Belg. J. Zool., 138 (2) : 149-153 July 2008

Protective action of peanut oil in rats exposed to gamma-rays

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ABSTRACT. 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 ã-radiation. Rats were subjected to a dose of 5Gy, over an exposure time of 133sec, at a dose rate 3.759rad/sec. Prior to irradiation, rats received peanut oil subcutaneously, (0.75mL/kg) over a one month period, on three days/ week. Serum and bone mineral contents were estimated, and serum protein, cholesterol and creatinine concentrations were determined. We also investigated some enzyme activities as well as hormonal calcium control. It seems that the deleterious effects of exposure to ã-radiation on most estimated parameters affecting Ca metabolism can be controlled to some extent by peanut oil administration prior to irradiation.

KEY WORDS : Radiation, Peanut oil, Calcium metabolism.

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 radio-activity 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 damage.

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 8.7-10.7mg/dL (BROZOSKA & MONIUSZKO-JAKONIUK, 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 & kidney), Ca^{++} sensing receptors, and calcium regulating hormones: parathormone, calcitonin and 1,25-dihydroxy vit D_3, (HURWITZ, 1996). Parathormone and calcitonin positively regulate renal 1,α-hydroxylase gene expression (a key enzyme for $1,25(OH)_2D_3$ synthesis) which is found mainly in the kidneys (MURAYAMA et al., 1999). ENDO et al., (2000) suggested that $1,25(OH)_2D_3$ has the potential to alleviate hypocalcemia, through the inhibition of bone resorption. COLMAN et al., (2002) reported that calcitonin inhibits bone resorption by acting directly on osteoblasts, as it binds to high affinity osteoclastic receptors, and inhibits osteoclastic activity.

Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction (BERROUD et al., 1996). SHFRANOVSKAIA (2002) found that the combined effects of acute gamma-irradiation and thyroparathyroidectomy change the structure-functional state of sarcoplasmic reticulum membranes of rat skeletal muscles conditioned by the disturbance of hormonal regulation of molecular-cellular mechanisms of Ca^{++} exchange. 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-trihydroxystilbene is a phytochemical present in peanuts, grapes and wine with beneficial effects such as protection against cardiovascular disease and cancer prevention. We measured several parameters in male albino rats subjected to ã-radiation, in order to clarify the role of peanut oil in protection against oxidative stress and bone injury.

MATERIALS AND METHODS

Male albino rats weighing 150±20g were divided into four categories each with six animals:

- (i) Normal control.
- (ii) γ-irradiated group [^{60} Co (5Gy) exposure time 133sec, at a dose rate 3.759rad/sec] at the Middle Eastern Regional Radioisotope centre for Arab countries.
- (iii) Peanut oil treated group (0.75mL/kg) for one month, 3 days/week.
- (iv) Peanut oil followed by γ-irradiation.

After the experimentation period (1 month), animals were placed in metabolic cages for 24h. For urine collection a few drops of HCl were added to avoid fermentation. Rats were then decapitated, blood samples collected, urine 4-hydroxyproline determined, and sera were separated for estimation of Ca, P, Mg, creatinine, total protein, cholesterol, alkaline phosphatase, acid phosphatase, $1,25(OH)_2D_3$, PTH, calcitonin (BERG, 1982). The right femur of each animal was cleaned from surrounding tissues, weighed and crushed, then completely homogenated in 3mL distilled water, and kept frozen at -20°C till examination. A known volume of the homogenate was used for estimation of Alkaline and Acid phosphatises (KIND & KING, 1954). Samples digested with concentrated nitric

acid were used for mineral estimation using an atomic absorption spectrophotometer.

RESULTS

As shown in Table (1), exposure to γ-radiation caused significant increases in serum Ca, P, and Mg concentrations. Meanwhile a decline was detected in estimated bone minerals.

Table (2) shows that irradiation increased serum and bone acid phosphatase activity as will as serum creatinine levels, and decreased that of alkaline phosphatase. In addition, Table (3) shows that levels of parathormone, calcitonin, $1,25(OH)_2D_3$ as well as serum cholesterol were elevated in the irradiated group. Also urine hydroxyproline significantly increased following irradiation, but serum total protein declined significantly. In the peanut oil pre-irradiated group all these parameters tended to approach the values found in the controls.

TABLE 1

Serum and bone mineral content in control and treated rats ($\bar{x}$±SE).

Estimated / Animal group	Serum Ca mg/dL	Bone Ca g/g wt.	Serum P mg/dL	Bone P g/g wt.	Serum Mg mg/dL	Bone Mg g/g wt.
Control	8.62±0.22	0.59±0.013	3.9±0.22	87.0±1.6	3.3±0.14	104.4±5.4
Peanut oil	8.94±0.11	0.63±0.014*	5.1±0.1***	72.0±1.4***	3.1±0.04	95.9±1.1
Irrad.	12.9±0.14***	0.35±0.014***	6.3±0.18***	32.3±1.3***	5.6±0.3***	68.2±1.9***
Peanut oil + irradiated	°°°9.74±0.12***	°°°0.47±0.011***	°°°5.2±0.25***	°°°39.7±0.7***	°°°3.1±0.2	°°°82.7±1.4**

* Significant at P<0.05
** Highly significant at P<0.02
*** Very highly significant P<0.01 relative to control group
°° Highly significant at P≤0.02
°°° Very highly significant P≤0.01 relative to irradiated group

TABLE 2

Metabolic and enzymatic activity in serum or bone in control and treated rats.

Estimated / Animal group	Serum creatinine mg/dL	Serum alk. pase KAU/dL	Bone alk. pase KAU/ g wt.	Serum acid pase KAU/dL	Bone acid pase KAU/ g wt.
Control	0.7±0.03	57.8±0.6	44.8±0.5	3.1±0.3	9.8±0.3
Peanut oil	0.6±0.02**	59.4±0.7	47.5±0.7*	3.7±0.2	9.8±0.2
Irrad.	2.1±0.11***	26.0±0.4***	23.8±1.05***	7.0±0.4***	12.2±0.25***
Peanut oil + irradiated	°°°0.8±0.02*	°°°37.8±0.4***	°°°37.5±0.3***	°°4.9±0.3**	°°°8.7±0.3*

* Significant at P<0.05
** Highly significant at P<0.02
*** Very highly significant P<0.01 relative to control group
°° Highly significant at P≤0.02
°°° Very highly significant P≤0.01 relative to irradiated group

TABLE 3

Serum cholesterol, total protein and some hormonal content in control and treated rats.

Estimated / Animal group	Serum Cholesterol mg/dL	Serum total protein g/dL	Parath ornone PTH (pg/mL)	1.25 $(OH)_2$- D_3 pg/ml	Urine Hydroxyproline (µg/mL)	Calcitonin pg/mL
Control	203±5.8	7.1±0.3	22.9±0.15	19.8±0.8	5.13±0.08	3.7±0.06
Peanut oil	211±3.5	6.6±0.15	22.4±1.2	20.8±0.6	5.3±0.15	3.9±0.07
Irrad.	509±7.2***	4.5±0.3***	27.8±0.42***	32.2±1.4***	7.25±0.11***	4.0±0.04**
Peanut oil + irradiated	°°°364±4.5***	°°6.1±0.12*	°°°22.8±0.08	°°°23.1±0.4**	°°°5.8±0.1***	4.1±0.12*

* Significant at P<0.05
** Highly significant at P<0.02
*** Very highly significant P<0.01 relative to control group
°° Highly significant at P≤0.02
°°° Very highly significant P≤0.01 relative to irradiated group

DISCUSSION

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 pro-oxidant and antioxidant activities, ultimately resulting in cell death (SRINIVASAN et al., 2006). Numerous attempts have been made to investigate different means for controlling and protection from radiation hazards using chemical, physical and biological means.

In our experiments, a marked increase was noted in serum calcium content with concomitant decrease in the bone calcium content of irradiated groups. The observed increase may be attributed to an increase in parathyroid hormone as mentioned by FUJIWARA et al. (1994), and/or to an 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 FUKUDA & 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 $1,25(OH)_2D_3$-stimulated bone metabolism and/or due to protection against free radicals generated by irradiation, (GLASCOTT et al., 1996).

An increase in serum inorganic phosphorus concomitant with a decrease in bone phosphorus in irradiated groups (Table 1) is in accordance with the results of FILIPOV et al. (1991), and may be due to bone demineralization after irradiation, and/or to 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 & PETERSON, 1984) and release of magnesium ion from bone (ZOFOKOVA & KANCHEVA, 1995), as well as acceleration of bone resorption (CHAVELLY & RIZZOLI, 1999). Retention of magnesium levels to near normal in the peanut oil pre-treated group may be attributed to the protection of the sulfhydral group (SH) from oxidative damage through inhibition of peroxidation of membrane lipids in the liver and kidney of rats (UPASANI & BALARMAN, 2001).

The increased serum creatinine in the irradiated group indicates development of nephritis and renal dysfunction, a result in agreement with BORG et al. (2002). This result may be attributed to impairment of glomerular selective properties caused by irradiation (BERRY et al., 2001). In the peanut oil protected group, serum creatinine level remained close to normal; this may be explained by the ability of some antioxidant in peanut oil to scavenge free radicals generated by irradiation, which would otherwise cause kidney damage (NATH et al., 1994).

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 cells, (GERACI et al., 1991), and/or injury to the intestinal mucosa after irradiation as mentioned by FAHIM 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 effect on membrane permeability leading to the maintenance of a higher level in serum (JUAN et al., 2002). In addition the presence of the strong antioxidant resveratrol may increase alkaline phosphatase in osteoblastic cells to stimulate bone resynthesis, a view which is in accordance with MIZUTANI et al. (1998).

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 lesions in the developing lysosomal membrane, through the action of oxygen free radicals, increasing membrane permeability and allowing acid phosphatase to escape (BECCIOLINI et al., 1982). The elevated bone acid phosphatase may also be due to the release of enzyme from osteoclast 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.

A decline was registered in serum protein of the ã-irradiated group (Table 3), possibly caused by DNA damage after irradiation, resulting in subsegment changes in m-RNA causing impairment in gene transcription that could inhibit protein synthesis (LAI & SINGH, 1996). In addition, the formation of free radicals can cause breakage of chemical bonds and destruction of protein molecules (TENG & MOFFAT, 2000). The maintenance of more normal protein levels in the peanut oil pre-irradiation group may be due to trapping of free radicals by reservatrol, thus preventing DNA damage (CADENAS & BARJA, 1999).

Elevated serum cholesterol after irradiation (Table 3) may be due to the increased ability of the liver to biosynthesise cholesterol (CHEN & THACKER, 1985), as well as to the decreased activity of cholesterol 7-hydroxylase, the key enzyme involved in degradation of cholesterol in the liver, as mentioned by CHUPUKCHAROEN et al. (1985).

In addition, irradiation caused increases in serum LDLc by lipid peroxidation (NATH, 1996), leading to transport of cholesterol to extrahepatic tissue through LDLc receptor as mentioned by ABD-EL MOMEIN et al. (1989).

The considerably lower level of cholesterol in the peanut pre-irradiated group compared to the irradiated group may be attributed to the monounsaturated fatty acids (MUFA) present in peanut oil, which lowered the concen-

tration of circulating triglycerides and cholesterol (FELDMAN, 1999).

As presented in Table (3), 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 adenoma 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 the active metabolite, vitamin D (SOTORNIK, 1997). The reduction in parathyroid damage following radiation in the peanut oil pretreated group could be due to presence in the oil of phytosterols, which have an 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 of calcitonin level in the peanut oil protected group may be due to antioxidant activity, which tends to improve bone formation and decrease bone resorption and hence reduce serum calcium levels (ARJMANDI et al., 2002).

The increase in $1,25(OH)_2D_3$ seen in the irradiated groups may be an indication of increased half life of the compound due to disturbances in lipid solubility, rather than to an increase in its secretion (AMIZUKA et al., 1999). The increase in serum parathormone level after irradiation may also have led to this result (CHAVELLY & RIZZOLI, 1999). The increase in serum magnesium ion may also have contributed to the increase in $1,25(OH)_2D_3$ (RUDE et al., 1985). The amelioration in the serum level of $1,25(OH)_2D_3$ in the peanut oil protected group may be due to improvement in parathyroid activity assisted by the antioxidant properties of peanut oil (AWAD et al., 2000) and/or the restoration of the process of bone formation (ARJMANDI et al., 2002).

Hydroxyproline is the specific amino acid of collagen and is considered as a suitable marker for bone metabolism, reflecting its resorption. The increase in urinary hydroxyproline observed in the irradiated group is expected, and may be related to the destruction of bone collagen and bone resorption following irradiation (LIESESGANG et al., 1998). In addition, wounds caused by irradiation may also play a role in increasing hydroxyproline as reported by YANG et al. (2001). The lack of elevation in hydroxyproline levels seen in the peanut oil pretreated group may be due to inhibition of collagenase genes, by the vitamin E component of peanut oil, a view which is in accordance with (AZZI et al., 2001).

In conclusion single whole body Gamma irradiation of rats resulted in disturbances in calcium metabolism and the hormones influencing it. Peanut oil treatment pre-irradiation may play a protective role against these abnormalities.

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Received: May 16, 2007
Accepted: January 4, 2008