EFFECT OF LOW DOSES OF CRUDE HYDATID CYST FLUID ON SOME BLOOD AND LIVER FUNCTION TESTS IN RABBITS

Akhtar Tanveer, Tayyaba Shaheen and Zaheer Anwar
Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

ABSTRACT

A study was conducted to find out some haematological and biochemical alterations in rabbits after inoculating crude hydatid cyst fluid (CHCF) of sheep origin. The rabbits were inoculated with increasing doses 10, 20, 30, 40, and 50 μL of CHCF for 14 weeks. It was noted that due to continuous inoculation of CHCF, the rabbits look weak, pale with loss of body weight and hairs. Fluctuations were noted in the red blood cells counts while their white blood cell counts stimulated initially but later on deviations were noted. Packed cell volume of experimental animals increased as compared to controls except after 20 and 30 μL doses of CHCF. Results of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and haemoglobin were similar in the sense that reduction in these values was common after inoculating 40 μL of CHCF till the end of experiment. However, erythrocyte sedimentation rate fluctuated throughout this period. Activity of transaminases (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) decreased with few alterations ($P<0.001$). Among other biochemical components of blood, plasma proteins increased ($P<0.001$), glucose and bilirubin level decreased in the experimental animals.

INTRODUCTION

Despite of wide prevalence and heavy medical and economic losses, the field of experimental hydatidosis remained neglected in Pakistan, till Tanveer *et al.* (1997 a, b) reported some haematological and biochemical alterations in rabbits after inoculating high and low doses of filtered hydatid cyst fluid of sheep origin. The previous work was further extended to find out the haematological and biochemical alterations after inoculating crude hydatid cyst fluid in rabbits as mammalian model.

MATERIALS AND METHODS

Animal Maintenance

Eleven healthy adults rabbits (*Lepus nigricollis*) were acclimatized for two weeks to the optimal conditions of animal house. They were provided with green fodder and tapwater *ad libitum* along with few crystals of $KMNO_4$. They were weighed individually in the start and then fortnightly after the completion of each dose regime.

Collection of Hydatid Cyst Fluid (HCF)

Sheep slaughtered at local slaughter house were examined for the presence of cyst in their liver and lungs and fluid contained in them was collected into air tight sterile glass vials and stored in the freezer for further use.

Dose Inoculation

Increasing doses (10, 20, 30, 40, and 50 μL) of CHCF were inoculated with the help of microlitre glass syringe by slow intravenous (ear vein) injections to six rabbits. Each dose was daily inoculated upto two weeks while the last dose was kept constant upto six weeks. The remaining five control rabbits were inoculated with distilled water in the same way.

Haematological Studies

After the completion of each dose regime blood samples with anticoagulant (EDTA) were pooled after making a cut on the marginal vein of the ear. Red blood cells (RBC) and white blood cells (WBC) counts were made by using Neubauer haemocytometer. Packed cell volume (PCV) was analysed by microhaematocrit method. Haemoglobin content was estimated by Drabkins reagent. Erythrocyte indices including MCV, MCH and MCHC were calculated by the formulae given by Swarup *et al.* (1986). ESR was estimated according to Westergren's method (Dacie and Lewis, 1991).

Biochemical Studies

Blood (4 mL) was collected directly from the jugular vein and was allowed to clot at 4°C. Serum was separated by centrifugation at 3000 rpm and was further

used for estimating different enzyme activities and metabolites.

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were estimated according to Reitman and Frankel (1957). Glucose was estimated by using the oxidase method. Total proteins were estimated according to Biuret method. Bilirubin contents were determined in the presence of caffeine by the reaction with diazotised sulfanilic acid. All these estimation were made by using Randox kits (U.K.).

Results were statistically analysed by Student 't' test (Steel and Torrie, 1981) between means in control and experimental groups of animals.

RESULTS AND DISCUSSION

Results of present investigation have shown that continuous inoculation of CHCF did not affect the survival of rabbits but apparently they became pale and weak. They showed loss of epitite and agility, shedding of body hair and decreased body weight with few exceptions where the increase may be attributed to the pregnancy (Dix *et al.*, 1977). In another investigation conducted by Tanveer *et al.* (1997a) survival and general appearance of the experimental rabbits was not affected when similar doses of CHCF of sheep origin were used after filtration (protoscoleces were not present) but decreased body weight noted due to such inoculations was similar with our present findings (Fig.1).

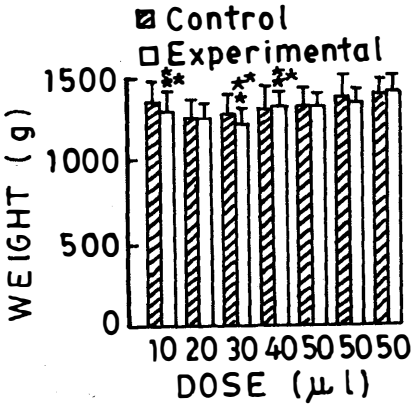

Fig. 1: Body weight (g) changes (%) in rabbits due to parenteral administration of different doses of crude hydatid cyst fluid (CHCF) under low dosage. Values given are mean±S.D. of 5 control and 6 experimental rabbits.

In natural hydatidosis such decrease in body weight can be attributed to that fluid which is seeping out of the syst wall (Pandey, 1971). Various toxic substances present in it (Aziz, 1966; Sanchez and Sanchez, 1971; Frayah and Haddad, 1980) were in turn responsible for the lowering of basic protein and carbohydrate metabolism that ultimately resulted in the reduced yield of meat and other stored products. A condition finally responsible for heavy economic losses (Schwabe, 1986; Iqbal *et al.*, 1986, 1989).

Besides weight reduction, the rabbits inoculated with CHCF, became anaemic, due to significant fall in erythrocyte number either due to their excessive damage or inhibition of their formation that may have adversely affected by the toxicity of CHCF (Fig.2). It is further suggested that the toxic enzymes present in CHCF may also have reduced their survival time thereby decreasing their number. In blood, decreased RBC count also tallies with the decreased haemoglobin contents. Our results are in accordance with the findings of Aminzhanov (1977), Wangoo *et al.* (1989) and Tanveer *et al.* (1997a).

In the present investigation, increased WBC counts, resulted after inoculating low doses of CHCF is an indication of body's invasion by some foreign compounds (Fig. 2). Similar findings have earlier been reported by Aminzhanov (1977), Wangoo *et al.* (1989) and Tanveer *et al.* (1997a) in sheep, mice and rabbits, respectively infected with *E. granulosus*.

Repeated inoculation of CHCF, resulted in increased PCV and MCV with the corresponding decrease in the number of circulating RBC and haemoglobin contents (Fig. 2). It is an obvious indication that despite decreasing RBC counts, increase in PCV and MCV is due to the increase in erythrocyte volume. Since the number of circulating RBC is decreasing thereby resulting in a decreased area for oxygen consumption. In order to fulfill the high demand of oxygen for respiration the volume of RBC has increased. Present results showed condition of macrocytic hypochromic anaemia, because PCV and MCV increased but MCH and MCHC decreased. It is suggested that increased MCV may also be attributed either to anaemia or toxicity of CHCF or haemolysis. In the present findings (Fig.2), sudden increase in MCV in the 2nd week (after inoculating 20 µL of CHCF) may be due to pregnancy (Dix *et al.*, 1977).

Increase in MCH (Fig. 2) in the present work also indicated that the RBC are not fully saturated with haemoglobin because inside the corpuscle, haemoglobin synthesis was inhibited due to the toxicity of CHCF or due to the iron deficiency (Eastham, 1985).

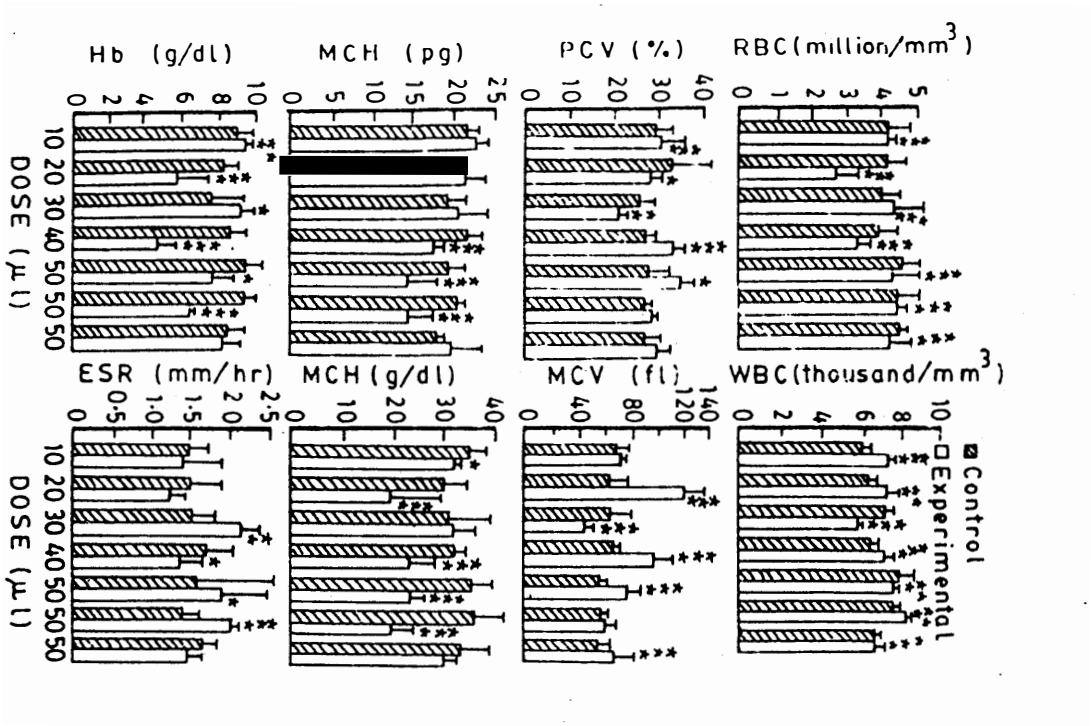

Fig. 2: Some haematological changes in rabbits due to parenteral administration of different doses of crude hydatid cyst fluid (CHCF) under low dosage. Values given are mean ± S.D. of 5 control and 6 experimental rabbits.

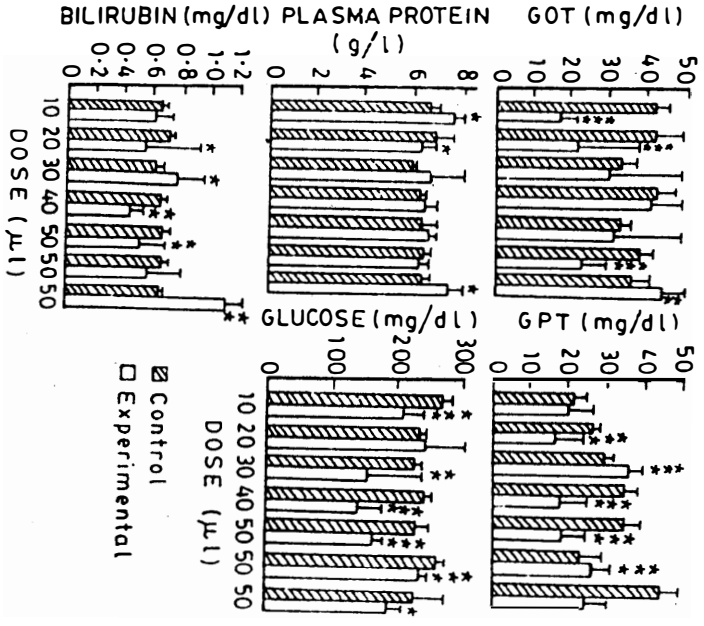

Fig. 3: Some biochemical changes in rabbits due to parenteral administration of different doses of crude hydatid cyst fluid under low dosage. Values given are mean ± S.D. of 5 control and 6 experimental rabbits.

CHCF have reduced the RBC count that have ultimately decreased the haemoglobin concentration (Fig. 2). Breakdown of RBC and impaired synthesis of haemoglobin are also responsible for the decreased haemoglobin contents. Haemolysis is another reason for decreased level of haemoglobin that leads to binding of haemoglobin to plasma hepatoglobins. During this process haemoglobin is excreted through urine (Gowenlock *et al.*, 1988).

Increased amount of globulins through the incoming CHCF have increased their ESR (Fig. 2). It might be suggested that toxicity of CHCF have increased the tendency of erythrocytes to clump together and they form clusters which settle down to their own weight (Eastham, 1985).

Fig. 3 showed reduced GOT and GPT activity (with few exceptions). Decreased activity of transaminase indicate abnormal liver function while increasing trend showed liver necrosis (Zimmerman, 1974) or gluconeogenesis through which amino acids may be transmitted and utilized for energy requirement. However, increased GOT and GPT activity, when filtered hydatid cyst fluid was inoculated in rabbits at the same dose (Tanveer *et al.*, 1997a).

An overall increase resulted in the plasma proteins can be attributed to the necrotic or damaged liver parenchymal cells which have permitted the outflow of soluble proteins (including enzymes) in their surroundings thereby increasing their level (Fig.3). Increased proteins level may also be attributed to the formation of antibodies against the antigens present in the incoming CHCF. Another strong reason for the increased proteins contents is the heavy inflow of proteins through the regularly inoculated CHCF. Increased proteins contents have also been reported by Tanveer *et al.* (1997a and 1997b) in rabbits inoculated with filtered hydatid cyst fluid. However, low proteins values (at 20 μL and 2nd dose of 50 μL CHCF) also indicate the influence of direct proteolytic effect of CHCF, as it contained many lytic enzymes (Frahya and Haddad, 1980) or due to some interference in the process of synthesis in liver or due to some malfunctioning of kidney (Khan, 1990). Most probably due to similar reasons in natural infections hydatidosis results in heavy economic losses through reduced quantity and quality of yield.

In the present investigation, rabbits faced a continuous stat of hypoglycemia (except in the 4th week of experiment) (Fig. 3) that was most probably related to the increased utilization required for induction of liver enzymes and microsomal proteins (Ali *et al.*, 1988) or to face the stress conditions imposed by aerobic and anaerobic enzymes present in the incoming CHCF (McManus and Smyth, 1982). It is also important to note that under stress condition the animal minimize its food uptake thereby decreasing its dietary proteins and energy. This also happened in the present investigation. Liver necrosis is another consideration for this decrease in glucose contents.

The reduced serum bilirubin level in rabbits after inoculating doses of CHCF showed disturbed liver functioning (Fig.3). However, increased bilirubin level (after 30 μL and last dose of 50 μL CHCF) may be attributed to the kidney or liver cells damage that have increased its level due to enormous RBC damage and disintegrating Hb molecules and obstruction of biliary tract (Khan, 1990).

Keeping in view the present results, it can be concluded that different haematological and biochemical alterations induced by low doses of CHCF are even more severe as compared to the previous findings (Tanveer *et al.*, 1997a) where filtered hydatid syst was inoculated at the same dose level. Further work in this connection is needed.

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