويدات - مخاطر الكسر- فئران .