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Possible Role of Garlic Oil and Parsley Extract in Ameliorating Radiation-Induced Bone Loss in Female Rats

L. Ramadan, W. El-Sabbagh and S. Kenawy*

*Drug Radiation Research Dept., National Center for Radiation Research and Tecchnology (NCRRT), P. O. Box; 29 Nasr City, Egypt and *Pharmacology and Toxicology Dept., Faculty of Pharmacy, Cairo University, Egypt.*

TO INVESTIGATE the possible protective effect of garlic oil and parsley extract against bone loss resulted in female virgin rats exposed to fractionated doses of γ-radiation (1Gy 3 times weekly for 5 weeks). Urinary calcium (uCa), calcium to creatinine ratio (Ca/Cr), hydroxyproline and serum phosphorus were measured as bone resorption biomarkers, while serum osteocalcine (OST) and serum alkaline phosphatase (ALP) were measured as bone formation biomarkers. Furthermore, nitric oxide (NO) which represents the balance in bone remodeling was measured. Malondiadehyde level (MDA) as well as superoxide dismutase activity (SOD) was measured as oxidative stress biomarkers.

Female irradiated rats in the present study had significant increases in both bone resorption and bone formation biomarkers after 6 weeks from the last exposure to γ-radiation. Irradiated rats also had significant decreases in plasma NO indicating imbalance in bone remodeling as well as significant increase in oxidative stress biomarkers. Daily treatment with garlic oil extracted in olive oil improved all measured parameters except OST level, while the vehicle used for garlic oil (extra virgin olive oil) significantly decreased bone resorption biomarkers. Parsley extract induced normalization to all bone resorption and formation parameters measured in irradiated rats. Daily administration of garlic oil and parsley extract protected the bone from degeneration induced by exposure to fractionated doses of gamma radiation.

Keywords: γ-irradiation, garlic oil, parsley extract.

Bone is often subjected to high doses of radiation in an effort to control cancer; so irradiation is one of the factors that exert a negative effect on osteogenesis. In clinical studies, atrophy and high susceptibility to fractures of the bone in radiation exposure field have been reported by Ergun and Howland (1980).

Jenkins *et al.* (1995) recorded hip fractures complications in patients who received chemo-radiotherapy for carcinoma of the vulva or anus. In young mice, haematological and skeletal changes have been observed after long term continuous external whole body irradiations; this was explained by alteration in bone remodelling which resulted from radiation injury to osteoclast precursors in the haematopoietic compartment as shown by Anderson *et al.* (1997). On the other hand, Matsumura *et al.* (1998) demonstrated that DNA content and total calcium content in osteoblast cell cultures were decreased by irradiation, leading to decreases in whole matrix calcification.

Gamma radiation affected sex hormones which play a role in bone metabolism. It was shown by Lee and Yoon (2005) that gamma radiation induced apoptotic and inflammatory degeneration of ovarian follicles in mice. The naturally occurring antioxidants are part of the natural biochemical defence system that evolved to protect cells against free radicals and reactive oxygen species arising from normal metabolic processes. A large number of plants containing antioxidant phytochemicals were reported to be radio protective in various model systems. The aim of the present study is to investigate the possible protective effects of certain natural products against bone loss that normally results in female rats after exposure to fractionated doses of γ-radiation, and the possible role played by antioxidants in protection from the damaging effect of radiation on bone.

Material and Methods

Animals

Female albino virgin rats, matched for age and weight (180-200 g), were obtained from the animal house of the Research Institute of Ophthalmology, Giza, Egypt. Animals were kept at room temperature and allowed free access to water and food (standard pellet diet). The study was carried out according to the guidelines of the ethical committee in Faculty of Pharmacy, Cairo University.

Drugs

Garlic oil extracted and suspended in pure extra virgin olive oil, (Nature's way products Inc., USA), and Parsley leaves extract (Nature Answer Inc., USA). Oestradiol benzoate (Misr Company for pharmaceutical Industries, Egypt) used as reference standard drug.

Irradiation

Whole body γ-irradiation was carried out using the facilities provided by NCRRT. Cesium-137 irradiation unit (Gamma cell-40) produced by the Atomic Energy of Canada Limited was used at a dose rate of 0.84Gy/ min at the time of experiment. A pilot test was carried out to estimate the degree of bone loss in rats exposed to fractionated doses of γ-irradiation (1Gy 3 times a week (2 days gap) for 5 weeks equivalent to 15 Gy). Urine samples were collected 4, 8, 11 and 15 weeks after the first dose of irradiation for measuring Ca and hydroxyproline levels to evaluate rate of bone resorption.

Experimental design

A total of 36 rats were divided into 6 groups each of 6 rats as follows: Rats in the 1st group were non-irradiated and served as normal group, while in the 2nd group rats were exposed to 15 Gy γ-radiation (1Gy 3 times weekly for 5 weeks). The 3rd group consisted of irradiated rats treated daily with oral doses of garlic oil (100 mg/ kg) in olive oil according to Gupta (1996) for 5 weeks. Irradiated rats in the 4th group were treated with extra virgin olive oil calculated to be equivalent to the amount used as a vehicle with garlic oil (2g/ kg for 5 weeks/ orally). In the 5th group, irradiated rats were treated as in the 3rd comprised group but using parsley extract instead of garlic oil. The 6th group irradiated rats injected s.c. daily for 5 weeks with oestradiol benzoate (30µg/ kg) according to the study of Shen *et al.* (1993).

Laboratory measurements

Urinary calcium (Ca), urinary calcium to creatinine ratio (Ca/Cr), urinary hydroxyproline and serum phosphorus were measured as bone resorption biomarkers. Calcium, creatinine, phosphorus and hydroxyproline were measured according to Gindler and King (1972), Schirmeister *et al.* (1964), El-Merzabani *et al.* (1977) and Woessner (1961). Serum osteocalcin (OST) and serum alkaline phosphatase (ALP) activity were measured as bone formation biomarkers according to Power and Fottrell (1991) and Belfield and Goldberg (1971). Nitric oxide (NO) was estimated in plasma as it reflects the balance in bone remodeling according to Montgomery and Dymock (1961). Plasma malondialdehyde (MDA) and blood superoxide dismutase activity were measured as oxidative stress biomarkers according to the methods of Yoshioka *et al* (1979) and Marklund and Marklund (1974), respectively.

Statistical evaluation

All values are expressed as means± S.E. Data were analyzed using one way ANOVA followed by Tukey-Kramer multiple comparison test. The p value was considered significant at $p< 0.05$. Graphpad software instant (version 2) was used to carry out these statistical tests.

Results

Pilot experiment

Exposing female rats to γ-radiation (15 Gy fractionated at three doses a week, each of 1 Gy for 5 weeks) induced a gradual increase in urinary calcium excretion along a period of 15 weeks.

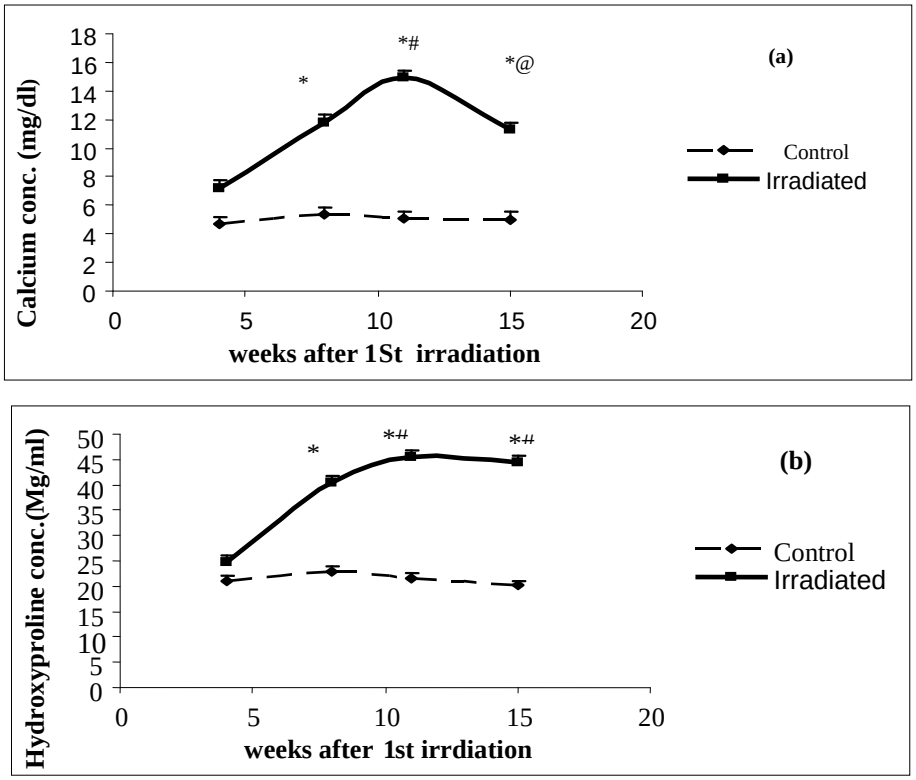

Fig. 1. Changes in urinary calcium conc. (mg/dl) (a) and hydroxyproline conc. (µg/ml) (b) of female irradiated rats (15 Gy; 1Gy 3 times a week for 5 weeks) after 4, 8, 11 and 15 weeks from the first dose of irradiation.

*. significantly different from value after 4 weeks at P≤0.05. #. significantly different from value after 8 weeks at P≤0.05. @. significantly different from value after 11 weeks at P≤ 0.05.

The 1st significant increase in urinary calcium was detected after 8 weeks from the 1st exposure to γ-radiation. However, the peak of calcium excretion was detected at the 11th week (Fig. 1).

On the other hand, at the 8th week, the urinary hydroxyproline level increased significantly by 63.1% compared with that in the 4th week. Furthermore at the 11th week, excretion of hydroxyproline significantly increased reaching the maximum; this level remained nearly constant till the 15th week (Fig. 1).

Urinary Ca and Ca/Cr ratio

Fig. 2. Showed that γ-rays induced a significant increase in urinary calcium excretion by 199.44% compared to control non irradiated rats. Administration of garlic oil, suspended in extra virgin olive oil, to irradiated rats showed a significant decrease in urinary calcium level by 70.1%.

On the other hand, daily administration of extra virgin olive oil alone to irradiated rats inhibited the increase in urinary calcium level induced by γ-rays only by 48.3%.

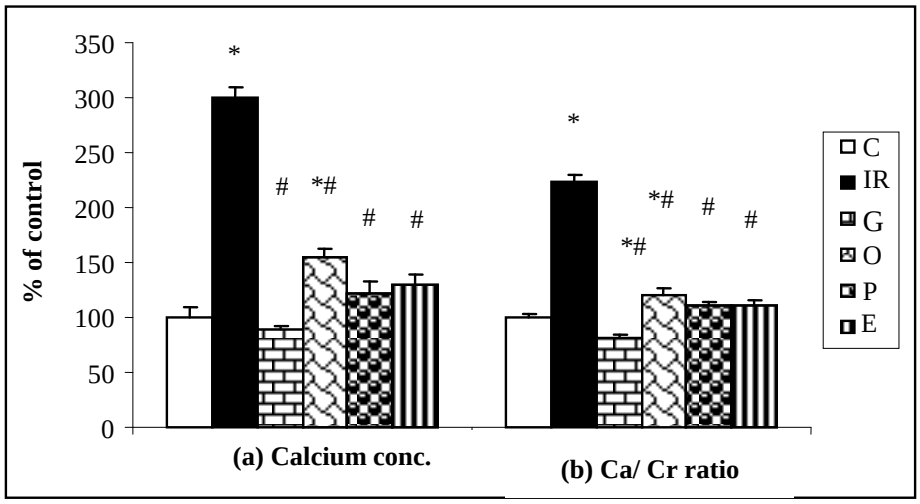

Fig. 2. Effect of garlic oil (100 mg/kg), extra virgin olive oil (2 g/kg), parsley extract (100 mg/kg) and oestradiol benzoate (30 µg/kg) on calcium conc. (mg/dl) (a) and calcium to creatinine ratio (b) in urine of female irradiated rats.

Legends as in Fig. 1. All data taken at 11 weeks post first irradiation, C= control normal group, IR= irradiated group, G= garlic oil treated group, O= olive oil treated group, P= parsley treated group, E= oestradiol treated group.

Parsley extract also significantly decreased the calcium level in urine of irradiated rats by 59.5%, while a significant decrease in urinary calcium level by 56.6% in irradiated rats was observed after daily injections with oestradiol benzoate. The administration of garlic oil extracted in olive oil, extra virgin olive oil, parsley extract and oestradiol benzoate to irradiated rats significantly decreased the Ca/ Cr ratio by 63.9%, 46.2%, 50.6% and 50.2% respectively (Fig. 2).

Urinary hydroxyproline and serum phosphorus levels

Irradiation induced a significant increase in urinary hydroxyproline level by 147%. Garlic oil extracted in olive oil, extra virgin olive oil, parsley extract and oestradiol benzoate significantly decreased the urinary hydroxyproline level in irradiated rats by 44.6%, 39.04%, 50.7% and 53.4% respectively (Fig. 3). Fig. 3. demonstrated that γ-irradiation significantly increased serum inorganic phosphorus level by 62.7%. Both garlic oil and parsley extract normalized the serum inorganic phosphorus level in irradiated rats. Extra virgin olive oil and oestradiol benzoate significantly decreased serum phosphorus level by 26.5% and 22.5% respectively in irradiated rats.

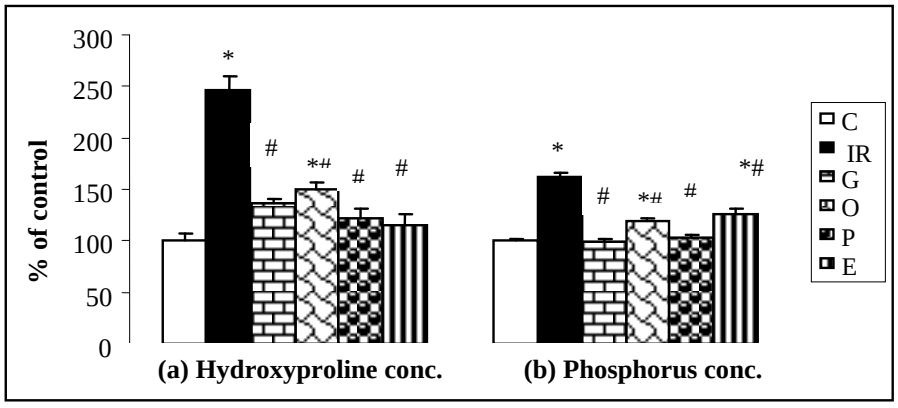

Fig. 3. Effect of garlic oil (100 mg/kg), extra virgin olive oil (2 g/kg), parsley extract (100 mg/kg) and oestradiol benzoate (30 µg/kg) on urinary hydroxyproline conc. (µg/ml) (a) and serum phosphorus conc. (mg/dl) (b) of female irradiated.

Legends as in Fig. 2.

Serum OST level, serum ALP activity and plasma NO

After 11 weeks from the 1st exposure to γ-radiation, serum osteocalcin was significantly increased by 22.9% when compared with control values.

Parsley extract and oestradiol benzoate significantly decreased serum osteocalcin level in irradiated rats by 31.9% and 22.2% respectively (Fig. 4).

Fig. 4. Showed an increase in ALP activity of irradiated rats by 78.5% compared with control rats. Daily administration of garlic oil normalized the activity of serum ALP.

Extra virgin olive oil, parsley extract and oestradiol benzoate induced a significant decrease in ALP activity of irradiated rats by 23.15%, 33.04% and 35.4% respectively. Fig. 4. showed that female irradiated rats showed a significant decrease by 59.4% in plasma nitric oxide level. However, daily administration of garlic oil, extra virgin olive oil, parsley extract and oestradiol benzoate induced significant increases in NO levels by 205.4%, 116.4%, 191.42% and 147.8% respectively.

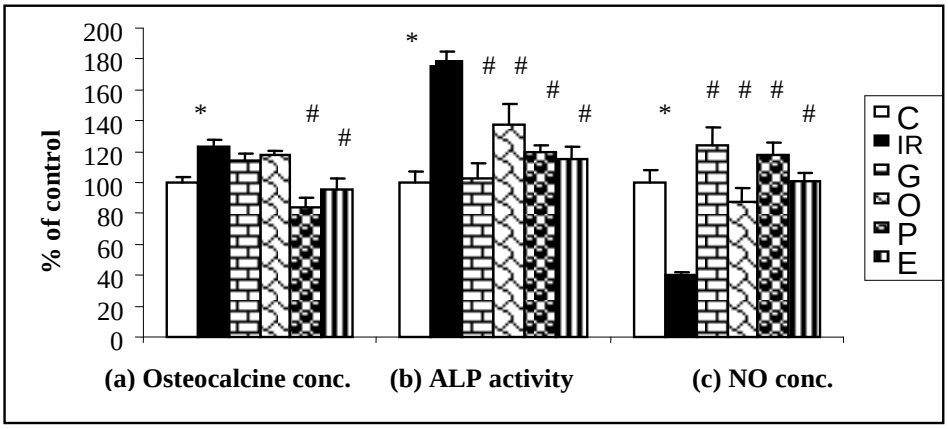

Fig. 4. Effect of garlic oil (100 mg/kg), extra virgin olive oil (2 g/kg), parsley extract (100 mg/kg) and oestradiol benzoate (30 µg/kg) on serum osteocalcine conc. (ng/dl) (a), serum alkaline phosphatase activity (IU/l) (b) and plasma nitric oxide conc. (µmol/l) (c) of female irradiated rats.

The legends as in Fig. 3.

Plasma MDA level and blood SOD activity

Exposure to fractionated doses of γ-irradiation (three doses a week each of 1 Gy for 5 weeks) induced a significant increase in MDA level by 85.8%.

Data showed that garlic oil, extra virgin olive oil, parsley extract and oestradiol benzoate exerted a significant decrease by 44.1%, 22.3%, 28.1% and 40.4% respectively in MDA levels of irradiated rats (Fig. 5).

Results showed that 6 weeks after exposure to 15 Gy a significant decrease in blood SOD activity by 49.2% was detected in irradiated rats. Administration of garlic oil extracted in olive oil induced a significant increase in SOD levels in irradiated rats by 81.4%, whereas extra virgin olive oil could not induce a significant increase in blood SOD level. Moreover, parsley extract and oestradiol benzoate induced significant increase in SOD levels in irradiated rats by 60% and 82.8% respectively (Fig. 5).

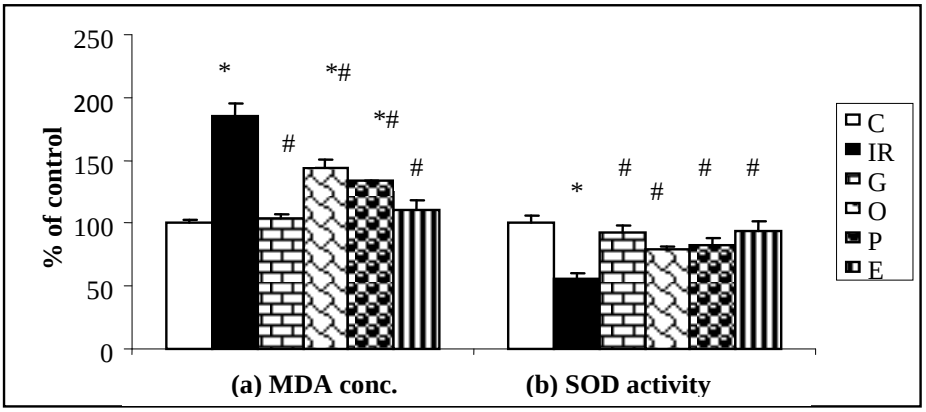

Fig. 5. Effect of garlic oil (100 mg/kg), extra virgin olive oil (2 g/kg), parsley extract (100 mg/kg) and oestradiol benzoate (30 µg/kg) on plasma MDA conc. (nmol/ml) (a) and blood SOD activity (U/ml) (b) in female irradiated rats.

The legends as in Fig. 3.

Discussion

Irradiated rats showed a significant increase in bone resorption biomarkers (Ca, Ca/Cr, hydroxyproline and phosphorus) after 6 weeks from the total exposure to fractionated 15 Gy dose. Present results also showed significant increases in serum OST and ALP levels in irradiated rats indicating high bone turnover rate after exposure to γ-irradiation. This was in accordance with the study of Chicarelli *et al.* (2007) who pointed out that irradiation affected the beginning of bone formation as well as acceleration of bone resorption in rats exposed to γ-radiation. However, several factors may be involved in the pathogenicity of osteoporosis after exposure to fractionated doses of γ-irradiation; as thyrotoxicosis (Nishiyama *et al.*, 1996) which could be linked to osteoporosis (Lakatos, 2003). Additionally, Girinsky *et al.* (1994) demonstrated that total body irradiation with a dose of 10 Gy induced a massive increase in

levels of bone resorbing cytokines in blood accompanied by a dramatic increase in levels of adrenocorticotrophic hormone (ACTH) inducing an increase in serum cortisol that has adverse effects on bone metabolism.

Daily treatment with garlic oil extracted in olive oil in the present experiment showed a possible protection against bone loss in irradiated rats. The significant decrease in Ca/Cr ratio as well as normalization of both urinary hydroxyproline and serum phosphorus levels could be linked to the ability of garlic oil in suppression of bone resorption induced by irradiation. Previously mentioned results of the use of garlic oil on irradiated rats revealed its radio protective effects. Singh *et al.* (1996) has reported the radio protective efficacy of garlic extract against irradiation damage in mice. However, the presence of trace elements such as selenium having antioxidant effects in garlic plant (Ip and Lisk, 1995) may explain one of radio protective properties of garlic plant. Our results showed that although extra virgin olive oil induced significant decrease in urinary Ca/Cr ratio, hydroxyproline and serum phosphorus level in irradiated rats, their levels were still significantly more than control values. On the other hand, although extra virgin olive oil significantly decreased serum ALP, it did not affect serum OST level. It can be suggested that the selected dose of extra virgin olive oil (2 g/kg) in the current study was not sufficient for complete protection from damage in bone of irradiated rats.

Parsley is rich with apigenin flavonoid which has a radio protective effect (Rithidech *et al.*, 2005) current results showed that daily administration of parsley extract induced a significant decrease in Ca/Cr ratio as well as hydroxyproline level in urine of female irradiated rats. In the current study, oestradiol benzoate could maintain the normal bone turnover rate in irradiated rats, this may be related to inhibition of both production and activity of bone resorbing cytokines by oestrogen (Manolagas and Jilka, 1995). Our results showed that exposure of rats to 15 Gy γ-rays (1Gy 3 times a week for 5 weeks) significantly decreased plasma NO level when compared with non irradiated rats. This may be explained by the study of Tishkin *et al.* (2007) who showed that whole body gamma irradiation induced endothelium dysfunction by suppressing calcium-activated potassium channels in rats which control the driving force for Ca2+ entry and therefore Ca2+ dependent NO synthesis in endothelial cells.

Garlic oil resulted in normalization of plasma NO level of irradiated rats. Morihara *et al.* (2002) demonstrated that aged garlic extract increased production of plasma NO by 30-40%. Extra virgin olive oil showed a significant increase in plasma NO value compared with that in non-treated group. The capability of virgin olive oil in modulating endothelial activity may be responsible for the increase in NO production by olive oil (Perona *et al.,* 2006). Daily administration of parsley extract induced normalization of plasma NO level of irradiated rats. This could be attributed to the presence of genistein in parsley, depending on results mentioned by Altavilla *et al.* (2004) who demonstrated that genistein flavonoid increased plasma NO breakdown products, reduced endothelin-1 levels and improved endothelial dependent vasodilatation. Moreover, oestradiol benzoate maintained the normal level of plasma NO in irradiated rats. 17-β oestradiol could modulate vascular function by rapid release of NO and up-regulation of eNOS through mitogen-activated protein (MAP) kinase-dependent mechanisms (Chen et al., 1999).

In this study, a significant increase in plasma MDA level as well as a significant decrease in SOD activity was observed in irradiated rats. It has been suggested that irradiation caused a marked change in the plasma total antioxidant capacity and large increase in oxidative stress (Chevion *et al.,* 1999). Rats supplemented with garlic oil in our study exhibited a significant decrease in MDA level and normalization of SOD activity. However, it has been reported before that garlic oil enhanced antioxidant and detoxifying enzyme systems (Saravanan and Prakash, 2004). Extra virgin olive oil showed a significant decrease in plasma MDA level; this was in accordance with Bogani *et al.* (2007) who reported that extra virgin olive oil could significantly increase serum antioxidant capacity. Parsley extract induced a significant decrease in MDA level and a significant increase in SOD activity in irradiated rats. It has been found by Nielsen *et al*. (1999) that supplementation of parsley rich with apigenin flavonoid for 2 weeks was sufficient to induce a significant increase in erythrocyte glutathione reductase and SOD activities in plasma.

Oestradiol benzoate induced a significant decrease in MDA level of irradiated rats as well as a significant increase in SOD activity. Ruiz-Larrea *et al.* (1994) suggested that the antioxidant properties of oestrogens are due to the presence of phenolic group in their steroid structure.

The beneficial effects of garlic oil and parsley extract against irradiation induced bone loss in female rats are demonstrated in the present findings. However, further studies are needed to investigate the factors participating in the development of osteoporosis after long exposure to γ-radiation.

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الدور المحتمل لزيت الثوم ومستخلص البقدونس فى تعديل نقص كثافة العظام المحدثه بالأشعاع فى اناث الجرذان

ليلى رمضان ، و ولاء الصباغ ، و سناء قناوى*

قسم البحوث الدوائية الإشعاعية ، المركز القومى لبحوث وتكنولوجيا الأشعاع ، ص ب ٢٩ مدينة نصر ، مصر ، و **قسم الأدوية و السموم ، كلية الصيدله ، جامعة القاهره ، مصر.

استهدفت هذه الدراسة إستعمال اثنين من المنتجات الطبيعية شائعة الإستخدام وهما زيت الثوم وخلاصة البقدونس، وإختبارهما و تجربتهما فى إمكانية الوقاية من مرض هشاشة العظام المحدث فى إناث الجرذان البيضاء عن طريق تعريض الحيوانات لجرعات مقسمة من الإشعاع الجامى (١جراى/ ٣ مرات إسبوعيا / ٥ أسابيع). تم تجميع عينات البول والدم من الفئران قد إستخدمت عينات البول لقياس الكالسيوم، الكرياتينين، نسبة الكالسيوم الى الكرياتينين و مستوى الهيدروكسي برولين، بينما تم قياس مستوى الفوسفور، انزيم الألكالين فوسفاتيز و الؤستيوكالسين فى مصل الدم. أيضا تم قياس مستوى كلا من النيتريك أوكسيد، المالون داى الداهيد فى البلازما ونشاط انزيم السوبرأكسيد ديسميوتيز فى الدم. وقد تم تجميع العينات بعد مرور٦ أسابيع من التعرض لآخر جرعة إشعاعية من الجرعات المقسمة (١٥ جراى). وقد أظهرت الفئران المشعة ازديادا ملحوظا فى مستوى الكالسيوم، نسبة الكالسيوم الى الكرياتينين و الهيدروكسي برولين فى البول و الفوسفور، الؤستيوكالسين ونشاط انزيم الألكالين فوسفاتيز فى مصل الدم بالإضافة الى المالون داى الداهيد فى البلازما. و من ناحية أخرى تناقص مستوى النيتريك أوكسيد فى البلازما ونشاط انزيم السوبرأكسيد ديسميوتيز فى الدم. وقد أظهرت النتائج أن الفئران المشعة والمعالجة بزيت الثوم مع زيت الزيتون قد أبدت تحسنا ملحوظا فى كافة القياسات فيما عدا مستوى الؤستيوكالسين فى مصل الدم. بينما أظهر العلاج بزيت الزيتون تحسنا فى القياسات لم يصل الى المعدلات الطبيعية، ولم يبد أى تحسن ملحوظ فى مستوى الؤستيوكالسين ونشاط انزيم السوبرأكسيد ديسميوتيز فى الدم. ومن ناحية أخرى أظهر العلاج بخلاصة البقدونس و الإيستراديول بنزوات الى تحسين جميع المؤشرات.