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Immunopathological Study of the Effect of Phytohaeagglutinin on Secondary Hydatidosis Development in White Mice

Anfal Kadem jalab Inam Bader Faleh
Department Pathoology –veterinary college- university of Baghdad

Abstract
This study was carried out to investigate the immunopathological effects of phytohaeagglutinin(PHA) on the immune response against hydatid cyst infection in mice.The treated groups of mice dividedinto two groups,the first group immunized with 0.2ml of10mg/mlof PHA and the second group immunized with 0.4ml of 25mg/ml of PHA in 0 time after two weeks the same doses as repeated against secondary hydatidatid cysts, then delayed type hypersensitivity test was done on these groups and compared with the third group(that had been inoculated with 0.2 ml of sterile phosphate buffer saline as a control group)at day 27 post immunization using soluble PHA.At day 30 half number of animals from each group were scarified to perform humoral immunity tests (ELISA).Then challenge with 2000 protoscolices were done for the remaining half ofall 3groupsand left them for three months then kill them for histopathological results post challenge showed marked growth of cysts in the livers of the control infected group, with some degenerative and necrotic lesions accompanied by amyloidosis in spleen. While the immunized groups with PHA revealed presence of focal mononuclear cells in liver ,kidney and lung tissue with lymphoid hyperplasia in spleen. Finally, this study showed that the PHA were highly immunogenicand this may related to the fact that one of the componentsh of bile salts hasDeoxycholic acid init´s structure has the lysis effect on the protoscolsis against hydatid cyst infection in mice.
Keywords: phytohaeagglutinin, hydatid cyst, histopathological, mice.

1-Introduction

Echinococcosis / hydatidosis a worldwide zoonotic infection, disease that affects humans and livestock, the causative agent of cystic hydatid disease is Echinococcus granulosus (CHD)(metacestode stage) [1,2]. Exposure to infection occurs accidentally by rupture of cysts or rupture during surgery of cysts may result in an important medical problem [3].. *Echinococcus granulosus* enhances both humoral and cellular responses in it's intermediate host [4,5]. Humoral responses will result in the production of immunoglobulins, which are important for the diagnosis of patients [6, 7 , 8, 9, 10]. However, cellular responses also will take place on the infected host which are important criteria for the prevention of the disease [11, 12]. In particular, Th1 cell activation seems to be more related to protective immunity, while Th2 cell activation is linked to the susceptibility to the disease [13]. Phytohaemagglutinin (PHA), derived from extracts of Phaseolus vulgaris seeds, has been used for a number of years, on account of its twin properties of causing erythroagglutination and of stimulating progressive lymphocyte mitosis in cell culture[14] Both agglutinating and mitogenic activities appear to be associated with the protein fractions of crude extracts, which are sufficiently alike in their physico-chemical properties to have caused difficulty in attempts to separate them[15]One other factor complicating fractionation procedures has been the slow and imprecise method of assay for the mitogenic activity. Fortunately, relatively crude extracts selected for their ability to yield good mitotic pictures (as in reagent grade Phytohaemagglutin) have proved entirely satisfactory for use in routine lymphocyte culture for chromosome studies. PHA has proved of interest in the study of the immune response[2,6]lymphocyte [15] and bone marrow dynamics[10,12] and for these purposes it is desirable that a substance of more closely reproducilble qualities and known potency should be used. Improvements in the technique of assaying mitogenic activity[16] have made it possible to quote a value for each batch of Purified Phytohaemagglutinin in terms of a reference standard preparation.

Aim of study: Study the effect of the Phytohaemagglutinin on the immunopathlogical change in albino mice that infected with secondary *Echinococcus granulosus* .

2-Meterials and Methods

Experimental design:

Seventy white mice were randomly divided into three groups as following :

1. The first group (25) mice were immunized intraperitonealy with (0.2 ml) containing (10mg /ml) of Phytohaemagglutinin for two doses with 14 days intervals (first immunization at day 0 and followed by booster immunizations at the second (day 14)).
2. The second group (25)mice immunized intraperitonealy with(0.4ml) containing(25mg /ml)of PHA for two doses with 14 days intervals (first immunization at day 0 and followed by booster immunizations at the second (day 14).
3. The third group(20 mice) were inoculated with 0.2 ml of sterile PBS as a control group ,then these 3

groups were infected with hydatid cysts after 2 weeks from adaptation (were injected with (1ml/mouse) containing 2000 protoscolices PCS intraperitonealy).

Parameter of the study:

- Cell mediated immune response of experimental mice determined by skin test : Delayed type hypersensitivity test was done to detect the cellular immune response according to[16] on all animals at 27 day post immunization and post challenge.
- Humoral immune response detected by ELISA(enzyme linked immunosorbent assay): This test was done according to manufacturer (immunological consultents laboratory, Inc.).
- histological examination: At the end of the each period of experiment, five animals from each groups were sacrified under deep anesthesia and specimens from liver and kidney fixed in buffered formalin, sectioned (5 mm thickness) and stained with Hematoxylin and Eosin according to [17].

3-Results and discussion

1.Humoral immune response detected by skin test (delayed type hypersensitivity-DTH)

The results of our study was revealed that the skin thickness after 24 h. was showed a higher means in the groups that immunized with PHA in both concentration(25mg/ml)and(10mg/ml)(2.3±0.06, 1.7±0.04mm) compared with control group (PBS) (0.11±0.05mm) with a significant difference ($p<0.05$). Also after 48h. the results were showed a significant difference ($p<0.05$) in the group that immunized with bile salts in both concentrations (25mg/ml and10mg/ml) (**1.9±0.02**,**1.2±0.06**mm) compared with control group (PBS) (0.09±0.03mm) without significant difference ($p>0.05$).

(Table1): Showed the mean values of skin thickness in 1st and 2nd immunized groups and 3rd control group:

time	24 hours	48 hours
1st group animal immunized with PHA at *concretions* (25 mg /ml)	**2.3±0.06** Aa	**1.9±0.02** **Ab**
2nd group animal immunized with PHA at concretions(10mg /ml)	**1.7±0.04** **Ba**	**1.2±0.06** **B b**
3th control group	**0.11±0.05** **Ca**	**0.09±0.03** **Cb**

Small different letters denoted that significant differences between period (P≤0.05).
Capital different letters denoted that significant differences between groups (P≤0.05).

Current study of mean values skin test was virable according to different Ultra centrifuge Ags that used in skin test.According to above results of skin test in immunized animal with PHA referred that both dose stimulated cellular immune response via activation of chemokines & cytokines mainly that cause increates the release of neutrophil IL-8 both in vitro & in vivo this agree with with earlier work by [18] who fined that high concentration of 1-hydroxy phenazine 75 & 100 had suppressive effect on specific immune response T cell with high significant $p<0.01$ after 5 week p.challenge with PSC our observation of skin test also showed high mean value of PHA mainly at 25µgm/ml this results are related to the nature of this agent as mitogenic & blastogenic properties as well as immunostimulant that result in a complex series of biochemical events that enhance antigenic stimulation of immunocompenant (19).

2. Humoral immune response detected by ELISA(enzyme linked immunosorbent assay):

ELISA test was showed the mean of antibodies (IgG) titer.The results revealed a irrelevant alterations($p<0.05$) in the means values of groups that immunized by PHA for both concentrations(10mg/ml)and (25mg/ml)which were (48.83±0.88) and (41.56±0.99) respectively as compared with control group (PBS)(10.12±0.60).respectively as compared with control group (10.12±0.60).

Table:(4-4) : Mean values and standard error of antibody (IgG) titers of the immunized groups and the control group at 30th days:

time	24 hours Mean±SE /
1st group animal immunized with PHA at concretions(25 mg /ml) .	48.83±0.88 A
2nd group animal immunized with PHA at concretions(10 mg /ml)	41.56±0.99 B
3th control group	10.12±0.60 C

Different capital letters in column denote significant differencesbetween ($p<0.05$)

PHA produced no depression of The the delayed hypersensitivity response to tuberculin; nor did it suppress adjuvant arthritis in the rat measured blood-circulating T lymphocytes and proteins associated with the cellular and innate immune responses supposedly elicited by the PHA-immune challenge[20]. Circulating T lymphocytes produced in the thymus, which are characterised by their expression of special T cell receptors (TCR), are responsible for the cell-mediated immune response in vertebrates. Briefly, T-cells are a group of very distinct subsets among which the most abundant are $CD4^+$ (active lineages), $CD5^+$ (adyuvant lineages), and $CD8^+$ cells (memory or antigen presenting cells). The first two subsets are implicated in cellular based defence, while $CD8^+$ constitute the most common memory subset. The $CD4^+$ subset is implicated in the production of several active substances, such as cytokines, interferon and several types of interleukin, such as interleukin -6 (IL-6). The $CD4^+$ subset participates in the first phase of the skin swelling response (i.e., 6–12 h after injection), where there is exudation of plasma from surrounding vascular tissues and edema at the injected site, by activating local innate cell populations (mainly basophils and macrophages) [21]

4-Histopathological findings:

A.microscopical examination of immunized group with PHA.

A.1:immunized with 10 µgm/ml PHA.

The wall of bronchus & bronchial of immunized animal were hyperplastic, both for its epithelial & muscular tissue with mild cellular infiltration as well as mononuclear cells aggregation either perivascular & / or per bronchiolar (fig: 1).also granulomatous lesion may observed in liver tissue consist mainly of PMNCs (fig:2) tubular epithelial lining were swollen & hydropic with moderate perivascular MNCs.H istopathological change microscopical splenic section showed peri arteriolar sheath lymphoid hyperplasia ,associated with appearance of apoptosis in some lymphoid follicle also focal amyloidosis occur in other section(Fig:3) The predominant neural lesion was mild neuronal swelling & edema while other section showed purnkenji cell degeneration .

A.2:immunized with 25 µgm/ml PHA.

pulmonary vessels showed moderate vasodilation & congestion with focal collection of mature lymphocyte seen in adjacent acinar tissue as well as shows dilation of inter lobular septa by fibrinous exudate was noted(Fig:4) in other section also some pulmonary alveoli were filled with eosinophilic proteinaceous substances moderate to severe MNCs infiltration were observed in liver tissue mainly in portal area & around dilated central vein(Fig:5). The majority of blood vessels in renal tissue were thickened caused by well-hypertrophied muscular media with focal mono nuclear cells aggregation The splenic lesion characterized by reactive lymphoid hyperplasia (Fig:6),together with moderate MNCs infiltration mainly in dilation splenic sinuses associate with moderate thickening of adjacent trabeculae while no clear changes were recorded in other section. There is moderate dilation and congestion of cerebral blood vessels associated with mild perivascular cellular aggregation together with evidence of gliosis.

B.Non immunized infected animals (positive control).

The bronchi & bronchioles were obstructed by mucopurulent exudate in which there are many inflammatory cells mostly MNCs ,focal sloughing of their epithelia,also fibromuscular hypertrophy of their wall occur ,together with extensive MNCs aggregation mainly seen around bronchi The main characteristic lesion in the infected hepatic tissue was presence of multiple hydatid cysts with its identified layers some of cysts have thickened wall with narrowing lumen containing daughter cyst(Fig:7) seen within cyst content focal & diffuse necrosis were noticed in liver parenchyma that express nuclear pyknosis & karyolysis with focal PMNCs aggregation together with extensive vacuolation other secretion showed granulomatous like lesion in liver tissue consist mainly of PMNCs with slight sinusoidal congestion also there is hemorrhage of kidney interstitial tissue was occur associated with sever degeneration of renal tubules as well as splenic tissue express focal & diffuse amyloidosis with red pulp congestion ,also the splenic lesion include include focal sclerosis with cellular infiltration of splenic capsul (Fig:8) with evidence of lymphoid depletion, in addition the degenerated cardiac

myocytes were swollen, hyalinized, deeply eosinophilic together with focal muscular fragmentation

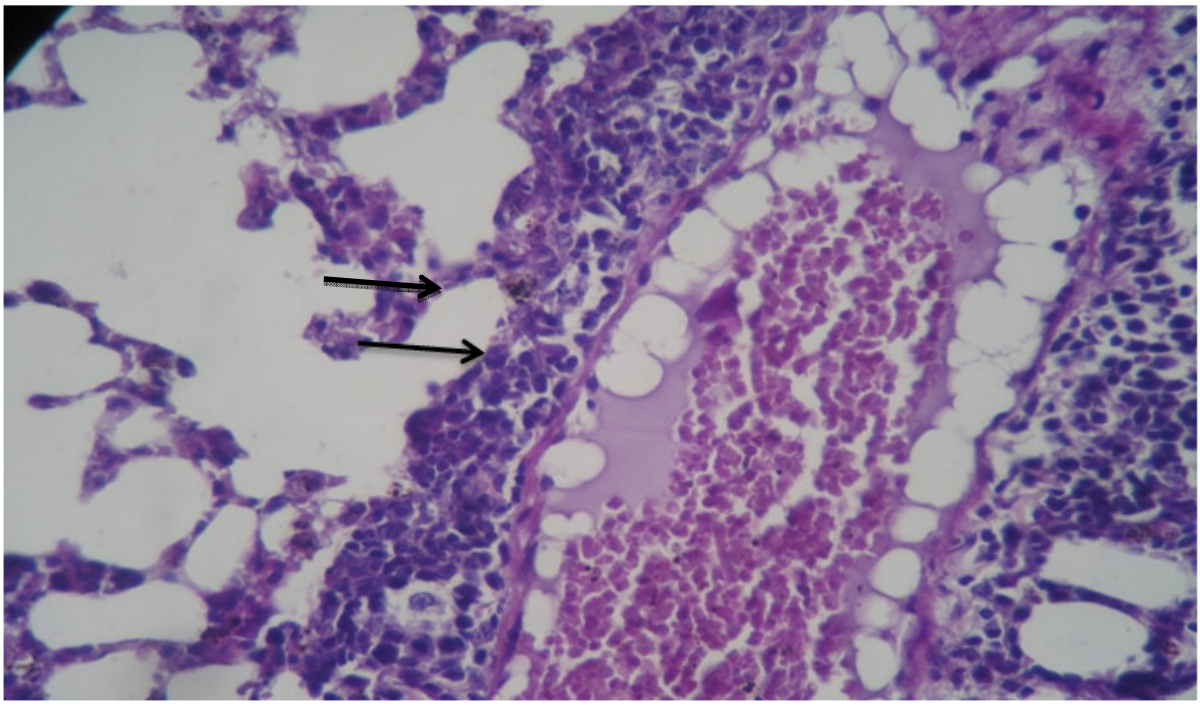


Fig.(1): Histological section in the lung of immunized group with 10 µgm/ml PHA show intense MNCs perivascular aggregation → (H&E stain)

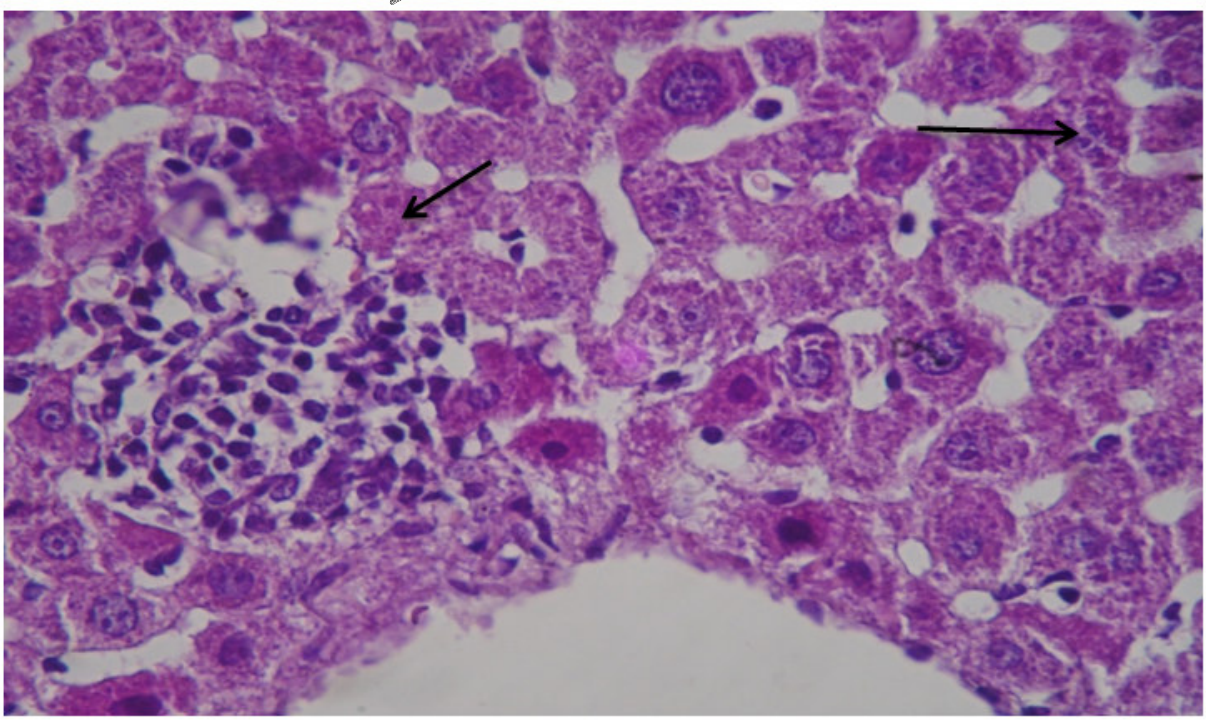


Fig.(2):Histological section in the liver of immunized group with 10 µgm/ml PHA show pyogranulomatous lesion ← (H & E stain 40x).

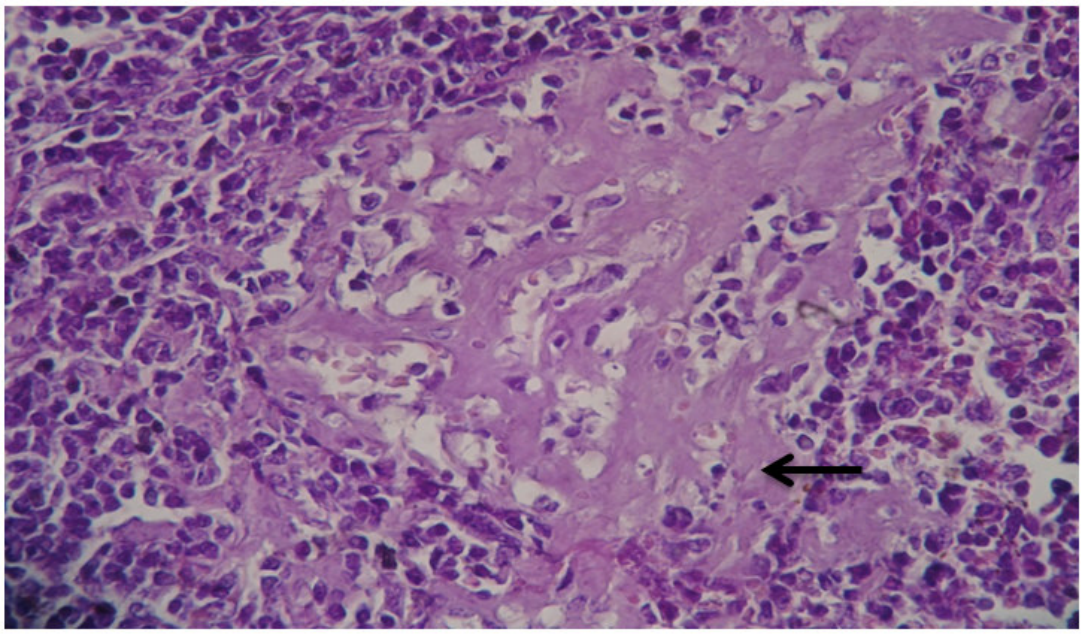
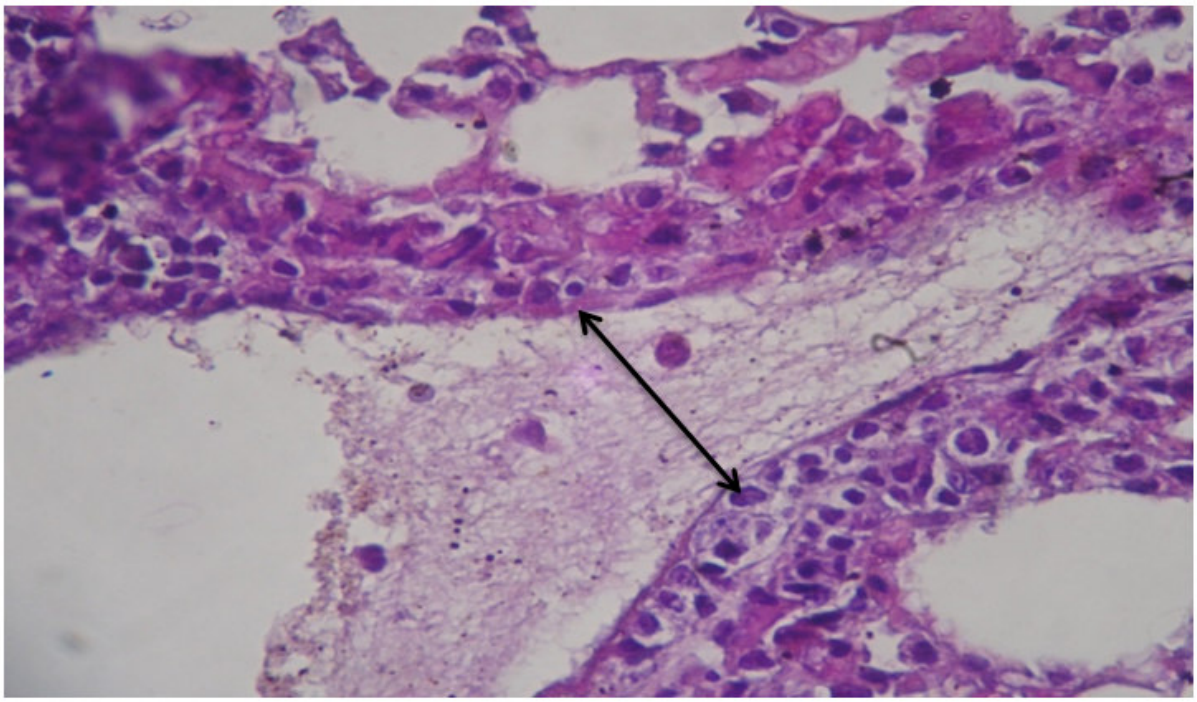


Fig.(3): Histological section in the of spleen immunized group with 10 µgm/ml PHA shows focal deposit of amyloid like substance ←



Fig(.4): Histological section in the lung of immunized group with 25 µgm/ml PHA shows dilation of inter lobular septa by fibrinous exudate ↔ (H&E stain 40X).

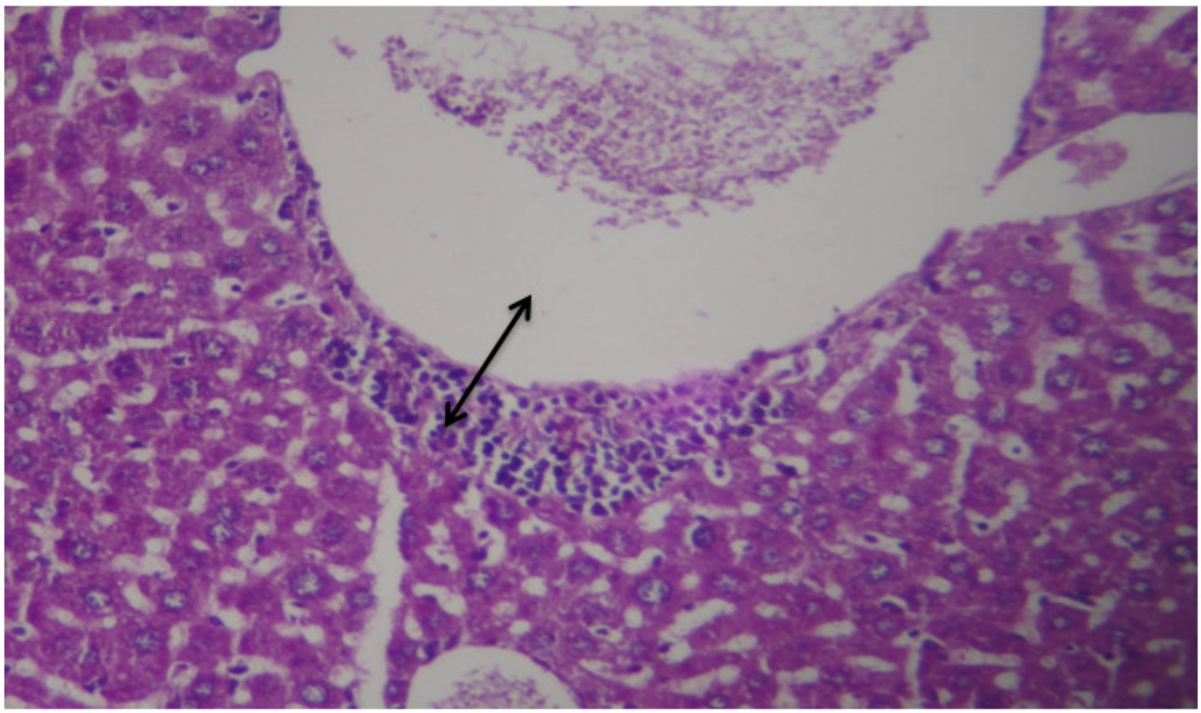


Fig:(5).Histological section in the liver of immunized group with 25 µgm/ml PHA shows MNCs infiltration around dilated central vein ⟷ (H&E stain 20X).

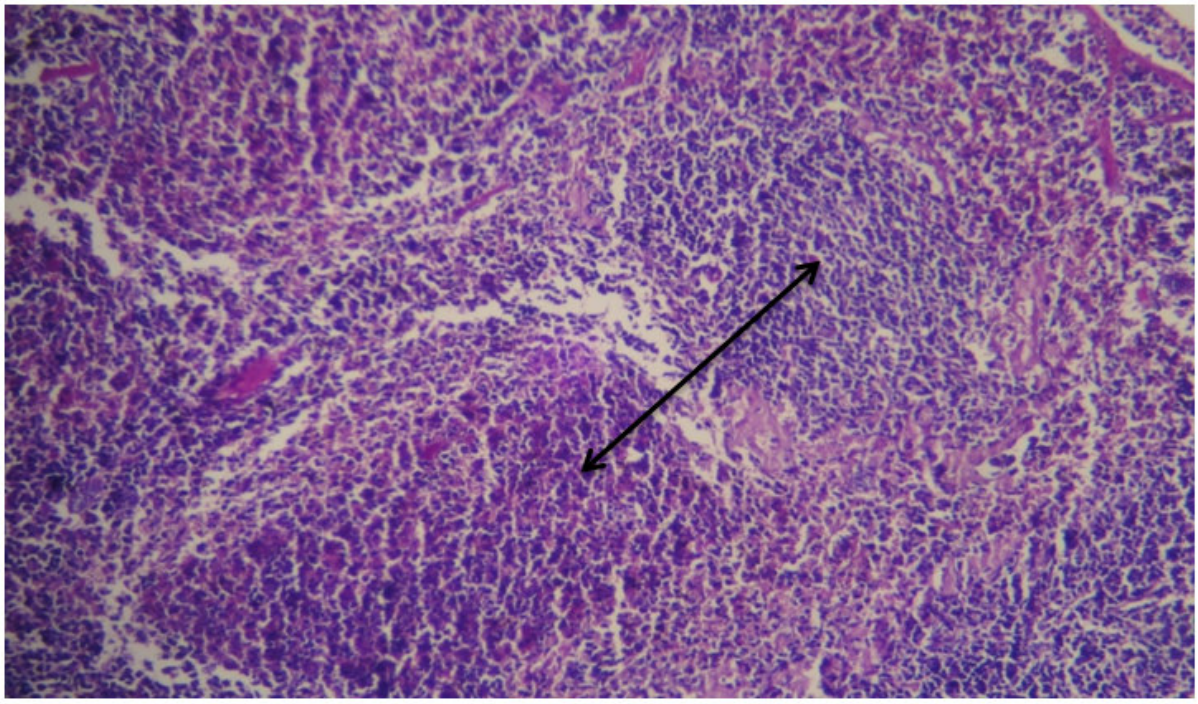


Fig:(6).Histological section in the spleen of immunized group with 25 µgm/ml PHA shows reactive lymphoid hyperplasia ⟷ (H&E stain 20X).

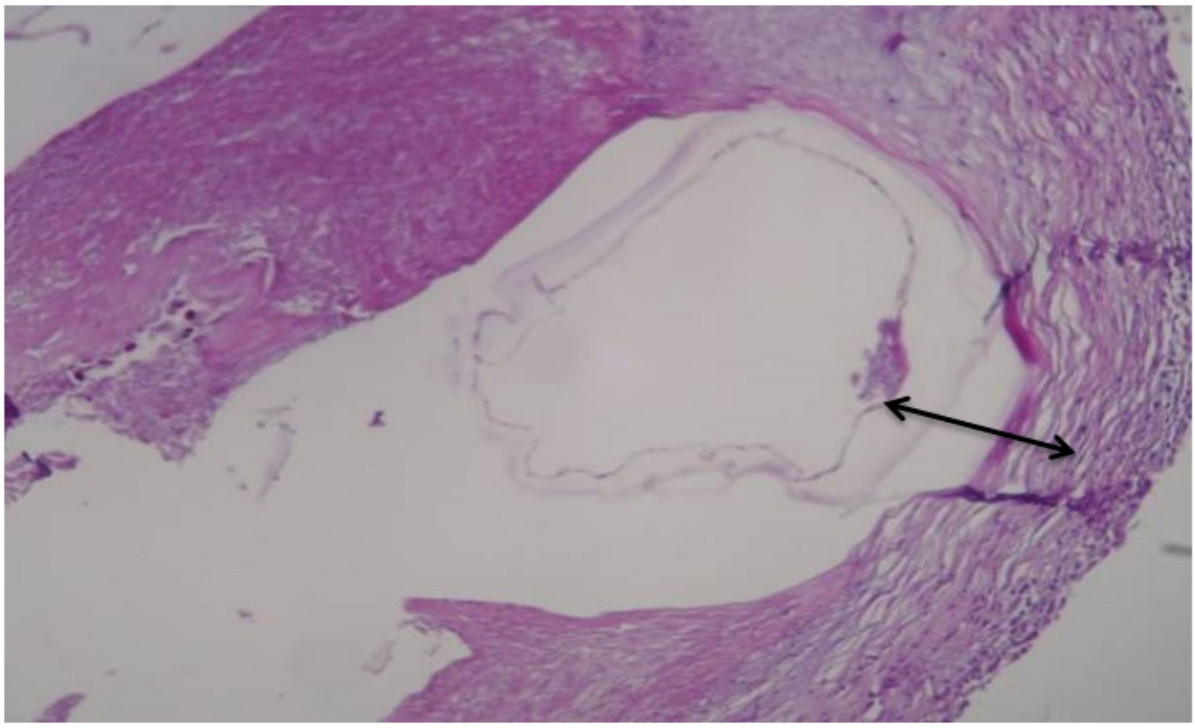


Fig:(7). Histological section in the liver of non-immunized infected group shows cystic structure with laminating fibrous wall & inner germinal layer ↔ (H&E stain 20X).

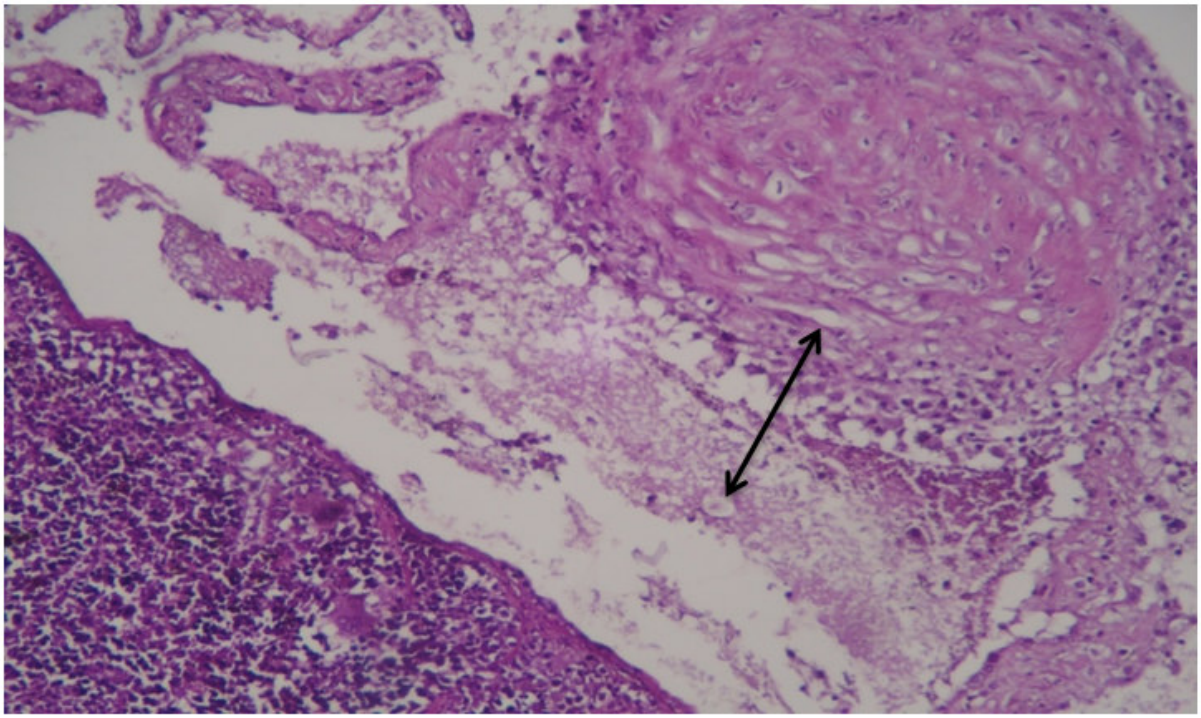


Fig:(8). Histological section in the spleen of non-immunized infected group shows focal sclerosis with cellular infiltration of splenic capsul ↔ (H&E stain 20X).

The results revealed(figure9,10) to the appearance of hydatid cysts in the liver of control infected group associated with vacuolar and necrotic changes The result of current study showed that all samples (germinal layer ,protoscolece) indicate positive for *E. granulosus* and agreement with the results of[22],as well as atrphy of splenic white pulp indicate that PSC reached the liver through the periton developed and parasite overcome the host defense with its secretion of immune complexes that inhibit immune mechanism of infected host according to [23].Liver is the common site of cystic development.Other study was mentioned that the oncosphere is trapped in the central veins of the hepatic lobules and the resulting cyst may be deep or superficial

[21].

intradermal inoculation of wing webs with PHA resulted in a heterophilic response in the peripheral blood of great tit nestlings, (ii) nestlings treated with PHA did not reveal suppressed growth, and (iii) the ability of nestlings to produce cutaneous swelling in response to PHA inoculation correlated positively with subsequent growth during the second half of the nestling period.

Histological examination of the lymphoid organs of PHA treated mice revealed reactive hyperplasia. Similar histological changes were seen in mice injected at the same time with a strong antigen such as horse ferritin. The depressive effect ofPHA on IgG antibody formation and the absence of an effect on IgM antibody formation, delayed hypersensitivity, and adjuvant arthritis .

Perhaps theMNCs infiltration in different organs tissues belonged finding of parasite inside celss of tissues. This disscutions agreement with[23].While amyloidosis in spleen(figure11) due to immunoglobulin deposition in this organ so these result is identical to[24].

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