

Evening Primrose Oil Attenuates Certain Radiation Induced Functional and structural Disorders in Female Rats.

Fatma, L.R. and Rezk R. G.

Radiation Biology, Department . NCRRT.
Radiation Health Research Department NCRRT.

Abstract

The aim of the present study was to evaluate the modulator role of evening primrose oil (EPO) on the radiation induced changes in certain biochemical and histological abnormalities.

Female rats were exposed to 5 Gy whole body γ -irradiation delivered as single dose. EPO was orally administrated to rats (9 ml/100g b.wt) 7 days before irradiation exposure. Animals were sacrificed 1 day (oestrus stage), 6 days (one estrus cycle) and 12 days (two estrus cycle) after irradiation. The results obtained revealed that treatment with EPO diminished the increase in total cholesterol, urea and creatinine levels in plasma in comparison with the levels recorded in the plasma of irradiated rats. Significant amelioration of the radiation induced changes in RBC's WBC's count and the haemoglobin concentration, calcium level, FSH and LH hormones were also recorded in the plasma of EPO treated and irradiated rats. Histological observations of photomicrograph of kidney and ovary sections showed that irradiation resulted in ruptured, dilated, haemorrhage glomerulei dissolution of the majority of primary follicles, and atrophy in ovary size. All these changes were obviously improved in animals supplied with EPO.

It could be concluded that EPO could be useful adjunct for maintaining the integrity of biochemical functions and restoring the original histological architecture of kidney and ovary after irradiation.

Introduction

Evening Primrose oil derived from plant seeds was shown to possess antidiabetic and anti-inflammatory properties (Garland *et al.*, 1997 and Schalin-Karrila *et al.*, 1987). The oil contains the essential fatty acids Gamma linolenic acid (GLA) and linoleic acid (LA) vital to body health (Barcelli *et al.*, 1990). GLA is a precursor of prostaglandins a group of highly reactive molecules which plays a role in regulation of growth and reproduction (Horrobin, 1983), as well as in treatment of breast pain, tenderness and fluid retention occurred in premenstruation.

Radiation interacts with matter by direct and indirect processes to form ion pairs, some of which may be free radicals that lead to molecular damage translated to biochemical damage that may be then

amplified and expressed as biological injury (Cockerham *et al.*, 1994).

One of the systems most sensitive to irradiation is the hematopoietic system which is characterized by a depression in the peripheral blood levels after irradiation (Monroy, 1987).

The kidney was reported to be relatively radiosensitive compared to other abdominal organs. Pathophysiological and histopathological changes are dose and time dependent.

Pathologic changes in endothelial cells of renal microvasculature appears soon after exposure to radiation and later tubule and glomeruli degeneration are observed (Mettler & Moseley, 1985 and Yeas, 1992).

Radiosensitivity of the ova depends

on the maturity of the follicle. Irradiation of radiosensitive mature and intermediate follicles results in periods of temporary sterility followed by fertility due to maturation of surviving immature follicles (Robertson, 1989). Exposure to radiation reduces the formation of ovarian steroid hormones (Littly *et al.*, 1991). Exposure to large radiation doses produce premature menopause (Walden and Farzaneh, 1990) and elevate gonadotropin levels (FSH and LH) (Wallace *et al.*, 1989).

Due to the vital importance of EPO, the present work was undertaken to evaluate its role in minimizing radiation induced changes in hematopoietic system, the blood levels of total cholesterol, calcium, kidney functions, the blood levels of FSH and LH. Histological examination of kidney and ovary tissues were performed in parallel.

Material And Methods

Female albino rats weighing about (120-170g) were obtained from the animal farm of the Egyptian Organization for Vaccine and Biological Products. The animals were housed in cages and maintained under standard conditions of ventilation, temperature, and humidity. Food and water were available adlib. The animals were examined daily using the vaginal smear technique to determine the suitable estrus cycle according to Mahesh and Brann (1992).

Animals were categorized into four equal groups (each of 18 rats) as follows: Group 1: Control; Group 2: Evening Primrose oil; Group 3: Irradiated ; Group 4: Evening Primeose Oil and irradiated.

Irradiation was carried out using a ¹³⁷Cs source (Gamma cell-40) at National Center for Radiation Research and Technology (NCRRT, Cairo, Egypt) with a dose rate of 1 Gy/1.2 min at the time of experiment. Rats were exposed to 5 Gy whole body gamma irradiation delivered as a single dose. The rats were irradiated at proestrus stage (for measuring FSH and LH). The blood was collected in oestrus

stage one day, 6 days and 12 days after irradiation.

EPO after dilution in corn oil before use (9 ml/100g body weight) was orally administered to rats by means of a stomach needle for 7 consecutive days before irradiation exposure.

EPO was purchased from Glaxowellcome (Egypt). Animals were fasted over night and five rats were sacrificed from each group 24 hrs, 6 days and 12 days after irradiation.

Blood samples were collected into dry EDTA tubes and plasma separated by centrifugation. Kidney and ovary were removed for histological analysis.

1- Biochemical analysis:

Kidney functions were evaluated in separated sera using commercial kits for colourimetric measurements of creatinine and urea nitrogen (Henry, 1974). Blood samples were collected in dry EDTA tubes used for the investigation of erythrocytic counts (RBC's), total leucocytic count (WBC's) and estimation of haemoglobin concentration according to Dacie and Lawis (1993).

Serum total cholesterol content was determined according to the method of Allain *et al.*, (1974). Calcium level was evaluated according to Stem and Lewis (1957).

FSH and LH were assayed using radioimmunoassay technique (Radbard, 1974 and Garza *et al.*, 1975).

2- Histological analysis:

For the histological preparations, serial paraffin sections of the kidney and ovary were prepared. The kidney and ovary were immediately excised, fixed in buffered formol, processed routinely for paraffin embedding and sectioned at 6µm. All sections were simultaneously stained with haematoxylin, and eosin (HE) in an automatic slide stainer and mounted with Canda balsam. Sections were examined by Zeiss light microscope to detect the histopathological changes.

The students T-test (Senedecor and Cochran, 1978) was applied for the

statistical analysis of the results. The results were considered significant at $P < 0.05$.

Results

1- Biochemical Analysis:

Data on experimental animals showed that normal healthy rats treated with 9ml EPO/100g body weight did not show any obvious harmful deleterious side effects but showed normal RBC's, WBC's count and haemoglobin concentration (Table 1). Normal total cholesterol, calcium (Table 2). Normal levels of urea and creatinine (table 3) and normal levels of FSH and LH contents in serum (table 4).

Whole body gamma irradiation of rats exposed to 5 Gy single dose resulted in significant drop in RBC's, WBC's count and haemoglobin concentration (table 1), significant increase in the level of total cholesterol, significant decrease in calcium level (table 2). A significant increase in plasma urea nitrogen and creatinine levels (table 3). A significant elevation in serum FSH and LH was recorded in all time intervals after irradiation as compared with control values (table 4).

However, treatment of irradiated rats with 9ml/100g body weight of EPO had significantly modulated the radiation induced decrease in RBC's, WBC's count and haemoglobin concentration (table 1). EPO ameliorated and reduced significantly the induced changes recorded in the above investigated parameters (table 2, 3 and 4).

2- Histological observations:

Kidney:- sections of control rats showed a normal renal cortex with normal renal corpuscles and the surrounding proximal and distal convoluted tubules. This section includes both vascular pole and the tubular pole of the renal corpuscle. At the vascular pole, normal lumen of an arteriole (a) and the macula densa (md) in the wall of the distal tubule could be distinguished. Some lacis cells could also be seen between the arteriole and the macula densa. At the tubular pole, the capsular space is continuous with the lumen of the proximal convoluted tubule (Pt) and normal proximal convoluted tubule (PCt). In contrast, cross sections of

the distal convoluted tubule (dct) exhibited more nuclei in the polar staining walls and lack a distinct striated border (Fig.1). Kidney section of rats treated with EPO showed normal renal cortex with normal corpuscle and the surrounding proximal and distal convoluted tubules (Fig. 2).

Whole body exposure of rats to 5Gy gamma irradiation delivered as single dose, resulted in loss of normal renal cortex with loss in corpuscle structure, widened, dilated and rupture proximal and distal convoluted tubules (Fig.3) After 6 & 12 days radiation showed extremely widening and dilation, ill-defined structure of glomerulai, haemorrhage, high content of inflammatory cells and fibroblasts in the glomerulai and surrounding it, ill defined structure of proximal and distal convoluted tubules (Fig. 4 & 5).

Treatment of rats with Evening Primrose Oil led to improvement of renal cortex and preserved renal corpuscles architecture and the surrounding proximal and distal convoluted tubules. The changes recorded after 24 hrs, 6 and 12 days post irradiation were less pronounced as compared to those recorded with irradiated rats (Fig. 6 , 7 & 8).

Ovary sections of control rats showed normal developing ovarian follicles. Primordial follicles, with one layer of flattened follicular cells. Normal corpus luteum with granulosa cells and theca cells (fig.9)

Ovary:- section of rats treated with Evening Primrose Oil showed normal developing ovarian follicles. Mature corpus luteum with granulosa cells and theca cells (fig. 10).

The effect of irradiation on ovarian tissue is clearly shown in Fig.11 in the form of primary and secondary follicles with abnormal shape and dissolution of the majority of the follicles. Absence of mature follicles. Fibroblasts filled the section. These features were after 24 hrs post irradiation.

After 6 and 12 days post irradiation, the section showed extremely dissolution and ill-defined shaped follicles, absence of immature follicles, whereas high content of fibroblasts filled the sections (Fig. 12 & 13).

A significant protection was afforded when animals were supplied with EPO pre irradiation exposure. The ovary sections showed well defined germinal epithelium, Tunica albuginea, different stages of follicles formation as perimordial follicles, primary follicles and mature corpus luteum

with granulosa cells, well defined thecal cells. These feature were seen after 24 hrs after irradiation (Fig. 14). While after 6 and 12 days regeneration of primordial, primary and secondary follicles with granulosa cells and regeneration of normal thecal cells (Fig. 15 & 16).were seen.

Table (1): Haematological values of female rats at various time entervals after whole body gamma irradiation (5 Gy) and / or Evening Primrose treatment.

Group	days	Haemoglobin g/dl	RBC's count x 10 ⁶ /ml	WBC's count x 10 ³ / ml
C		Mean ± SE 14.97 ± 0.002	5.45 ± 0.009	5.99 ± 0.008
R	1	12.02 ± 0.007*	4.42 ± 0.302*	3.90 ± 0.130*
	6	10.45 ± 0.126*	3.75 ± 0.008*	3.28 ± 0.142*
	12	11.25 ± 0.003*	3.55 ± 0.105*	4.40 ± 0.005*
EPO	1	14.80 ± 0.006	5.45 ± 0.006	5.900 ± 0.122
	6	14.89 ± 0.005	5.33 ± 0.005	5.88 ± 0.185
	12	14.50 ± 0.109	5.40 ± 0.208	5.98 ± 0.125
EPO+R	1	12.45 ± 0.13 ^{*a}	4.49 ± 0.009*	5.16 ± 0.172 ^a
	6	12.80 ± 0.005 ^{**}	4.99 ± 0.299 ^{*a}	5.31 ± 0.004 ^a
	12	12.65 ± 0.004 ^{*a}	4.45 ± 0.007 ^{*a}	5.46 ± 0.291 ^a

Each value represents mean of 5 observations ± SE

(*) mean significantly different from control group P ≤ 0.05

(a) mean significantly different from irradiated group P ≤ 0.05

Table (2): Serum cholesterol and calcium levels in blood of female rats at various time intervaes after whole body gamma irradiation (5 Gy) and / or EPO treatment.

Group	days	Total cholesterol mg/ml	Calcium mg/ml
C		Mean ± SE 58.72 ± 0.008	11.60 ± 0.010
R	1	91.91 ± 0.0003*	8.50 ± 0.114*
	6	108.40 ± 0.009*	8.02 ± 0.12*
	12	89.46 ± 0.006*	7.16 ± 0.004*
EPO	1	58.75 ± 0.007	11.59 ± 0.007
	6	58.87 ± 0.355	11.65 ± 0.123
	12	59.07 ± 0.491	11.51 ± 0.007
EPO+R	1	73.55 ± 0.107 ^{*a}	10.60 ± 0.001 ^{*a}
	6	76.08 ± 0.0007 ^{*a}	10.85 ± 0.004 ^{*a}
	12	74.46 ± 0.116 ^{*a}	10.50 ± 0.007 ^{*a}

Each value represents mean ± SE of 5 observations + SE.

(*) mean significantly different from control group at P ≤ 0.05

(a) mean significantly different from irradiated group P ≤ 0.05

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Table (3): Serum Urea and Creatinine levels in blood of female rats at various time intervals after whole body gamma irradiation (5 Gy) and / or EPO treatment.

Groups	days	Serum Urea mg/dl	Serum Creatinine mg/dl
C		16.42 ± 0.007	0.86 ± 0.05
R	1	40.90 ± 0.264*	1.25 ± 0.005*
	6	48.70 ± 0.181*	1.40 ± 0.007*
	12	38.11 ± 0.259*	1.07 ± 0.001*
EPO	1	17.51 ± 0.11*	0.92 ± 0.001
	6	17.01 ± 0.13*	0.99 ± 0.004
	12	17.74 ± 0.003*	1.02 ± 0.002
EPO+R	1	30.05 ± 0.005 ^{*a}	1.19 ± 0.002*
	6	25.31 ± 0.113 ^{*a}	1.12 ± 0.003 ^{*a}
	12	20.01 ± 0.004 ^{*a}	1.04 ± 0.007 ^{*a}

Table (4): The concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the sera of female rats at various time intervals after whole body gamma irradiation and / or Evening Primrose oil treatment

Groups	days	FSH ml μ/ml	LH ml μ/ml
C		6.25±0.005	7.086±0.0005
R	1	9.33±0.008*	8.42±0.004*
	6	11.43±0.139*	8.99±0.0009*
	12	18.01±0.0001*	11.59±0.007*
EPO	1	6.07±0.001*	7.91±0.0001*
	6	7.20±0.007*	7.21±0.007
	12	7.70±0.001*	6.75±0.007*
EPO+R	1	8.15±0.003 ^{*a}	8.27±0.003 ^{*a}
	6	9.35±0.112 ^{*a}	8.39±0.007 ^{*a}
	12	10.53±0.177 ^{*a}	9.22±0.006 ^{*a}

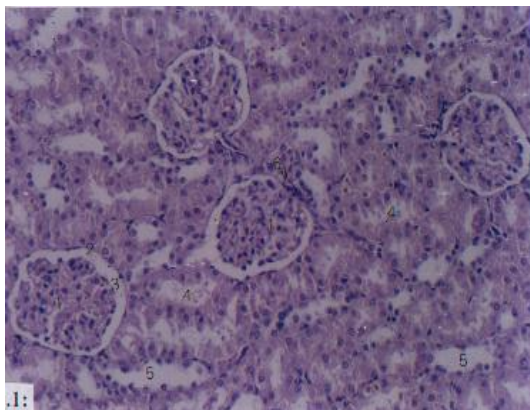


Fig.1: Photomicrograph of a section in the kidney of control rat showing normal renal cortex architecture with normal 1. glomerulus 2. glomerular capsule 3. urinary space between the glomerulus and the capsule .4. proximal convoluted tubules are dark staining.5. distal convoluted tubules are lighter staining .6. macula densa (X.200)

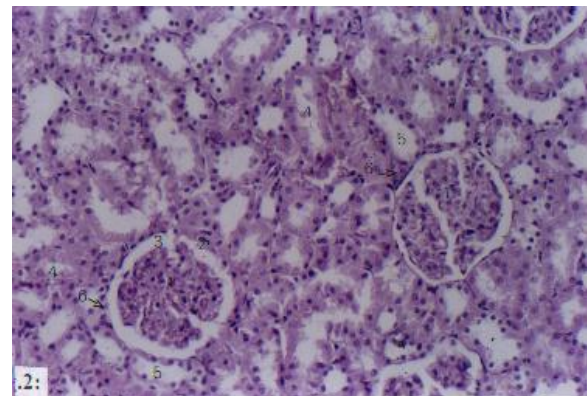


Fig.2: Photomicrograph of a section in the kidney of Evening Primrose Oil treated rat showing normal renal cortex architecture with normal. 1. glomerulus. 2. glomerular capsule 3. urinary space between the glomerulus and the capsule. 4. proximal convoluted tubules are dark staining and 5. normal distal convoluted tubules are lighter staining.6. macula densa. (X200).

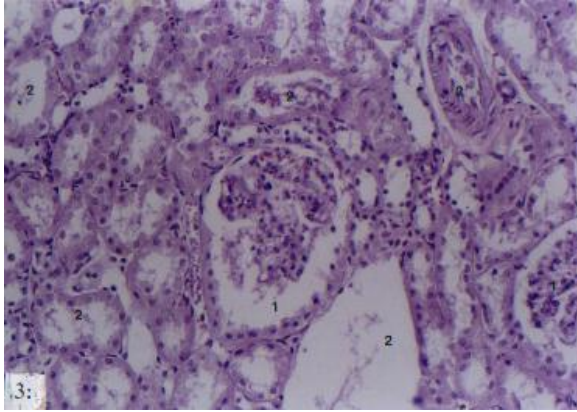


Fig.3: Photomicrograph of a section in the kidney of rats 24 hrs after irradiation showing loss of normal renal cortex, loss renal corpuscle structure. 1. Widened, dilated glomerulus .2. ruptured and dilated proximal and distal convulated tubules (X 200).

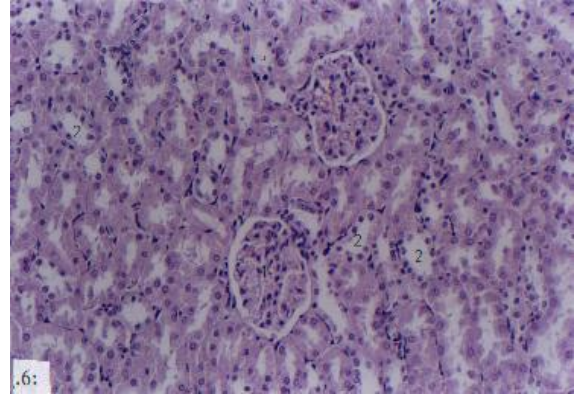


Fig.6: Photomicrograph of a section in the kidney of Evening Primrose Oil treated rat 24 hrs after irradiation showing normal renal cortex with 1. well-defined glomerulus structure. 2. regeneration of distal and proximal convulated tubules (X 200).

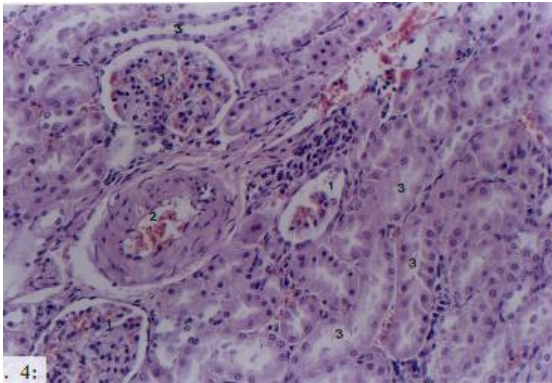


Fig. 4: Photomicrograph of a section in the kidney 6 days after irradiation showing 1. Ill-defined glomerulai.2. glomerulus sclerosis.3. Extremely widened, dilated and ill-defined proximal & distal convulated tubules (X 200).

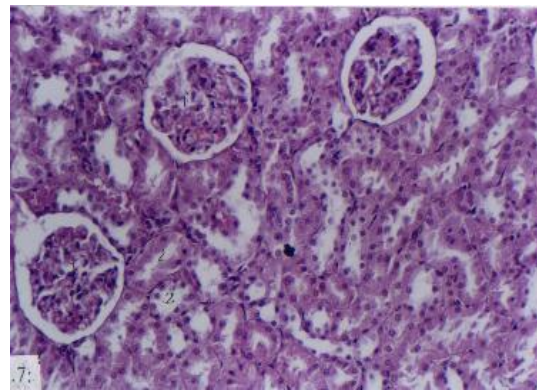


Fig.7: Photomicrograph of a section in the kidney of Evening Primrose Oil treated rat 6 days post irradiation showing 1. improved glomerulai structure 2. regeneration of distal and proximal convulated tubules (X 200).

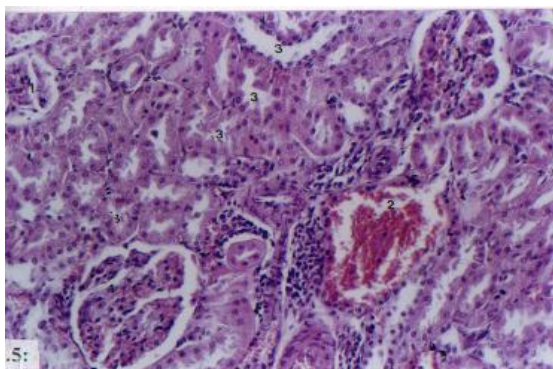


Fig.5: Photomicrograph of a section in the kidney 12 days after irradiation showing 1. ill-defined glomerulai. 2. high content of inflammatory cells and fibroblasts, haemorrhage in the glomerulai and surrounding it. 3. ill-defined structure of proximal and distal convulated tubules.

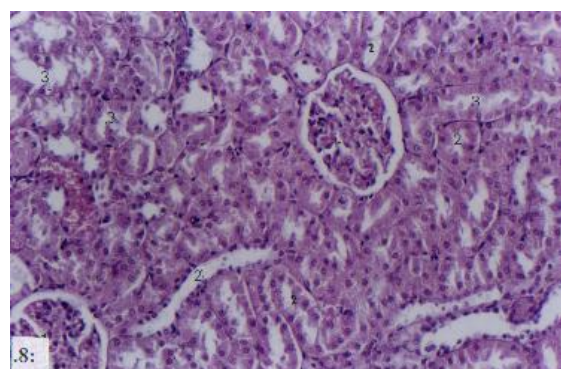


Fig.8: Photomicrograph of a section in the kidney of Evening Primrose Oil treated rat 12 days post irradiation showing 1. well-defined structure of glomerulai. 2. Some proximal and distal convulated tubules were regenerated and. 3. Some still ill-defined structure.

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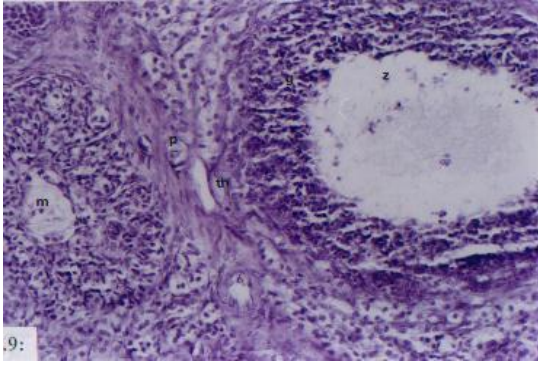


Fig.9: Photomicrograph of a section in the ovary of a control rat showing normal primordial follicles with flattened follicular cells (P). 2. normal ripening follicle with stratified follicular epithelium (granulosa) (g), and nature corpus luteum (m). zona pellucida (z) and developing theca (th).

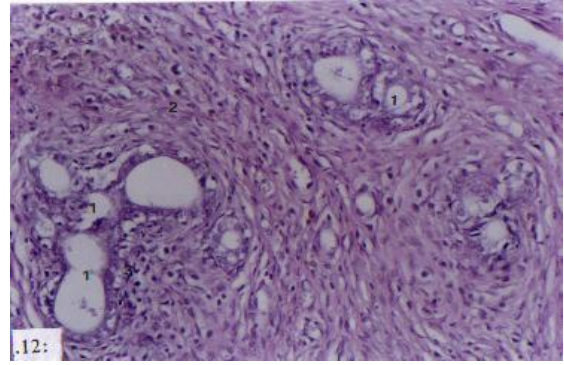


Fig.12: Photomicrograph of a section in the ovary of rats 6 days post irradiation showing 1. dissolution of the ovarian follicles. 2. high content of fibroblasts filled the section .3. inflammatory cells around the dissolved follicles (X200).

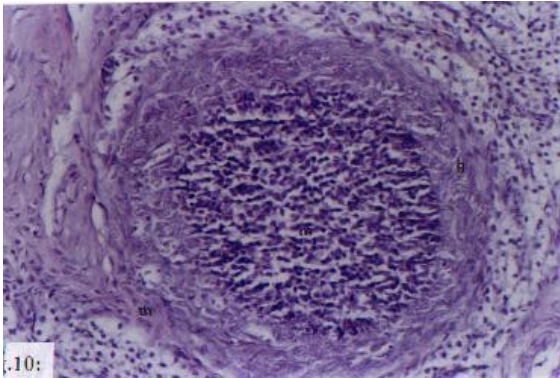


Fig.10: Photomicrograph of a section in the ovary of Evening Primrose Oil treated rats showing normal developing ovarian follicle with mature corpus luteum (m), normal granulosa cells (g) and theca cells (th) (X200)

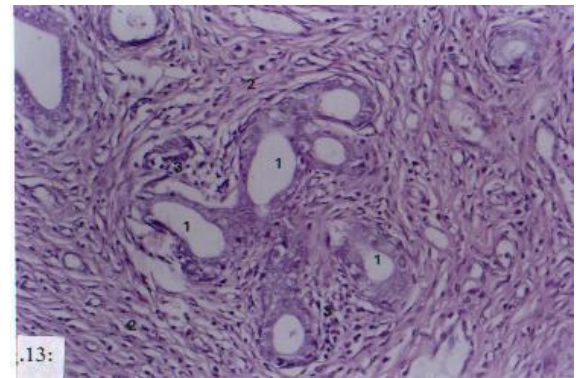


Fig.13: Photomicrograph of a section in the ovary of rat 12 days post irradiation showing 1. dissolution and abnormal shaped ovarian follicles .2. high content of fibroblasts filled the section .3 inflammatory cells surrounded the dissolved follicles (X 200).

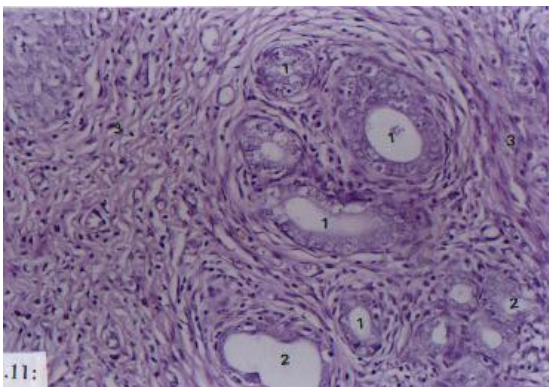


Fig.11: Photomicrograph of a section in the ovary of rats 24 hrs after irradiation showing 1. abnormal shaped primary and secondary follicles .2. dissolution of follicles . 3. high content of fibroblasts filled the section (X200).

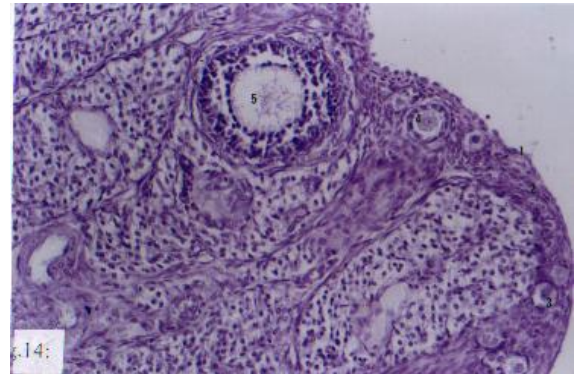


Fig.14: Photomicrograph of a section in the ovary of Evening Primrose Oil treated rats after 24 hrs post irradiation showing 1. well-defined germinal epithelium, 2. normal tunica albuginea. 3. normal primordial follicles. 4. normal primary follicles. 5.will-defined corpus luteum with normal granulosa cells and thecal cells (X200).

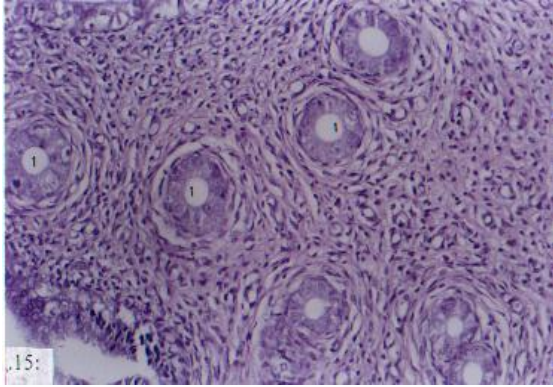


Fig.15: Photomicrograph of a section in the ovary of Evening Primrose Oil treated rats after 6 days of irradiation showing 1. regeneration of different stages of ovarian follicles (X 200)

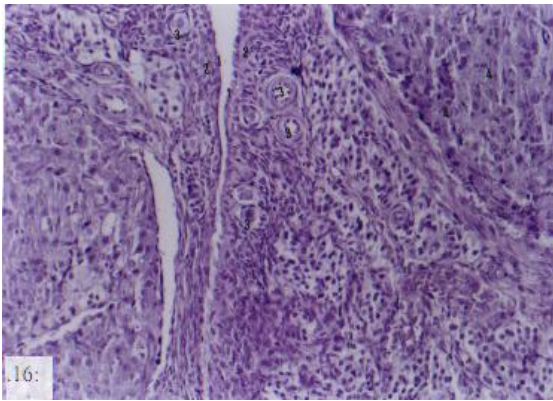


Fig.16: Photomicrograph of a section in the ovary of Evening Primrose Oil treated rats 12 days post irradiation showing 1. normal germinal epithelium. 2. normal tunica albuginea .3. regeneration of perimordial and primary ovarian follicles. 4. well-defined shaped corpus luteum with granulosa cells and theca cells (X 200).

Discussion

Exposure to ionizing radiation is characterized by excessive production of reactive oxygen species (ROS) associated with inflammatory process (Beyzadeoglu *et al.*, 1997).

The results obtained in the present study showed that whole body exposure of rats to γ -irradiation resulted into significant drop in RBC's , WBC's and haemoglobin concentration, significant increase in the level of total cholesterol, significant decrease in calcium level, significant increase in plasma urea and creatinine level and significant elevation in serum FSH and

LH. Histological abnormalities in the kidney and ovary tissues were also observed in irradiated rats.

Evening primrose oil administered pre-irradiation, ameliorated the disturbance, in the parameters under investigation.

In this work, the significant decrease in haemoglobin concentration recorded in irradiated rats could be related to a decrease in haemoglobin affinity for oxygen (Saad El-Deen *et al.*, 1996), atrophy of bone marrow and destruction of RBC's (Abu-Seif *et al.*, 2003).

The decline in total leucocytic count (T.L.C.) might be due to change in lymphocytes and neutrophils count (Roushdy & Ashry, 1979 and Fekry *et al.*, 1999), as well as mitotic changes ; lymphocytes share with the oocytes the property that they die in the interphase after irradiation (Abou-Safi *et al.*, 1999).

Administration of EPO prior to irradiation induced a significant amelioration of these changes, which might be attributed to the presence of (GLA) n-3 and (LA) n-6. Stone *et al.*, (1979) reported that EPO minimizes antithrombotic changes and increase the capacity of platelets to synthesize prostaglandin E₁ (PGE₁), Urakaz *et al.*, (1987) explained that n-3 fatty acid supplementation increases platelets and red blood cells counts 5 times due to its effect on platelets aggregation and fatty acids composition in platelets and red blood cell membranes (De La Cruz *et al.*, 1997).

Furthermore, EPO has an inhibitory effect on cyclooxygenase activity and increase arachidonic acid in erythrocytes membranes (Corbett *et al.*, 1991).

The significant elevation in plasma total cholesterol content might be due to the stimulation of cholesterol synthesis in the liver after 10-40 min of radiation (Kaznacheev *et al.*, 1985). Moreover, Iiling worth *et al.*, 1995 contributed that irradiation induced increased number of low density lipoprotein (LDL). Also Cerin *et al.*, 1993 showed that cholesterol which was higher in the follicular phase in women with premenstrual syndrome. The high level of cholesterol in irradiated rats that were accompanied by significant amelioration in cholesterol level observed in EPO

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treated irradiated rats might be due to the presence of (GLA) n-3 and (LA) n-6 of EPO that inhibit the increasing of plasma total cholesterol (Fukushima *et al.*, 1997).

In this work, whole body γ -irradiation of Ca⁺⁺ rats (5 Gy) resulted into a significant decrease in plasma calcium level that might due to Ca ions activation by absorbed radiation energy and (Ca) reaction with free radicals. Furthermore, hypocalcemia might be due to increase in renal calcium excretion. The results obtained showed that a high level of cholesterol content was accompanied by a low level of calcium.

This correlate with the vice versa relation of calcium and cholesterol (Garland *et al.*, 1997).

In the present study, the increased level of plasma urea and creatinine after irradiation reflected renal damage. This elevation might be related to slight increase in tissue hydration at the estrus stage (Abou-Safi, 1998), also associated with increased estrogen level in blood and adrenal corticosteroids showed heterogeneous sensitivity to irradiation and may also result from reduction in effective renal plasma flow and glomerular filtration rate (Robbins *et al.*, 1989 and Robbins *et al.*, 1991).

Pathologic changes in endothelial cells of the renal microvasculature, tubules and glomeruli degeneration were seen after exposure to radiation (Yaes, 1992). The vital role played by the proximal and distal convoluted tubules in selective reabsorption of Na, Ch, Ph, Ca, amonia, creatinine and para-amino hippuric acid is injured. This may be due to after exposure radiation subchronic changes as intimal necrosis, subendothelial fibrinoid thrombosis, atrophy of tubules and replacement of collagen (Mettler & Moseley, 1985).

In the present study, sections of the kidney tissue of irradiated rats showed loss of normal renal cortex with loss in corpuscle structure, widened, dilated, atrophy and ruptured proximal and distal convoluted tubules, ill-defined glomeruli, haemorrhage, inflammatory cells and

fibroblasts in the glomeruli surrounding and filling it.

The results obtained from the present study depicted that the protection with EPO pre irradiation played an effective role in improving disorders induced by irradiation. Barcelli *et al.*, 1986, found that EPO and other polyunsaturated fatty acids when fed to partially nephrectomized rats have favorable effects on the progression of renal failure. The author found that changes induced in platelets production of thromboxane A₂ and the renal production of various eicosanoids act as protective role of oils.

The protective role of EPO might be mediated by its efficiency to minimize nephrotoxicity (Darlametsos and Varonos, 2001).

EPO reduces inflammation and caused a significant rise in the amount of dihomogammalinolenic acid in plasma phospholipid fatty acids (schalin-Karrila *et al.*, 1987). Also dietary EPO offset nephrotoxic effects and may be useful in minimizing hypertension (Mills *et al.*, 1992).

Furthermore, diet prepared with EPO had a clear beneficial effect on glomerular sclerosis and tubular abnormalities (Barcelli *et al.*, 1990).

In the present study, there was reversible relationship between secretion of gonadotropin and the period of time, where LH level was greater in the follicular phase.

On the contrary, FSH level was greater in the follicular phase than during luteal phase. Also, hypersecretion of Fsh and LH as observed after whole body gamma irradiation might result either from activation of hypothalamic pituitary axis or as a positive feed back mechanism in response to the lowered levels of estradiol and progesteron in serum. Also radiation induced damage of central nervous system may be reflected on hormonal secretion (Saleh *et al.*, 1988).

Administration of EPO before irradiation ameliorates the radiation induced hormonal changes that might be mediated by prostaglandin, which play a role in the pathophysiology of premenstrual syndrome and ovulation.

This work showed that radiation induced injuries on ovarian tissue in the form of abnormal shape and dissolution of the majority of the follicles and absence of mature follicles. Furthermore, the theca interna and granulosa cells of the ovarian follicles and the corpus luteum had ruptured and ill-defined shaped.

Changes after radiation exposure severely reduces the formation of steroid hormones, even to the point of gonadal failure (Littley *et al.*, 1991 and Wallace *et al.*, 1989). Persistently elevated gonadotropin levels (FSH and LH) and amenorrhea were associated with the reduced ovarian hormones (Littley, *et al.*, 1991). This might be related with injured theca interna and granulosa cell of ovarian follicles Ganong, 1989. Abdominal radiation of females in childhood resulted in pubertal failure or premature menopause (Wallace *et al.*, 1989).

In this work significant protection and amelioration was afforded when animals were supplied EPO pre-irradiation. The ovarian sections showed mature corpus luteum, well-defined shaped granulosa and thecal cells of ovarian follicles.

Essential fatty acids in EPO are found in the structural lipids of the cells, are concerned with the structural integrity of the mitochondrial membrane and occur in high concentration in the reproductive organs (Harper *et al.*, 1979).

Horrobin, 1983 demonstrated that EPO is a highly effective treatment for the depression and irritability, the breast pain and tenderness, and the fluid retention associated with the premenstrual syndrome. Nutrients known to increase the conversion of essential fatty acids to prostaglandin, E₁ include magnesium, zinc, niacin and ascorbic acid. The clinical success obtained with some of these nutrients may in part be related to their effects on essential fatty acid metabolism.

It could be concluded that EPO rich in essential fatty acids gamma linolenic acid and linoleic acid might play a role in the modulation of radiation-induced biochemical and histological damage.

References:

1. **Abou-Safi, H. M. (1998):** Physiological impairment of irradiation drugs estrus cycle in rats and role of combined treatment with vitamins A and B group. *Egypt. Med. Sci.* 19 (1), 157.
2. **Abou-Safi, H. M.; Hussein, A. H. and El-Sayed, N. M. (1999):** Efficacy of Vitamin A and / or E as antioxidants against serum glucose, liver glycogen and lipid discrepancy induced by gamma radiation during the estrus cycle of rats. *Egypt. J. Rad. Sci. Applic.* 12 (2), 51.
3. **Abou-Seif, M. A.; El-Naggar, M. M.; El-Far, M.; Ramadan, M. and Salah, N. (2003):** Prevention of biochemical changes in gamma irradiated rats by some metal complexes. *Clin. Chem. Lab. Med.* 41 (7), 926.
4. **Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W. and FU, P. C. (1974):** Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20 (4), 470.
5. **Barcelli, U. O.; Miyata, J.; Ito, Y.; Gallon, L. and Pollakve, U. (1986):** Beneficial effects of polyunsaturated fatty acids in partially Nephrectomized rats. *Prostaglandins* 32 (2), 211.
6. **Barcelli, U. O.; Weiss, M.; Beach, D.; Motz, A.; Thompson, B. (1990):** High linoleic acid diets ameliorate diabetic nephropathy in rats. *Am. J. Kidney Dis.* 16 (3), 244.
7. **Beyzadeoglu, M.; Balkan, M.; Demiriz, M.; Birican, B. (1997):** Protective effect of Vitamin A on acute radiation injury in the small intestine. *Rad. Med.* 15 (1), 1.
8. **Cerin, A.; Collins, A.; Landgren, B. M. and Eneroth, P. E. (1993):** Hormonal and biochemical profiles of premenstrual syndrome. Treatment with essential fatty acids. *Acta Obstet. Gynecol. Scand* 72 (5), 337.
9. **Cockerham, L. G.; Mickly, G. A.; Walden, T. L.; Jr. and Stuart B. O. (1994):** Ionizing radiation In: Principles and Methods of toxicology. 3rd. Ed. By A. Wallace Hayes. Raven press, Ltd. New York. Ch. 13 p. 456.
10. **Corbett, R.; Menez, J. F.; Floch, H. H. and Leonard, B. E. (1991):** The effect of chronic Ethanol administration on rat liver and erythrocyte lipid composition modulatory role of Evening Primrose oil. *Alcohol* 26 (4), 459.

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11. **Dacie, J. V. and Lewis, M. S. (1993):** Practical haematology 6th Ed., Ch.5 churchill livingstone, London and New York. 37.
12. **Darlametsos, I. E. and Varonos, D. D. (2001):** Role of prostanoids and endothelins in the prevention of cyclosporin-induced nephrotoxicity. Prostaglandins Leukot. Essent. Fatty Acids 64 (4-5), 231.
13. **De La cruz, J. P.; Martin-Romero, M.; Carmona, J. A.; Villalobos, M. A. and Se La cuesta, F. (1997):** Effect of Evening Primrose oil on Platelets aggregation in rabbits an atherogenic diet. Thromb. Res. 87 (1), 141.
14. **Fekry, A. E.; Samia, E. and Hegazy, R. A. (1999):** Influence of Methandienone on serum protein fractions and some physiological parameters in gamma irradiated rats. Egypt. J. Rad. Sci. Applic. 12 (2), 117.
15. **Fukushima, M.; Matsuda, T.; Yamagishi, K.; Nakano, M. (1997):** Comparative hypocholesterolemic effects of six dietary oils in cholesterol fed. Rats after long-term feeding. Lipids 32 (10), 1069.
16. **Ganong, W. F. (1989):** Review of Medical physiology, 14th ed. Appleton and Lange, San Maleo, CA.
17. **Garland, H. O.; Forshaw, A. G. and Sibley, C. P. (1997):** Dietary essential fatty acid supplementation urinary calcium excretion and reproduction performance in the diabetic pregnant rat. Endocrinol. 153 (3), 357.
18. **Graz, R.; Buras, B. J.; Manlimos, F. and Abraham, G. (1975):** Analytical letters 8, 225 cited from saleh (1988).
19. **Harper, H. A.; Rodmell, V. W. and Mayes, P. A. (1979):** Metabolism of lipids In: Review of physiological chemistry, 17th ed. Middle East Edition, Librairie du Liban, Lange Medical publications. P. 333.
20. **Henry, J. B. (1974):** Clinical diagnosis and measurement by laboratory methods. Todd. Sanford, Davidsohn, 16th ed. W. B. Saunders. Philadelphia, P. A. P. 260.
21. **Horrobin, D. F. (1983):** The role of essential fatty acids and in the premenstrual syndrome. J. Reprod. Med. 28 (7), 465.
22. **Illingworth, D. R.; Duell, P. B. and Connor, W. E. (1995):** Disorders of lipid metabolism. Edit by Felig, P.; Baxter, S. D. and Forhman, L. A. 3rd Internation. Edition Mc Crow-Hill, Inc. 1315.
23. **Kaznacheev, Y. U. S.; Kulagina, T. P.; Markovich, L. N. and Kalonytseva, I. K. (1983):** Radiation induced changes in lipid content of liver and thymus chromation of gamma irradiation rats Radiobiol. 25 (2), 174.
24. **Littley, M. D.; Shalet, S. M. and Beardwell, C. G. (1991):** Radiation and the hypothalamic-pituitary axis In: Radiation injury to the nervous system, edited by P. H. Gutin, S. A. Leibel, and G. E. Sheline, P. 303 Raven press, New York.
25. **Mahesh, V. B. and Brann, D. W. (1992):** Interaction between ovarian and adrenal steriods in the regulation of gonadotropin secretion. J. Steroid. Biochem. Molec. Biol. 41 (3-8), 495.
26. **Mettler, F. A.; Jr. and Moseley, R. D.; Jr. (1985):** Medical effects of ionizing radiation. Grune and stratton, New York.
27. **Mills, D. E.; Ward, R. P.; Me Cutcheon, D.; Dixon, H.; Ly, H. and Scholey, J. (1992):** Attenuation of cyclosporine-induced hypertension by dietary fatty acids in the borderline hypertensive rat. Transplantation 53 (3), 649.
28. **Monroy, R. L. (1987):** Radiation effects on the lymphohematopoietic system: Compromise in immune competency In: Military Radiobiology, edited by J. J. conklin and R. I. walker, 113, Academic Press, San Diego.
29. **Robertson, J. B. (1989):** Toxicology of ionizing radiation In: A Guide to general toxicology, 2nd ed., edited by J. K. Marquis, P. 141. S. Karger A. G., New York.
30. **Robbins, M. E.; Campling, D.; Rezvani, M.; Golding, S. J. and Hopewell, J. W. (1989):** Radiation nephropathy in mature pigs following the irradiation of both kidneys. Int. J. Radiat. Biol. 56, 83.
31. **Robbins, M. E.; Bymaters, T.; Rezvani, M.; Colding, S. T. and Hopewell, J. W. (1991):** Residual radiation-induced damage to the kidney of the pig as assayed by retreatment. Int. J. radiat. Biol. 60, 917.
32. **Roushdy, H. M. and Ashry, M. A. (1979):** Haematopoitic response to the interaction between protein malnutrition and gamma irradiation Isotope and Radiat. Res. 11.
33. **Saad El-Deen, A.; Abdel-Fattah, K. T. and Roushdy, H. M. (1996):** IR spectral modification of gamma irradiated haemoglobin of rat and prophylactic role of immidazole. J. Egypt. Ger. Soc. Zool. 21 (A) conf. Physol., 203.
34. **Saleh, S.; Roushdy, H. M.; Abde Aziz, M. T. and Ramadan, L. A. (1988):** Radiation-induced disorders in ovarian function. Egypt. J. Rad. Sci. Applic. 5 (1), 75.

35. **Schalin-Karrila, M.; Mattila, L.; Tansen, C. T. and Uotila, P. (1987):** Evening primrose oil in the treatment of atopic eczema: Effect clinical status, plasma phospholipid fatty acids and circulating prostaglandins. *Br. J. Dermatol.* 117 (1), 11.
36. **Senedecor, G. W. and Cochran, W, G. (1978):** Statistical methods 8th ed., Univ.Press, USA.
37. **Stem, J. and Lewis, W. H. P. (1957):** *Clin. Chem. Acta.* 2, 576. Cited from saleh (1988).
38. **Stone, K. J.; Willis, A. L.; Hart, W. M. and Kernoff, P. B. (1979):** The metabolism of dihomo-gamma-linolenic acid in man. *Lipid* 14 (2), 174.
39. **Urakaze, M.; Hamazaki, T.; Hakuta, M.; Ibuki, F.; Yano, S. and Kumagai, A. (1987):** Effect on platelet aggregation and fatty acid composition in phospholipids of plasma. platelets, and red blood cell membranes in rabbits. *Am. J. Clin. Nutr.* 46 (6), 936.
40. **Walden, T. L.; Jr. and Farzaneh, N. K. (1990):** *Biochemistry of Ionizing Radiation* Raven. Press, New York.
41. **Wallace, W. H.; Shalet, S. M.; Crowne, E. C.; Morris. Jones, P. H. and Gattamaneni, H. R. (1989):** Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. *Clin. Oncol. (R. Coll. Radiiol.)* 1, 75.
42. **Yaes, R. J. (1992):** Radiation damage to the kidney. In: *Advances in Radiation Biology*, Vol. 15. Relative Radiation sensitivities of Human organ systems, Parrrts III, edited by K. I. Altman and J. T Lett, P. 1-35 Academic Press, San diego.

استخدام زيت البريمروز في الحد من بعض الاختلالات الوظيفية والتركيبية التي يحدثها في إناث الجرذان

*** فاطمة لطفى رمضان - ** رينيه جورجي**

***قسم البيولوجيا الإشعاعية - **قسم البحوث الصحية الإشعاعية
بالمركز القومي لبحوث وتكنولوجيا الإشعاع**

يهدف هذا البحث إلى تقييم الدور الإستشفائي لزيت البريمروز (زيت زهرة الربيع) في الحد من التغيرات التي تحدث في بعض المعايير البيوكيميائية والهستولوجية في الجرذان المعرضة كليا لأشعة جاما 5 جراى جرعه واحدة وأختير وقت التبويض وما قبل الدورة الشهرية. وقد تناول الجرذان زيت البريمروز بمقدار 9 مللى / 100مجم من وزن الجسم سبعة أيام قبل التعرض للإشعاع. وقد تم في هذه الدراسة إختيار بعض المعايير كدلائل لتأثير أشعاع وهى عدد كريات الدم الحمراء والبيضاء ونسبة الهيموجلوبين بالدم - قياس تركيز الكالسيوم والكوليسترول فى الدم - وظائف الكلى من حيث مستوى اليوريا والكرياتينين وقياس الهرمونات الأنثوية المحفزة لتكوين الحويصلات بالمبيض وذلك من خلال القياس أثناء 3 أزمنة مختلفة أثناء الدورة الشهرية وقبلها. ذلك بالإضافة إلى إجراء الفحص النسيجي لخلايا الكلى والمبيض.

وأوضحت النتائج أن تعرض الجسم الكلى لأشعة جاما يؤدي إلى زيادة معنوية فى جميع القياسات فى البلازما يصاحبه نقص معنوى فى الكالسيوم ومعايير الدم (عدد كريات الدم الحمراء والبيضاء ومستوى الهيموجلوبين بالدم) فى معظم أوقات التجربة. كما أوضح الفحص النسيجي لقطاعات الكلية والمبيض أن الإشعاع أحدث تدمير ، تمددات ، نزيف بالحافظات الكلوية وكذلك الحال فى المبيض حيث وجدت أغلب الحويصلات من النوع الإبتدائى كما حدث تقلص أيضا فى حجم المبيض كله وتدمير للخلايا المسؤولة عن إفراز الهرمونات الأنثوية.

أما مجموعة الجرذان التى تناولت البريمروز بمقدار 9 مللى / 100 جم من وزن الجسم لمدة سبعة أيام قبل التعرض للإشعاع فقد حدث تحسن ملحوظ فى جميع المعايير البيولوجية المقاسة. كما أن الفحص الهستولوجى لأنسجة الكلى والمبيض أكدت هذه النتائج حيث حدث تحسن ملحوظ فى إرجاع الشكل الطبيعى للحافظات والأنابيب الكلوية وكذلك وجود حويصلات ناضجة بالمبيض كما أعيدت الخلايا المسؤولة عن إفراز الهرمونات الأنثوية إلى طبيعتها بعد تداعيها من الإشعاع. وهذه النتائج تدل على أن زيت البريمروز يلعب دورا هاما فى الحد من أضرار التعرض الإشعاعى للإناث فى المجالات الإشعاعية وكذلك إستخدامه من خلال فترة التبويض وقبل فترة الدورة الشهرية للتخفيف من ألامها.