



RESEARCH ARTICLE

PHYSIOLOGICAL, IMMUNOLOGICAL AND HISTOPATHOLOGICAL COMPARISON OF ECHINOCOCCUS GRANULOSUS (G6) CAMEL STRAIN BY DIFFERENT VIABILITY STATUS USING SECONDARY CYST DEVELOPMENT IN RAT

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Abstract

The current study aimed to demonstrate the differences in hydatid cysts of *Echinococcus granulosus* (G6) camel strain by different viability status using secondary cyst development in the rat and to study physiological, immunological and histopathological changes associated with. A comparative study was conducted along time of exposure in different groups of rats which intraperitoneally injected with three different status of the viability of hydatid cyst fluid of camel origin (G6). The groups were: low viability protoscolices (1), high viability protoscolices fluid (2), not viable and not completely transformed to the calcareous status of protoscolices fluid (3), and one negative control group. The immunobiology of host-*E. granulosus* interactions were evaluated by measuring IL-10 a cytokine associated with immunoregulation of effector responses. Histopathological changes were conducted in liver, lung, and kidney because these organs are the target of infection and play an important role in the immune status of the host. Results showed that the secondary infection by hydatid cyst fluid in the experimental model caused severe pathological changes which increased in rats of groups 2 and 3 along with the time of infection, in addition to considerable changes that revealed by hematological and biochemical tests. The median IL-10 level in rats of group 1 was 6.77 pg/ml compared to 5.74 pg/ml in control group, while the third group recorded the highest level equal 10.45 (above 10.0 pg/ml). The rats of the second group reached to 9.11 pg/ml, but no more survival of rats above 10.0 pg/ml. Elevated IL-10 levels were correlated with poor survival. We concluded that the most effective stage of hydatid cyst development was the stage before transformation to calcareous phase and the most effective changes in serological, biochemical, immunological and pathological changes occur during this stage.

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Introduction:-

Cystic echinococcosis is a chronic endemic helminthic disease caused by infection with metacestodes (larval stage) of the tapeworm *Echinococcus granulosus*. It is one of the most widespread parasitic zoonosis in animals and humans and one of the hygienic complications in both developing and developed countries (Budke *et al.*, 2006; Carmena *et al.*, 2008; Craig *et al.*, 2007; Menezes da Silva *et al.*, 2010). Hydatid cyst parasitizes many mammals including horses, camels, sheep, cattle, and man, in many organs (Rueda *et al.*, 1995) causes severe problems in different animal species especially in animals come in close contact with the final host (Namjoo and Arzanipour, 2014). In man, these cysts cause several complaints as pains or heaviness in abdomen and liver, cough, fatigue or anemia, and are often accompanied by hepatomegaly. It can also result in many acute complications such as anaphylactic responses depending on the number and location of the cysts (Rueda *et al.*, 1995). Ten strains of *E. granulosus* (G1–10) have been described with molecular biology techniques using mitochondrial DNA sequences (McManus and Thompson, 2003). The sheep strain (G1) and camel strain (G6) have a worldwide geographical distribution, specifically in North Africa, the Middle East and in some European countries (Dakkak, 2010; Khalifa *et al.*, 2014; Varcasia *et al.*, 2011). Comparative studies on the development of various isolates of the parasite have proved particularly useful, not only for speciation and taxonomy but also in the epidemiology and control of hydatidosis (Smyth & Davies, 1974; Thompson, 1977; Thompson, 1982).

On hydatid disease two alternative tools have been developed, one is in vitro culture and the other is laboratory animal models (Nakaya *et al.* 2006; Zak and Sande, 1999). Various species of animals (sheep, baboons, monkey, rabbits, mice, gerbils) and various ways of infection (intragastric administration of eggs, intraperitoneal, intravenous or subcutaneous injection of activated oncospheres and intraperitoneal inoculation of protoscolices or cysts) have been described (Zak and Sande, 1999). In this study, an attempt was made to develop hydatid cysts (G6 genotyping) in rats following intraperitoneal injection of protoscolices (different viability status) and following different changes occurred in rats of different groups along time of the experiment.

Materials and Methods:-

Collection of protoscolices:-

Naturally infected organs (lungs and livers) with hydatid cysts (G6) from camels were collected from El-Bassatein and El-Moneib abattoirs. Cyst fluids were aspirated using sterile disposable syringes and examined for fertility and viability. Cysts which contained no protoscolices (PS), as well as heavily suppurate or calcified, were considered infertile. Fertile cysts were subjected to viability test by centrifugation at 5,000 rpm for 30 min, a drop of the sediment containing the protoscolices was placed on the microscope glass slide and covered with the coverslip and observed for amoeboid-like peristaltic movements (flame-cell activity) with $\times 40$ objective (Salih *et al.*, 2011). When it became doubtful or confusing to observe such movements, a drop of 0.1% Gentian violet dye solution was added to equal volume of protoscolices in hydatid fluid on a microscopic slide with the principle that viable protoscolices should completely or partially exclude the dye while the dead ones take it up (Macpherson 1985; Smyth and Barrett 1980). Protoscolices were isolated from the fertile cysts washed three times with phosphate buffer saline (PBS pH=7.2) and preserved in labeled sterile tubes contain the same volume of warm Hanks' Balanced Salt Solution (HBSS) for the experiment.

Animals:-

Seventy-two male albino rats of *Sprague Dawley* strain, with average weight 160- 200 g were obtained from National Research Center, Dokki, Giza, Egypt. Animals were examined to be free from parasitic infection to avoid production of the antibodies before the experiments and kept in Animal house, Faculty of Science, Al-Azhar University, Cairo, Egypt under standard laboratory care of 21°C, 16% moisture, with water available *ad-libitum* and diet contains 20% protein, 3% fat, and 22% fiber. Rats were acclimatized for 7 days before the start of the experiment. Animal experiments were carried out according to Institutional Animal Care and Use Committee, National Research Centre Animal Care Unit.

Experiment design:-

Animals were randomly divided into four groups (18 rats/ each) as follows: the first group intraperitoneally (IP) injected with low viable protoscolices (380 PS in 0.5 ml HBSS/rat), the second group IP injected with high viable protoscolices (1000 PS in 0.5 ml HBSS/rat), the third group injected with 0.5 ml/ rat of semi-calcareous fluid, before completely calcification, and has no protoscolices, whereas the fourth negative control group administrated with only HBSS. After injection, rats daily examined to monitor external and morphological changes for 3 weeks before

scarifying. Three animals per group were sacrificed at the end of each week from the 3rd to the 8th weeks (6 weeks) post infection for post-mortem examinations. Before scarifying, rats of the second and third groups died earlier at the 7th and 6th weeks respectively.

Blood collection and preparation:-

Rats were anesthetized using cotton wool soaked in chloroform and the abdominal cavity was opened until the sternum using medical scissors. The blood samples were directly drawn from the heart using 5ml sterile syringe into clean, dry centrifuge tubes that allowed to stand for 10 minutes at room temperature, then centrifuged at 3000rpm for 15 minutes using laboratory centrifuge (SM 800B, Surgifriend Medicals, England). Sera were carefully removed and stored frozen at -80° C until used for biochemical and immunological analyzes. Blood used for hematological analysis was collected into heparinized sample tubes containing EDTA to prevent the blood from clotting and taken for analyzes within 24 hours of collection.

Hematological studies:-

Hematological parameters including red blood cells (RBCs), white blood cells (WBCs) and platelets count in addition to hemoglobin level, hematocrit, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) were accomplished. The samples were analyzed using the automated hematologic analyzer SYSMEX, KX-21 (Japan) as described by Dacie and Lewis, (1991).

Biochemical analysis:-

Serum samples were analyzed for total protein (Biuret method) (Smith, 1985), albumin (BromoCresol green (BCG) (Doumas *et al.*, 1971), cholesterol (Cholesterol Oxidase/ Peroxidase method) (Allain *et al.*, 1974), Bilirubin (Ballisteri and Shaw, 1987) and glucose (Caraway and Watts, 1987). The activity of liver enzymes Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT) was measured by (Gella *et al.*, 1985) method, acid phosphatase (AcP) (Hilman and Klin, 1971), alkaline phosphatase (ALP) (Rosalkiet *et al.*, 1993) and Gamma-glutamyl-transpeptidase (GGT) by SZASZ method (Orlowski and Meister, 1963). All biochemical parameters were determined using a Technicon RA-2000 random access, automated analyzer.

Measurement of cytokines in serum:-

Rat IL-10 ELISA kit is an in vitro enzyme-linked immuno-sorbent assay for the quantitative measurement of rat IL-10 in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for rat IL-10 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-10 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and the biotinylated anti-rat IL-10 antibody is added. After washing away unbound biotinylated antibody, HRP- conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution (12 ml of 3, 3',5,5'- tetra-methyl-benzidine in buffer solution) is added to the wells and color develops in proportion to the amount of IL-10 bound. The stop solution (8 ml of 0.2 M Sulfuric acid) changes the color from blue to yellow, and the intensity of the color is measured at 450nm. The data was calculated by measuring the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. The standard curve was plotted on log-log graph paper or using Sigma plot software, with a standard concentration on the x-axis and absorbance on the y-axis. (RayBio® ELISA Kits).

Histopathological preparation:-

At the end of each week, as shown earlier, portions of liver, kidney, and lung were washed in saline solution and fixed in 10% formalin for 24-48 hours at room temperature, embedded in paraffin and sectioned at 4-6 micrometers. Sections stained with haematoxylin-eosin stain using standard histological technique according to Bancroft (1975) for histopathological examination by Olympus BX51 light microscope.

Statistical analysis:-

The results were expressed as Mean \pm standard deviation (SD) of the mean. The significance difference between means was evaluated by one-way analysis of variance (ANOVA) followed by post hoc test for the comparison of significance using the Statistical Package program (SPSS version 23). Values of $P < 0.05$ were considered as statistically significant.

Results:-

Parasitology Results:-

The current study revealed several external changes that represented in weight loss, loss of body hair and blindness in the infected groups at the end of the experiment, however, the visual inspection after the autopsy showed different changes as internal bleeding and splenomegaly. Autopsy results detected the appearance of laminated layer stage in livers and kidneys of groups 1, 2 and 3 that started at weeks 7, 6 and 3 after injection respectively (Fig. 1a, 1b) while, protoscolices formation appeared in the last rat of the first group at the end of the experiment. Cysts formation showed in all the infected groups as it appeared at week 7 and 8 in the livers and kidneys of rats in group 1. On the other hand, the second group showed cysts formation in rats' liver at the 7th week of infection, while all rats of group 3 showed a formation of cysts in both kidneys and livers starting from the third week of infection until the death of animals earlier at the 5th week of infection. No cysts formation were observed in other organs along the experiment. Nucleated layer (N. L.), brood capsule (B. C.), and protoscolices formation (P.) stages were reported in kidneys of group 1 at 8th week of the experiment. Also, a smear provoked from hydatid cyst showed hooks (Fig. 1c).

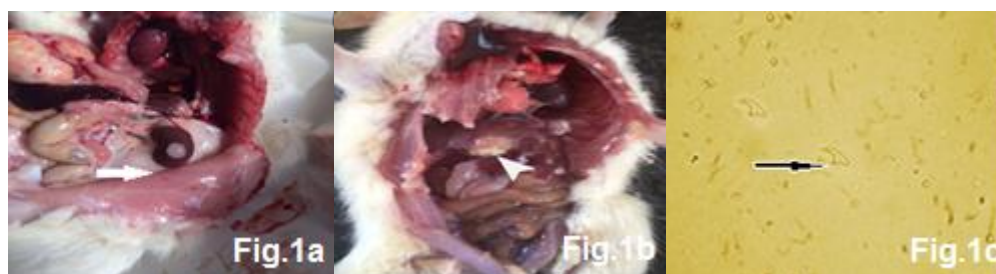


Fig 1:-Showing the four stages in the development of secondary hydatid cysts

Hematological and biochemical results:-

In the current study, the effect of *E. granulosus* infection with different viability status on the hematological and serum biochemical parameters was studied for 6 weeks except for groups 3 and 2 where all animals died earlier at the 6th and 7th week of administration was summarized in tables (1) and (2) respectively.

Hematological parameters in the control and *E. granulosus* infected groups were recorded after 3 weeks of infection at the end of every week revealed that there was a gradual increase in the white blood cells (WBC) along with time in all studied groups as compared to control. On the other hand, the red blood cells (RBC), hemoglobin (HB) and packed cell volume (PCV) were gradually and significantly decreased all over the infected groups in a dose and time-dependent manner as compared to the control. The lowest values were estimated after 8 weeks of infection in the 1st group as all animals in both of group 2 and 3 died at the beginning of the 7th week of infection. Likewise, platelets showed a significant decline all over the infected groups in a time-dependent manner with some fluctuations as compared to the control. Mean corpuscular volume (MCV) recorded increased values in groups 1 and 3 except for group 2 that showed unchanged values until the 6th week of infection where it recorded a significant decrease as compared to the control values. Meanwhile, mean corpuscular hemoglobin concentration (MCHC) exhibited a significant decline in all treated groups at the beginning of the study that restored to be very close to normal at the 4th week except for group 3. Unfortunately, the values could not be followed for both group 2 and 3 as all animals died, but group 1 values showed a continuous decline in MCHC throughout the remaining time of the experiment. However, no significant difference in the mean corpuscular hemoglobin (MCH) between the values of treated groups and those of control was detected (Table 1).

Total protein values showed a significant increase after infection by *E. granulosus* throughout the experiment as compared to the control (Table 2). Meanwhile, albumin exhibited an insignificant decline in the different infected groups with some fluctuations especially in group 2. Moreover, the average globulin levels recorded a significant elevation that becomes clearly noticed from the 4th week of the experiment with the highest level in the third group as compared to the normal control. Infected rats showed a gradual significant increase in the glucose levels analog with time after exposure to *E. granulosus* in the different treated groups as compared to control. However, cholesterol levels showed great fluctuations that end in elevation in some groups. Peritoneal administration of *E. granulosus* showed an overall increase in the ASAT and ALAT activities in all groups in a time-dependent manner where the highest levels were obtained after 6 weeks in group 2 as compared to the control. However, *E. granulosus* infection did not show any significant change in the GGT activities in groups 1 and 2, its activity recorded gradual

significant increased values in group 3. In addition, group 1 showed a slight increase in GGT values in the last two weeks of the experiment. Furthermore, *E.granulosus* (G6), camel strain, infected rats showed a marked increase in the levels of serum ALP in comparison with that of control rats as shown in Table (2). No significant difference was detected in AcP levels in group 2, however, its level in group 1 and group 3 showed a gradual significant change that was inversely related to time in group 1 as compared to control. On the other side, the effect of *E.granulosus* showed an increased trend in bilirubin levels that was more pronounced in group 1 as compared to control.

Table 1:-Hematological parameters of the infected rats with different doses of *Echinococcus granulosus* as compared to control group.

Week/ Group		RBCs	WBCs	Platelets	HB	MCV	MCH	MCHC	PCV
3	C	8.12 ±1	13.8 ±1	783±1	14.8± 0.2	59.2± 0.2	18.2±1	30.8±.2	48.1±1
	G1	7.42±1	12.4±0.2*	408±2*	12.9±0.1*	64.4±0.1*	17.4± 0.5	26.9±0.1*	47.9± 0.1
	G2	7.11±2	17.3±0.3*	490± 3*	12.3±0.2*	58.4±1	17.3±1	29.6±0.5*	41.5±0.5*
	G3	5.97±0.3	16.9±0.5*	632±1*	10.1± 1*	61±0.5*	16.9± 0.1	27.7±0.3*	10.1±1*
4	C	8.12±2	13.8±0.2	783± 2	14.8± 1	59.2± 1	18.2± 2	30.8± 2	48.1± .1
	G1	5.3±0.2*	19.8±0.3*	407± 2*	10.9±0.1*	69.1±0.8*	20.4± 2	29.7± 0.3	36.2±0.2*
	G2	6.65±1	17.3±0.3*	485± 5*	11.3±0.5*	58.3± 0.2	16.9± 0.6	31.8± 0.2	35.5±0.5*
	G3	4.3±0.3*	21.2± 2*	309± 2*	9.2± 1*	74.5±0.5*	21.2± 2	28.4±0.4*	9.2± 2*
5	C	8.1± 0.5	13.8± 1.5	783± 3	14.8± 0.1	59.2± 0.1	18.2± 0.2	30.8± 0.5	48.1± 0.5
	G1	5.1±0.5*	21.9±0.1*	408± 2*	10.4±0.5*	68.9± 2*	20.3±0.2*	29.4± 1	35.3±0.3*
	G2	5.98± 1*	18.3± 2*	465± 4*	11± 0.7*	58.3± 0.2	18.3± 0.5	31.5± 0.5	34.9±0.1*
	G3	4.2±0.1*	22.3±0.2*	300± 6*	8.5± 0.9 *	74.2±1.2*	20.1± 1*	29.6±0.3*	8.4± 0.5*
6	C	8.1± 1.5	13.8± 0.5	783± 2	14.8± 0.5	59.2± 3	18.2± 1.5	30.8± 1	48.1± 0.8
	G1	5.1±0.1*	22.7± 2*	487± 1*	10± 0.9*	68.6±0.3*	19.6± 0.4	28.6± 1	35± 1*
	G2	5.6±0.5*	22± 0.6 *	407± 3*	10.8±0.2*	51.2±0.3*	16.26±2	31.8±0.5	34± 0.6*
7	C	8.12± 1	13.8± 2	783±4	14.8± 2	59.2± 0.5	18.2± 0.3	30.8± 0.9	48.1± 2
	G1	5± 1*	27± 0.5*	491± 1*	9.3± 0.5*	69.2±0.5*	18.6± 1	27.1±0.1*	34.3± 2*
8	C	8.1± 0.6	13.8± 0.8	783± 3	14.8± 1.5	59.2± 2	18.2± 0.8	30.8± 3	48.1± 1.5
	G1	4.9±0.5*	28± 1*	465± 2*	8± 2*	69.8±0.2*	16.4±0.2*	23.5±0.5*	34± 0.5*

The values are expressed as mean ± SD. * P< 0.05 level compare with control group. C: control group.

Immunological results:-

The first signs of immune reaction can be observed with the naked eye in the liver, as the small accumulation of cells and appearance of hydatid cyst, as early as 3 weeks after infection. In the early phase of echinococcosis infection (21 days post infection), the concentrations of IL-10 in the sera of the infected rats of group 1 was the same as that of healthy control rats. However, comparing IL-10 concentration for the other two groups recorded increased levels that reached maximum level in rats of group 3. By progression, in the time of the experiment, we noticed a gradual increase in IL-10 levels (Table 3). We also observed when measured IL-10 levels in serum of infected rats; Group (1) still in low levels by comparison with other groups until the end of the experiment (56 days). Group (2) showed gradually increasing until 42 days after the rest of rats died. Group (3) started by an approximately high level of IL-10 and increased until reached to peak (10.45) in 35 days in the last rat of group 3.

Table 2: - Biochemical parameters of the infected rats with different doses of *Echinococcus granulosus* as compared to control group.

Week/ Group		Total protein	Albumin	Globulin	Glucose	Cholesterol
3	C	7.6± 1	4.1± 1	3.5± 0	120± 1	63± 2
	G1	7.47± 0.8	4.5± 0.5	2.97± 0.4	137± 1.1*	62± 1
	G2	8.47± 1.7	3.33± 0.3	5.13± 1.4*	118± 0.4	49.2± 1.5*
	G3	8.37± 0.85	4.6± 0.5	3.77± 0.4	163± 1.5*	35.5± 0.5*
4	C	7.6 ± 0.5	4.1 ± 0.5	3.5 ± 0	120 ± 0.5	63 ± 1
	G1	9± 1*	4.4± 1	4.6± 0	142± 0.9*	72± 0.2*
	G2	9.3± 0.6*	3± 2	6.3± 1.4*	118± 2	53.2± 0.1*
	G3	9.1± 0.6*	3.84± 0.9	5.3± 0.3*	195± 2*	54± 0.5*
5	C	7.6± 2	4.1± 2	3.5± 0	120± 2	63± 0.5
	G1	9.6± 0.5	4± 0.5	5.6± 0*	168± 1*	72.7± 0.2*

6	G2	10.5± 1.5*	2.57± 0.3	7.9± 1.2*	127± 2.3*	59.16± 1.8*
	G3	9.2± 0.2	3.6± 0.1	5.6± 0.1*	331± 1*	59.33± 1.3*
	C	7.6± 0.1	4.1± 3	3.5± 2.9	120± 0.1	63± 0.1
	G1	10.53± 1.18*	3.9± 2	6.63± 1.09*	190± 1.1*	72.9± 0.28*
7	G2	11.2± 0.5 *	2.52± 0.5	8.68± 0.03*	148± 0.5*	67.9± 0.9*
	C	7.6± 3	4.1± 2	3.5± 1	120± 3	63± 3
8	G1	11.3± 0.2 *	2.22± 0.2	8.98± 0.03*	210± 0.8*	72.9± 0.28*
	C	7.6± 1.5	4.1± 2	3.5± 0	120± 1.5	63± 0.6
	G1	11.3± 0.2*	2.22± 0.2	8.98 ± 0.03*	241± 1.2*	72.9 ± 0.28*
	C	7.6± 1.5	4.1± 2	3.5± 0	120± 1.5	63± 0.6

Weeks/ Group		ALP	AcP	ALAT	ASAT	GGT	Bilirubin
3	C	18.03± 0.15	0.03± 0.01	18± 0.5	72.3± 1.1	8 ± 2	0.24±0.01
	G1	32± 1*	0.9± 0.03*	32± 1*	170± 2.2 *	6.6± 0.2	0.45± 0.02*
	G2	20± 1.5*	0.02± 0.01	58± 0.8*	212± 3.4*	10± 1	0.29± 0.02*
	G3	27± 0.9*	0.06 ± 0.02	25± 5*	140.67± 1.5*	17± 0.6*	0.31± 0.03*
4	C	18.03 ± 0.5	0.03 ± 0.0	18 ± 2	72.3 ± 1.1	8 ± 0.5	0.24 ± 0.01
	G1	34± 2*	0.7± 0.04*	33.8± 0.1*	184± 0.1*	6± 3	0.67± 0.02*
	G2	22.9± 3*	0.02± 0.01	58± 0.7*	380± 2*	9.3± 0.3	0.3±0.1
	G3	27± 0.7*	0.2± 0.05*	48± 2*	255± 0.5*	25± 0.8*	0.35±0.01 *
5	C	18.03± 0.1	0.03± 0.01	18± 1	72.3± 1.1*	7.97± 0.65	0.24±0.01
	G1	26± 0.2*	0.5± 0.1*	34± 0.4*	304± 0.6*	7.0± 0.9	0.32± 0.01*
	G2	24± 0.3*	0.04± 0.43	70± 0.1*	480± 5*	9± 3	0.3± 0.01*
	G3	29± 0.7*	0.18± 0.01*	85± 5*	469.3± 1.5*	26± 0.1*	0.4± 0.04*
6	C	18.1± 0.3	0.03± 0.01	18± 5	72.3± 1.1	8± 0.01	0.24± 0.01
	G1	26± 0.2*	0.5± 0.05*	36.9± 0.1*	412± 2.5*	8.6± 0.2	0.54± 0.04*
	G2	24.3± 0.15*	0.01± 0	90± 0.4*	527± 3*	8± 1	0.42 ± 0.03*
7	C	18.1 ± 0.2	0.03± 0.01	18± 0.4	72.3± 1.1	8± 1	0.24± 0.03
	G1	27± 0.4*	0.3± 0.03*	37.8± 0.2*	409± 5*	10.2± 0.5*	0.43± 0.01*
8	C	18.1 ± 0.3	0.03± 0.01	17.7± 1.2	72.3± 1.1	8± 0.4	0.24± 0.2
	G1	27 ± 1*	0.3 ± 0.01*	38 ± 2.5*	432 ± 3.3*	11.0000*	0.28 ± 0.2

The values are expressed as mean ± SD. * P< 0.05 level compare with control group. C: control group.

Histopathological results:-

The present study showed the histopathological changes produced mainly in kidneys, liver and to a lesser extent in lungs of rats which appeared as morphological changes, and also autopsy results showed the presence of hydatid cysts especially in both liver and kidney organs:

Table 3:- The change in Interleukin-10 (pg. /ml) levels in infected rats with different doses of *Echinococcus granulosus* as compared to control group.

Groups	Control	G1	G2	G3
Week 3	5.74± 0.02	5.74± 0.04	6.93 ± 0.03*	7.64± 0.03*
Week 4	5.74± 0.03	5.89 ± 0.01*	7.57 ± 0.05*	8.99 ± 0.04*
Week 5	5.74 ± 0.04	6.05 ± 0.05*	8.08 ± 0.08*	10.45 ± 0.04*
Week 6	5.74 ± 0.05	6.12 ± 0.06*	9.11 ± 0.06*	-----
Week 7	5.74 ± 0.04	6.45 ± 0.04*	-----	-----
Week 8	5.74 ± 0.04	6.77 ± 0.03*	-----	-----

The values are expressed as mean ± SD. * P< 0.05 level compare with control group

Liver:-

The histological sections in the liver of rat group3 (21days post-infection) showed focal hepatic necrosis associated with inflammatory cells infiltration as well as apoptosis of hepatocytes in addition to sinusoidal leukocytosis (Fig.2a, b,c). Also the histological sections in the same group but in 28 days post infection showed severe pathological changes which increased with post-infection (Fig.3a). These changes were Kupffer cells activation and small focal hepatic necrosis associated with inflammatory cells in addition to portal infiltration with eosinophils and cystic dilatation of bile duct and fibroplasia in the portal triad around the bile duct (Fig.3b), and at end of experiment the

liver of the last ratin group 1 showed dissociation of hepatic cords and cytoplasmic Vacuolation of hepatocytes (Fig.3c).

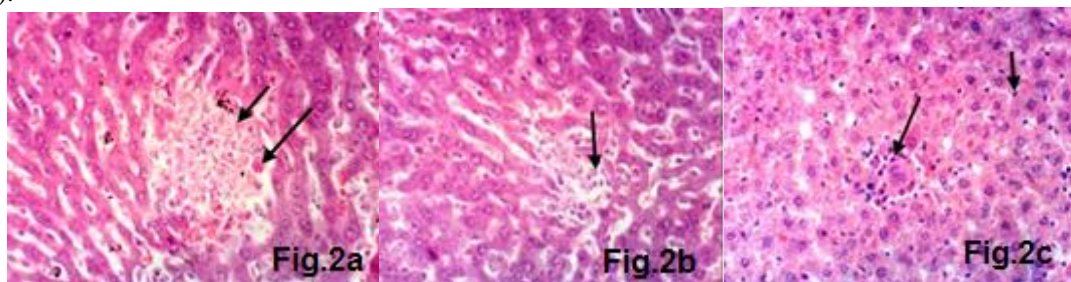


Fig 2:-Sections of rat livers of group 3 after 3 weeks of infection showing focal hepatic necrosis associated with inflammatory cells infiltration as well as apoptosis of hepatocytes (a), sinusoidal leukocytosis (b), and Kupffer cells activation and small focal hepatic necrosis associated with inflammatory cells infiltration (c) (H & E X 400).

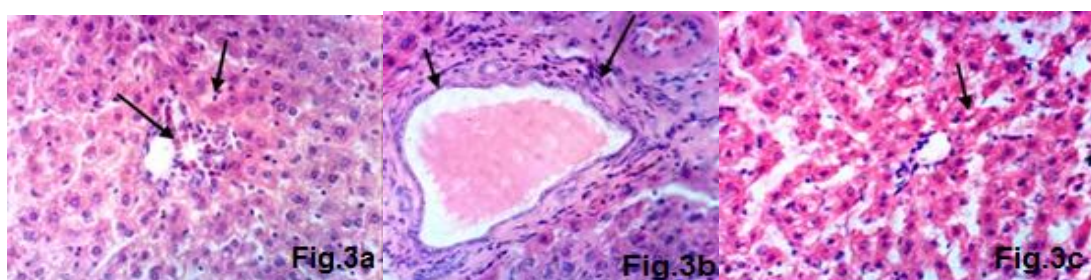


Fig 3:-Sections of rat livers of group 3 after 4 weeks post infection showing Kupffer cells activation and portal infiltration with eosinophil (a), and dilatation of bile duct and fibroplasia in the portal triad around the bile duct (b). Whereas, liver of rat from group 1 in 56 day post infection showing dissociation of hepatic cords and cytoplasmic Vacuolation of hepatocytes (c) (H & E X 400).

Kidney:-

The histological sections in the kidney of infected rat groups are demonstrated in Figures 4a-c and 5a-g. Kidney of rats in group1 revealed inflammatory cells infiltration in the renal capsule, atrophy of some glomerular tuft, congestion, and hypertrophy of another glomerular tuft, also results showed hyper-cellularity of glomerular tuft. Histopathological changes which occurred in 5 weeks post infection in the rats of groups (2 & 3) which increased by increasing the time represented in the eosinophilicproteinaceous material in the lumen of renal tubules. Hyper-cellularity of the glomerular tuft and the eosinophilicproteinaceous material in the lumen of renal tubules. Thickening of the glomerular basement membrane and focal inflammatory cells infiltration. Vacuolation of tubular epithelium, atrophy of glomerular tuft and distension of Bowman's space. Slight distension of Bowman's space, and thickening of glomerular basement membrane with congestion of glomerular tuft.

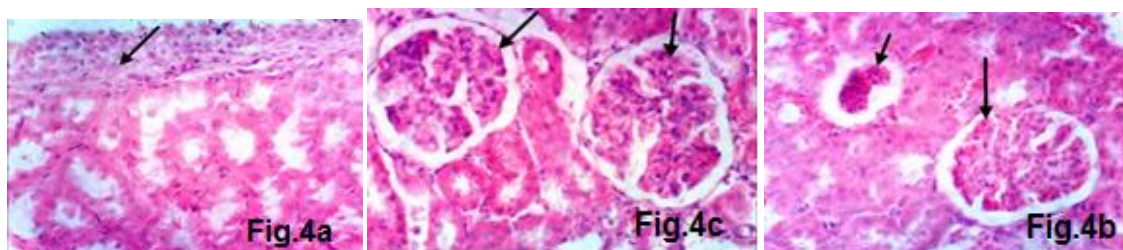


Fig 4:-Sections of rat kidneys of groups 2 & 3 after 5 weeks post infection showing inflammatory cells infiltration in the renal capsule (a), atrophy of some glomerular tuft, congestion and hypertrophy of other glomerular tuft (b), and congestion and hypertrophy and hyper-cellularity of glomerular tuft (c) (H & E X 400).

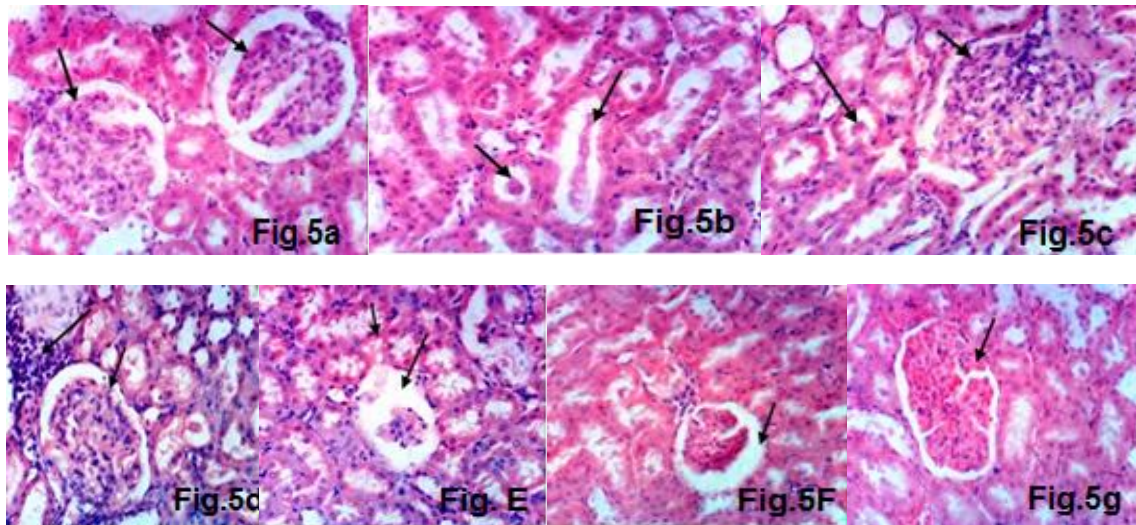


Fig. 5:-Sections of rat kidneys of group 1 after 6 weeks post infection showing hyper-cellularity of glomerular tuft (a), eosinophilic proteinaceous material in the lumen of renal tubules (b, c), thickening of the glomerular basement membrane and focal inflammatory cells infiltration (d), Vacuolation of tubular epithelium, atrophy of glomerular tuft and distension of Bowman's space (e), slight distension of Bowman's space, and thickening of glomerular basement membrane with congestion of glomerular tuft (f).

Lung:-

Pathological examination of alveolar tissues in the present study revealed that chronic interstitial pneumonia, interstitial pneumonia, and haemosiderosis, in the rat of group 1 in 8 weeks, while the last rat of group 3 at 5 weeks showed the same results in addition to perivascularitis (Fig. 6a-c).

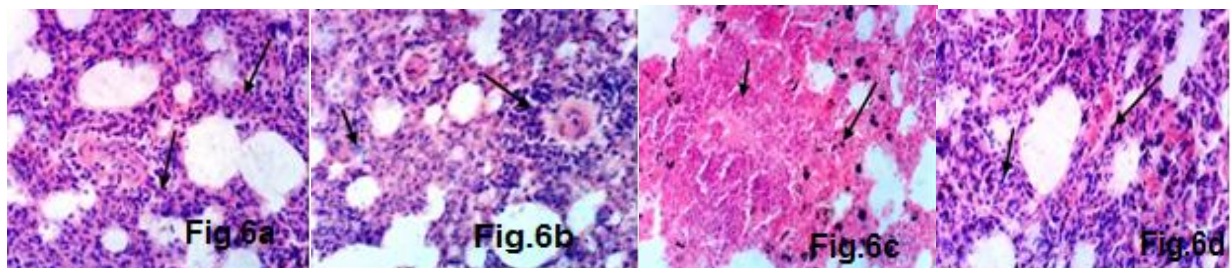


Fig. 6:- Sections of rat lungs of group 3 after 6 weeks post infection showing chronic interstitial pneumonia (a), perivascularitis (b), haemosiderosis (c). Lung of rat from group 1 in 56 day post infection showing chronic interstitial pneumonia and haemosiderosis (d) (H &E X400).

Discussion:-

Although the last decade has clarified many aspects of host-parasite relationship in human cystic echinococcosis, establishing the full mechanisms that cause the disease in other intermediate hosts requires more studies (Siracusano *et al.*, 2012). It is generally accepted that *Echinococcus* is unaffected by the immune response during the developing stage. However, natural infections in sheep indicate that some cysts can be killed during the latter stages of development (Zhang and Zhao, 1992), with the relatively frequent occurrence of dead, calcified metacestodes or necrotic cysts. In our experimental study, Hydatid cysts of *E. granulosus* developed in internal organs (mainly the liver and kidney) of the three group rats as unilocular fluid-filled bladders that consist of two parasite-derived layers, an inner nucleated germinal layer and an outer a cellular laminated layer surrounded by a host-produced fibrous capsule. Brood capsules and protoscolices (PSC) bud off from the germinal membrane. Some cysts indicate enlargement in size, but in some portions active small-cysts takes place. These enlarged cysts have a close relation to brood capsule and scolex formation. In addition, no calcification could be detected in those lesions growing rapidly along this short cycle in early infection until the end of the experiment. A previous study by Holcman and Heath (1997) showed that secretions from the penetration glands of hatched and activated oncospheres of *E. granulosus* because lysis of host tissues may protect the parasite against the host immune response while the

laminated layer develops. Another study by Rogan (1988) explained that as the primary cyst having degenerated, leaving the cavity full of host leukocytes and protoscolex-derived daughter cysts. In our opinion, the infection is in progress not in the abortive form of the disease. Moreover, the progression of cyst degeneration does take place in group 3, then 2 and after that group 1 in ascending order may signify increased immunological stimulation with cyst progression. This in contrast to the reports by Wang *et al.* (2011) that they considered the mild calcification was easy to be detected in those lesions growing rapidly in short cycle.

In the present study, the increasing trend of WBCs may be considered as a defense mechanism against the inflammatory processes which may be produced in the body (especially in the liver) and lead to an increase of WBCs production of the stimulated bone marrow. Likewise, Younuset *al.*, (2016) have been related these changes to the lytic effect of the newly developing *E. granulosus* hydatid cysts. These results are in agreement with the findings of Al-Nasiri, 2006; Moraitakiet *al.*, 2010; Khalaf, 2013. Previous studies in experimentally induced secondary infections in mice, intraperitoneally injected PSC are surrounded by a considerable cellular infiltration within 3 days, initially involving activated macrophages and subsequently including neutrophils, eosinophils, and lymphocytes (Richards *et al.*, 1983; Riley *et al.*, 1986; Riley *et al.*, 1985). As far as, red blood cells, platelets, hemoglobin and packed cell volume are usually kept in normal levels through a balance between the new formation and the destruction of old cells in the control group. Our investigation recorded a significant decrease in these parameters which come in a dose and time-dependent manner that may be attributed to increased hemolysis or disturbance in the hemopoietin system which slows of its production (Guyton, 1993). Moreover, Frayha and Haddad, 1980 and Akhtar *et al.*, 1998 related these changes to HCF enzymes that cause an increase in the blood cells destruction. Similar results were obtained from previous studies by Tanveeret *al.*, 1997 and Akhtar *et al.*, 1998 whereas Khalaf, 2013 reported no changes in these parameter concentrations in rats with *E. granulosus*. Increased MCV and decreased MCHC that observed in the inoculated groups of this study may indicate a macrocytic hypochromic anemia that cope with the increasing demand of oxygen requirement. The erythrocyte enlargement may be related to an accumulation of fluids after disturbance of cell membrane permeability. Similar findings have also been previously reported (Akhtar *et al.*, 1998; Xhemollariet *al.*, 2016).

Determination of plasma proteins reported having significant prognostic and diagnostic values, especially in liver diseases. Results of the present study revealed a marked increase in serum total protein that may be imputed to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite-induced cell membrane disruption (Orhue and Nwanze, 2004). Similar findings have been reported by Tanveeret *al.* (1997) and Radfar and Iranyar (2004) but they attributed this increase in protein to incoming CHCF that already contained different kinds of proteins. Rueda *et al.*, 1995 and Anwar and Tanveer, 2000 interpreted this elevation in protein concentration with time by the formation of antibodies against antigens present in hydatid cyst fluid (HCF). In agreement with this, our findings revealed a statistically significant elevation in globulins. As far as serum globulins were concerned, several reports determined its alterations with various parasites and revealed its increase specifically in the endoparasites infection Ayazet *al.*, 2007; Dede *et al.*, 2014). On the other hand, our results disagree with those reported by Smyth (1979) and Meduriet *al.* (1990) that reported a decline in protein level and related this to its use by the parasite, since the protoscolices of *E. granulosus* can digest host proteins. A drop in albumin levels was determined in our results in spite of there were no statistical difference as compared to control that may be attributed to the long half-life of albumin, therefore, a decrease in serum albumin is usually not apparent early in the course of liver diseases (Halsted and Halsted, 1981; Cheesbrough, 1998). This hypoalbuminemia may imply a combination of impaired synthesis and loss via the gut, kidney or both as revealed by several studies in various parasite diseases (Yeruhamet *al.*, 1998, Turgut, 2000; Zygnaret *al.*, 2007).

Our biochemical results revealed a marked increase in glucose in the experimentally infected animals that may be due to the effect of the infection on the liver which play an important role in the glucose metabolism (Khalaf, 2013) or occurrence of glycolysis and gluconeogenesis cycles which related to energy production within parasite cyst (El-Bayati *et al.*, 2010). This in accordance with Shaafie *et al.*, 1999 and Muhsin *et al.*, 2015 who recorded high levels of glucose in the primary intermediate host. As far as the activity of enzymes is concerned, ALP, ALAT and ASAT activities were also measured showed an increase among the treated groups compared to the negative control group. Several studies have also previously reported significant increases in ALP, ALAT, and ASAT activities in *E. granulosus* infected animals (Abdulla, 2007; Shindalaet *al.*, 2007). These studies explained that the liver infection with Cestodes tapeworms led to hepatocyte destruction and enzyme release or even indicates a cellular response. On the other hand, AcP values were closely related to the control values at the beginning of the experiment then trend to decrease with time. Rahmanet *al.* (1992) suggested that the decrease in the activities of AcP might be due to the

increased permeability of plasma membrane or cellular necrosis that showing the stress condition of the treated animals. Taken together, the serum total bilirubin concentrations were dramatically increased in *E. granulosus* infected groups that may be related to additional enzymes produced by the protoscolices and/ or brood capsule (Anwar and Tanveer, 2000). Increased quantity of total bilirubin also may indicate the increased breakdown of hemoglobin that interprets the decreasing levels in hemoglobin and RBCs levels of our investigation.

During cystic echinococcosis (CE) the host-parasite relationship is interactive and the outcome of infection depends on the balance achieved by the combination of the different variables involved with the host immunity and the *E. granulosus* avoidance strategies (Siracusano *et al.*, 2008). There is no direct evidence that the viability, death or calcified of such cysts is due to an immunological phenomenon, but it is a likely possibility. In addition, there are no detailed studies of immunological events associated with the degeneration of different types of the cyst, and it is, therefore, unknown which mechanisms may be involved. Our experimental study in rat supported the possible local immunosuppression mediated by IL-10 as a possible mechanism that helps the parasite in escaping the host cell-mediated response in agree with Mondragón-De-La-Peña *et al.* (2002).

The co-expression of IL-10 and IFN- γ at high levels in human hydatidosis suggests that the immune response to *E. granulosus* infection may be due to the very complex mixture of antigens in hydatid cyst fluid (McManus and Bryant, 1995) which probably contain distinct epitopes for each T-cell subset. Like other helminthic infections (Finkelman *et al.*, 1991; Lange, *et al.*, 1994; Pearce *et al.*, 1991), echinococcosis induces two very distinct Th1 and Th2 cytokine secretion patterns. It is not understood why hydatid infection can induce high levels of both Th1 (produce IL-2, IFN- γ , and lymphotoxin), and Th2 cytokines (express IL-4, IL-5, IL-6, IL-10) since they usually down-regulate each other (Pearce *et al.*, 1991). In our results, elevated levels of IL-10 that produced in vivo experimental infection were correlated with poor survival in rats and accompanied with severe pathological changes which increased in rats of high and calcareous groups and period post-infection. This is in agreement with Haralabidis *et al.* (1995) who reported that primary and secondary infections elicit similar responses, which include elevated levels of IL-10. And the increased production of IL-10 in hydatid disease patients corresponds to high levels of IgE (Babba *et al.*, 1994).

Conclusion:-

The effective stage of hydatid cyst development was the stage before transformation to calcareous stage. The most effective changes in serological, biochemical, immunological and pathological changes occur during this stage. Secondary infections by hydatid cyst fluid in experimental rats caused an alteration in liver functions in addition to changes in blood and body chemistry which showed by hematological and biochemical tests. Elevated IL-10 levels were correlated with poor survival in rats. Microscopically examination of livers and lungs of animals of different groups showed severe pathological changes which increased in rats of low and calcareous groups and period post-infection. The change of physiological parameters could be considered as a good diagnostic and reliable indicator to monitor the progress and severity of *E. granulosus* infection.

Ethical approval:-

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interests:-

The authors declare that they have no conflict of interests regarding the publication of this paper

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