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Protective role of Peanut oil in rats exposed to two different doses of gamma radiation that produced oxidative stress and bone injury

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Abstract

Introduction: Exposure to ionizing radiation represents a genuine, increasing threat to mankind and our environment. The steadily increasing applications of radiation in clinical practice, industrial and agricultural activities, on top of residual radioactivity resulting from nuclear test explosions, have a measurable impact contributing to possible radiation hazards in humans. Control of radiation hazards is considered as one of the most important challenges in order to protect our lives from radiation damages.
The trans-3,4,5-trihydroxystibeneis a phyto-chemical present in peanuts and grapes with beneficial effects such as protection against cardiovascular disease and cancer prevention.

Purpose: The present study aims to clarify the role of peanut oil as a radioprotector in male albino rats against oxidative stress and bone injury induced by two different doses of gamma irradiation.

Material and Methods: Rats were subjected to a dose of 5 Gy (group 3) or 10 Gy (group 4) (single dose/ whole body) in comparison with control group (group 1). Prior to the two doses of gamma radiation, rats received peanut oil subcutaneously, (0.75 ml/kg) over one month period, on three days/week (group 5 and 6). Group 2, rats received peanut oil subcutaneously, (0.75 ml/kg) as group 5, but without exposure to radiation.

Results: The results showed that whole body gamma irradiation revealed significant acceleration in the level of lipid peroxide (MDA), with significant depletion in glutathione content (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. Meanwhile. Also, the study showed significant increase in serum calcium with concomitant decrease in the bone calcium and significant increase in serum inorganic phosphorous concomitant with a decrease in bone phosphorous after radiation exposure.
Administration of peanut oil pre-irradiation has significantly ameliorated the radiation induced disturbance in all the investigated parameters.

Conclusion: Metabolism can be controlled to some extent by peanut oil administrated prior to irradiation.

Keywords: Peanut oil - Ionizing radiation - Antioxidants – Calcium - Phosphorous.

Introduction

Exposure to ionizing radiation represents a genuine, increasing threat to mankind and our environment. The steadily increasing applications of radiation in clinical practice, industrial and agricultural activities, on top of residual radioactivity resulting from nuclear test explosions, have a measurable impact contributing to possible radiation hazards in humans. Control of radiation hazards is considered as one of the most important challenges in order to protect our lives from radiation damages.

Ionizing radiation provokes the decomposition reaction of water producing a variety of reaction oxygen species (ROS) **(Sadani and Nadkami, 1997).** ROS such as hydroxyl radicals (OH), super-oxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are extremely reactive and react with the molecule of cell membranes that are composed of a double layer of lipids with proteins dispersed throughout **(Ho *et al.*, 1998)**. Under normal conditions, there is a balance between the generation of ROS and the cellular antioxidant systems (Timothy and Sharma, 1991). Exposure to ionizing radiation produces significant alterations in the oxidant activity in tissues and causes overproduction of ROS leading to oxidative damage of the lipids, proteins and DNA. The oxidation of polyunsaturated fatty acids in membrane induced by ROS is called lipid peroxidation (LPO). However, organisms have protective systems against ROS, like endogenous antioxidant enzymes. Superoxide dimutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) constitute primary enzymatic defense system **(Halliwell and Gutteridge, 1990)**. Reduced glutathione (GSH) is a major antioxidant that provides reducing equivalents for the GSH-Px **(Galeotti *et al.*, 1991)**.

Calcium is one of the essential elements for normal functioning of an organism and its concentration in serum is kept within the narrow range of 8.7-10 mg/dl **(Brozoska and Manuszko, 1996)**. Because of the importance of calcium in regulating vital cellular and tissue functions, the concentration of calcium ions in body fluids is regulated by an effective feedback control system including a Ca^{++} transporting subsystem (bone and kidney), Ca^{++} sensing receptors and calcium regulating hormones: parathormone, calcitonine and 1,25-dihyroxy vit D_3 **(Hurwitz, 1996)**. Parathormone and calcitonin positively regulate renal 1,α hydroylase gene expression (a key enzyme for 1,25 $(OH)_3$ D_3 synthesis) which is found mainly in the kidneys **(Murayama *et al.*, 1999)**.

Endo *et al.* (2000) suggested that 1,25 $(OH)_3$ D_3 has the potential to alleviate hypocalcemia, through the inhibition of bone resorption.

There is evidence that gamma radiation damages bone tissue via free radical attack on the collagen **(Akkus *et al.*, 2005)**. Therapeutic doses of radiation have been shown to have deleterious consequences on bone health, occasionally causing osteoradionecrosis and spontaneous fractures **(Hamilton *et al.*, 2006)**.

Juan *et al.* (2002) reported that trans-3,4,5-trihydroxystibeneis a phyto-chemical present in peanuts and grapes with beneficial effects such as protection against cardiovascular disease and cancer prevention.

In the present study, several parameters were measured in male albino rats subjected to two different doses of gamma radiation, in order to clarify the role of peanut oil in protection against oxidative stress and bone injury.

Material and Methods

Male albino rats weighing 150 ± 200 g were divided into six categories each with six animals. They were kept under the same controlled laboratory conditions of temperature, lighting and ventilation. All rats were fed on standard casein diet and water *ad libitum*.

i) Normal: Control group.
ii) Peanut oil group: Rats were received peanut oil subcutaneously, (0.75 ml/kg) over one month period, on three days/week.
iii) γ- irradiated group: Rats exposed to single dose (5 Gy) of whole body gamma irradiation was carried out using ^{60}Co

source at National Centre for Radiation Research and Technology (NCRRT), Egypt.
iv) γ- irradiated group: Rats exposed to single dose (10 Gy) of whole body gamma irradiation was carried out using ^{60}Co source at National Centre for Radiation Research and Technology (NCRRT), Egypt.
v) Peanut oil followed by γ- irradiation group: Rats were received peanut oil subcutaneously, (0.75 ml/kg) over one month period, on three days/week before exposed to single dose (5 Gy) of whole body gamma irradiation.
vi) Peanut oil followed by γ- irradiation group: Rats were received peanut oil subcutaneously, (0.75 ml/kg) over one month period, on three days/week before exposed to single dose (10 Gy) of whole body gamma irradiation.

Radiation facilities:
Irradiation of animals (5 and 10 Gy) were performed at the National Centre for Radiation Research and Technology, Cairo, Egypt, using Cs-137 (Gamma Cell-40) giving a dose rate of 0.57 Gy/min at the time of experiment.

Biochemical analysis:
After the experimentation period, rats were then decapitated, blood samples were collected from heart by using disposable syringes and transferred to both dry and EDTA sterile test tube, Lipid peroxidation as malondialdehyde (MDA), reduced glutathione content (GSH), super oxide dismutase activity (SOD) and catalase (CAT) were assayed in ETDA blood **(Yoshioka *et al.*, 1979 ; Beutler *et al.*, 1963 ; Minami and Yoshikawa, 1979 and Johansson and Borg, 1988, respectively)**, sera were separated for estimation of Calcium (Ca.) **(Kaplan and Pesce, 1996),** Phosphorous (P.) (**Robert and Paul, 1937)**, Magnesium (Mg.) **(Willis, 1959)**, alkaline phosphatase and acid phosphatase **(Stephan *et al.*, 1977** and **Diana and Moss, 1961, respectively).**

The right femur of each animal was cleaned from surrounding tissues, weighed and crushed, then completely homogenated in 3 ml distilled water and kept frozen at – 20^{o}C till examination **(Kind and King, 1954)**. Samples, digested with concentrated nitric acid, were used for mineral estimation using an atomic absorption spectrophotometer.

Results

Table (1) presented MDA level, GSH content and SOD and CAT activities of rats exposed to two different doses of radiation with and without peanut oil administration.
Irradiated group recorded highly significant elevation ($P<0.001$) in MDA level, as compared with the corresponding non-irradiated group. In addition, it is evident from table (1) that irradiation caused significant depletion in GSH content and both SOD and CAT activities ($P<0.001$) on both doses (5Gy and 10Gy). Groups treated with peanut oil pre-irradiation turned the value of MDA level to its normal value, also peanut oil caused amelioration in GSH content and SOD and CAT activities.

The data in table (2) revealed that whole body gamma irradiation resulted in significant increase in serum calcium, inorganic phosphorous and magnesium contents with concomitant decrease in the bone Ca., P. and Mg. contents. Groups treated with peanut oil pre-irradiation resulted in sufficient amelioration in all investigated parameters.

Table (3) showed that, whole body gamma irradiation resulted in significant elevation in PTH and calcitonine with percentages of 73.9% and 63.1%, respectively. Treated groups by peanut oil pre-irradiation turned the values of PTH and calcitonine to their normal values in the group irradiated by 5 Gy and showed amelioration in their values in the group irradiated by 10 Gy.

The data in table (4) revealed that, irradiation caused significant decline in the

activities of alkaline phosphatase in both serum and bone with percentage of 44.5% and 53.1%, respectively. Also irradiation resulted in significant elevation in the activities of acid phosphatase in both serum and bone with percentage of 225% and 173%, respectively. Groups treated with peanut oil pre-whole body gamma irradiation resulted in sufficient amelioration.

Table (1): Lipid peroxides as malondialdehyde (MDA) level, glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activities in rats after whole body gamma irradiation and/or peanut oil administration.

Group	TBARS(MDA) (µmol/l)	GSH (mg/ml)	SOD (µg/ml)	CAT (µg/ml)
Control group	86.6 ± 4.7 (100%)	45.5 ± 3.1 (100%)	61.0 ± 3.6 (100%)	23.6 ± 1.1 (100%)
Peanut oil G.	87.0 ± 6.0 (100.4%)	46.0 ± 3.5 (101.0%)	62.2 ± 4.3 (101.9%)	24.0 ± 1.8 (101.6%)
Irradiated G.(5 Gy)	*** 122.0 ± 10.8 (140.8%)	*** 31.3 ± 2.6 (68.7%)	*** 34.6 ± 2.2 (56.7%)	*** 16.0 ± 1.0 (66.0%)
Irradiated G.(10 Gy)	*** 141.0 ± 11.2 (162.8%)	*** 28.2 ± 1.7 (61.9%)	*** 30.0 ± 1.9 (49.1%)	*** 11.2 ± 0.86 (46.6%)
Peanut oil + Irradiated G.(5 Gy)	90.8 ± 5.7 (104.8%)	40.4 ± 3.6 (88.7%)	54.4 ± 4.1 (89.1%)	* 20.0 ± 1.4 (84.7%)
Peanut oil + Irradiated G.(10 Gy)	102.6 ± 6.8 (118.4%)	** 33.0 ± 1.9 (72.5%)	** 48.3 ± 2.8 (79.1%)	** 18.3 ± 1.0 (77.5%)

G. = group
S. serum
Each value is the mean of 6 rats ± S.E.
Significant difference from control at * P <0.05, ** P <0.01 and *** P <0.001 as judged by Student t-test.

Table (2): Calcium (Ca), phosphorous (P) and magnesium (Mg) (both in serum and bone) concentrations in rats after whole body gamma irradiation and/or peanut oil administration.

Group	S. Ca. (mg/dl)	Bone Ca. (g/gwt)	S. P. (mg/dl)	Bone P. (g/gwt)	S. Mg. (mg/dl)	Bone Mg. (g/gwt)
Control group	8.6 ± 0.32 (100%)	0.6 ± 0.03 (100%)	3.8 ± 0.21 (100%)	88.0 ± 5.4 (100%)	3.4 ± 0.16 (100%)	105.0 ± 6.4 (100%)
Peanut oil G.	8.94 ± 0.4 (103.9%)	0.63 ± 0.02 (105.0%)	4..0 ± 0.3 (105.2%)	86.5 ± 4.4 (98.2%)	3.2 ± 0.2 (94.1%)	100.0 ± 7.1 (95.2%)
Irradiated G.(5 Gy)	** 12.9 ± 0.8 (150.0%)	** 0.45 ± 0.04 (75.0%)	*** 6.3 ± 0.36 (165.7%)	*** 48.5 ± 3.4 (55.1%)	*** 5.6 ± 0.36 (164.7%)	** 78.5 ± 5.9 (74.7%)
Irradiated G.(10 Gy)	*** 19.4 ± 0.7 (225.5%)	*** 0.29 ± 0.02 (43.3%)	*** 9.4 ± 0.54 (247.3%)	*** 32.3 ± 2.2 (36.7%)	*** 6.7 ± 0.7 (197.0%)	*** 56.0 ± 4.1 (53.3%)
Peanut oil + Irradiated G.(5 Gy)	9.3 ± 0.64 (108.1%)	* 0.52 ± 0.03 (86.6%)	4.5 ± 0.23 (118.4%)	79.0 ± 4.3 (89.7%)	3.1 ± 0.21 (91.1%)	96.3 ± 7.3 (91.7%)
Peanut oil + Irradiated G.(10 Gy)	10.2 ± 0.7 (118.6%)	** 0.42 ± 0.02 (70.0%)	** 5.2 ± 0.32 (136.8%)	** 61.6 ± 4.8 (70.0%)	** 4.8 ± 0.38 (141.1%)	* 88.0 ± 6.4 (83.8%)

Legend as in Table (1).

Table (3): Parathormone (PTH) and calcitonin levels in rats after whole body gamma irradiation and/or peanut oil administration.

Group	PTH (pg/ml)	Calcitonin (pg/ml)
Control G.	23.0±1.1 (100%)	3.8±0.17 (100%)
Peanut oil G.	22.8±1.4 (99.1%)	3.9±0.2 (102.6%)
Irradiated G.(5 Gy)	** 31.2±2.2 (134.4%)	** 5.0±0.32 (131.5%)
Irradiated G.(10 Gy)	*** 40.0±2.9 (173.9%)	*** 6.2±0.39 (163.1%)
Peanut oil + Irradiated G.(5 Gy)	24.6±2.0 (106.9%)	4.1±0.28 (107.8%)
Peanut oil + Irradiated G.(10 Gy)	** 30.0±2.6 (130.4%)	** 5.1±0.41 (134.2%)

Legend as in Table (1).

Table (4): Alkaline phosphatase (Alk. Ph.) and Acid phosphatase (Acid Ph.) (both in serum and bone) activities in rats after whole body gamma irradiation and/or peanut oil administration.

Group	S. Alk.Ph. (KAU/dl)	Bone Alk. Ph. (KAU/dl)	S. Acid Ph. (KAU/dl)	Bone Acid Ph. (KAU/dl)
Control G.	58.4±4.0 (100%)	45.3±3.2 (100%)	3.2±0.18 (100%)	9.7±0.6 (100%)
Peanut oil G.	59.0±3.4 (101.0%)	46.1±3.0 (101.7%)	3.4±0.2 (106.2%)	9.8±0.71 (101.0%)
Irradiated G.(5 Gy)	*** 34.3±2.0 (58.7%)	** 34.0±2.1 (75.0%)	*** 5.6±0.38 (175.0%)	* 12.2±0.94 (125.7%)
Irradiated G.(10 Gy)	*** 26.0±1.8 (44.5%)	*** 23.8±1.1 (53.1%)	*** 7.2±0.43 (225%)	*** 16.8±0.98 (173.1%)
Peanut oil + Irradiated G.(5 Gy)	52.2±3.9 (89.3%)	40.0±3.0 (88.3%)	* 3.9±0.24 (121.8%)	10.2±0.84 (105.1%)
Peanut oil + Irradiated G.(10 Gy)	** 44.5±3.2 (76.1%)	* 37.5±2.4 (82.7%)	*** 4.9±0.28 (158%)	** 13.4±0.76 (138.1%)

Legend as in Table (1).

Discussion

The use of ionizing radiation to kill tumor cells is a common treatment for cancer. Exposure to ionizing radiation causes radiolysis of water in tissues leading to generation of ROS, which are known to affect the antioxidant defense system and induce LPO **(Riley, 1994)**. The consequence of this increased free radical generation and imbalances in antioxidant defense is oxidative stress, which leads to oxidative damage, resulting in increased lipid peroxide levels. TBARS is used as an indicator of the rate of LPO which is accepted as tissue chain reaction **(Ohkawa *et al.*, 1979)**.

Gamma rays act either directly or by secondary reactions to produce biochemical lesions that initiate series of physiological symptoms. Ionizing radiation is known to induce oxidative stress through the generation of reactive oxygen species (ROS) resulting in imbalance of the prooxidant and antioxidant activities, ultimately resulting in cell death **(Srinvasan *et al.*, 2006)**. Numerous attempts were made to investigate different means for controlling and protection from radiation hazards using chemical, physical and biological means.

The results revealed significant acceleration in the oxidation of lipid associated with depletion in GSH level due to radiation exposure. It was argued that the oxidant/antioxidant imbalance due to oxidative stress is the main cause of the excessive formation of peroxides as MDA **(Oliinyk *et al.*, 2001)**. This response of antioxidant activity was attributed to the acute period of inflammatory processes developed during radiation exposure, which is characterized by the accumulation of lipid

peroxidation products **(Davydov *et al.*, 2000)**. The decrease in antioxidant enzyme activities and increase in the free radicals may be the main cause of irradiation – induced peroxidation and damage of cell activities. The significant acceleration in lipid peroxidation is attributed to peroxidation of the membrane unsaturated fatty acids due to free radicals propagation concomitant with the inhibition in bio-oxidase activity **(Pollack and Leeuwenburgh, 2000)**.

According to the present findings, peanut oil provided subcutaneously protects against biological effects caused by gamma irradiation. This protection may be due to its stimulating effects on antioxidant system or ability to prevent and/or react with the free radicals to convert them into non-harmful forms. Due to vitamin E content of peanut oil, it prevents lipid peroxidation chain reaction in the cell membrane. It was suggested that vitamin E undergoes this in two ways : by interaction with unsaturated fatty acid and protecting the polypeptide chain of proteins **(Cadenas and Barja, 1999 and Muazzez *et al.*, 2007)**.

A marked increase was noted in serum calcium content with concomitant decrease in the bone calcium content of irradiated group. The observed increase may be attributed to an increase in parathyroid hormone as mentioned by **Fujwara et al. (1994)**, and/or increase in the intestinal brush border membrane cation permeability **(Hizhnyak, 1997)**. On the other hand, the decline of bone calcium content may be due to bone demineralization after irradiation as reported by **Fukunda and Lida (1999)**.

The reduction in the calcium disturbances following irradiation in the peanut pre-irradiated group may be due to the vitamin E content of peanut oil providing a suitable level of zinc **(Farag, 1999)**, which enhances 1,25 $(OH)_3$ D_3 stimulated bone metabolism and/or protection against free radicals generated by irradiation **(Glscott *et al.*, 1996 and Yao *et al.*, 2005)**.

An increase in serum inorganic phosphorus concomitant with a decrease in bone phosphorous in irradiated groups is in accordance with the results of **Filipov *et al.* (1991)** and may be due to bone demineralization after irradiation, and/or the destruction or arrest of the activities of bone cells such as osteoblasts. The administration of peanut oil pre-irradiation seems to reduce radiation damage possibly due to its antioxidant effect **(Chen *et al.*, 2002)**.

The increase in serum content and concomitant decrease in bone content of magnesium may be a result of increased levels of parathyroid hormones, which stimulate magnesium absorption from the gut **(Hulter and Peterson, 1984)** and release of magnesium ion from bone **(Zofokova and Kancheva, 1995)**, as well as acceleration of bone resorption **(Chavelly and Rizzoli, 1999)**. Retention of magnesium levels to near normal in the peanut oil pre-treated group may be attributed to the protection of the sulfhydryl group (SH) from oxidative damage through inhibition of peroxidation of membrane lipids of rats **(Upasni and Balarman, 2001)**.

Whole body gamma irradiation produced elevation in the level of rat serum parathyroid hormone relative to the control group, a result which may indicate parathyroid odenoma and carcinoma caused by irradiation **(Christmas *et al.*, 1988)**. This increase may also be attributed to defective calcium absorption mechanism resulting from impaired hepatic and renal function, and consequently the formation of active metabolite, vitamin D **(Sotornik, 1997)**. The reduction in parathyroid damage following radiation in the peanut oil pretreated group could be due to presence of phytosterol, which have anticancer effect **(Awad *et al.*, 2000)**.

The increased calcitonin in the irradiated group may result from a feedback mechanism to overcome the increase in calcium and parathormone levels **(Fujiwara *et al.*, 1994**). The alteration in calcitonin level in the peanut oil protected group may be due to antioxidant activity, which tends to improve bone formation and

decrease bone resorption, thus, reduce serum calcium levels **(Arjmandi *et al.*, 2002)**.

The observed decline in serum and bone alkaline phosphatase in the irradiated group may be due to early decline in the intestinal alkaline phosphatase isoenzyme activity **(Stephan *et al.*, 1977)**. This decrease may also be attributed to a transitory reduction in the release of alkaline phosphatase to the enzymatic circulation by rapidly metabolizing cell **(Geraci *et al.*, 1991)**, and/or injury to the intestinal mucosa after irradiation as mentioned by **Faheim *et al.* (1993)**. The decrease in bone alkaline phosphatase in the irradiated group implies bone deformity resulting from an excess of resorption over formation **(Aitsula, 1986)**, as bone alkaline phosphatase is more specific as an important bone formation marker than is total alkaline phosphatase **(Khosla *et al.*, 1999)**.

The amelioration in alkaline phosphatase activity resulting from peanut oil pre-irradiation may be due to a beneficial effects on membrane permeability leading to the maintenance of a higher level in serum **(Juan *et al.*, 2002)**. In addition, the presence of strong antioxidant resveratol may increase alkaline phosphatase in osteoblastic cells to stimulate bone resynthesis, a view which is in accordance with **Mizutani *et al.* (1998)** and **Xu *et al.* (2010)**.

The elevated serum and bone acid phosphatase levels in the irradiated group may be attributed to the breakdown of lysosomal membranes by the lipid peroxidation effect of radiation, resulting in release of the enzyme **(Kumar *et al.*, 2003)**. In addition, irradiation may lead to lesion in the developing lysosomal membranes, through the action of oxygen free radicals, increasing membranes permeability and allowing acid phosphatase to escape **(Becciolni *et al.*, 1982)**. The elevated bone acid phosphatase may be due to the release of enzyme from osteoplast lysosomes as a result of bone resorption after irradiation **(Aitsula, 1986)**. The maintenance of more normal serum and bone acid phosphatase levels in the peanut oil protected group should be attributed to the free radical scavenging ability of vitamin E, which can suppress bone resorption and prevent membrane lesions.

Conclusions

It could be concluded that, administration of peanut oil before whole body irradiation resulted in sufficient amelioration against radiation effects on the biochemical aspects examined in the present study.

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الدور الوقائى لزيت الفول السودانى فى الجرذان المعرضه لجرعتين مختلفتين من اشعاعات جاما التى تنتج الضغط التأكسدى و إصابات العظام

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يهدف هذا البحث إلى توضيح دور زيت الفول السودانى كواقى إشعاعى فى ذكور جرذان الألبينو، مضاد للجهد المؤكسد و إصابات العظام المسببة بواسطة جرعتين مختلفتين لإشعاعات جاما.

تم تعريض الجرذان لجرعة إشعاعية مقدارها خمس جراى (المجموعة الثالثة) أو عشرة جراى (المجموعة الرابعة) (جرعة منفرده/الجسم كلة) بالمقارنة بمجموعة ضابطة (المجموعة الأولى).

قد أعطيت الجرذان زيت الفول السودانى عن طريق الحقن تحت الجلد قبل تعريضها للجرعتين الإشعاعتين، بمقدار(75, مل/كجم) لفترة قدرها شهر واحد مقسمة على ثلاث جرعات فى الأسبوع (المجموعة الخامسة و السادسة).

قد أعطيت الجرذان زيت الفول السودانى عن طريق الحقن تحت الجلد بجرعة مقدارها (75, مل/كجم)، و لكن بدون تعريضها للإشعاع (المجموعة الثانية).

تم قياس بعض مستويات أنظمة مضادات الأكسدة فى مصل الدم و الدم مثل: مستوى أكسدة الدهون (ثيوباربيتيوريك أسيد) و نشاط الجلوتاثيون و السوبر اكسيد ديسميوتيز و الكتاليز و الجلوتاثيون بير اكسيديز. و تم تقدير محتوى المعادن فى العظام و مصل الدم. و تم استقصاء بعض أنشطة الإنزيم مثل الكالسيوم الهرمونى الضابط.

يمكن التحكم فى التمثيل الغذائى إلى حد ما عن طريق إعطاء زيت الفول السوداني قبل التشعيع.