HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN RABBITS DUE TO HIGH DOSES OF CRUDE HYDATID CYST FLUID OF SHEEP ORIGIN

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ABSTRACT

Studies were conducted on the haematological and biochemical changes in rabbits due to high doses of crude hydatid cyst fluid (CHCF) of sheep origin. Significant reduction in the body weight of rabbits was noted due to continuous inoculation of CHCF ($P<0.001$). However, a considerable increase in their red blood cell counts (27.28%), white blood cell counts (58.58%) and erythrocyte sedimentation rate (18.30%) was noted alongwith reduced packed cell volume ($P<0.001$). The values of erythrocyte indices (MCV, MCH, MCHC) and Hb contents correspondingly increased ($P<0.001$).

Estimation of enyzme activity showed increased values for glutamic oxaloacetate transaminase while the activity of glutamic pyruvic transaminase was decreased ($P<0.001$) when compared with their respective controls. Among the biochemical metabolites, bilirubin and total proteins increased ($P<0.001$) with decreased glucose level ($P<0.001$).

INTRODUCTION

In Pakistan, larval stages of *Echinococcus* have been recognized as producing hydatid cysts in man (Chaudhry *et al.*, 1992; Naveed *et al.*, 1993; Junejo *et al.*, 1995) and livestock (Pal and Jamil, 1986; Hussain, 1987; Iqbal *et al.*, 1989). Hydatid cysts of variable sizes in liver, lung, spleen, heart, bones and other body organs adversely affect the normal functioning of these organs as indicated by the biochemical changes due to hydatidosis reported by Aminzhanov (1977, sheep), Lau *et al.* (1985, sheep), Wangoo *et al.* (1989, albino mice) and Tanveer *et al.* (1997, 1997a rabbits).

Considering the medical and veterinary importance of hydatidosis, it was thought prudent to find out some haematological and biochemical changes produced in rabbits due to parenteral administration of high doses of crude hydatid cyst fluid of sheep origin.

MATERIALS AND METHODS

Maintenance of Rabbits

Thirteen adult, apparently healthy rabbits (Lepus nigricolis) were acclimatized under optimal conditions of the Animal House of the Zoology Department, Punjab University, Lahore. They were fed on green fodder *ad libitum* alongwith tap water mixed with few crystals of KMNO4. The food and water pots were daily washed with KMNO4 solution. Whenever required, Gentamycin injections were given to save them from different infections. Electric heaters were used to maintain the room temperature at 25±2°C. Each rabbit was weighed weekly upto 14 weeks.

Collection of Hydatid Cyst Fluid

With the help of disposable syringe and needle (18 G), hydatid cyst fluid was aspirated from the unilocular cysts located in liver, lungs and spleen of sheep obtained from the local slaughter house. The fluid was transferred to sterile vials and placed in the ice boxes containing water at 4°C. It was brought to the laboratory and stored in a deep freezer for further use. Flame cell activity of protoscoleces was always observed before using the fluid.

Dose Administration

For this purpose, ear vein was cleared by removing hair and increasing doses (0.1, 0.15, 0.2, 0.25, 0.3, 0.3 and 0.3 ml) of crude hydatid cyst fluid (CHCF) were daily inoculated upto 2 weeks to 8 experimental rabbits. After two weeks the dose was increased (as mentioned above) while the last three doses were kept constant. The remaining 5 rabbits served as control and distilled water was inoculated with similar protocol.

Blood Sampling

Blood samples with EDTA 2.0 ml and without EDTA, 3.0 ml, were collected from ear vein of each rabbit. Samples without EDTA were allowed to clot in a refrigerator, and centrifuged at 2500 rpm for 20 minutes. Clear serum was separated and kept at -20°C till analysis for biochemical studies. Blood samples with EDTA were used for haematological studies.

Haematological Studies

Red blood cells (RBC), white blood cells (WBC), packed cell volume and erythrocyte sedimentation rate were estimated according to Dacie and Lewis (1991). Erythrocyte indices i.e., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as mentioned by Dacie and Lewis (1991).

Biochemical Studies

Unhaemolysed and clear serum was used for estimating glutamate pyruvic transaminase (GPT), glutamate oxaloacetate transaminase (GOT), glucose, bilirubin and plasma protiens. GOT and GPT were estimated according to Reitman and Frankel (1957). Glucose was estimated according to oxidase method. Bilirubin was estimated in the presence of caffeine by the reaction with diazotised sulfanilic acid. All these estimations were made by using Randox kits (U.K.). Plasma proteins were estimated by using Biuret method (Henry *et al.*, 1974).

Statistical Analysis

The data were analysed by single factor analysis of variance and Student's 't' test (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The results showed that uninterrupted inoculation of CHCF not only altered the haematology and blood biochemistry of rabbits but also affected their general appearance by making them sluggish, agile alongwith loss of hair and appetite. Present findings showing significant reduction in the body weight (Fig. 1A) due to CHCF inoculation are in accordance with the results reported by Pandey (1971) and Tanveer *et al.* (1997, 1997a). They have suggested that fluid seeping out of the cyst wall is responsible for damaging the surrounding cells thereby resulting in organ condemnation and weight reduction. In the present investigation besides weight loss, the experimental rabbits also showed loss of appetite and became slugglish and inactive with the passage of time.

In health, RBC count and Hb concentration are kept at normal level through a balance between the new formation and the destruction of old ones by the nature. In the present invetigation, the normal RBC level deviated on both sides (Fig. 1B). Increased RBC counts may be due to oxygen tension in the blood or some severe heart disease (Eastham, 1985) or disturbance in the stable haemopoetic system of the animal due to incoming CHCF. While their decrease can be attributed either to the slow production (due to disturbed haemopoetic system) or increased haemolysis (Guyton, 1993), increased blood cell destruction due to the enzymes present in the incoming CHCF (Farayha and Haddad, 1980) or most probably, CHCF has reduced the survival time of RBC (Aminzhanov, 1977; Wangoo *et al.*, 1989).

It was further noted that experimental rabbits showed an overall increase in the Hb level (Fig. 1 C) alongwith some fluctuations, that can be attributed to increased haemolysis (Charles and Norman, 1966; Eastham, 1985; Dacie and Lewis, 1991). Continuous inoculation of CHCF in rabbits lead to leukocytosis in the present studies by incrased WBC counts (Fig. 1D). Our findings are in accordance with the results reported by Aminzhanov (1977), Wangoo *et al.* (1989) and Tanveer *et al.* (1997, 1997a).

Present studies showed a continuous decline in PCV (Fig. 1E) throughout the stipulated time period. In experimental rabbits maximum increase in MCHC was 37.54 per cent. Increased MCV and MCHC (Fig. 1F,G) observed due to inoculation of CHCF indicated macrocytic normochromic anaemia (Benjamin, 1985; Swarup *et al.*, 1986) i.e., enlargement of erythrocytes (hypertrophic) to hold greater amount (37.5%) of haemoglobin, to cope with the increasing demand of oxygen requirement. Rapid increase in the ESR in experimental rabbits was probably due to the toxicity of CHCF (Fig. 1H).

As far as the activity of enzymes is concerned, it was noted that in the beginning the experimental rabbits showed high GOT values (Fig. 2A). This is also confirmed by the decreasing GOT level with the pasage of time and increasing dose of CHCF. The higher levels in the start may be due to the presence of GOT already present in the incoming CHCF (Farayha and Haddad, 1980). Decreasing trend in GOT and increasing GPT levels (Fig. 2A, B) in experimental rabbits might be due to the increased selective permeability of hepatocytes or more damage of GOT (Animal's own and invading) than GPT due to the presence of lytic enzymes in CHCF (Jenssen and Bryant, 1969). However, increased GOT and GPT also indicates cellular response (necrosis of hepatic cells) or gluconeogenesis through which amino acids may be transmitted and utilized for energy requirements.

Glucose level of the experimental rabbits continuously declined (Fig. 2C) in response to the increasing dose and time which could be due to stress condition during which glucose is oxidized for energy production to cope the stress impact (Tufail *et al.*,

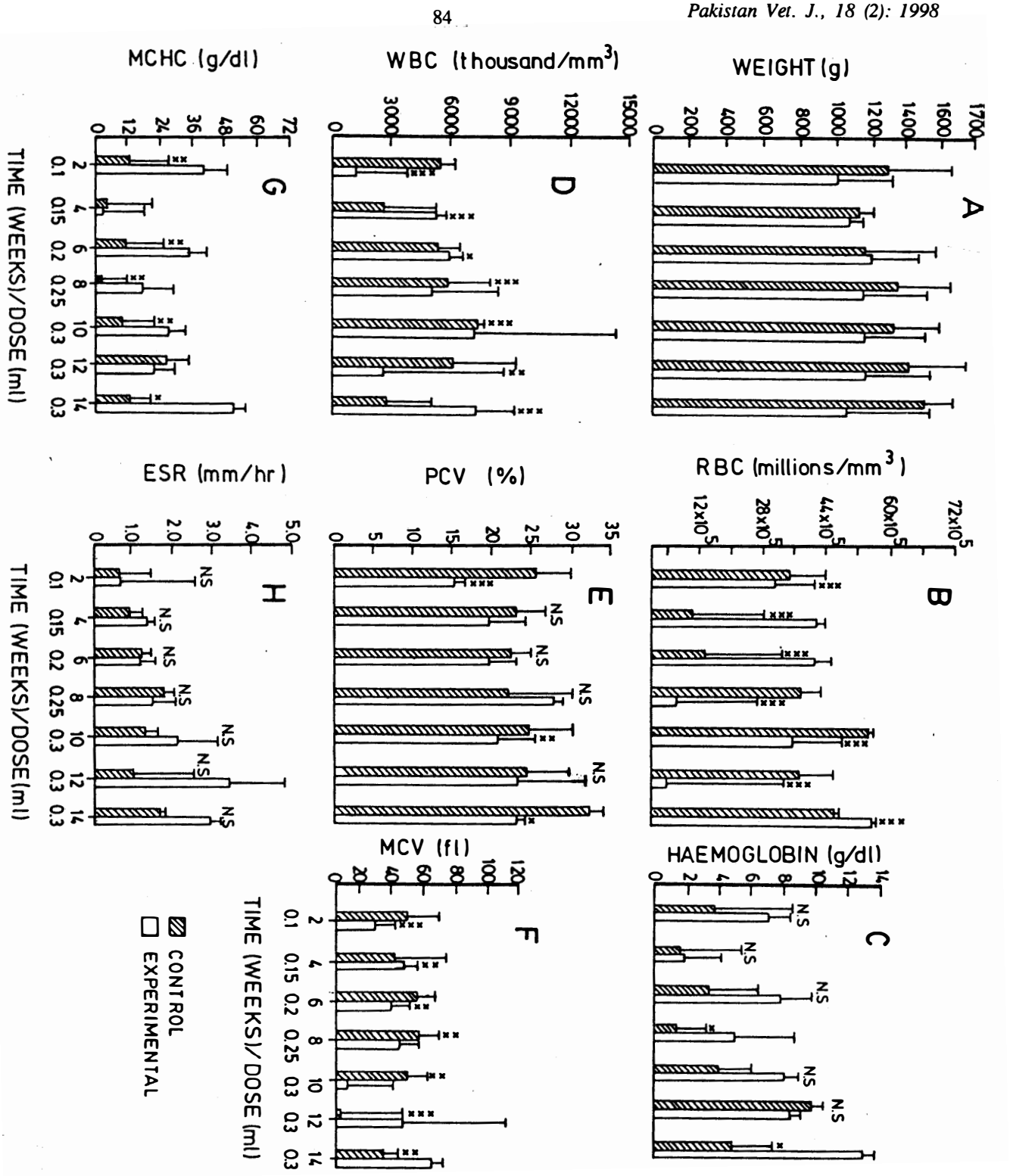

Fig. 1: Body weight and haematological changes in rabbits due to parenteral administration of different doses of crude hydatid cyst fluid. Values given are mean ± S.D. of 5 control and 8 experimental rabbits for 14 weeks. (*P<0.05; **P<0.01; ***P<0.001; NS Non-significant)

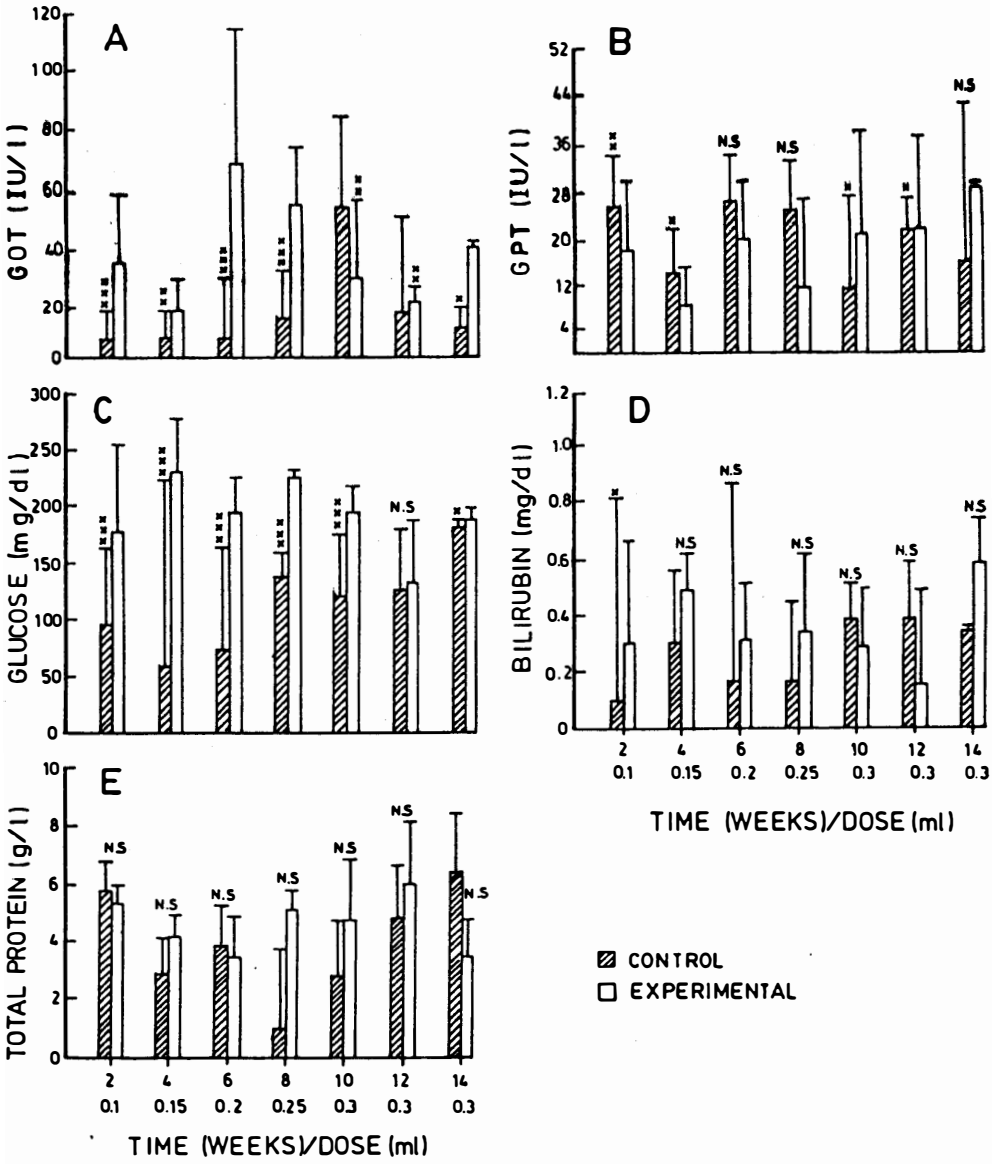

Fig. 2: Some biochemical changes in rabbits due to parenteral administration of different doses of crude hydatid cyst fluid. Values given are mean ± S.D. of 5 control and 8 experimental rabbits for 14 weeks. (*P<0.05; **P<0.01; ***P<0.001; NS Non-significant)

1994). It has been further suggested that glucose might be forcefully catabolized by aerobic and anaerobic enzymes (Agosin *et al.*, 1957) present in the protoscolces. They have found kinase, myokinase, mannose isomerase and phosphatase in the protoscoleces. Our findings are in accordance with the work reported by Tanveer *et al.* (1997, 1997a).

Decreased levels of some metabolites like bilirubin and plasma proteins (Fig. 2D,E) in the experimental rabbits were noted in the beginning but later on gradually increased. Decrease in these values may be in response to some proteolytic effect of CHCF, as it contained many lytic enzymes (Farayha and Haddad, 1980). Increase in the protein contetns may be attributed to incoming CHCF that already contained different kinds of proteins. Similar findings have earlier

been reported by Tanveer *et al.* (1997, 1997a) in rabbits infected with *E. granulosus*.

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