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HAEMATOLOGICAL AND BIOCHEMICAL STUDY ON RABBIT POST WHOLE BODY X- IRRADIATION AND TREATMENT BY NIGELLA SATIVA OIL OR OLIVE OIL

By

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ABSTRACT

Haematological and biochemical changes following cumulative doses of whole body X- irradiation (5Gy) and treatment by Nigella sativa oil and olive oil were evaluated in adult male domesticated rabbits. Irradiation caused a significant decrease in white blood cell count, red blood cell count, haemoglobin content, haematocrit percentage, mean corpuscular haemoglobin, mean corpuscular haemoglobin corpuscles and blood platelets. Whereas, mean corpuscular volume and lymphocyte percentage were significantly increased. Irradiation exposure also caused significant increase of urea, uric acid and creatinine concentration in rabbit's blood serum compared to the control level. Irradiation also showed significant increase in glucose, cholesterol and triglycerdies contents. Signs improvements in haematological parameters and serum of non-protein nitrogen constituents e.g. urea, uric acid, creatinine, glucose, cholesterol and triglycerides of rabbit were noticed after oral administration of Nigella sativa oil or olive oil prior to irradiation.

INTRODUCTION

It is known that over-exposure to ionizing radiation may cause destructive changes beside various systemic injuries, these include production of malignancies, reduction infertility and life span (Ward *et al.*, 1976; Roushdy *et al.*; 1985.; Walash *et al.*, 1988 & Eissa, 1992).

The kidney is the principal organ for excretion of toxins and the large number of nephrons provide extensive surface of endothelium for exposure to toxins (Goyer, 1990) .

The magnitude of urinary outputs of creatinine, creatine and uric acid have been taken as sensitive parameters indicating the degree of radiation damage to the metabolism of the tissues especially of muscles (Gerber *et al.*, 1961). Increase of creatinine are the graver prognostic signficances than those other nitrogenous substances and their estimation has sometimes been used for diagnosis of renal disease. These substances together with urea, form the major part of the non-protein nitrogen of the blood which is sometimes determined instead of urea (Varley, 1976). Willett *et al.*, (1986) found an elevation in serum creatinine of patients with upper abdominal malignancies secondary to radiation treatment. Recently, different radioprotective substances either chemically prepared or occurring naturally are under investigation (Myazaki *et al.*, 1990 and Azza, 1990). Evaluation of the radioprotective effects is determined by changing in survival time post-irradiation or improving the histopathological or ultrastructural changes produced by ionizing radiation. The black seed (*Nigella sativa*) is a herb which has been in use for 2000 years as a natural remedy for a wide variety of illnesses. Recent clinical and animal studies have shown that extracts of black seed have a bronchodilating antibacterial, blood pressure regulating and bile flow stimulating effects (Wilson, 1983) (Khamis *et al.*, 1988) . Ahmed and Osamà (1987) reported that black seed has an enhancing effect on the immune system. Ibrahim *et al.*, (1992) reported that the alcoholic extract of Nigella sativa produced a significant increase in the life span of mice bearing carcinoma. In 1959, the crystalline active principle "Nigellone"

having the chemical formula $C_{18}H_{22}O_4$ was isolated from the oil of Nigella sativa seeds (Ahmed and Osama, 1987). A series of long-chain a, B-unsaturated aldehyde have been characterized as antimicrobial agents from the olive Olea europea fruit and its oil flavor. These volatile compounds exhibited a broad antimicrobial spectrum (Kubo *et. al.*, 1995).

Little information was available about the prophylactic action of olive oil against radiation impairment (Waer *et. al.*, 1994) reported that olive oil gave good results against radiation damage.

In the present study, the possible radioprotective effect of Nigella sativa oil and olive oil on haematology and biochemistry of adult rabbit's serum is tested.

MATERIALS AND METHODS

Thirty-six male healthy rabbits of similar age (50-55 days) and weight about 1300 gm were used for the experimental work. The animals were kept during treatment under good ventilation and adequate standard diet. The animals were categorized as follows:

1- The first group (untreated animal) served as normal control.

2- The second group received intermittent X- radiation dose level at 1Gy increments delivered every other day up to a cumulative dose of 5Gy.

3- The third group received orally Nigella sativa oil at dose 1ml/kg. b.w. daily for 20 days, then these, animals were categorized into two subgroups; subgroup one, animals served as experimental control, while animals of the second subgroup received cumulative doses up to 5Gy X-irradiation.

4- The fourth group received orally olive oil at dose 1ml/kg b.w. daily for 20 days, then these animals were categorized into 2 subgroup, subgroup

1, animals served as experimental control, while animals of the second subgroups received cumulative doses up to 5 Gy X- irradiation.

At the end of experiment, blood samples were collected from jugular vein (24 hr's post treatment) as recommended by Shakoori *et al.*, (1992) for haematological examination (Jain, 1986). Clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min and then collected and stored in a deep freeze at (-20c) for different biochemical analysis .

Chemical analysis : Serum samples were analyzed for glucose, triglycerides and total cholesterol by the methods described by Trinder (1969), Fossati and Prencipe (1982) and Allain (1974), respectively. Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for urea, Fossati *et al.*, (1980) for uric acid and Bartels and Bohmer (1972) for creatinine.

The statistical analysis for T- test was performed by using SPSS.

RESULTS AND DISCUSSION

The data presented in Table (1) summarize the effect of whole body X-irradiation and the role of Nigella sativa oil or Olive oil as the natural protective agent on haematological parameters. After 3 days of X-rays exposure, there was a significant decrease in the total red cell count, and haemoglobin concentration was significantly decreased. There was also a significant decrease in the total leucocytic count, whereas the lymphocytic cell count was highly significant increase .

Mean corpuscular haemoglobin (MCH) also exhibited a significant decrease. Almost all the haematological parameters were drastically affected after X- irradiation exposure. The decrease in red blood cell count and haemoglobin lowered the oxygen supply to different tissue, thus resulting in low energy production. The significant decrease in RBC

count can be explained on the basis of inhibitory effect of X- irradiation on histogenesis. Decrease in haemoglobin content and MCH can be explained due to decreased size of RBC or impaired biosynthesis of heme in bone marrow.

The significant increase e in WBC count indicated the activation of defense mechanism and immune system of rabbit. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals (Kollar and Roan, 1980). Leukocytosis was manifested by lymphocytosis, which was the main features of the differential leukocytic count. The increase in lymphocytes may be due to the toxic action of X- irradiation exposure which stimulates the haemopoietic system to release more lymphocytes causing an increase in their number in blood stream . On the other hand, the lymphocytic count showed a significant increase. Jonderko *et al*., (1984) found an increase in lymphocytic count with positive reaction to acid phosphatase following exposure of fodders to different concentrations of Propoxur for 13 weeks.

The present results also showed a significant decrease in haematocrit value (packed cell volume, PCV) after X- irradiation with a value of 5.52%.

Mean corpuscular volume (MCV) showed 23% significant increase after X- ray exposure . Results under the current investigation may be concomitant with the changes of RBC count and hemoglobin levels, the MCV and MCH were significantly changed. These changes may correlated with some pathological changes that developed in blood forming organs with destruction of a number of red cells due to lytic substances or with both factors. In this regard Naylor (1971) concluded that the anemia results from hemodilution extra vascular hemolysis as well as from toxic dyshemopoiesis

However, present results showed a significant decrease in the mean corpuscular hemoglobin concentration (MCHC) after X-irradiation with a value (MCHC) after X-irradiation with a value of 6%. The blood

platelet (PLT) count showed a significant decrease when compared with the control level (Table 1) .

Table (1) : Hematological study on the rabbit post whole body X-irradiation and treatment by Nigella sativa oil or Olive oil .

Parameters	Control n = 6	Experimental groups				
		Cumulative doses up to 5Gy n = 6	Nigella sativa oil n = 6	Nigella sativa oil + cumul-ative 5Gy n = 6	Olive oil n = 6	Olive oil + Cumul-ative 5 Gy n = 6
WBC count (x10 cells/Ul)	8.62 ± 0.31	6.90 ± 0.55*	12.78 ± 3.18	9.95 ± 0.85	9.3 ± 0.25	7.79 ± 0.30*
RBC count (x10 cell/Ul)	5.68 ± 0.18	4.32 ± 0.17*	6.22 ± 0.15*	5.27 ± 0.23	5.62 ± 0.20	5.10 ± 0.22*
Hb (g/dl)	11.25 ± 0.17	10.16 ± 0.30*	11.73 ± 0.29	10.95 ± 0.22	10.77 ±0.38	10.18 ± 0.21*
Hematocrit %	35.13 ± 0.47	33.19 ±0.25*	36.32 ± 0.51	34.30 ± 0.55	33.6 ± 0.90	32.95 ±0.40*
MCV (fl)	62.33 ± 0.85	76.93 ± 0.63*	59.49 ± 2.6	65.25 ± 0.35*	59.93 ±0.92	64.81 ± 0.69*
MCH (pg)	20.10 ± 0.36	23.82 ± 0.34*	18.96 ± 0.37	20.98 ± 0.21*	19.38 ±0.31	19.99 ± 0.25*
MCHC (g/dl)	32.65 ± 0.45	30.85 ± 0.22*	32.60 ± 0.68	32.02 ± 0.70	32.17 ±0.80	30.95 ± 0.43*
Lymphocyte %	3.08 ± 0.13	4.91 ± 0.19*	4.83 ± 0.24*	3.82 ± 0.35*	3.6 ± 0.15*	3.45 ± 0.11*
PLT (x10 6/Ul)	390 ± 21.41	331.18 ±16.60*	468.33 ± 46.5	435.29 ± 21.30	381± 22.70	353.1± 20.7

All values expressed as mean + SE

*** Significant differences at P < 0.05**

Table (2) :Biochemical study on the blood of rabbit post whole body X- irradiation and treatment by Nigella sativa oil or Olive oil

Parameters	control n = 6	Experimental groups				
		Cumulative doses up to 5Gy N = 6	Nigella sativa oil n = 6	Nigella sativa oil +cumulative 5Gy n = 6	Olive oil n = 6	Olive oil +cumulative 5 Gy n = 6
Urea (mg/dl)	49 ± 2.51	65.17 ± 2.28*	62 ± 3.30*	53.6 ± 2.16	61.33± 2.10*	51.19 ± 3.31
Uric acid (mg/dl)	0.18 ± 0.05	0.61 ± 0.03*	0.28 ± 0.04	0.22 ± 0.04	0.30± 0.01*	0.27 ± 0.05
Creatinine (mg/dl)	1.17 ± 0.04	1.98 ± 0.31*	1.32 ± 0.04*	1.19 ± 0.04	1.33± 0.03*	1.21 ± 0.05
Glucose (mg/dl)	121.43 ± 13.2	195.4 ± 15.25*	104.17 ± 10.9	125.31 ±12.8	123.5± 11.41	131.39 ± 13.10
Cholesterol (mg/dl)	76.16 ± 7.7	101 ± 8.35*	118 ± 9.5*	90 ± 6.41	220.33 ±14.3*	94.48 ± 9.60
Triglycerides (mg/dl)	338.54 ± 29.56	480.5 ± 28.8*	218.67 ±23.7*	359 ± 21.60	150.17 ±14.65*	381.44 ± 24.10

All values expressed as mean + SE

*** Significant differences at P < 0.05**

Singh *et al.*, (1992), also reported significant decreases in the total erythrocyte, leucocyte and thrombocyte counts, and haematocrit and haemoglobin content after exposure of the Indian catfish to a low concentration of Propoxur for 96 hours. So it might be concluded that

cumulative X-rays exposure had caused a direct toxic effect on blood parameters similar to that caused by some insecticides.

The data presented in Table (2), summarize the effect of whole body X- irradiation and the role of Nigella sativa oil or Olive oil as the natural protective agents on some parameters of blood constituents of rabbits. In the present study, a significant increment of urea contents were observed after cumulative doses up to 5 Gy of X- irradiation.

Urea is the end product of protein catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. The elevated serum urea levels observed in the present study may be due to the destruction of red blood cells during treatment. In addition, the elevation of blood urea is a good indicator for kidney diseases. On the other hand, Zamurovic *et al.*, (1964) reported that whole body gamma irradiation of rats (6.1/Gy) revealed no detectable changes in the levels of blood urea, either immediately after irradiation or after 1,2,5 and 30 days post-exposure.

In general uric acid content in rabbit's blood serum increased after X- ray exposure. Uric acid concentration in rabbit's serum treated by X-irradiation were 3.39 time greater than control level (Table 1). Uric acid is the end product of the catabolism of tissue nucleic acid, i.e. purine and pyrimidine bases metabolism (Wolf *et al.*, 1972). Loyda (1984) found increase in serum uric acid of 16 day old chicken embryo 8 Gy radiation exposure.

In the present work, the serum creatinine levels exhibited significant increment in the treated rabbits. A marked increase in blood creatinine and urea nitrogen of rabbits was recorded after X- irradiation with cumulative doses up to 5Gy. These findings are in agreement with those observed of Thyagarajan *et al.*, (1977). Increased serum creatinine levels is an evidence of marked impairment of kidney function (Best and Taylor, 1961). Roushdy *et al.*, (1985) noticed that radiation exposure of

albino rats (7.8, 11.7 Gy) caused significant increase in uric acid in both plasma and urine as well as in plasma creatinine but with decreased level in urine. Willett *et al.*, (1986) found elevation in serum creatinine and decrease in creatinine clearance of patient with upper abdominal malignancies secondary to radiation treatment. Results under the current investigation were in agreement with the findings given by Bakshwan and Dighidy., (1995) who reported that whole body gamma irradiation resulted in significant increase in creatinine concentration and blood urea nitrogen and in appearance of certain histopathological changes in the kidney of rats.

In general, the glucose content of rabbit's blood serum, Table (2) increased significantly in response to the X- rays exposure. Radiation may directly or indirectly play a specific role in pancreatic secretions, glyconeogenesis process, glycogen metabolism or glucose oxidation. It means that there was a disturbance in carbohydrate metabolism of rabbits by the X- irradiation exposure. Different opinions were reported regarding the source of post irradiation glycogen. Blood glucose was considered the main source of liver glycogen under irradiation by Becciolini *et al.*, (1974). Glyconeogensis from the metabolites which were released from the destroyed radiosensitive tissues were considered by Kendysh and Moraz (1979). Cloes *et al.*, (1986) reported reduction in both the cell size and number of Langerhans cells of the pancrease after irradiation .

In general results in Table (2), indicated a significant increase in serum cholesterol, and triglycerides. Cholesterol and triglycerides concentration in rabbits serum treated by X- irradiation exposure were 1.33, 1.42 and 1.6 times greater, respectively, than the control level. The observed changes in serum triglycerides, and cholesterol contents take place in the liver due to imbalance between the normal rates of lipid synthesis, utilization and secretion (Glaser and Mager, 1972). On the other hand, lipid deposition in irradiated rabbits may be attributed to decreased proteins available for triglyceride transport from the liver, since irradiation causes lesion to rER (Moussa *et al.*, 1984), alters its ribosomal

concentration (Barker *et al.*, 1963) and interferes with its normal functions. (Porter, 1961) .Regarding the effect of Nigella sativa oil or Olive oil on the blood biochemical parameters of the kidney and liver functions.

The present writer agreed with Bakshwan and El-Dighidy (1995) who indicated that administration of Nigella sativa oil before radiation exposure resulted in detectable radioprotective effect. Our investigation agreed also with Waer *et al.*, (1994) who reported that both Thiola and olive oil gave good results against radiation damage.

Administration of Nigella sativa oil or Olive oil as a source of unsaturated fatty acids might have a repair effect producing peroxides which start a chain of autoxidant and free radical formation leading to the transfer of electron to disulphide groups of any other antioxidant (Othman *et al.*, 1990).

Finally it could be concluded that, Nigella sativa oil and Olive oil may act as protective agent for cumulative doses of X- irradiation.

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الملخص العربى

دراسة أثر تعرض الارانب كيون تجارب للأشعاع و كذلك المعالجة بزيت حبة البركة او زيت الزيتون على بعض القياسات الدموية والبيوكيماوية

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يهدف هذا البحث الى دراسة تأثير تعرض الجسم الكلى للأرنب لأشعة اكس المجزئة (٥ جراى) على القياسات البيوكيماوية فى سيرم الدم . أحدثت أشعة اكس المجزئة تغيرات مختلفة وملموسة فى قياسات الدم للأرانب مثل نقص ملحوظ فى عدد كرات الدم البيضاء و الحمراء و الهيموجلوبين و الهيماتوكريت، وكذلك MCHC , MCH بألاضافة الى نقص فى عدد الصفائح الدموية . بينما أرتفع عدد الكرات الليمفاوية بالاضافة الى MCH عند مقارنتها بحيوانات المجموعة الضابطة. أما بالنسبة لمستوى سكر الجلوكوز و بعض الليبيدات و المكونات النيتروجينية غير البروتينية فى سيرم دم الارانب ،فقد تبين ان اشعة اكس المجزئة أحدثت زيادة ملموسة فى تركيز اليوريا و حمض ألبوليك و الكرياتنين، بالاضافة الى سكر الجلوكوز و ألكوليسترول و الجليسريدات الثلاثية ، ومن ناحية اخرى فقد ادى إعطاء زيت حبة البركة او زيت الزيتون للأرانب قبل التعرض لجرعة الاشعاع، فى حدوث تحسن ملحوظ فى حالة الارانب المعاملة.