Study of the pathological changes induced by irradiated protoscolices (from sheep hydatid cysts) after intra peritoneal injection in white male mice

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Summary

In the present study , we investigate the effect of Gamma radiation of wavelength 81.58416X10^-13 on the viability of protoscolices , the larval stage of *Echinococcus granulosus* , which was isolated from liver hydatid cysts of invested sheep .

The study was accomplished on 80 white male mice , the mice were divided randomly into four equal groups , group 3 and 4 were invested intra peritoneally with 2000 Gamma irradiated protoscolices , with 5 and 25 Gray of Gamma rays for 1.2 and 6.6 minutes respectively .

The experimental irradiation of protoscolices was Gamma rays cobalt 60 (Co-60) . Then the ability of protoscolices was checked before and after the irradiation. Group 1 and group 2 were used as negative and positive control respectively.

After one month group 2 , 3 and 4 were given challenge dose of 2000 protoscolices intraperitoneally for every single mouse in every group. Mice were killed after 2 , 4 , 6, 8, and 12 weeks after the challenge .

Macroscopic and microscopic studies were done for all experimental groups .

The following findings were seen :-

1. Reduction of the violability of protoscolices by inverse of the radiation intensity incorrespondense with the time of exposure.
2. The macroscopic studies indicate reduction in the average number and diameter of the secondary hydatid cyst in the irradiated groups in comparison with the positive group .

3. The histopathological changes were characterized by presence of degenerated distracted hydatid cysts in the livers , especially in the irradiated group , with the presence of hyperplastic splenic white pulp lymphoid tissue in comparison with the positive control .

دراسة التغيرات المرضية الناجمة عن حقن الرؤيسات الأولية المشعة (المعزولة من أكياس عدرية في الأغنام) في التجويف الخلبي لذكور الفئران البيضاء

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الخلاصة

جرى خلال هذا البحث دراسة تأثير أشعة كاما ذات الطول الموجي X 81.58416 -13 ^10 على حيوية الرؤيسات الأولية (الطور اليرقي لطفيلي المشوكات الحبيبية) و المعزولة من أكياس عدرية كبدية من الأغنام المصابة . تمت الدراسة على 80 فأر، ذكر ابيض ، قسمت عشوائيا" و بالتساوي إلى أربع مجاميع ، المجموعة الثالثة و الرابعة حقنت بالتجويف الخلبي بـ 2000 راس أولي مشعع بأشعة كاما (25 Gray , 5 Gray) ولمدة 1.2 و 6.6 دقيقة على التوالي . أجريت تجارب التشعيع بأشعة كاما ، كوبلت 60 و قد تم فحص حيوية الرؤيسات الأولية قبل و بعد التشعيع .

المجموعة الأولى و الثانية عُدت مجموعتي سيطرة سالبة و موجبة على التوالي . و بعد شهر من الحقن ، أعطيت المجاميع الثانية ، الثالثة و الرابعة جرعة تحدي بـ 2000 راس أولي حيوي في التجويف الخلبي لكل فأر في المجموعة و قتلت الفئران بعد 12 , 8 , 6 , 4 , 2 أسبوع بعد جرعة التحدي ، وقد تم دراسة التغيرات المرضية العيانية و المجهرية لكافة المجاميع. وقد بينت النتائج ما يلي :-

1. اختزال حيوية الرؤيسات الأولية المشعة بأشعة كاما عكسيا" مع شدة جرعة التشعيع و المدة الزمنية .
2. بينت التغيرات المرضية العيانية اختزال بمعدل عدد و أقطار الأكياس العدري الثانوية في المجاميع المشعة مقارنة بمجموعة السيطرة الموجبة .
3. تميزت دراسة التغيرات النسيجية بوجود الأكياس العدرية المتنكسة في الكبد و لاسيما في المجاميع المشعة مع وجود فرط تنسج اللب الأبيض في الطحال مقارنة بمجموعة السيطرة الموجبة .

Introduction

Hydatid cyst or hydatid disease is a major zoonotic problem in Iraq , sheep, cattle, and man act as intermediate host with formation of hydatid cysts mostly in liver , lung .

While dogs act as final host for the actual parasite *Echinococcus granulosus* , which especially common in sheep raising areas such as the Middle East .(1)

The entire worm consists of 4 segments , including the scolex , and it is only 3 – 5 mm long . The scolex has retractable rostellum armed with a double circle of hooks and 4 suckers . Only a single ripe proglottid occurs at any one time and it is tiny.

The importance part of the life cycle so far as its pathogenecity is concerned, is the metacestode which may occur in man, cattle, sheep, camels, horses, and rabbits. These hosts ingest *Echinococcus granulosus* eggs, the oncospheres are liberated in the intestine and enter mesenteric veins and make their way to various organs , especially liver and lung (2 ; 3) .

In these organs , the oncospheres develops into a spherical cyst may grow to diameter of 15 cm . The size and shape of the cyst may be limited by the organ or the space in which it develops . The cyst's inner lining is germinative and gives rise to scoleces, brood capsules and daughter cysts.

Brood capsules and scoleces may become free from their attachments and may form a loss mass on the floor of the cyst, this mass is known as hydatid sand. The cyst is filled with hydatid fluid (4, 5).

The fluid in sheep hydatids is mixture of host serum components and parasite antigens. Host IgG molecules have been found in hydatid cysts from several species in mammals. As many as (2) million scoleces may be present in a large cyst and may contain (2) litters of fluid . These fluid filled cyst are unilocular and may persist for years .

The hydatid cyst grows at a rate of (1) mm/ month, or approximately (1) cm / year (6 ; 7) .

Clinical symptoms do not usually develop until the cyst at least (10) cm in diameter . Physical signs of tumor mass do not usually develop until the cyst reaches a diameter of (20) cm .

Symptoms of hydatidosis are due to (2) factors , localization with mechanical effects brought about by the space occupying growing cyst, and the generalized toxic and/or allergic reactions secondary to absorption of the toxic products of the parasites . Heavy infection may result in sever tissue damage and death (8 ; 9).

Damage by any cyst is, of course, related to its size and location, simple cyst seems to do little harm to animals, although pressure on surrounding organs may be consequence. Rupture of cyst in man may cause toxic reactions such as rash or weal, redness of the skin, fever, shortness of breath, cyanosis, vomiting, diarrhea, circulatory shock, and sudden death. Intravenous injections of sheep hydatid cyst fluid into animals cause similar cardiovascular responses. Enlarged cyst may destroy bone or may impair the normal functioning of other organs. Migrating cyst can be serious.

Host tissue reactions toxemia, eosinophilia, pressure effects, obstruction of blood vessels (10 ; 11) .

The previous microscopically studies showed that the invades oncospheres surrounding by leukocytic inflammatory cells, mostly lymphocytes and neutrophils after hours of infestation, then enclosed by fibrous adventitia and developing granulomatous inflammatory reaction especially in advanced host response, therefor, the larva either undergoing necrosis and degeneration due to host immune response or the developed larvae will over come the inflammatory response of infested tissue (12 ; 13 ; 14) .

Work with mice suggests a combination of humoral and cell mediated immunity factors in host resistant to echinococcus .

The only treatment for hydatid cyst is surgical excision, and even this procedure is often unsuccessful or impractical. Prevention

consists mainly of keeping dogs free from infection and avoiding ingestion of tapeworm eggs (14 ; 15 ; 16) .

The aim of this study is to irradiate protoscolices then injecting in white male mice, to find out, if these protoscolices lost their ability to form hydatid cyst, but at the same time, they are antigenically active, able to promote humoral and cellular immunity to protect the intermediate host from infection in case of a challenge by natural spontaneous infection. Then followed by macroscopic and microscopic studies.

Further more, the present research project is an attempt to develop a vaccine against hydatid disease in sheep and man by using Gamma irradiation.

Materials and Methods

1. Animals used in this experiment were white balbe mice of 28 – 35 days old. From Al – Razi Institute Research Center . with controlled temperature, light, and feeding .
2. Protoscolices were prepared and isolated from liver hydatid cysts of infested sheep slaughtered at Al – Karkh Slaughter House brought in sterilized containers to pathology laboratory of Baghdad Veterinary Medical College . They were prepared according to method of (17) . Choosing Protoscolices, which have 90% availability of living once .
3. Gamma irradiation . The viable living prepared Protoscolices were irradiated by Gamma irradiation by using cobalt 60(Gamma Cell 900) as radiation source which is phapha Atomic Research Center / Trompay / Bombays / India. Dose rate is 0.286563 kg/hr., for the irradiation experiment using wavelength 81.58416 X 10^-13 meter and energy average 1.25 MEV in College of Science department of atomic physics .

Design of the study

The study consists of 4 groups:-

1. (20) White male mice as untreated negative control.
2. (20) White male mice as treated positive control.
3. (20) White male mice injected intraperitoneally with Gamma irradiated protoscolices of 5 Gray for 1.21 minute.
4. (20) White male mice injected intraperitoneally with Gamma irradiated protoscolices of 25 Gray for 6 minute.

After (1) month, mice of groups 2, 3, and 4 challenged intraperitoneally with live / viable protoscolices / mice.

Mice of the first group injected intraperitoneally with normal physiological saline .

Pathological examination

Mice of all groups were killed after 2, 4, 6, 8, and 12 weeks of challenge dose, they were sacrificed and examined carefully macroscopically for any hydatid cyst formation and for any other abnormalities.

Tissue samples will taken from all visceral organs, including abnormalities, for histopathological processing. Selected samples were passed through paraffin processing procedure to make paraffin blocks, these blocks were be cut at 5 – 6 Microns, slides were stained with hematoxylin and eosin stain (18) .

Results

Macroscopic (gross) examination

5 Gray

The macroscopic examination showed enlarged liver (hepatomegaly) and spleen (splenomegaly), after 2 weeks until the 12 weeks after giving the challenge dose (Fig.-1-) it appeared development of small individual hydatid cyst in the intestine and peritoneum (5 cysts), after 4 weeks of giving the challenge dose, and after 8 weeks increase the number and size of the hydatid cyst, as it

become up to (10 cysts), after 12 weeks increase their number up to (13 cysts), but they were empty of living protoscolices .

25 Gray

After 4 weeks from giving the challenge dose, the number of hydatid cysts in the liver just one (Fig.-2-), and in the 8 week, the number of cysts was 2, after 12 weeks, the number was only one with diameter of 0.2 mm, in addition to Splenomegaly .

Positive control group

The macroscopical pathological picture of this group characterize by presence of secondary hydatid cysts in visceral organs (liver, spleen, kidney and intestine), as the number of these cysts went up to (133 cysts), with average diameter of 5 mm (Fig.-3-) .

Microscopic histopathological changes

5 Gray

The liver showed after one month of challenge, presence of inflammatory changes in hepatocyte result in infiltrate of mononuclear cells and lymphocytes in sinusoid, pericentral vein, in addition to dilatation of central and peripheral veins, which showed congestion .

The spleen showed evidence of lymphoid depletion in the white pulp, with congested red pulp, associated with increase of reticular cells and macrophages (Fig.-4-)

In the kidney, there was evidence of glomerulonephritis with tubular fatty degeneration which result in narrowing of tubular lumen and occurrence of cell edema in the epithelial lining of other tubules. (Fig.-5-)

Microscopic examination of the lung showed proliferation of bronchial epithelial cells (hyperplastic epithelial cells),

With peribronchial lymphoid aggregates and macrophages. With the progression of infection (after 2 months), the histopathological changes showed more severity manifested by heavy fatty degeneration

of the liver associated with diffuse lymphocytic infiltration and increase activity of the Kupffer cells with associated bile duct proliferation (Fig.-6-) . In addition to sever lymphoid depletion in splenic white pulp.

These pathological changes were continued to progress after 3 months from the study until the termination of the experiment, as it was found that liver section showed accumulation of degenerate protoscolices, with mononuclear cells infiltration (Fig.-7-) .

25 Gray

The histopathol0ogical changes of this group characterized as being more sever than those of 5 Gray irradiation, by presence of degenerated, secondary hydatid cysts characterized by necrosis of their germinal and laminated layers enclosed by granular necrotic zone (Fig.-8-), as well as, associated with focal hepatic degeneration and marked centric lobular fatty degeneration 2 months after challenge (Fig.-9-) . While the histopathological changes in the spleen characterized by lymphoid hyperplasia of white pulp with lymph follicle formation, with germinal center proliferation of small lymphocytes, 3 months after the challenge dose (Fig.-10-).

Positive control group

One month after infestation with protoscolices , there were degenerative necrotic changes in the liver characterized by presence of pyknotic nuclei and marked deep acidophilic cytoplasm, associated with infiltration of lymphocytes and mononuclear cells in liver parenchyma, also activation of kupffer cells (Fig.-11-), accompanied by presence of secondary hydatid cysts enclosed by fibrous capsule which infiltrated by lymphocytes, eosinophils and

macrophages (Fig.-12-) .Also there was evidence of steatitis, with associated infiltration of mixed inflammatory cells in the adjacent adipose tissue (perihepatic capsule peritoneal adipose tissue) .

The spleen showed atrophy of the white pulp lymphoid tissue .

In the kidney, there was evidence of degenerative changes with increase of glomerular cellularity .

In the lung, there were increase peribronchial and peribronchiolar aggregates of lymphocytes and mononuclear cells. After 3 months, there was secondary hydatid cysts with thickened fibrous capsule (Fig.-13-), with marked liver fibrosis especially in periportal areas accompanied by atrophy of hepatocytes (Fig.-14) and that was 3 months after infestation with protoscolices .

Discussion

Infestation of mice by intraperitoneal injection of Gamma irradiated protoscolices, the results if compared with the positive control, it showed increase number and diameter of the hydatid cysts formation in the positive control in comparison with groups of mice infested with Gamma radiated at 5 and 25 Grays respectively, and those changes were compared relatively with the dosage level of the Gamma irradiation of the protoscolices, as it was correspond with the irradiation dose and time of exposure, which were resulted in marked suppression of the viability of the protoscolices and it agreed what was reported and found by (19) whom they found that X- rays of (2 ; 5 ; 10) K- rad caused meaning full reduction in the total viability of the protoscolices .

The present experimental project, demonstrate that the histopathological changes in mice infested with Gamma irradiated protoscolices, had very strong relationship with the activity of the immunized protoscolices, which were found in these tissues and those results were in agreement with what was reported by (20) .

In another previous study, about the e4ffect of X – ray on the viability of the germinal membrane of the hydatid cyst (*Echinococcus multilocularis*), it appears that irradiation dose of 55-k rad was able to destroy the germinal cells (21) .

The groups of mice, which were infested with Gamma irradiated protoscolices of 5 Gray, they were characterized by decrease secondary hydatid cysts, which were formed in the organs, specially the liver and that could be due to the effect of the radiation on the protoscolices viability, especially the formation of hydatid cyst depend on the viability of the protoscolices and the strength of the host immune response (22) .

As well as, it was found that the degenerate protoscolices of this group were enclosed by thick fibrous capsule infiltrate by inflammatory cells and that was in agreement what was mentioned by (23), that formation of fibrous capsule depend on the host immune response as it protect the host from the toxic effects and also prevent availability of the nutrients for the parasite, which at the end result in destruction of the parasite . In addition, proliferation of bile ducts and increase activity of kupffur cells, which have a phagocytic activity, have an important role in controlling the infestation, as the Gamma irradiated protoscolices stimulate the inflammatory cells and their proliferation (24) .

On the other hand, the occurrence of marked fatty degeneration in hepatocytes, which was due to metabolic functional changes of the hepatocytes, which was due to the toxic effects of the Gamma irradiated protoscolices (24 ; 25) .

The increase activity of the splenic white pulp, which was characterized by lymphoid hyperplasia especially at dose of 25 Gray, as it was due to the fact the degenerate protoscolices were blocked and enclosed by fibrous capsule, then it's presence prepare and activate the lymphocytes, then this will cause proliferation of lymphocyte in splenic white pulp as it was reported by (26) .

The formation of the secondary hydatid cysts in the positive control, it was due to activity of protoscolices, which over come the immune response of the host, as it has the ability to resist the host antibodies and combining with them (Trap and mask) and that occurred for the advantage of the protoscolices and it made the antibodies unable to

differentiate the antigenicites of the protoscolices and therefor, those protoscolices will flourish and developed into secondary hydatid cysts (27) .

As well as, the arrival of parasitic antigen to the spleen, would result in depletion of T lymphocyte and in the end, this would result in weakening of the cellular immune response of the host and that would give rise to splenic white pulp atrophy, even though it was able to control the growth of hydatid cyst, but as the hydatid cysts develop and increase in it's size , it would become the cellular immunity, the one in control, as it was found by (28) .

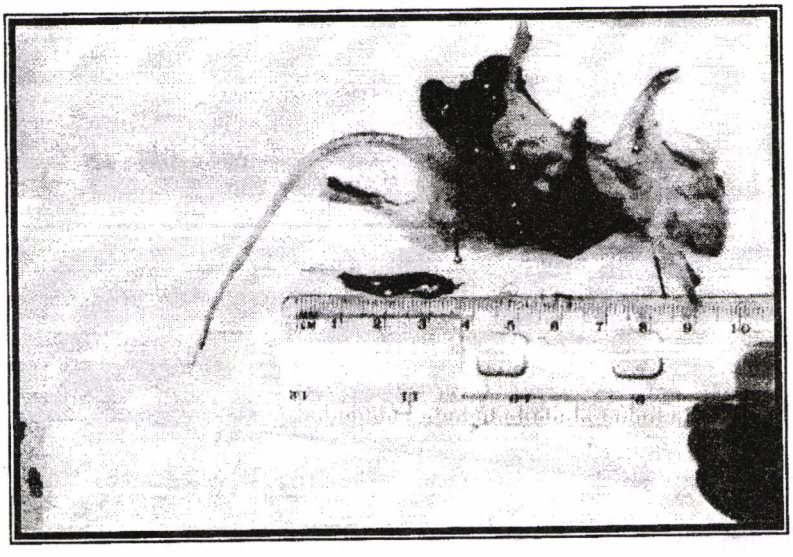

Figure 1 Gross section of abdominal cavity organs for 1 mouse of group '' 5 Gray '' showed: Splenomegally, after one month of challenge .

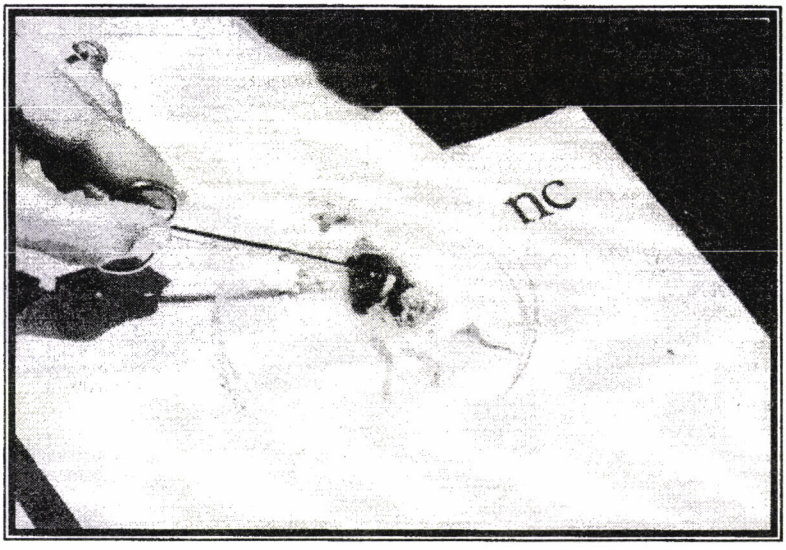

Figure 2 Gross sections of abdominal cavity organs for 1 mouse of group '' 25 Gray '' showed: Degenerated hydatid cyst with splenomegally, after 3 months of challenge.

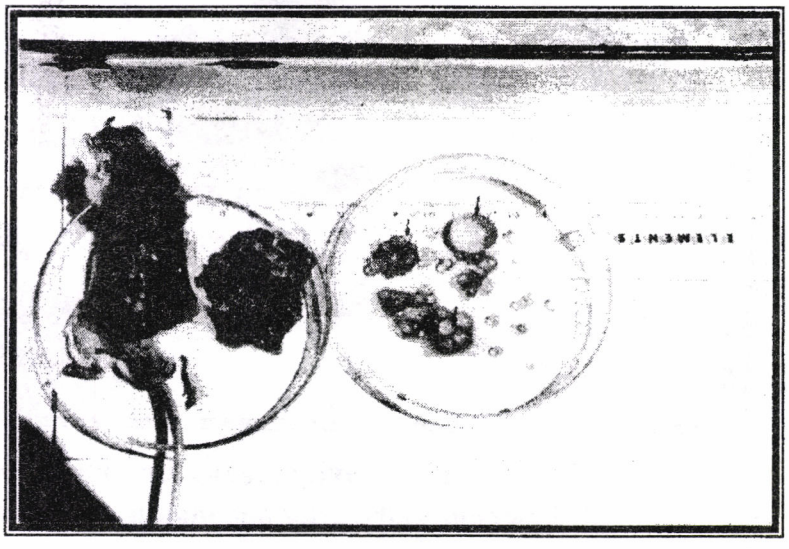

Figure 3 Gross section of abdominal cavity organs for 1 mouse of positive control showed: Development of many secondary hydatid cysts in liver, intestine and peritoneal cavity, after 2 months of challenge.

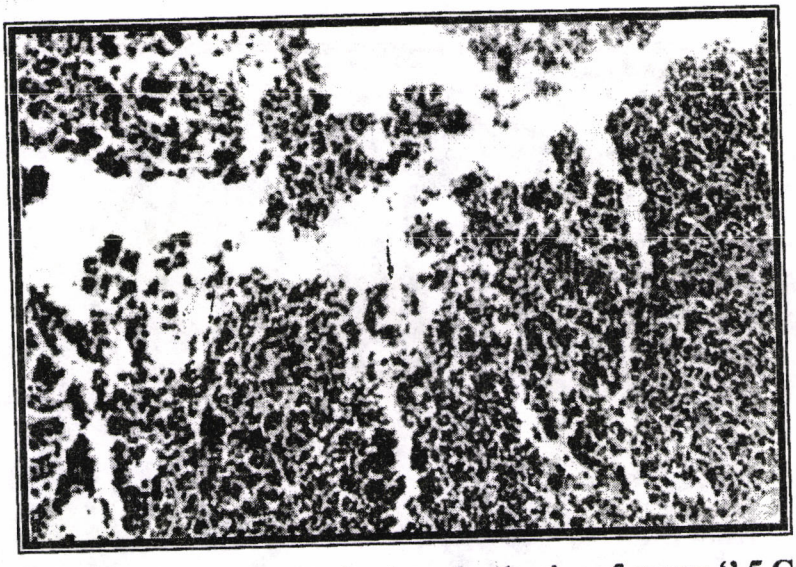

Figure 4 Microscopic section of spleen for 1 mice of group " 5 Gray " showed: Sever lymphoid depletion in white pulp, after 2 months of challenge (40X. H & E stain) .

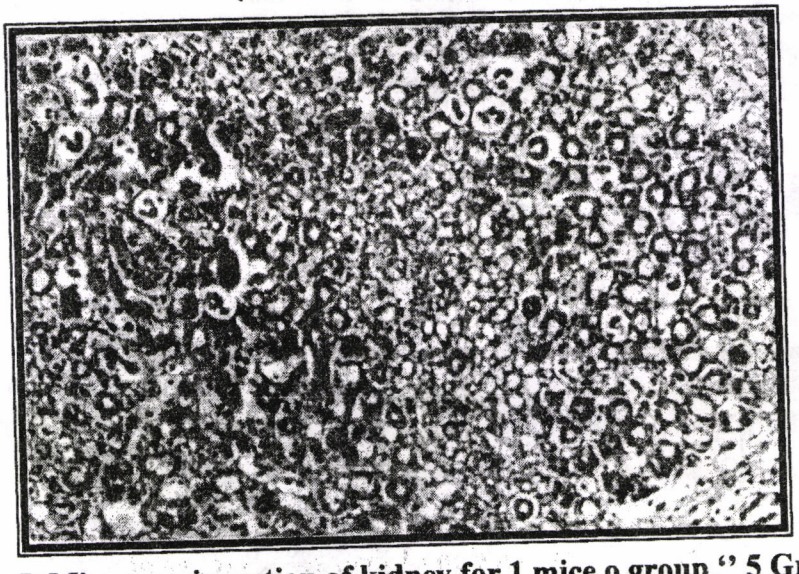

Figure 5 Microscopic section of kidney for 1 mice o group " 5 Gray " showed: Fatty changes with cell edema of epithelial lining of renal tubules, after one month of challenge . (10X. H & E stain)

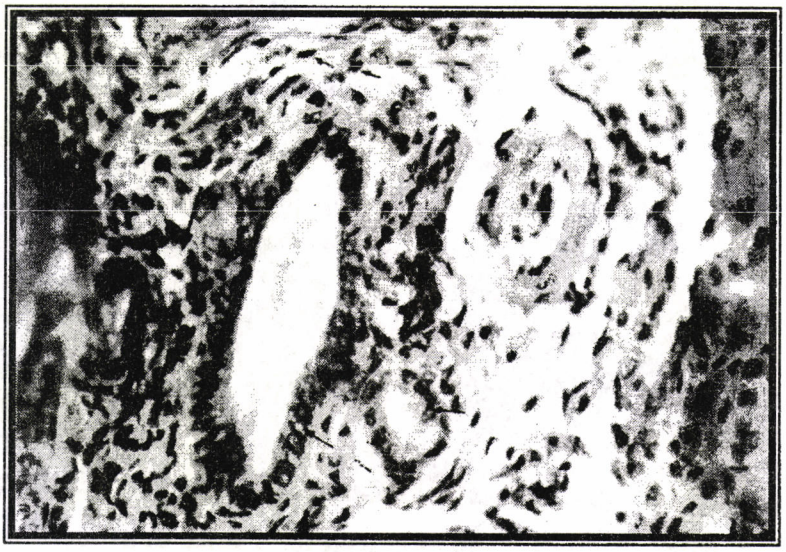

Figure 6 Microscopic section of liver for 1 mice of group '' 5 Gray '' showed: Dilatation and hyperplasia of bile duct with mononuclear cell infiltration and portal fibrosis, after 2 months of challenge . (40X H&E stain).

Figure 7 Microscopic section of liver for 1 mouse of group '' 5 Gray '' showed: Appearance of degenerated protoscolices which surrounded by inflammatory infiltration with fibrosis, after 3 months of challenge . (20X. H & E stain) .

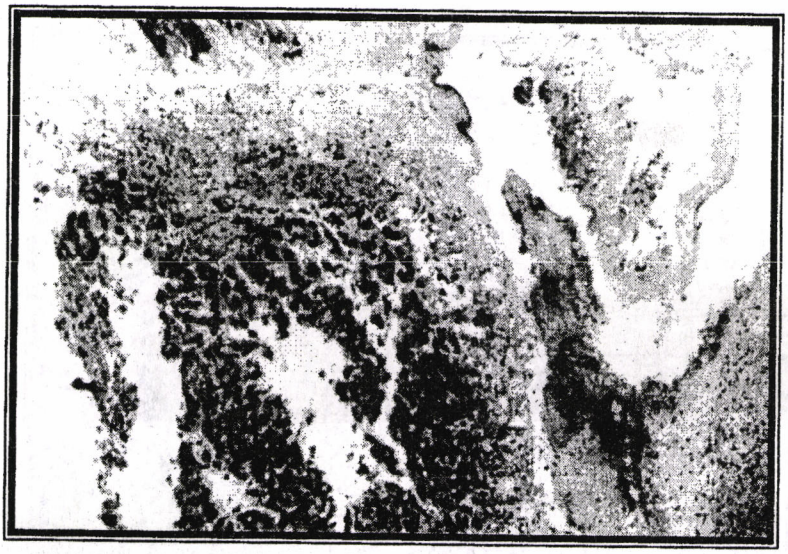

Figure 8 Microscopic section of liver for 1 mouse of group " 25 Gray " showed: Degenerated, liquifactive, secondary hydatid cyst, enclosed by eosinophilic necrotic zone, after 2 months of challenge. (20X. H & E stain) .

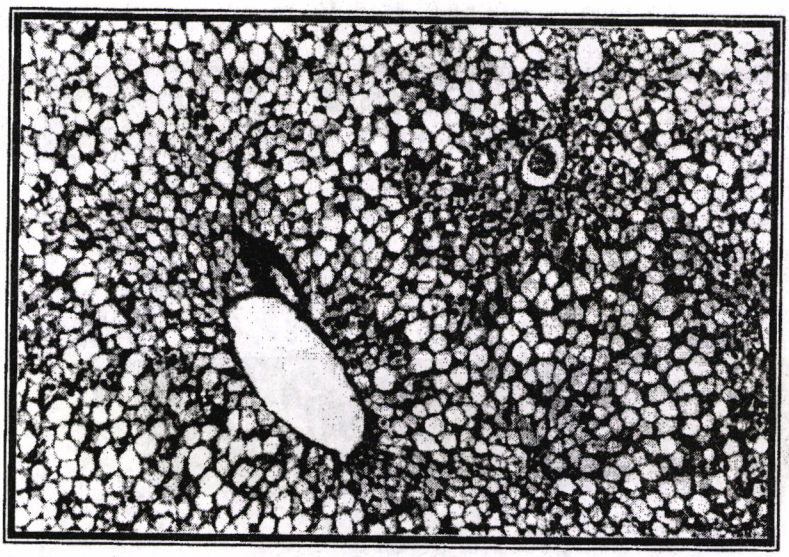

Figure 9 Microscopic section of liver for 1 mouse of group " 25 Gray " showed: Marked centri-lobular fatty degeneration, after 2 months of challenge . (20X. H & E stain) .

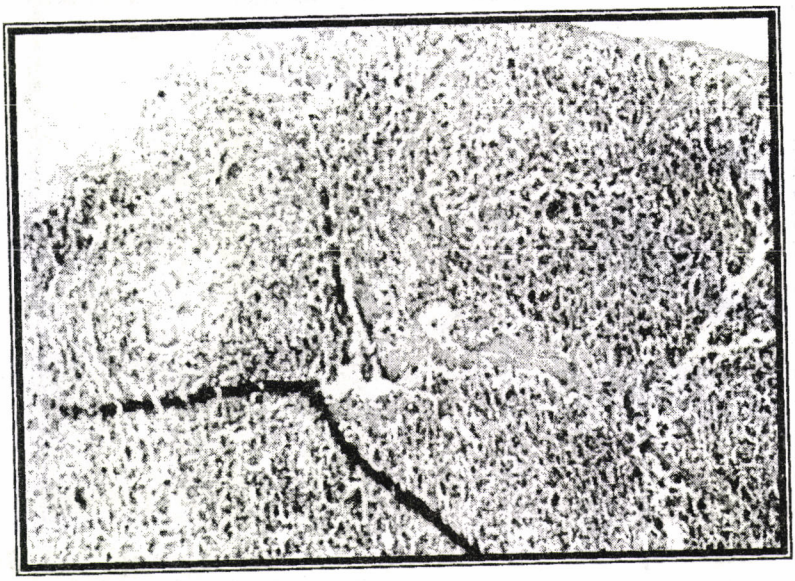

Figure 10 Microscopic section of spleen for 1 mouse of group '' 25 Gray '' showed: Lymphoid hyperplasia of white pulp, after 3 months of challenge . (10X. H & E stain) .

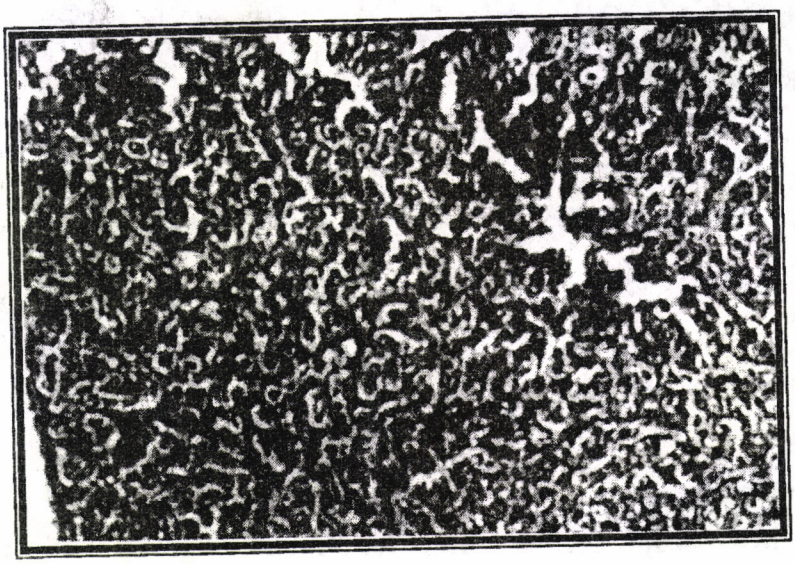

Figure 11 Microscopic section of liver for 1 mouse of positive control group showed: Infiltration of lymphocytes and mononuclear cells among hepatocytes, associated with degenerative changes, after one month of challenge . (40X. H & E stain) .

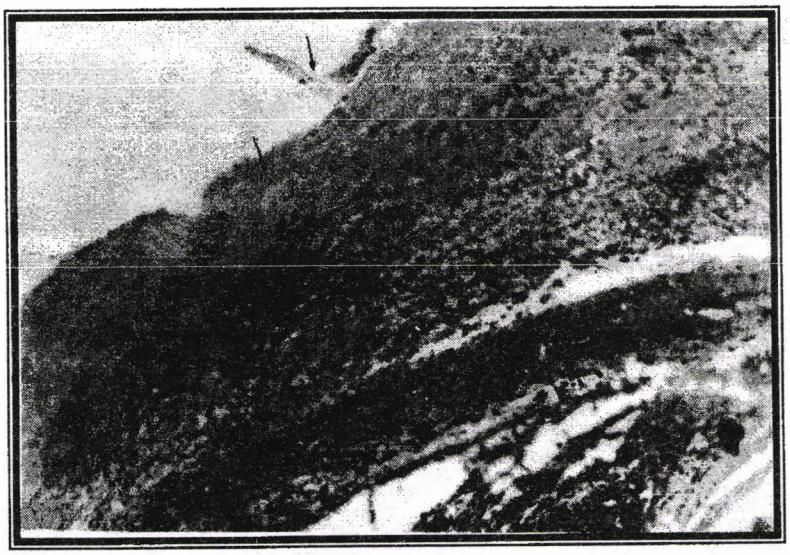

Figure 12 Microscopic section of liver for 1 mouse of positive control group showed: Secondary hydatid cyst associated with all their layers (germinal, laminated and adventitia) in liver parenchyma, after 2 months of challenge . (40X. H & E stain) .

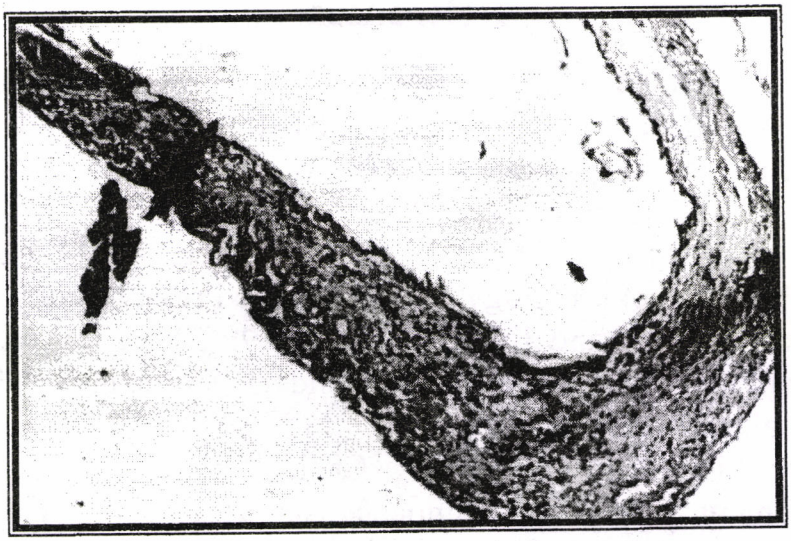

Figure 13 Microscopic section of liver secondary hydatid cyst of 1 mouse for positive control group showed: Degeneration of germinal layer with thickened fibrous capsule, after 3 months of challenge. (20X . H & E stain) .

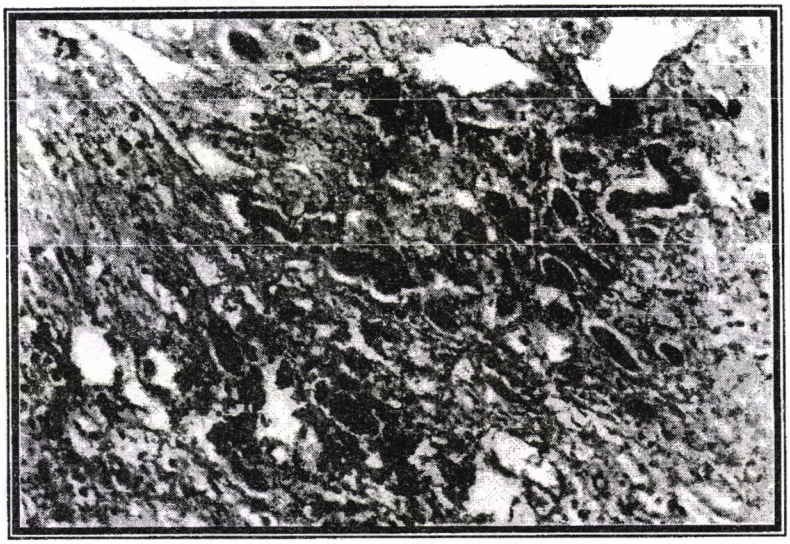

Figure 14 Microscopic section of liver for 1 mouse of positive control group showed: Marked fibrosis in liver parenchyma associated with atrophy of hepatocytes, after 3 months of challenge . (40X. H & E stain) .

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