ROLE OF IONIZING RADIATION ON CONTROLLING KIDNEY CHANGES IN EXPERIMENTAL INFECTION WITH *TOXOCARA CANIS*

MERVAT A. MOAWAD, MONA M. AMIN and EMAN N. HAFEZ
Health Radiation Research Dept., National Centre for Radiation Research and Technology (NCRRT),
Tel. +201068429907, Mailing P. O. Box: 29 Nasr City, Egypt.

Email: Eman_naser612@hotmail.com Assiut University web-site: www.aun.edu.eg

ABSTRACT

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Toxocara canis (*T.canis*) is an important parasite that infects many domestic and farm animals whose economical importance cannot be neglected. The aim of the present study is to detect the hematological, biochemical and histopathological changes on kidney of rats vaccinated with two doses of gamma radiation (600Gy and 800Gy) embryonated eggs. Eighty rats were divided into four groups group I: as normal control group (-ve control), group II: received 2500 infective embryonated *T. canis* eggs per ml/rat orally, as control infected (+ve control), group III: received 600 Gy irradiated *T.canis* eggs, group IV: received 800 Gy irradiated *T. canis* eggs, However, at 14th day post infection, rats were re-infected (challenged) with non-irradiated infective *T.canis* eggs. The study showed marked histopathological changes with significant decrease of glutathione (GSH) and superoxide dismutase (SOD) and remarkable increase of lipid peroxidation (MDA) in kidney tissue of infected control group. Also, marked increase in urea, creatinine and total proteins with decrease in albumin in control infected group. Vaccinated-challenged group III and IV showed amelioration in all histopathological, biochemical and hematological changes. Radiation exposure attenuated the larval migration from the gastrointestinal tract to other organs and controls the damaging effect on the kidney. The dose of 800Gy showed better results than lower dose.

Key wards: *Toxocara canis; Kidney; Antioxidents; gamma radiation; vaccination*

INTRODUCTION

Toxocara canis is a nematode whose biological cycle includes its definitive canine host, the environment and paratenic hosts, which include man, rats, rabbits, birds and pigs (Glickman, 1993). Toxocariasis is considered an aberrant infection because humans are incidental hosts, and the arasite cannot completely mature in the human body (Alderet *et al*., 1999 and Alonso *et al*., 2000). Instead the invasive larvae migrate for months through distant organs including brain, heart, lungs, kidney, muscles and eye (Fortenberry *et al.*, 1991) until they are overcome by the human inflammatory reaction and die. Some are destroyed in the liver or pass onto the lung and destroyed there (Rothenberg, 1998), while others can survive in tissues for at least nine years and possibly for life of the host (Pawlowski, 2007). The mode of transmission to humans is by oral ingestion of infective eggs (Glickman *et al.*, 1981). Three clinical syndromes have been associated with *Toxocara* infection in humans; visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis Taylor and Holland, 2001). The oxidative stress status was determined by measuring serum malondialdehyde (MDA) level as indicator of lipid peroxidation, erythrocytes superoxide dismutase (SOD) and glutathione (GSH) as indicators of endogenous antioxidant enzymes level. Potential mechanisms and roles of oxidative stress have also been investigated in a number of parasitic diseases. *Brugia malayi* has been found relatively resistant to nitric oxide (NO) mediated toxicity (Rajan *et al.*, 1996). Lipid peroxidase product has been measured showing a significant increase in *Schistosoma mansoni* infected mice, and values have been partially restored after antioxidant melatonin application (El-Sokkary *et al.*, 2002). Oxidative stress as a mediator of hepatic tissue damage related to *Leishmania chagasi* infection has been investigated, and a significant correlation between occurrence of oxidative stress and lipid peroxidation as a mechanism of liver damage and tissue injury has been reported in the chronic stage of infection (Oliveira and Cecchini, 2000). Enzymatic and non-enzymatic antioxidants have been reported from filarial

nematodes and this group has been suggested as relatively resistant to oxidative stress (Selkirk *et al.,* 1998). Visceral larva migrans (VLM) caused by *Toxocara spp*. larvae is an important systemic parasitic disease of humans. It affects most of the organs and causes responsive changes and tissue damage in infected individuals (Espinosa *et al.,* 2002). Most studies on irradiation of pathogenic organisms have been carried out primarily with the aim of establishing an attenuated vaccine. One of the developing and hopeful routes of vaccination is to use feeble irradiated parasite as a vaccinating antigen. The parasites studied for this purpose include *Toxocara canis* (Kamiya *et al.,* 1987).

This work aimed to detect hematological, biochemical and histopathological changes in kidney of toxocariasis infected mice and determine the effect of vaccination with 600 Gy and 800 Gy gamma radiation-irradiated *Toxocara canis* eggs in controlling these changes.

MATERIALS and METHODS

Animals
Male albino rats weighing 100–160 gm, each of the same colonies were used. 80 rats were kept in the laboratory at room temperature and housed in cages (10 rats in each cage). Rats received a diet of standard rodent pellets produced by the Cairo Company for Oil and Soap. Water and food were available ad libitum.

Preparation of embryonated eggs of parasite
T. canis embryonated eggs were obtained from the uteri of female nematodes collected from the naturally infected dogs. Eggs were incubated in 0.5% formalin solution at 28°C for 4 weeks. Embryonated eggs were kept at +4°C until used. Rat in the VLM groups were each infected with 2500 embryonated eggs (Galvin, 1964).

Radiation source:
Toxocara eggs were exposed to 600Gy and 800Gy gamma-radiations rays at dose rat 2.5 KGy/h at the time of experiment. This was done in national Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Infection of rats
The experiment was run in groups; each group consisted of 20 rats, first group was used as control group, second group: each animal received orally infective embryonated *T. canis* eggs (2500 infective eggs per ml/ rat), third group: received the same number of 600Gy irradiated infective *T. canis* eggs, fourth group: received 800Gy irradiated infective *T. canis* eggs. However, at 14th day post infection, rats were re-infected (challenged) with non-irradiated infective *T. canis* eggs (2500 eggs/rat). Five rats were scarified at the 7th and 14th days after first infection also, at the 7th and 14th day post challenge. Serum was separated, from blood by centrifugation at 3000 rpm for 10-15 minutes and utilized for the measurement of biochemical parameters. Kidney was removed for biochemical analysis and histopathological study. The homogenates of kidney tissues (10%) were prepared in normal saline for biochemical study.

Biochemical study:
Blood biochemistry values were measured by use of an automated spectrophotometer and included urea (mg/dl), creatine (mg/dl), total proteins (g/dl), albumin (g/dl). Values were taken as normal for blood biochemistry (Kaneko, 1989).

Measurement of the lipid peroxidation: Tissue TBARS (thiobarbituric acid reacting substances) levels as the marker of lipid peroxidation were determined with the spectrophotometric method. Stock solution: 15% trichloroacetic acid (TCA), 0.375% thiobarbituric acid (TBA), 0.25 of N hydrochloric acid (HCl). Samples were heated in a water bath for 20 min and after cooling, centrifuged at 2,000 rpm for 15 min. The formation of pink color, as a result of the reaction in-between one molecule of TBARS and two molecules of TBA, was measured at 560 nm spectrophotometrically (Buege and Aust, 1978).

Measurement of the tissue SOD activity: Tissue super oxide dismutase activity was measured by modified method. This assay for super oxide dismutase activity involved inhibition of nitroblue tetrazolium (NBT) reduction, with xanthine oxidase used as a super oxide generator. One unit SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Reaction mixture: 40 ml of 0.3 mmol/l xanthine solution, 20 ml of 0.6 mmol/l EDTA solution, 20 ml of 150 µmol/l nitroblue tetrazolium solution, 12 ml of 400 mmol/l Na2CO3 solution, and 6 ml of bovine serum albumin. The final concentration of xanthine oxidase was 167 U/l. The production of formazon was determined at 560 nm, spectrophotometrically. Measurement of glutathione was determined according (Sun *et al.,* 1988 and Beutler *et al.,* 1963).

Histopathology
After proper fixation of kidney tissues, thin pieces were processed in ascending grades of alcohol for dehydration and cleared in xylene. The paraffin embedded tissues were cut into 4-5 micron thick sections and stained with Haematoxylene and Eosin (H&E) as per conventional procedure (Akkiv and Nilsson 1999).

Statistical analysis
All data were expressed as mean ±SE (standard error). Data were assessed by using a one- way

ANOVA using SPSS 15.0 program and $p< 0.05$ was considered statistically significant.

RESULTS

Glutathione peroxidase activity was determined in all groups as shown in table 1. Significant difference was observed on all days post infection and post re-infection in infected group II as the mean values were 25.7±0.4, 23.1±0.3, 20.7±0.2 and 18.4±0.5 compared to normal control group I (31.9±0.2). Vaccinated groups II and IV showed significant increase in all days compared to control infected group II and significant decrease compared to normal control group I. The mean values increases more in group IV (800 Gy). Regarding SOD levels (table 2), there was significant decrease in control infected group II in days 7th, 14th, 7th (R) and 14th (R) as they were (3.8±0.1), (4.0±02), (3.5 ±0.3±0.3) compared to control normal group I (5.9±0.06). Vaccinated groups II showed significant decrease than normal control and significant increase than infected control group in all days as they were (5.1±0.1), (4.1±0.3), (3.9±0.2) and (3.7±±0.1) respectively. In group IV irradiated with 800 Gy the mean values level were (5.4±0.2), (4.7±0.1), (4.2±0.1) and (3.8±0.1) respectively. Malondialdehyde levels increased significantly in all days of infected group II as they were (145.4±1.5), (150.7±1.4), (164.6±2.1) and (171±2.9) in comparison with the control normal group I (117.5±2.1). Vaccinated group III gave significant increase than normal and significant decrease than infected group as the mean levels in days 7th, 21th, 7th (R) and 21th (R) were (125.1±2.4), (127.5±3.5), (130±3.7) and (157.1±1.2) respectively while group IV with the higher dose of radiation, the values were (122.1±2.3), (133.3±1.5), 136.7±2.8) and (146.2±2.5) respectively.

Biochemical studies table (4) shows the mean values of urea levels of group (II) it was 93.4±0.15[a] at 7th day post infection and 100.6±2.1[a] at day 14th post challenge which is highly significant increased ($P<0.01$) as compared to control normal group I (40.0±2.1). Vaccinated group III showed significant increase compared to the normal control group and significant decrease relative to infected control one in both 7th day and 7th (R) as they were 63.2±1.6 and 66.7±1.1 respectively. Also, group IV gave 58.4±2.1 and 89.5±2.4 with significant decrease compared to control infected group. Mean value of serum creatinine was 0.84±0.1 in control normal group I and in control infected group II it was 1.3±0.15 at 7th and 1.5±0.15at 7th (R) with significant increase compared to normal group. Vaccinated groups II and IV in both days 7th and 7th (R) showed 0.95±0.09, 1.1±0.07, 1.1±0.14 and 0.97±0.06 respectively with decrease in the levels than control infected group. Regarding total protein levels, there was significant increase in control infected group II in days 7th and 7th (R) as they were 6.6±0.3 and 7.3±0.1 respectively compared to normal group I (6.1±0.5). Vaccinated groups III and IV showed no significant decrease compared to infected control group at 7th day as they were 6.2±0.5 and 5.8±0.4 respectively while, the levels significantly decrease at day 7th (R) (6.4±0.3) and (6.3±0.3) respectively. Albumin level decrease in control infected group at both post infection and post challenge while globulin levels significantly increase in the same group compared to normal. Vaccinated groups III and IV showed slight elevation than normal level but less than control infected group in post infection and post challenge days.

Table 1: Glutathione (GSH) activities in kidney tissues of all exposed groups (U/gHb).

Groups \ Days		7th	14th	7th (R)	14th (R)
Group I	31.9±0.2				
Group II		25.78±0.4[a]	23.12±0.3[a]	20.73±0.2[a]	18.44±0.5[a]
Group III		26.3±0.1[ab]	24.89±0.1[a]	22.91±0.1[ab]	22.10±1.3[ab]
Group IV		29.14±0.2[ab]	28.08±0.8[ab]	27.52±1.2[ab]	25.94±0.9[ab]

Table 2: SOD activities in kidney tissues all exposed groups (U/gHb).

Groups \ Days		7th	14th	7th (R)	14th (R)
Group I	5.9±0.06				
Group II		3.82±0.1[a]	4.06±0.2[a]	3.50±0.3[a]	3.21±0.3[a]
Group III		5.13±0.1[ab]	4.16±0.3[a]	3.92±0.2[ab]	3.71±0.1[ab]
Group IV		5.40±0.2[ab]	4.76±0.1[ab]	4.27±0.1[ab]	3.80±0.1[ab]

Table 3: LPO activities in kidney tissues all exposed groups (U/gHb).

Groups \ Days		7th	14th	7th (R)	14th (R)
Group I	117.5±2.1				
Group II		145.4±1.5[a]	150.7±1.4[a]	164.6±2.1[a]	171±2.9[a]
Group III		125.1±2.4[ab]	127.5±3.5[ab]	130±3.7[ab]	157.1±1.2[ab]
Group IV		122.1±2.3[ab]	133.3±1.5[ab]	136.7±2.8[ab]	146.2±2.5[ab]

Values represented mean ±SE of 5 rat in each group.
a: significant at (P<0.05) compared to control. b: significant compared to infected group

Table 4: Effect of *T.canis* on kidney functions of different groups.

Groups	Days	Urea (Mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Group I		40.0±2.1	0.84±0.1	6.1±0.56	2.2±0.15	3.49±0.51
Group II (Infected)	7th	93.4±0.15[a] (133.5%)	1.3±0.15[a] (44.0%)	6.6±0.3[a] (24.5%)	2.0±0.12 (-8.0%)	4.1±0.38[a] (31.1%)
	7th (R)	100.6±2.1[a] (154.6%)	1.5±0.15[a] (58.5%)	7.3±0.15[a] (13.7%)	2.2±0.10[a] (-9.0%)	5.21±0.17[a] (46.5%)
Group III (600Gy IRR)	7th	63.2±1.6[ab] (58.0%)	0.95±0.09 [ab] (13.0%)	6.2±0.5 (1.6%)	2.6±0.13 (4.0%)	3.5±0.2 (1.44%)
	7th (R)	66.7±1.1[ab] (68.8%)	1.1±0.07[ab] (34.1%)	6.4±0.3[b] (10.3%)	2.4±0.20 (11.3%)	3.4±0.18[b] (6.25%)
Group IV (800 Gy IRR)	7th	58.4±2.1[ab] (46.0%)	1.10±0.14[ab] (30.9%)	5.8±0.43 (-4.9%)	2.3±0.15 (-8.0%)	3.2±0.13 (-8.3%)
	7th (R)	89.5±2.4[ab] (126.5%)	0.97±0.06 [ab] (18.2%)	6.3±0.3[b] (8.6%)	2.35±0.18 (4.5%)	3.3±0.2[b] (3.1%)

Values represented mean ±SE of 5 rat in each group.
a: significant at (P<0.05) compared to control. b: significant compared to infected group

Histopathology Histopathological section from kidney of normal rat group I (fig. 1) shows normal renal structure regarding gloeruli and their tufts, renal tubules, blood vessels and intra-tubular connective tissue. Group II of rat that infected with egg (+ve control) showed a larva migrans replaced the renal parenchyma which showing degenerative changes (fig. 2). Congestion of glomerular tufts and distension of Bowman'space was shown in (fig. 3). Fig. 4 shows no histopathological changes in vaccinated groups.

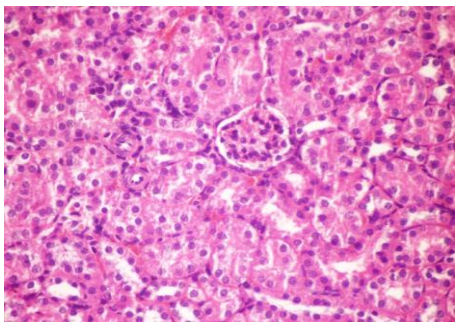

Fig. 1: Kidney of group I revealed normal renal structure regarding gloeruli, their tufts, renal tubules, blood vessels and connective tissue. (H&E X400)

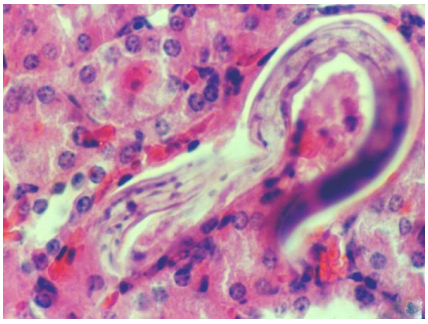

Fig. 2: Kidney of rat from control infected group II showing larva migrans replaced the renal parenchyma which showing degenerative changes. (H&E X400)

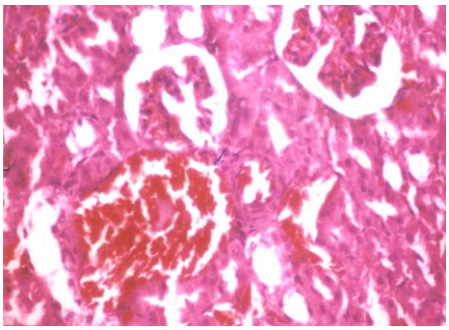

Fig. 3: Kidney of rat from control infected group II showing congestion of renal blood vessel and necrobiosis of tubular epithelium. (H&E X400)

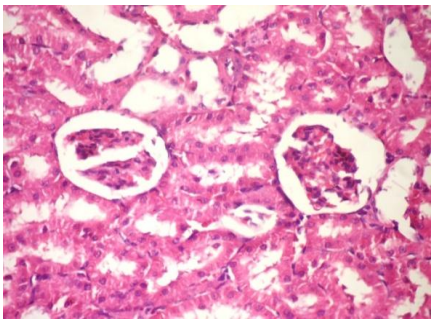

Fig. 4: Kidney of rat from vaccinated groups showing no pathological changes

DISCUSSION

The use of ionizing radiation in the production of irradiated attenuated vaccines against parasitic infestations has been taken into consideration since the last five decades. The irradiated attenuated vaccines represent a recent successful and promising approach in control of the parasitic diseases.

The present results indicated that gamma radiation can be used to attenuate *T. canis* eggs to produce vaccine against infection with effective stage of *T. canis* eggs. Also, in this study, gamma rays at dose of 800 Gy proved that it causes some sort of increasing immunity of the body against new infection, since histopathological changes observed in the kidney were much less in this group. This may be due to a reduction in the rate of migration of the larvae to the tissues of infected rats, or due to immunization of the body against infection by the attenuated eggs. This is in agreement with previous study used UV radiation for attenuating the infective stage of the parasite (Ruppel *et al.,* 1990). Also, another study stated that irradiation of infective *T. canis* larvae reduces their pathogenicity, inhibits their migration from liver and lungs, and kills some of the parasites during the first 3 weeks of infection (Barriga and Myser, 1987). Naguib and Amin, (2006) reported that damage in the eyes caused by infection with *T. canis* decreased by increasing the dose of irradiation of the infected stage.

Although oxidation reactions are crucial for life, they can also be damaging as they can produce reactive oxygen species that damage cells by chain reactions through lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair (Nakabeppu *et al.,* 2006). Oxidative stress is terminated by antioxidant system which stops these chain reactions by being oxidized themselves (Valko *et al.,* 2007).

Lipid peroxidation is a degenerative process which affects the polyunsaturated fatty acids of membrane phospholipids. The general mechanism of this process involves the formation of toxic aldehydes, which react with protein and non-protein substances and result in widespread changes in cellular membranes. These degenerative processes can be prevented molecularly (vitamin A, vitamin C, vitamin E, uric acid, ceruloplasmin) and enzymatically (Cu-Zn SOD, GSH-Px, and catalase). Therefore lipid peroxidation can be estimated directly by determination of reactive oxygen species (hydroxyl, superoxide anion, H_2O_2

and single reactive oxygen radicals) or MDA levels in plasma, tissues and erythrocytes (Yarsan, 1998).

In the present study, we evaluated the lipid peroxidation in mice which were infected with *T. canis*, and vaccinated with different doses of gamma irradiated eggs. The estimation was done by determining MDA levels, GSH-Px, and SOD activities in kidney homogenate on days 7^{th} and 14^{th} post infection and post challenge. The results showed that a significant increase kidney LPO tissue of group II infected with non irradiated *T. canis* eggs, accompanied by significant decreases in SOD and GSH content. This is due to parasitic infections are associated with significant degree of free radicals formation as indicated by significantly higher MDA and lower SOD levels among those rats. While, in irradiated (600 Gy and 800 GY) infected eggs groups III and IV respectively showed a significant decrease in LPO and increases SOD and GSH in kidney tissue as compared to infected control group II. The data obtained by Yarsan *et al.* (2003) who showed that *T. canis* infection stimulates lipid peroxidantion and decreased glutathione peroxidase, superoxide dismutase and catalase activity in erythrocytes.

Lipid peroxidation, super oxide dismutase and glutathione production are important under the oxidative stress conditions, and many pathological disorders and infections can cause oxidative stress. It was found that, *S. mansoni* infected mice and all immunized groups recorded significant increase in lipid peroxides, while significant decrease in glutathione content was observed, also they found that in post challenged groups, the antioxidant levels recorded significant improvement in mice immunized by saponin (Maghraby *et al.,* 2010).

Previous study reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxides. Also they showed that liver GSH was drastically depleted in *S. mansoni* infected mice (Pascal *et al.,* 2000 and Hamed 2006). This decrease was attributed to the increased cytotoxicity with H_2O_2 produced as a result of inhibition of glutathione reductase that keeps glutathione in the reduced state (Gharib *et al.,* 1999). *S. mansoni* infection impairs the antioxidant system since the level of GSH depletion was used as an index of oxidative stress and a sign that hepatic cells are utilizing more antioxidant defenses (Ip *et al.,* (2000). Also, significant reduction in the levels of activities of SOD, Catalase, total Glutathione peroxidase and vitamin E in fascioliasis cases in comparison to control (EL Shazly *et al.,* 2014). It was reported that most of the pathologic features associated with *Toxocara* infection resulted from the damage of tissue caused by immune-mediated inflammatory response and biologically active products such as proteases released by larvae (Buijs *et al.,* 1997 and Smith, 1993). This is harmony with the fact that re-infection with the results of the present work indicated that, the mean levels of serum creatinine, urea and protein activities have been found generally to increase significantly in most of the infected rat group II in comparison with that of the control normal group I. This may be attributed to complications arising from the extensive larval migration inside the body tissues especially the liver parenchyma and other tissues. Also, this was in accordance to a study that reported an increase in level of blood urea and serum creatinine in patient presented by severe asthma as a result of visceral larva migran associated with hyper eosinophilia (Feldman and Parker 1992). Although reported that people who were positive for *Toxocara* infection had higher alkaline phospatase but serum creatinine and blood urea found to be in normal range and they are not determining factors in *Toxocara* infection and renal dysfunction in heavy infection with toxocariasis (Dar *et al.,* 2009 and Pol Merkur, 2008).

Histopathological examination of kidney of rats, after infection with *Toxocara canis* eggs revealed various pictures of pathological affection. There was diffuse mesangioproliferative glomerulonephritis with mesangeal expansion of most of glomeruli. Some glomeruli showed severe hyalinosis with marked adhesions to the bowman's capsule. The lumina of glomerular capillaries were markedly obliterated. There was also cystic dilatation of tubules with proteinaceous casts. The intersitial tissue showed mild inflammatory oedema. Chronic inflammatory cellular infiltration to form granulomatous inflammation was noticed. This was in agreement with Casarosa *et al.* (1992) who reported that histological examination of kidneys from mice experimentally infected with *Toxocara canis* embryonated eggs demonstrated the presence of a segmental or diffuse mesangioproliferative glomerulonephritis. Immunohistochemical studies established that renal alterations were associated with glomerular deposits of IgG, IgM and third component of complement (C3) and these findings suggest that an immunomediated mechanism might possibly be involved in the genesis of kidney damage observed in mice infected with *T. canis* embryonated eggs.

These histopathological changes caused by infection with *T. canis* decreased by increasing the dose of irradiation of the infected stage, radiation exposure attenuated the larval migration from the gastrointestinal tract to the other organs. The above results showed that irradiated *T. canis* eggs give a degree of protection in kidney of rats that re-infected with *T. canis*. This may be due to the effect of irradiation on the parasite which retards the development of its larvae, these larvae might be too weak to produce comparable damages as those which were not irradiated.

Previous study on the effect of radiation on the viability and migratory ability of second-stage larvae of *Toxocara canis* in mice reported that most of the larvae irradiated with 80 or 160 Krad remained in the digestive tract, mainly in the stomach and the proximal half of the small intestine (Kamiya *et al.*, 1987). It was reported that irradiation of infective *T. canis* larvae, then, reduces their pathogenicity, inhibits their migration from liver and lungs, kills some of the parasites during the first 3 weeks of infection, but favors their late survival in the host (Barriga and Myser, 1987).

It was concluded that vaccination with *Toxocara canis* eggs irradiated with 800Gy gamma radiation ameliorate the biochemical, haematological and histopathological of renal toxocariasis.

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دور الأشعاع المؤين فى السيطرة على التغيرات الكلوية المصاحبة للعدوى التجريبية بالتوكسوكارا كانيس

ميرفت عبد الفتاح معوض ، منى محمد أمين ، ايمان ناصر حافظ

Email: Eman_naser612@hotmail.com Assiut University web-site: www.aun.edu.eg

توكسوكارا كانيس هو أحد الطفيليات الهامة التى تصيب الإنسان والحيوانات المنزلية وحيوانات المزارع التي لا يمكن تجاهل أهميتها الإقتصادية. الهدف من هذه الدراسة هو دراسة تأثير التوكسوكاراكانيس على كلى الجرذان المعدية ببويضات التوكسوكارا الغير مشعة أو المشعة بأشعة جاما بجرعة ٦٠٠ جراي أو ٨٠٠ جراي والتحدي لبويضات التوكسوكارا العادية. وقد شملت الدراسة التقييم البيوكيميائي لمضادات الأكسدة الدفاعية من خلال قياس مستوى الأنشطة الأنزيمية من ديسموتاز وبيروكسيداز والجلوتاسيون. كما تم دراسة وظائف الدم بالأضافة إلى التغيرات الهيستوباثولوجية فى الكلى. قسمت ثمانون جرذ إلى أربع مجموعات **المجموعة الأولي:** وهي المجموعة السليمة **المجموعة الثانية:** وهي المجموعة المعدية ببويضات التوكسوكارا العادية عن طريق الفم (١٥٠٠ بويضة/ مل) **المجموعة الثالثة:** وهى مجموعة معدية ببويضات توكسوكارا مشعة بأشعة جاما (٦٠٠جراي) (١٥٠٠ بويضة/ مل). **المجموعة الرابعة:** وهي معدية ببويضات مشعة بجرعة (٨٠٠جراي) (١٥٠٠ بويضة/ مل). بعد اسبوعين من عدوي الجرذان في المجموعات المختلفة تعاد عدوتهم مرة اخرى ببويضات توكسوكارا عادية كجرعة منشطة. وقد فحصت خمس جرذان من كل مجموعة بعد ٧ و١٤ يوم من العدوى الأولى وبعد ٧ و ١٤ يوم من العدوى الثانية أظهرت نتائج هذه الدراسة ان الجرذان المطعمة ببويضات التكسوكارا المشعة (٦٠٠ أو ٨٠٠ جراى) أفضل فى مستوى التغييرات البيوكيميائية والهستوباثولوجية وكذلك وظائف الدم. كما وجد أن التطعيم ببويضات التكسوكارا المشعة ب ٨٠٠ جراى كانت نتائجها أفضل.