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Effect of gamma irradiation on the development of hydatid cyst in mice

Dr. Ekram Ismail Fagiree Ismail.
Department of Biology, College of Science, University of Hafr Al Batin, P. O. Box 1803, Hafr Al Batin, Saudi Arabia. Alzaiem Al Azhari University. Sudan.
ik_fagiree@hotmail.com

Dr. Mohammed Bahaa Eldeen Ahmed Saad.
Alahlia University. Khartoum. Sudan

Dr. Aisha Abass Elsadig.
Department of radioisotopes of the Veterinary Research Center. Khartoum Sudan

Dr. Nahlah Makki Almansour.
Department of Biology, College of Science, University of Hafr Al Batin, P. O. Box 1803, Hafr Al Batin, Saudi Arabia. Nahlama@uhb.edu.sa

Abstract: The aim of this study was to evaluate the effect of Gamma irradiation on the development of hydatid cyst and to study the histopathological changes of hydrated cysts. The work was conducted at the Department of Biology, 'University of Khartoum, The hydatid cyst were opened and the protoscoleces were esexposed to different doses of irradiation (60, 150, 250 krads). The viability of the protoscoleces was measured by staining with Eosin and also they were intraperitenealy inoculation into mice (five for each dose) with a number of 400 protoscoleces for each mice.

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Four weeks later, the mice were challenged with a dose of 2000 unexposed protoscoleces for each mouse. The results showed that there was a difference in the establishment rate and the development of cysts inside the mice where the number was reduced with the irradiation doses compared to the control group which was not exposed to irradiation. Most of the cysts developed in the liver and peritoneal cavity. The effect of irradiation was obvious as the cysts were not well developed and small in size compared to the control groups. Histopathological sections were made from different organs of mice (liver, lungs, kidney and muscles) in the group exposed to irradiation and the control group. The results revealed the occurrence of inflammations and oedema in all examined organs, there was necrosis in the liver and narrowing in the portal vein. In the control group, the effect was very severe showing oedema and acute inflammation in different organs.

Keywords: Mice, hydatid cyst, irradiation, protoscoleces.

الهدف من هذه الدراسة هو تقييم تأثير اشعة جاما على تطور الاكياس العدارية ودراسة التغيرات النسيجية المرضية للكيسات المائية. تم إجراء العمل في قسم الأحياء بجامعة الخرطوم. تم فتح الاكياس العدارية وعرضت لجرعات مختلفة من الإشعاع (60 ، 150 ، 250 كراد). وأيضًا تم باستخدام حيويتها بصبغها بالايوسين تم حقن الفئران تحت الجلد (خمسة لكل جرعة) بعدد 400 جزيء أولي لكل فأر. بعد أربعة أسابيع ، تم تحدي الفئران بجرعة من 2000 جزيء أولي غير معرض للاشعاع لكل فأر. أظهرت النتائج وجود اختلاف في معدل التأسيس وتطور الأكياس داخل الفئران حيث انخفض العدد بجرعات التشعيع مقارنة بالمجموعة الضابطة التي لم تتعرض للإشعاع. تطورت معظم الأكياس في الكبد والتجويف البريتوني. كان تأثير التشعيع واضحاً حيث لم تكن الأكياس متطورة وصغيرة الحجم مقارنة بمجموعات التحكم. تم عمل أقسام الأنسجة المرضية من أعضاء مختلفة من الفئران (الكبد والرئتين والكلى والعضلات) في المجموعة

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المعرضة للإشعاع والمجموعة الضابطة. أظهرت النتائج حدوث التهابات ووذمة في جميع الأعضاء التي تم فحصها ، ووجود نخر في الكبد وضيق في الوريد البابي. في المجموعة الضابطة ، كان التأثير شديدًا جدًا مظهراً وذمة والتهاب حاد في أعضاء مختلفة.

الكلمات المفتاحية: اشعاع ،جزيئات اولية ,فئران، حويصلات مائية.

Introduction:

In the field of vaccination, the helminth parasites constitute special problems. They are large complex organisms which do not multiply within the host. A good deal of work is in progress on the fractionation and characterization of helminth antigens with view to recognizing those responsible for protective immunity and thus likely to provide effective vaccination. Many trials have also been carried out with dead helminth products prepared from whole parasites or extracts of parasites. In general, these approaches have been disappointing and, although significant protection has been demonstrated in a number of trials, it usually falls far short of the level necessary of practical application (IAEA, 1988).

One fraction (25kDa) separated by sodium dodecyle sulphate- polycrylamide gel electrophoresis (SDS-PAGE) from a crude preparation of onchosphereal antigens was also shown to stimulate similar level of resistance (Heath and Lawrence, 1996).

Lightowlers et al (1996) found that vaccination with Eg95 reduced the number of viable cysts by 99.3% compared with unvaccinated animals; these results suggested that the Eg95 vaccine could have wide applicability as a new tool for use in hydatid control campaigns.

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A recombinant vaccine of *Echinococcus. granulosus* in sheep has been successful in field trails. This vaccine has been licensed to a commercial group in the peoples Republic of China. Models suggest that livestock vaccines would be most effective if combined with testing and treatment of dogs (OIE report, 2011).

Arange of different antigens including cyst fluid, cyst membranes and PSC had been used as prototype vaccines against *E.granulosus* however, onchospheres or onchospheral antigens induce much higher levels of protection in sheep and mice against challenge (Zhang et al, 2012). Irradiation and nuclear techniques provide some of the most powerful analytical tools in research today, making possible the solution of fundamental biological problems in life sciences (Biology, Agriculture and Medicine).The introduction of these techniques has generated a diversity of experimental approaches leading to new and far-reaching insights into living systems and has helped to re-define and broaden our concepts of life processes. In medical diagnosis and therapy, in the production of irradiated vaccines, in the control of pests, in food preservation, crops, production improvement and many other programmes, these techniques have helped to alleviate human sufferings and have significantly contributed to our prosperity and to enhancing the quality of life in many parts of the globe (IAEA,1975,1988).

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The use of irradiation in parasitology research:

A study in1990 by *Charmy,etal*, white albino mice (Mus Musculus albinus) were intraperitoneally inoculated with aseptical normal and gamma irradiated protoscolices of *E. granulosus* at dose levels of 40, 60, 80 and 100 Krads. Mice, either normaly infected or infected with irradiated protoscoleces and sacrificed at intervals of 4, 6, 8, 10, 12, 14 and 16 weeks, showed a marked increase in the percentage of cyst number in the liver than in the peritoneal cavity or around spleen, although by increasing the dose level to 100 Krads. No cyst were developed around the spleen. Meanwhile, the % of developing cyst in the peritoneal cavity was time and dose level dependent. The mean cyst diameter showed a progressive increase with the increase of infection time and a successive decrease by increasing the dose level of gamma irradiation. An increase in the number of cyst was observed particularly in normal mice where a marked increase was noted on the 8th week post infection while a successive decrease in the mean number of cyst was observed by increasing the dose level of gamma irradiation.

Also, SINGH and DHAR. (1988) studied the effect of gamma irradiation on the protoscoleces of *E. granulosus* of sheep origin. *In vitro* and *in vivo* effects of varying levels of gamma irradiation on protoscoleces of *E. granulosus* of sheep origin were studied. Radiation doses of 100 Gy onwards caused a decrease in the viability of protoscoleces *in vitro*. However, infectivity of protoscoleces was not affected at radiation doses of 300 Gy in

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golden hamsters and 200 Gy in mice although number and size of cysts developing from infections with irradiated protoscoleces in these animals was small in comparison to cysts developing from infections with normal protoscoleces. Four hundred *E. granulosus* protoscoleces, normal or 100 Gy irradiated, proved fatal for mice.

A significant progressive decline in worm establishment was observed in pups given an infection of *E. granulosus* protoscoleces exposed to increasing levels of gamma irradiation from 100 to 600 Gy. No worms established in pups infected with protoscoleces irradiated at 400 and 600 Gy, respectively. Worms developing from irradiated infections in pups were stunted and showed developmental abnormalities. In another study,Al-Samarrae and Ahamed Jassim (2010) used mice as a model to investigate the chromosomal aberration due to gamma radiation, subjected to radiated and non-radiated protosoleces of *E. granulosus* . In this experimental infestation, gamma ray of 81.58416 x 10.3 wave length have been used. The protoscoleces were subjected to a high degree of 25 Gy and low degree of 5 Gy. The hydatid cyst and protoscoleces were subjected to gamma rays Co_{60} with varying dose of 5 Gy and 25 Gy for 1.2 and 6 minutes respectively. According to the method described by Smyth and Davies,(l974) two groups of mice were infested intra- peritoneally with irradiated hydatid cysts of 5 Gy and 25 Gy respectively and another two groups were infested intra- peritoneally with irradiated protoscoleces of 5 Gy and 25 Gy respectively. The other two groups were used as positive and negative controls.

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Mice of positive control group were inoculated with non-irradiated protoscoleces of the same dose. The negative control mice group was inoculated with sterile normal physiological saline. One month later, all the groups were challenged with the dose of 2000 protoscolex/animal intraperitoneally. Five mice from each group were killed after 2 , 4 , 8 and 12 weeks post challenge and tissue samples were collected and examined grossly and microscopically to investigate the macro and micro changes from cytological and genetic points of view in comparison to positive and negative controls. Bone marrow samples were collected from femur of mice and processed according to Cook and Pallister (2000). Processed cells were allowed to grow for 24 hours, collected by centrifugation. The precipitated cells were fixed on microscopical slides and stained with Giemsa for 5-7 minutes. The results revealed no significant variation in both M1 and chromosomal aberration were found in bone marrow cells of infested mice in comparison to control. While it was increased in bone marrow cells of mice infested with 5Gy and 25 Gy irradiated hydatid cyst. Such increase or decrease was found to be low and not significant.

Adam (1997) showed that the reaction of animal tissue against hydatid cyst was found to be in the form of cellular infiltration of lymphocyte and plasma cells. Alveolar oedema, atelectasis, mild congestion and compression of bronchioles adjacent to the cyst in the infected lungs. Atrophy of liver tissue around the capsule, hyperplasia of bile ducts and sinusoid dilatation were observed in infected liver.

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Microscopical examination revealed two different appearances specific to the cyst and the host. The outer wall of the cyst was detected to be light pinkish in color and to be formed of hyalinous cuticular membrane. The germinative layer was assessed to exit below the outer wall. Necrotic material and many giant cells, surrounded by an outer layer comprised of macrophages, lymphocytes and a few numbers of epithediliod histiocystes were present at the periphery of the cyst wall. The area in which the cyst was located was surrounded by a thin fibrous capsule from the external (Vural et al, 2005).

Saad et.al., (1986) found that histopathological variations in the arrangement of layers of the cysts were observed. The cellular reaction of the affected liver was less than in the lung tissue and often divided the fibrous tissue capsule into two adjacent layers.In the lung, pronounced infiltration was found to be adjacent to the lung tissue or in between the serous and fibrous layers, while no marked infiltration was seen at these locations in the liver. Also they found that the cellular infiltration consisted mainly of lymphocytes and plasma cells. They found that the histopathological (cellular infiltration) consisted mainly of lymphocytes and plasma cells. They reported hyalinization, focal necrosis and calcification in c.t capsule of old sterile cyst, diffuse and dense in fertile cysts with slight alveolar oedema, atelactesis and emphysema in the lung tissues and also atrophy of liver tissue surrounding the liver capsule. They reported also hyperplasia in bile ducts

Verma and Swammy (2009), microscopically, found that the lesions found in the liver were depending upon the stage of development and variability of the cysts.

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Some sections revealed echinococcal scoleces in the capsule with negligible reaction. Such scoleces were also found in the hepatic parenchyma showing slight hemorrhage, leucocyte infiltration and mild hepatocellular degeneration. In most of the cysts, the capsule was thick having from inside out a highly cellular zone rich in mononuclear cells with abundant fibroblasts and an outer thick fibrous zone of concentrically arranged collagen bundles. The cyst wall was formed of an eosinophilic laminated cuticular structure. There was infiltration predominantly with lymphocytes and macrophages, and occasionally neutrophils, eosinophils and giant cells. In fertile cysts, they showed that there was thick connective tissue layer , the outer part of which heavily infiltrated with lymphocytes along with some fibroblasts and eosinophils, while on the inner side, these was a sructureless hyaline layer followed by the germinal layer. It showed brood capsule containing scoleces in the various stages of development along with hooklets. The adjacent parenchyma was markedly congested and showed multiple small haemorrhagic areas containing erythrocytes. The junction between the hepatic parenchyma and the connective tissue capsule of the cyst revealed small numbers of mononuclear cells and lymphocytes. The nearly hepatocytes, particularly between cysts, showed engaged sinusoids, collapsed hepatocytes and fibrosis; further , the hepatocytes in the immediate vicinity of cyst wall showed atrophy.

Osman (2007) found that in histopathological study, all lung sections showed fibrous tissue reaction (capsules), cellular reaction, necrosis and collapsed lung tissue neighboring the cyst wall, while liver section shows fibrous tissue reaction, cellular infiltration, atrophy and necrosis of

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hepatocytes neighboring the cyst wall. Parasite membranes (laminated membranes and germinal layer) were obvious, some were continuous and intact containing brood capsule and protoscoleces and the other were disrupted. Some sections showed only remnant of these membranes in both lung and liver sections. Laminated membranes varied in the thickness and number of lamination.

Histopathologically, severity of calcification, fibrous capsule formation and giant cell layer were similar for multivesicular and unilocular cysts. However, the severity of subcapsular inflammation, inflammatory cell infiltration into adjacent organ parenchyma and eosinophil leucocyte infiltration into the cyst lumen was higher in multivesicular cysts (Kul and Yilidiz, 2010).

Heidarpoura et al (2012) had concluded that cystic echinococcosis in camel is associated with oxidative stress. The resulting oxidative stress seems to have a role in the injury of hepatocytes, changes of trace elements and destruction of erythrocytes.

Kul and Yilidiz (2010) also had concluded that, in histopathological examination, in unilocular cysts, parasitic structures were surrounded by foreign-body giant cells, dense eosinophil leucocytes, macrophages, lymphocyte infiltration capsule.

Histopathological examination of the lung tissue exhibited extensive tissue reaction characterized by various degrees of cellular infiltration and fibrosis along with numerous brood capsules attached to the cystic membrane.

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The bronchial epithelium was hyperplastic and peribronchiolar blood vessels were engorged with blood. Necrotic material and many giant cells, surrounded by an outer layer comprising of macrophages, lymphocytes and a few numbers of epitheloid histocytes, were seen. Outer wall of the cyst showed typical echinococcal hyalinous laminated membrane (Leishangthem et al, 2010).

Histopathological changes were the formation of fibrotic capsules around biliary tracts and portal vein and also leaky liver was marked in all the sections examined. Meanwhile, pre-malignant changes were seen in the different foci particularly around the biliary tracts and portal veins (Rashed et al, 2004).

The development of the larval stage of E.granulosus to form a hydatid cyst is slow andvariable. It depends on a number of factors; including species, strain of parasite and host, andthe degree of infection (Thompson, 1986). Other factors such as age, sex and immunological status of host have also been implicated (Baban, 1990). A number of investigators have carried out experimental infections of cystice chinococcosis, using albino mice as anexperimental animal model, but there has been disagreement about the effect of the parasite infective dose on the outcome of infection. These studies, which have been carried out in Iraq and other places, used protoscoleces (PSCs) as the causative agent of cystic echinococcosis, but their infective doses were variable. Kammerer and Perez-Esandi 1975, injected 1000 PSCs/mouse. Juma 1993 used 1500 PSCs/mouse. Al-Salami 2004, andHashemitabar *et al.2006,* used a challenge dose of2000 PSCs/mouse. Al-Kaisy 2005 used 3000PSCs/mouse. Finally, Al-Nasiri (2006).used differentdoses: 625,

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1000 and 2000 PSCs/mouse, and she found that 625 PSCs/mouse was a sufficient dose to establish an experimental infection. This study was established to shed light on the relationship between the number of injected PSCs and the pathological changes that consequently occur in the liver and spleen, since theseorgans have a role in the immune status of the host and are a target for such an infection.

Materials and Methods:

Twenty mice, 20-weeks of age were used in this experiment. Mice were divided into four groups of 5 each.Group1, 2 and 3 received intraperitoneally 400 irradiated protoscoleces100GY (60 krad), 300 GY(150 krad) and 600 GY(250 krad) respectively and group 4 received 400 un- irradiated protoscoleces and was considered as the untreated control.

Four weeks post-infection, all groups of mice were challenged intraperitoneally with equal amount of 2000 protoscoleces. All groups of mice were sacrificed after 6 months and established cysts were counted and examined.

Certain parameters were considered. These were:

1- Morphological changes of the protoscoleces.

2- Viability of the cyst.

3- Development of the cyst.

The establishment rate was determined using the formula:

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$$\frac{\text{Number of cysts in each group}}{\text{Number of cysts in the control group}} \times 100$$

Histopathology:

Representative samples from the infected organs were fixed in 10 % formal – saline for histopathological studies. After fixation, the tissue (cyst wall and surrounding tissue) was divided into three equal parts .Samples were dehydrated in descending grades of alcohol cleared in xyline and embedded in paraffin wax. After blocking, sections 5µm thick were cut using a rotary microtome. Tissue sections were floated in a water bath at 48ºC and collected on glass slide. Sections were further deparaffinized in xyline and dehydrated in descending grades of alcohol then washed in distilled water. Sections were stained using haematoxylin and eosin (H&E) as described by Drury and Wallington (1967).

Results:

Effect of Gamma rays irradiation of protoscoleces on cysts development in mice:

Three different irradiated doses were used (60,150 and 250 krad).The establishment rate compared to the control was found to be 19.3%, 45% and 48.4% respectively (Table 1)

The difference in establishment rates between the irradiated groups and the control was found to be statistically insignificant.

Irradiation of Co_{60} at 60 krads revealed establishment of 3 cysts in one mice and only one cyst in the remaining 3 mice. In this group, all the

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cysts encountered were sterile and of the small size (Table 2, Figure 1).They were located either in the liver (3) or peritoneum (1).

In mice irradiated with 150 krads, 2 mice showed 5 cysts each and 2 mice showed 2 cysts each. All cysts were of the small size and located in the liver (2) and liver + peritoneum cavity (2). All cysts encountered were sterile (table 3, Figure 2).

In mice irradiated with 250 krads, 6, 5, 3 and 1 cyst was counted in the 4 mice. All cysts were of the small size. All were located in the liver and + peritoneum. All cysts encountered were sterile (table 4, Figure 3).

The control group revealed the establishment of cysts as high as 11 in one mice and as low as 5 in another mice. They were all of the small size locating in the liver, peritoneal cavity and kidney. Only 9 cysts were sterile and the rest (22) were fertile (Table 5, Fig 4).

Table 1: Effect of irradiation with gamma in different groups

Irradiation dose(krads)	No of cyst	Establishment rate	Expected Responses	Residual	Probability
60 krads	6	19.30%	6.361	-.361	.106
150 krads	14	45.00%	11.079	2.921	.074
250 krads	15	48.40%	17.559	-2.559	.070
Control	31	100%	28.039	2.961	.904

Since the significance level is less than 0 .150, a heterogeneity factor is used in the calculation of confidence limits.

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Table 2: Effect of irradiation with gamma 60 krad on size & stage of the cyst:

Mice No.	Total number of cyst estableshed	site	size	Case
1	3	liver	small	Sterile
2	1	liver	small	Sterile
3	1	liver	small	Sterile
4	1	peritoneal	small	Sterile
Total	6	-	-	-

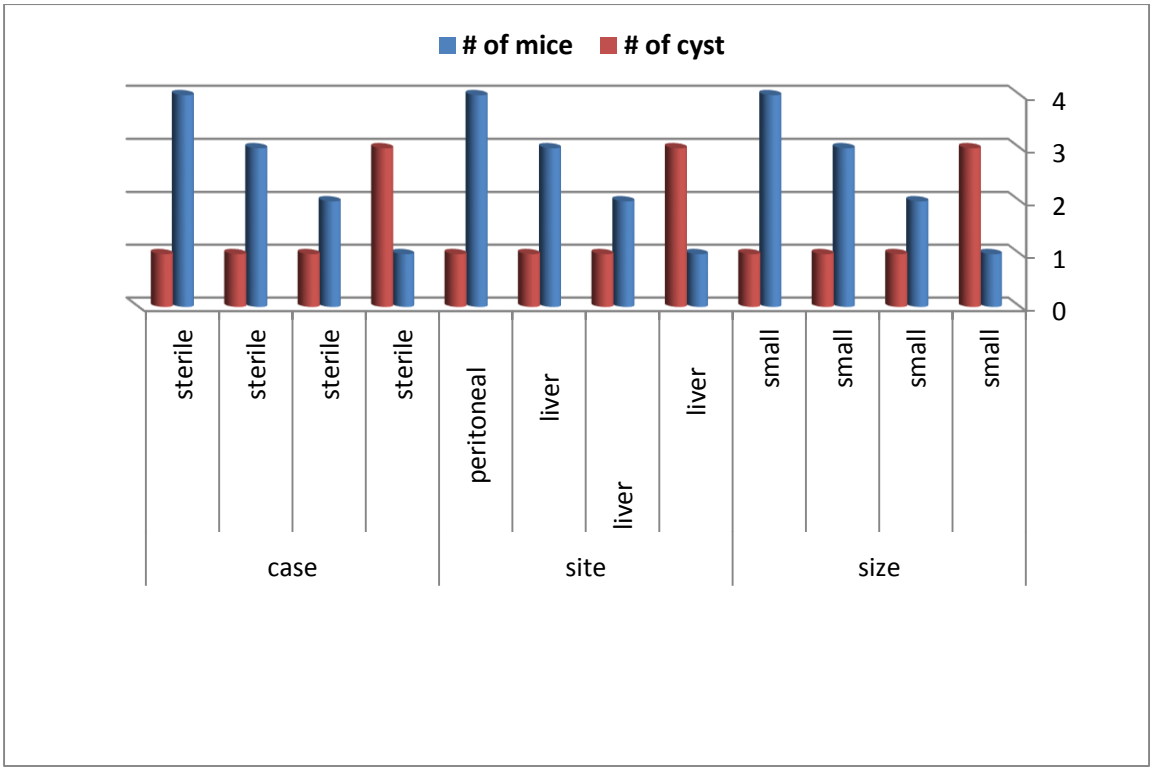

Figure1: Effect of irradiation with gamma 60 krads on size & stage of the cyst

Table 3:Effect of irradiation with gamma 150 krad on size & stage of the cyst:

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Mice No.	No. of cyst	site	size	Case
1	5	Liver & peritoneal	small	sterile
2	5	liver	small	sterile
3	2	liver	small	sterile
4	2	Liver & peritoneal	small	sterile
Total	14	-	-	-

Establishment rate=$\underline{14 \div 31 \times 100 = 45\%}$

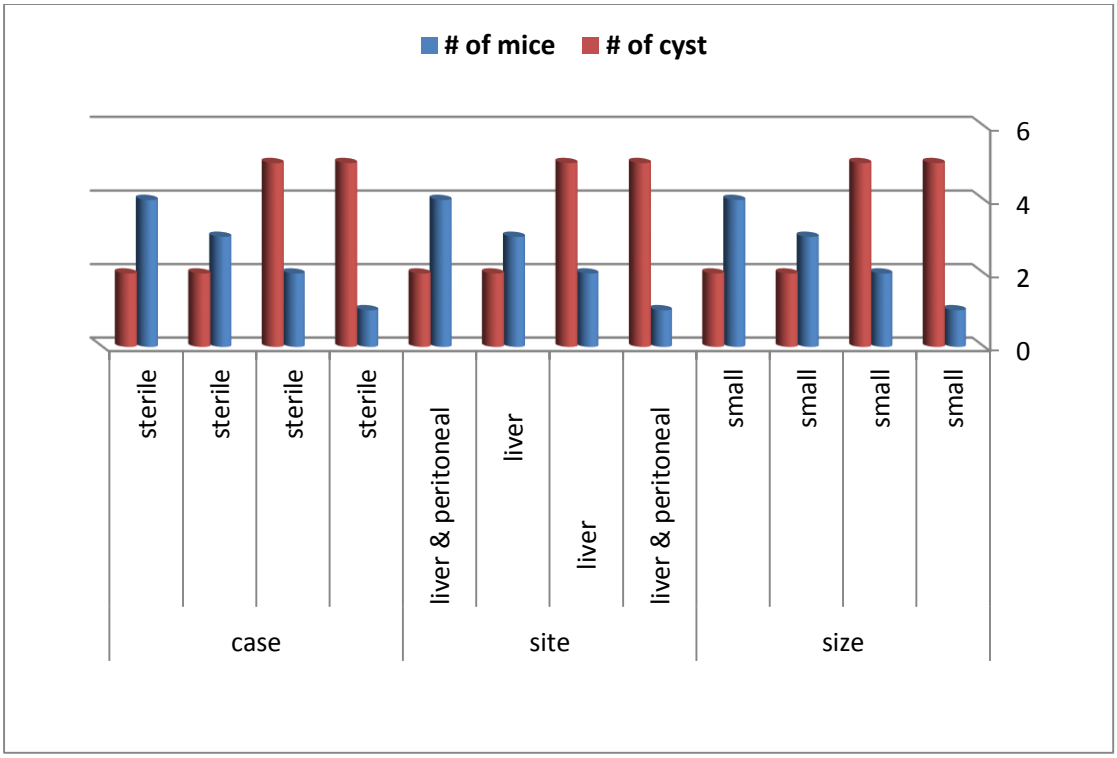

Figure 2: Effect of irradiation with gamma 150 krads on size & stage of the cyst

Table4: Effect of irradiation with gamma 250 krad on size & stage of the cyst:

Mice No.	No. of cyst	site	size	case
1	3	Liver	small	sterile
2	1	liver	small	sterile

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3	5	liver	small	sterile
4	6	Liver & peritoneal	small	sterile
total	15	-	-	-

Establishment rate=15 ÷ 31 × 100 =48.4%

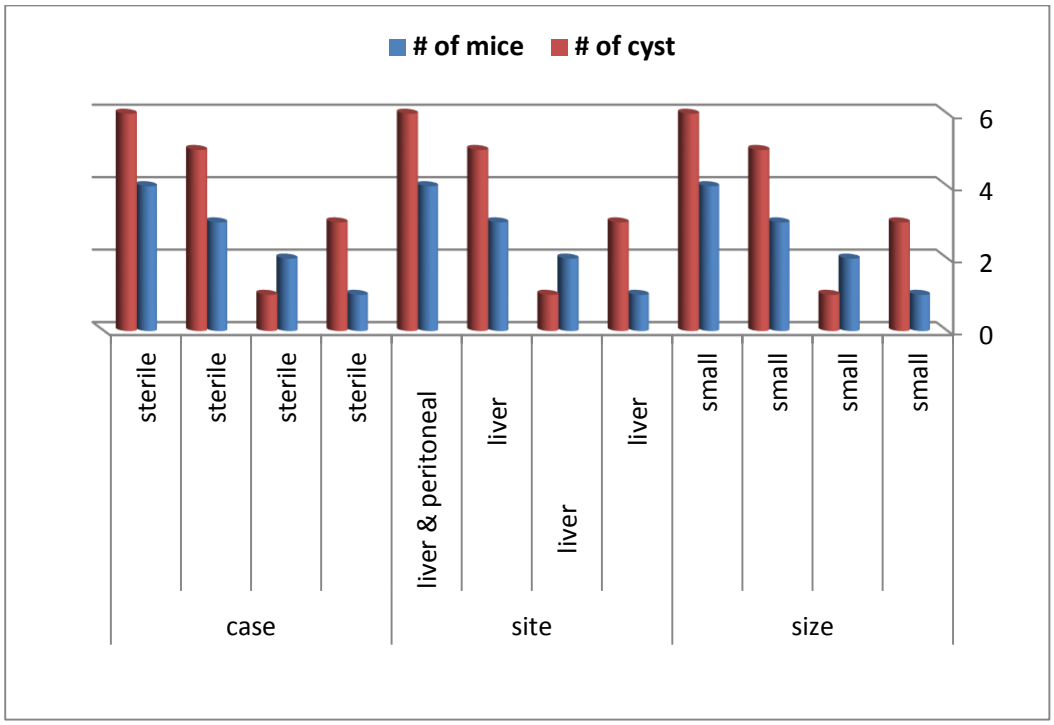

Figure 3: Effect of irradiation with gamma 250 krads on size & stage of the cyst.

Table 5: The Controls groups

Mice No.	No. of cyst	site	size	case
1	9	Liver & peritoneal	small	sterile
2	11	Kidney & liver& peritoneal	small	fertile
3	6	liver	small	fertile
4	5	peritoneal	small	fertile
total	31	-	-	-

Establishment rate=31 ÷ 31 × 100 =100%

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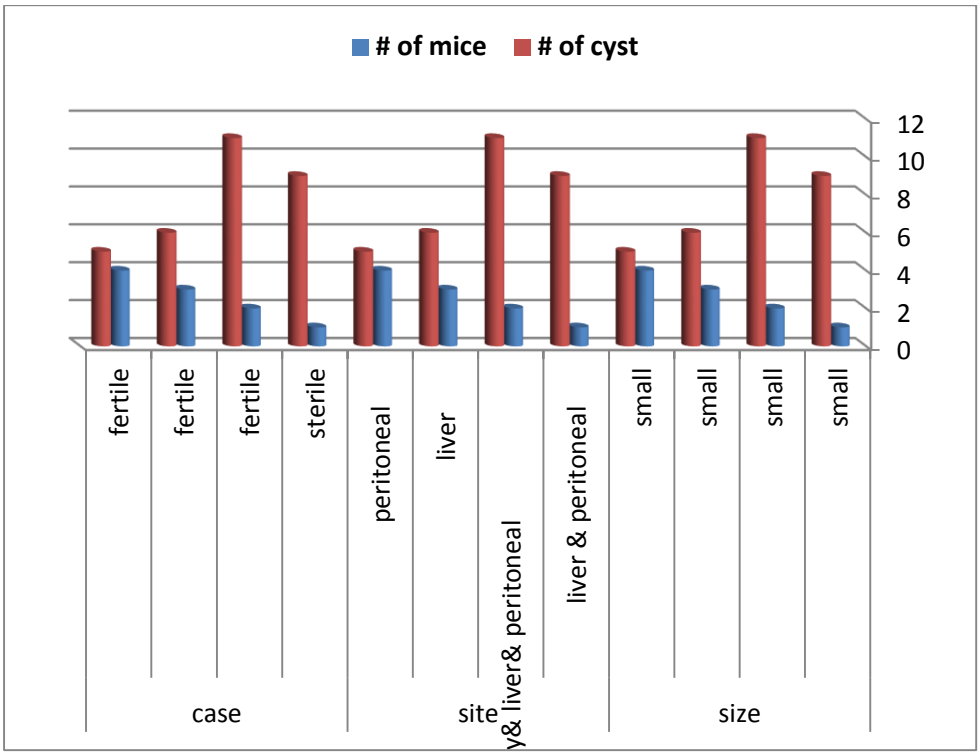

Figure 4: The Controls groups.

Histopathology :

The histopathological results in this study showed that:

Representative specimens were taken from different mice in the three groups exposed to 60,150 and 250 krads in addition to the control group.

Liver:

In group1 (60 krads) and group 2(150 krads), there was a normal hepatic cells tissues, some necrotic areas with very few scoleces and inflammatory area. In group 3 (250 krads), a huge destruction of hepatic tissues with infiltration of inflammatory cells that replaced hepatic tissue was observed. Necrotic hepatic cells at first stage of dissolution were also observed (Fig 5, Fig 6)

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Section of the control group showed wide area of coagulative necrosis boarded by a narrow zone of inflammatory cells infiltrates, tissue debris and extra vascular erythrocytes forming infarct like structure .In the control sections, large cyst and scoleces with hooks were very clear, size of central vein was changed (Fig 7).

Lung:

In group 1, there was a disintegrated inflammatory cells and oedema. In group 2, sand fluid with necrotic cells was observed. There was also disintegrated inflammatory cells, dead alveoli ,homogenous area in alveoli, very few scoleses ,oedema and emphysema (Fig 8, Fig 9). In group 3, oedema, inflamatory cells and few scoleses were observed. .The control group revealed very severe oedema , emphysema and atelectases, cyst fluid was not clear.

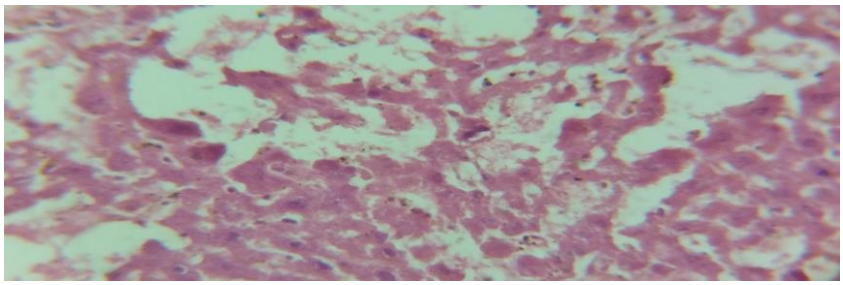

Figure 5 :Histopathology of liver exposed to 150 krads.

a : Degenerated and necrotic hepatic cells, b:Scoleces ,c:Cyst fluid.

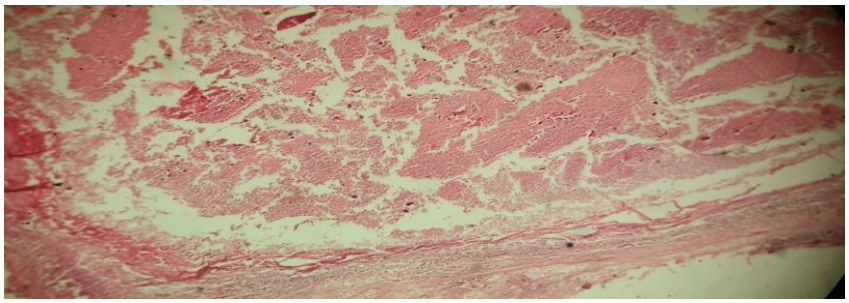

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Figure6:Histopathology of liver exposed to 250 krads.

a : Cyst fluid, b: Scoleces ,c: Cyst wall.

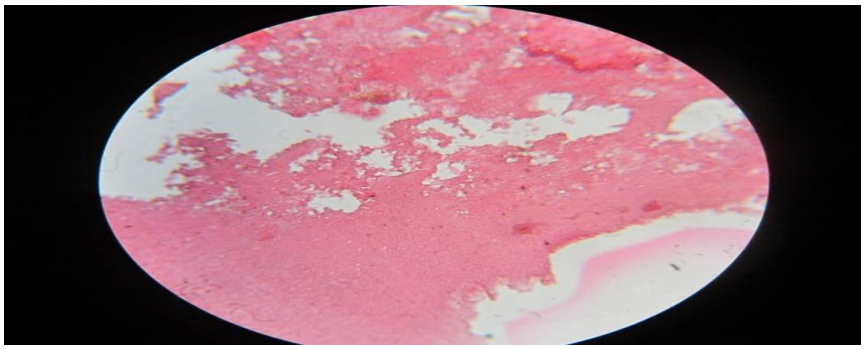

Figure7:Histopathology of a liver in the control group.

a : Coagulative necrosis, b: Cyst wall ,c: Cyst fluid ,d: Ruptured cyst.

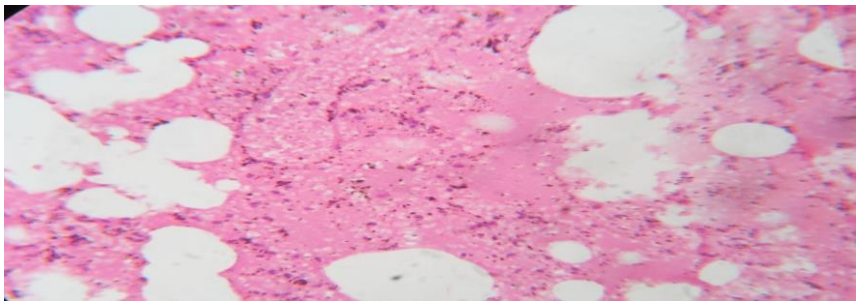

Figure8 :Histopathology of a lung exposed to 60 krads.

a : Oedema ,b : Emphysema

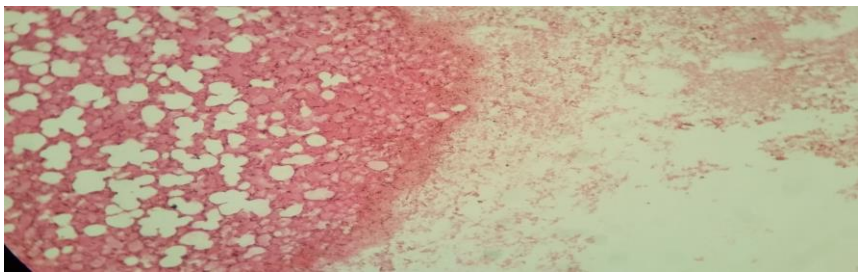

Figure9: Histopathology of a lung exposed to 150 krads.

a: Emphysema ,c : Sandy fluid.

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Discussion:

From the results, irradiation with different doses of cobalt 60 (60,150 and 250 krads) revealed that the establishment rates after challenge were 19.3%, 45% and 48% respectively. This finding was consistent with the work of Ayse (1977) who reported establishment rates of 50%, 42% and 40% for the doses of 60,150 and 250 krads respectively. Our results showed that the infection rate decreased in 60 and 150 krads dose clearly and the number of cysts was very low compared with the controls. This finding agrees with the results of Movsesjan et al (1967 a, b) who reported also in their study a decrease in the number of cysts. This investigation showed that there was a decrease in the mean number of cyst when increasing the dose level of irradiation compared with the control which showed that the number of cysts were very high. This was in line with the results of Charmy et.al (1990) and Wikrhauser, et.al (1974) who reported that the number of cyst decreased when the dose of irradiation was increased. In this study, the development of protoscoleces inside the cysts in case of being exposed to the three radiation doses is incomplete and very few compared to the control group. If present, it will be morphologically altered and incompletely developed. In many cases, we find only the hooks in large numbers compared to the control. This in our opinions may be attributed to the effect of the irradiation dose on the scoleces and their development rate inside the cyst fluid. These results agree with the results of Ayse (1977) who reached the conclusion that irradiation doses with Co_{60} has an effect on the cysts and the scoleces as well clearly as that all cysts were sterile while the fertility rate in the controls group was 60%.

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. Histopathological changes in mice:

From the results of our study in group 1(60 krads) and group 2 (150 krads) in the liver, there was a normal hepatic tissues. In group 3 (250 krads), a huge distruction of hepatic tissues with inflammatory cells that replaced hepatic tissues was observed. In the control group, there was a wide area of coagulative necrosis boarded by anarrow zone of inflammatory cells infiltrates, tissue debris and extra vascular erythrocytes forming infarct like structure. In the control group, large cyst and scoleces with hooks were very clear, size of central vein was changed. These results agree with the work of Azhar,et.al (2009) who showed that the histopathological changes of liver of mice injected with different doses of PSCs were dependent on the dose, as well as the post-infection period. The most severe changes were recorded in livers of animals injected with 2000- 2500 PSCs/mouse. It is generally accepted that the liver is the essential body organ responsible for detoxifying and withdrawal of many injurious substances in the body (Cohen, 1982). and the hepatic cells account for the formation of much of the blood proteins, but not gamma globulins (Ham, 1965). The histopathological changes may be due to the connection between the immune status of the host and liver status. Thus, it is quite reasonable to detect great pathological consequences in this organ in mice subjected to the effects of *E. granulosus* PSCs or their antigens. Similar studies done by Guo *et al.*(2000), Hanada *et al.* (2003), Fayed *et al(2004)*, Rashed *et al.(2004)* and Al- Kaisy (2005) showed the same pathological changes.

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The infiltration of the portal areas by lymphocytes and increase in Kupffer cells indicates an inflammatory reaction in liver tissue and has a role in defense mechanisms (Schumann et al 2000).

The finding of histopathological changes in the lung in group 1(60krads) showed a disintegrated inflammatory cells and oedema, group 2 (150)showed sand fluid with necrotic cells, there was also disintegrated inflammatory cells, dead alveoli ,homogenous area in alveoli, very few scoleces ,oedema and emphysema, group 3(250) showed oedema, inflammatory cells and few scoleces. The control group revealed very severe oedema , emphysema and atelectases, cyst fluid was not clear. The infiltration of cells was consistent with the finding of Saad et al, (1985) who almost got the same observations.

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