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BLOOD BIOCHEMISTRY OF RABBITS AFTER INTRAVENOUS INOCULATION OF UNILOCULAR HYDATID CYST FLUID OF SHEEP ORIGIN

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Abstract: Unilocular, hydatid cyst fluid of sheep origin was inoculated to five group of rabbits. The doses given were (1) Crude Low Dose (CLD), (2) Filtered Low Dose (FLD), Crude Medium Dose (CMD), (3) Filtered Medium Dose, (FMD) and (4) High Dose (HD of filtered hydatid cyst fluid). Control group was inoculated with similar doses of distilled water. Blood samples were pooled fortnightly and biochemical analysis made were glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) bilirubin, glucose, and plasma protein content. To face stress conditions through energy production, all the groups showed elevation in their GOT, GPT activities and depletion in glucose and protein contents of blood. High dose group, however, behaved differently, which showed depletion in both of these transaminases, probably due to interference in the synthesis or post synthetic destruction of these enzymes. Bilirubin content increased significantly only in the "high dose" group probably due to enhanced degradation of haemoglobin content.

Key words: Hydatidosis, blood biochemistry, rabbit.

INTRODUCTION

Hydatidosis adversely affect the normal body functioning by producing haematological changes (Amizhanov, 1977; sheep, Hinz and Gehring, 1987; mice, Alkarmi and Behbiani, 1989; Bressen-Handi *et al.*, 1989; man, Wangoo *et al.*, 1989; mice, Tanveer *et al.*, 1996, a,b, rabbits). In addition to these biochemical alterations have also been reported by Davydov and Smirnov (1982, pigs), Kroeze and Tanner (1985, rats) and Tanveer *et al.* (1997, 1998, 1998a).

Considering the medical, veterinary and economic importance of hydatidosis (FAO, 1985) the present study is aimed to work out the effects of hydatid cyst fluid on blood biochemistry of rabbits as a mammalian model.

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MATERIALS AND METHODS

Rabbits (*Oryctoragus cuniculus*) maintained in the optimal condition of animal house were acclimatized for two weeks prior to inoculation with hydatid cyst fluid of sheep origin (Tanveer *et al.*, 1996). Different doses of hydatid cyst fluid were given according to following schedule.

Time (day)	GROUPS			
	CLD (n=7) FLD (n=7)	CMD (n=7) FMD (n=7)	HD (n=7)	
	Dose (ml/day)	Dose (ml/day)	Dose (ml/day) 1st week	2nd week
1-14	10	0.1	0.1	0.15
15-28	20	0.15	0.2	0.25
29-42	30	0.2	0.3	0.35
43-56	40	0.25	0.4	0.45
57-70	50	0.3	0.5	0.55
71-84	50	0.3	0.55	0.55
85-98	50	0.3	0.55	0.55

Abbreviatinos used:
CLD, Crude low dose; FLD, Filtered low dose; CMD, Crude medium dose; FMD, Filtered medium dose; HD, High dose filtered hydatid cyst fluid.

About 4.0 ml of blood pooled out fortnightly in small sterilized test tubes was allowed to clot at 4°C and centrifuged at 3500 RPM for 20 minutes. Clear serum was separated and was further used for various biochemical analyses like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), bilirubin, glucose, and plasma protein content. GOT and GPT activity was estimated by using RANDOX Kit based upon the method of Reitman and Frankel (1957), Bilirubin by Jendrassik and Grof (1938), Glucose by Glucose oxidase per oxidase method of Trinder (1969), modified by Teuscher and Richterich (1971), and Barham and Trinder (1972). Plasma protein content was estimated by using Biuret method designed by Henry *et al.*, (1974). Statistical significance was computed according to Student's 't' test (Steel and Torri, 1981).

RESULTS AND DISCUSSIONS

Fig.1 showed that GOT activity remained almost unchanged in control group through out the study period. The increase in GOT activity was statistically non significant through out crude hydatid cyst fluid (CHCF) treatment in CLD group. FLD group also showed an average increase in GOT activity that was statistically significant only after 42 ($P<0.05$), 56 ($P<0.01$) and 70 ($P<0.001$) days. GOT activity increased with dose and time in CMD group and was found statistically significant after 14 ($P<0.05$), 28 ($P<0.05$), 56 ($P<0.05$), 70 ($P<0.05$) and 98 ($P<0.01$) days in this group. Increasing trend in GOT activity was also observed in FMD group but it was found statistically non significant through out the HCF treatment in this group. HD group however, showed an average decrease of GOT activity after CHCF

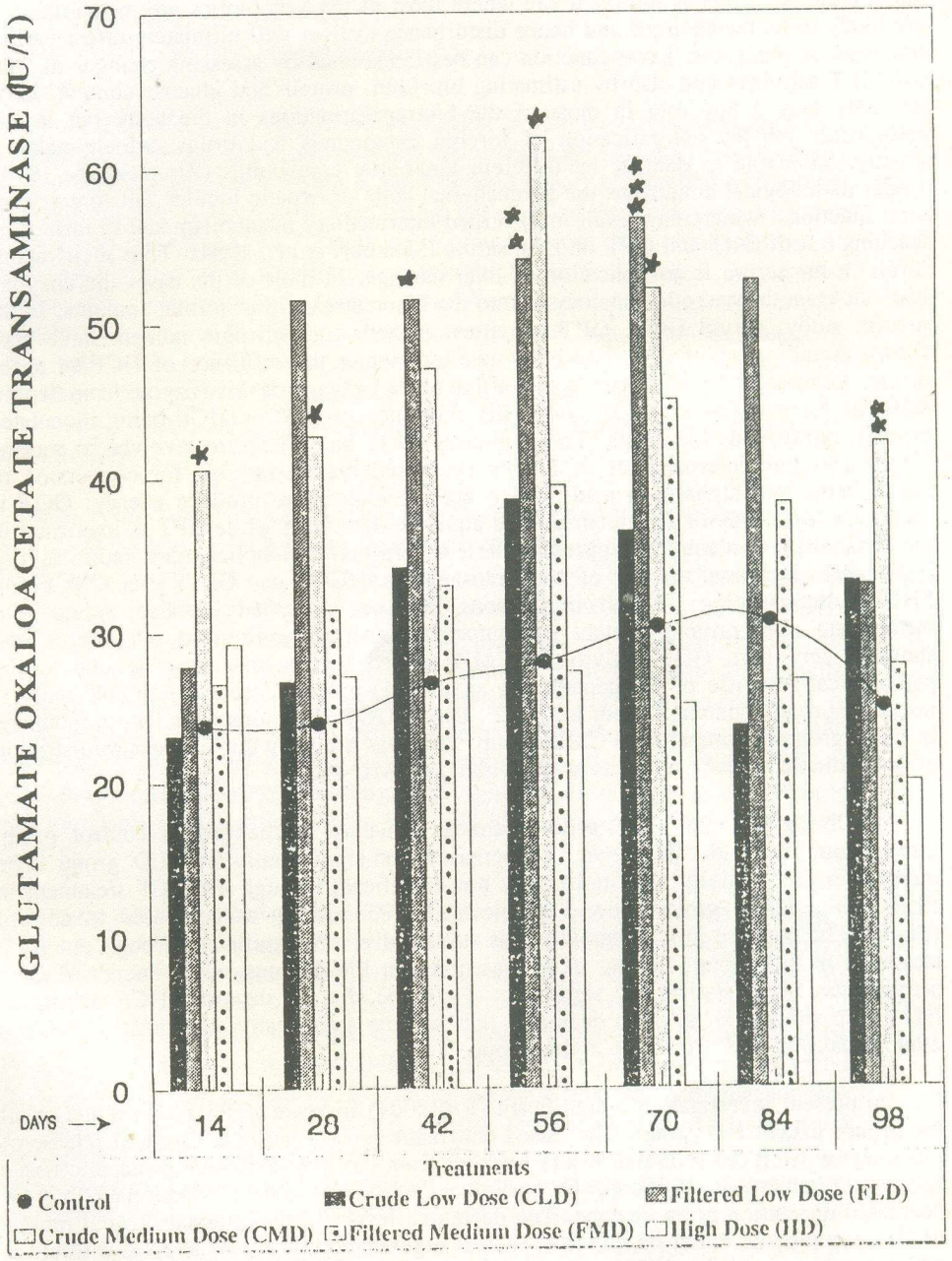

Fig. 1: Change in Glutamate Oxaloacetate Transaminase (U/l) of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses like crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) (please see materials and methods). Statistical significance has been determined by student's "t" test and the probability represented by stars; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

administration. Liver is the main site where most of the xenobiotics are metabolized or are likely to be metabolized and hence disturbance to liver will ultimately effect various biochemical processes. Liver function can best be studied by assessing changes in GOT and GPT activities and also by estimating bilirubin, protein and glucose content. Liver not only play a key role in most of the biotransformations in the body but is also responsible for the detoxification of foreign compounds and drugs, which makes it greatly vulnerable to damage by different xinobiotic compounds (Zimmerman, 1974). Under pathological conditions the parenchymal cells of hepatic lobules fail to carry out vital functions, which may result in disturbed intermediary metabolism and in turn cause leaching out of GOT and GPT into the serum (Shakoori *et al.*, 1984). Thus their raising levels in the serum is an indication of liver damage. In most of the cases the enzymes leak out from the necrotic hepatocytes into the blood stream in abnormal amounts. In the present study, serum GOT, GPT activities, protein and bilirubin content have been shown as indicators of disturbed liver functions under the influence of HCF of sheep origin. Increase in GOT activity is either due to (i) Leakage of this enzyme from hepatic cells (ii) Increased synthesis of GOT (iii) Presence of GOT in HCF being inoculated through intravenous injections. Transaminases (GOT and GPT) are involved in routing amino acid into intermediates of Kreb's cycle and are responsible for conversion of amino acids into alpha keto acids which are metabolized to produce energy. GOT is important for metabolizing glutamate and alpha ketoglutarate while GPT is important in the metabolism of alanine and pyruvate these in tern may metabolize other amino acid of importance. Increased activity of both transaminases (GOT and GPT) after CHCF and FHCF administration through intravenous injection indicated elevated amino acid metabolism after protein content to compensate energy requirement. Present results showed, very high GOT activity in CMD treated rabbits that may be due to the pathological response of the hepatocytes against (1) protoscoleces, (2) soluble and (3) insoluble protein content present in CHCF. Serum GOT elevations were less pronounced in FMD group as compared to CMD group. This was probably due to the administration of only soluble types of antigens to the rabbits of FMD group.

Fig.2 showed that GPT activity almost remained unchanged in control group through out the study. However, it decreased non-significantly in CLD group. The increase in GPT activity was statistically non-significant through out HCF treatment in FLD group. CMD group showed an elevated GPT activity as compared to control (Fig.2). The increase in GPT activity was statistically non significant through out HCF treatment in this group. Similar trend was noted in FMD group where increased GPT activity was found statistically significant ($P<0.05$) after 70 days of FHCF treatment. HD group showed an average decrease, that became statistically significant ($P<0.01$) after 70 days of HCF treatment in this group.

In present investigation a significant ($P<0.001$) increase in GPT was noted in all the groups except HD group. The raised enzymatic levels may be a result of release of this enzyme from the cells due to (1) Leaking from the liver cells due to hepatic tissue necrosis (Zimmerman, 1974). (2) Or enzyme induction and release (Street, 1969) (3) Or decreased deposition of an enzyme. The decreased level of GPT in experimental rabbits of HD group could either be due to (1) Great regeneration power of liver as a result of which leaking out of the GPT in the serum become minimal (2) The biosynthetic activity which implies routing of all the biochemical components towards this activity in liver (Knox and Greengard, 1965; Bhatia *et al.*, 1972) (3) Enzymatic inhibition (Hendrickson and Bowden, 1976; Meany and Pocker, 1979) (4) Development of the host resistance against the foreign and excessive GPT.

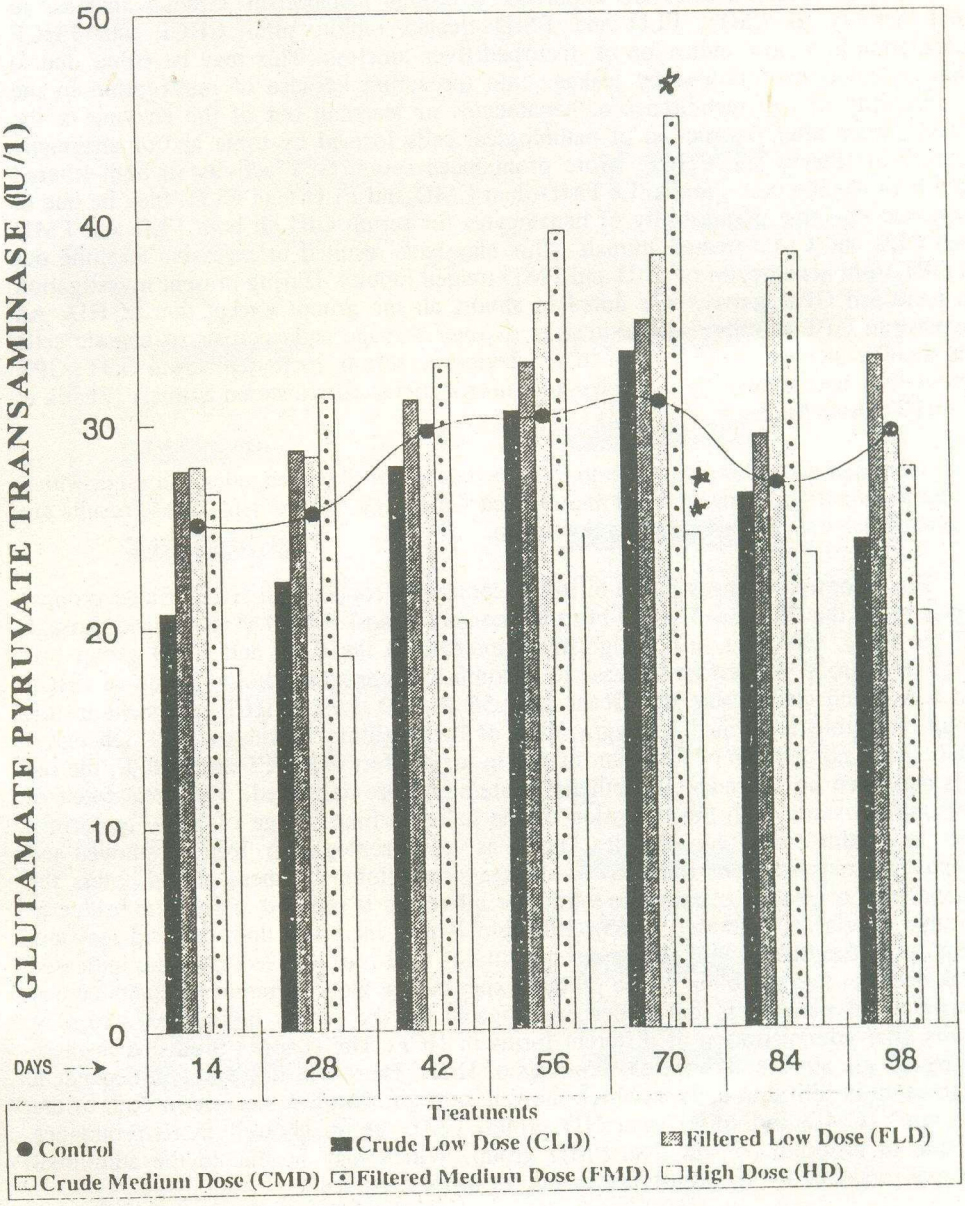

Fig. 2: Change in Glutamate Pyruvate Transaminase (U/l) of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses like crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) (please see materials and methods). Statistical significance has been determined by student's "t" test and the probability represented by stars; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Both the transaminases are important in protein metabolism. Gradual increase in GPT activity in CMD, FLD and FMD treated rabbits after CHCF and FHCF inoculation is a clear indication of disturbed liver function. This may be either due to enzyme induction followed by leakage into the serum because of interruption in the permeability of cell membranes of hepatocytes or leaching out of the enzyme in the blood stream after destruction of pathological cells formed by toxic and/or enzymatic activity of filtered and CHCF. More pronounced serum GPT activity in both filtered then both of the crude groups (i.e FMD than CMD and FLD than CLD) may be due to increased selective permeability of hepatocytes for serum GPT in both FLD and FMD than CLD and CMD treated animals. This may have resulted in excessive leaching out of GPT from hepatocytes of FLD and FMD treated rabbits. During present investigation an increased GPT activity was noted in almost all the groups except that of HD. An increase in GPT activity is an indication of liver damage and necrosis of hepatic cells and thereby increasing the level of these enzymes in serum. Increased serum GOT, GPT activity has been shown by many workers in insecticide administered animals (Bhatia *et al.*, 1973; Abdelsalam *et al.*, 1982).

Increased activities also indicate cellular damage or gluconeogenesis through which amino acids may be transaminated and utilized for energy requirement. These results are in accordance with Kroeze and Tanner (1985).

Fig.3 showed changes in the bilirubin content of control and HCF treated groups. It was noted that in control group bilirubin content almost remained unchanged through out the study. However, a non-significant increase in the CLD and FMD group was noted. HD group showed an increase in bilirubin content after administration of FHCF and was found statistically significant after 56 and 70 days of HCF treatment in this group. Bilirubin is formed by degradation of haemoglobin (Cantarow and Schepartz, 1967). After completion of life span, or due to toxic effect of CHCF and FHCF, the red cells are taken up by reticuloendothelial system and are destroyed. The breakdown of haemoglobin starts with the oxidation of the delta-methine bridge of heme to form a green biliverdin-iron globin complex known as verdohaemoglobin. Iron is removed and attached to transferrin for transport to storage sites, globin is liberated and enters the general pool of protein metabolism while the biliverdin is reduced at methine bridge to bilirubin (Datta and Ottaway, 1965). Bilirubin is also increased due to blood loss and haemolysis (Eastham, 1985). Increased quantity of total bilirubin indicates the increased break down of haemoglobin. In the present experimental work different biochemical and hematological parameters have been investigated on the whole blood and serum of rabbits after administration of different forms of HCF. The changes produced in these parameters are attributed to the toxic effects of HCF. There was noted a dose dependent relationship in bilirubin content which showed more pronounced increase in both of the "medium" (CMD and FMD) and HD group. CMD group showed more pronounce increase in bilirubin content than FMD group. Which may be due to the additional enzymes produced by the protoscoleces and/ or brood capsules.

Fig.4 showed changes in glucose content of control and HCF treated groups. It was noted that these content remained unchanged through out the study in control group. CLD group showed an average decrease in glucose content after administration of CHCF. When analyzed by Student's "t" test decrease in glucose content was found statistically significant ($P<0.05$) after 14 and 56 days of CHCF treatment in CLD group. Decrease in glucose content was found statistically significant ($P<0.05$) after 56 and 70 days of HCF treatment in FLD group. FMD group also showed a general decrease that was found statistically significant ($P<0.05$) after 56 days of HCF treatment in this group. In the HD group glucose content decrease was more pronounced

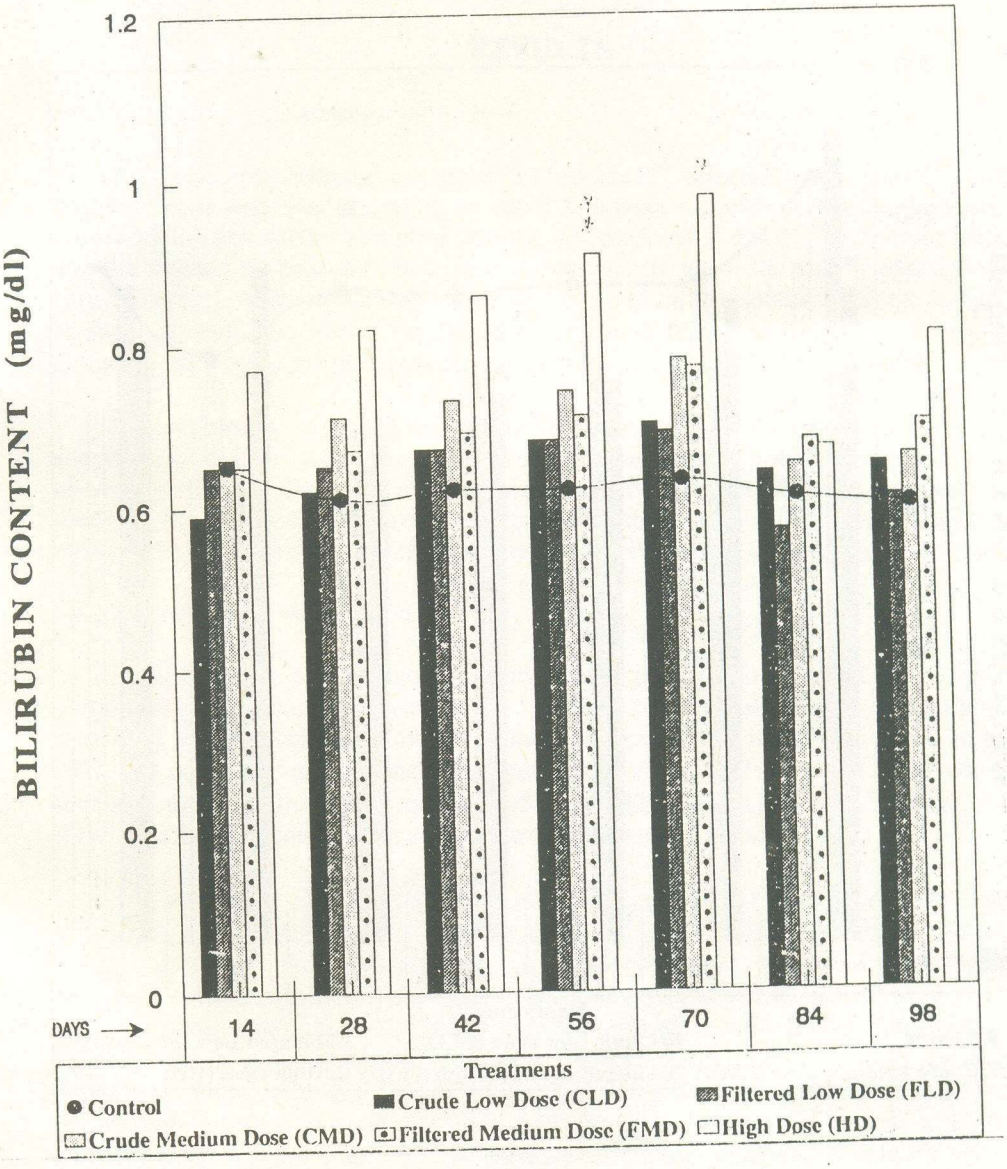

Fig. 3: Change in Bilirubin Contents (mg/dl) of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses like crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) (please see materials and methods). Statistical significance has been determined by student's "t" test and the probability represented by stars; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

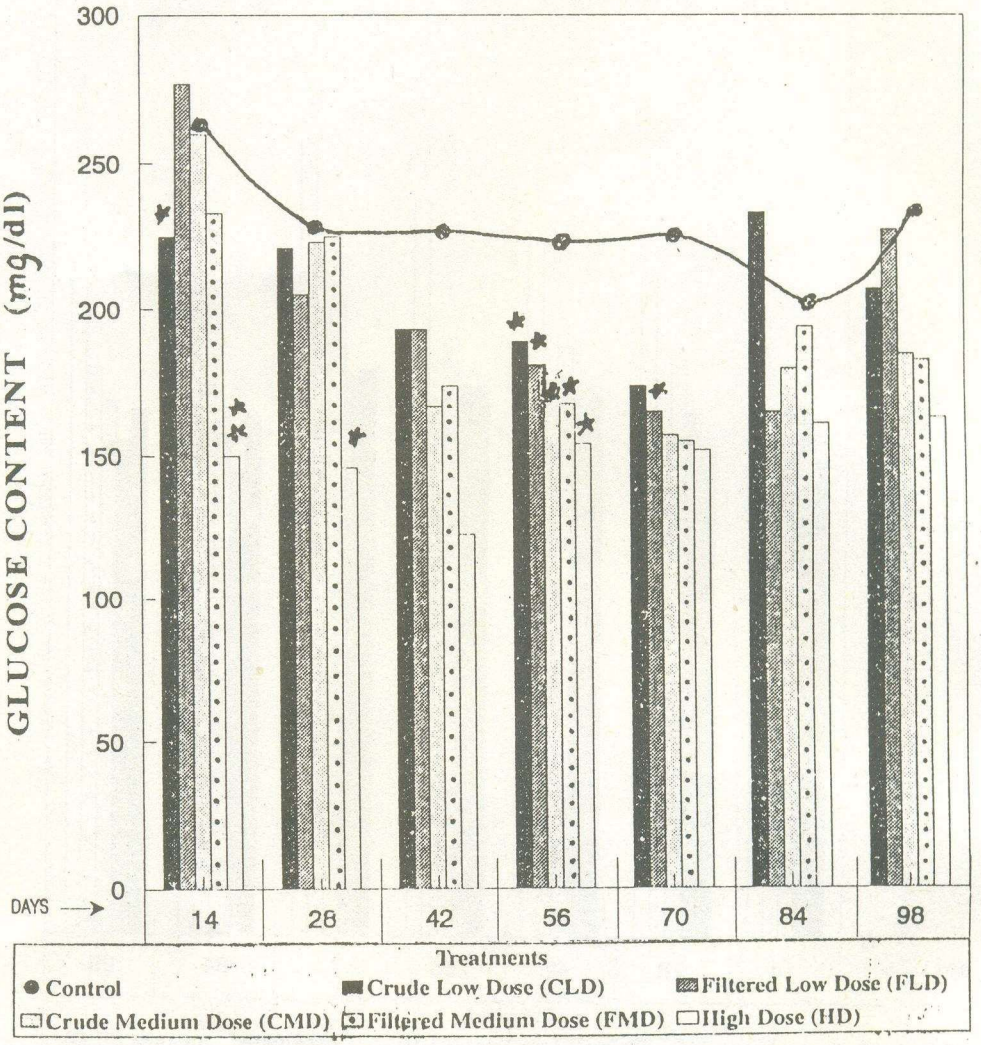

Fig. 4: Change in Glucose Contents (mg/dl) of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses like crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) (please see materials and methods). Statistical significance has been determined by student's "t" test and the probability represented by stars; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

and found statistically significant after 14 ($P<0.01$), 28 ($P<0.05$) and 56 days of HCF treatment in this group. Glucose is the main source of carbon for making different compounds in the body. It is the instant source of energy. The sequence of reactions by which glucose is degraded anaerobically is called glycolysis which produces ATP molecules, which is the high energy compound. The reverse process of glycolysis is called gluconeogenesis during which glucose molecules are produced from amino acids and other macromolecules. Surplus amount of glucose is also stored in the body in liver of animals as glycogen. Whenever there is deficiency of glucose in body then animal utilizes its stored glycogen for producing energy and making different compounds (Conn and Stumps, 1987). In the present findings, depletion in glucose content is common in all the groups and that the depletion was dose dependent. Glucose depletion in the present study may be due to (1) Decrease gluconeogenesis or increased glycolysis (2) Liver damage (3) Inhibition of liver enzymes (4) Stressed conditions (5) Tubular dysfunctioning in kidney, which resulted in reduced glucose reabsorption from nephron. Tufail *et al.*, (1984) have suggested that glucose level decreases because it might be forcefully catabolized by the aerobic and anaerobic enzymes which are also present in the protoscoleces (Agosin *et al.*, 1957). They have found kinases, myokinase, mannose, isomerase and phosphate in protoscoleces. In the present investigation FHCF contained enzymes of protoscoleces that are toxic. McManus and Smyth (1982) measured the substrates and enzymes of glycolysis and associated enzymes both in *Echinococcus granulosus* and *E. multilocularis*. They have determined that phosphorylase, hexokinase, phosphofructokinase and pyruvate kinases, were regulators of carbon flow in glycolysis. Glucose depletion in this study may be due to the aerobic and anaerobic enzymes present in the HCF. Agosin and Repetto (1963, 1965) found that scoleces and whole homogenate oxidize a range of tricarboxylic acid intermediates as well as of glutamate, glyoxylate, glycolate, acetate and lactate. Continuously low glucose content of CHCF and FHCF treated rabbits was noted with increase in time and dosage. This also indicated the stress condition of treated animals. Glucose is mostly oxidized for energy production to cope with the stress impacts. Similar findings have also reported by Tanveer *et al.* (1997, 1998, 1998a).

Fig.5 showed changes in Plasma protein content of control and HCF treated groups. It was noted that plasma protein content remained almost unchanged through out the study in control group. The decreased protein content in CLD group were found non significant through out this treatment. FLD group showed a slight increase that was found statistically significant ($P<0.05$) after 84 days of FHCF treatment. CMD and FMD groups showed decreased protein contents after administration of CHCF. HD group also showed decrease in protein content after FHCF administration. Decrease in protein content was found statistically significant ($P<0.05$) after 56 and 70 days of FHCF treatment in this group. Low protein content recorded in the eperimental rabbits (as compared to that of control) is an indication of direct proteolytic effect of CHCF and FHCF as they contained many lytic enzymes as reported by Faryha and Haddad (1980). Benjamin (1985) suggested that decrease in protein might be due to increased protein catabolism from strong condition like fever, infection etc. GOT and GPT activities are present in cystic fluid and are also involved in the interconversion of amino acids (Sanchez and Sanchez, 1971; Frayha and Haddad, 1980). These amino acids then can move to oxidative pathway to cope with energy requirement. *In vitro*, hydatid cyst took protein from surroundings and catabolize (Coltorti and Varela-Dias, 1975; Hustead and Williams, 1977). Increased protein content, in all the groups from 84 to 98 days, may be due to the formation of antibodies against the antigens present in hydatid cyst fluid. Faryha and Haddad (1980) indicated the presence of albumin, globulin, many enzymatic proteins, lactate dehydrogenase, phosphatase activity GOT and GPT in hydatid cyst fluid, which may also be the cause of elevated protein content at the end of experiment

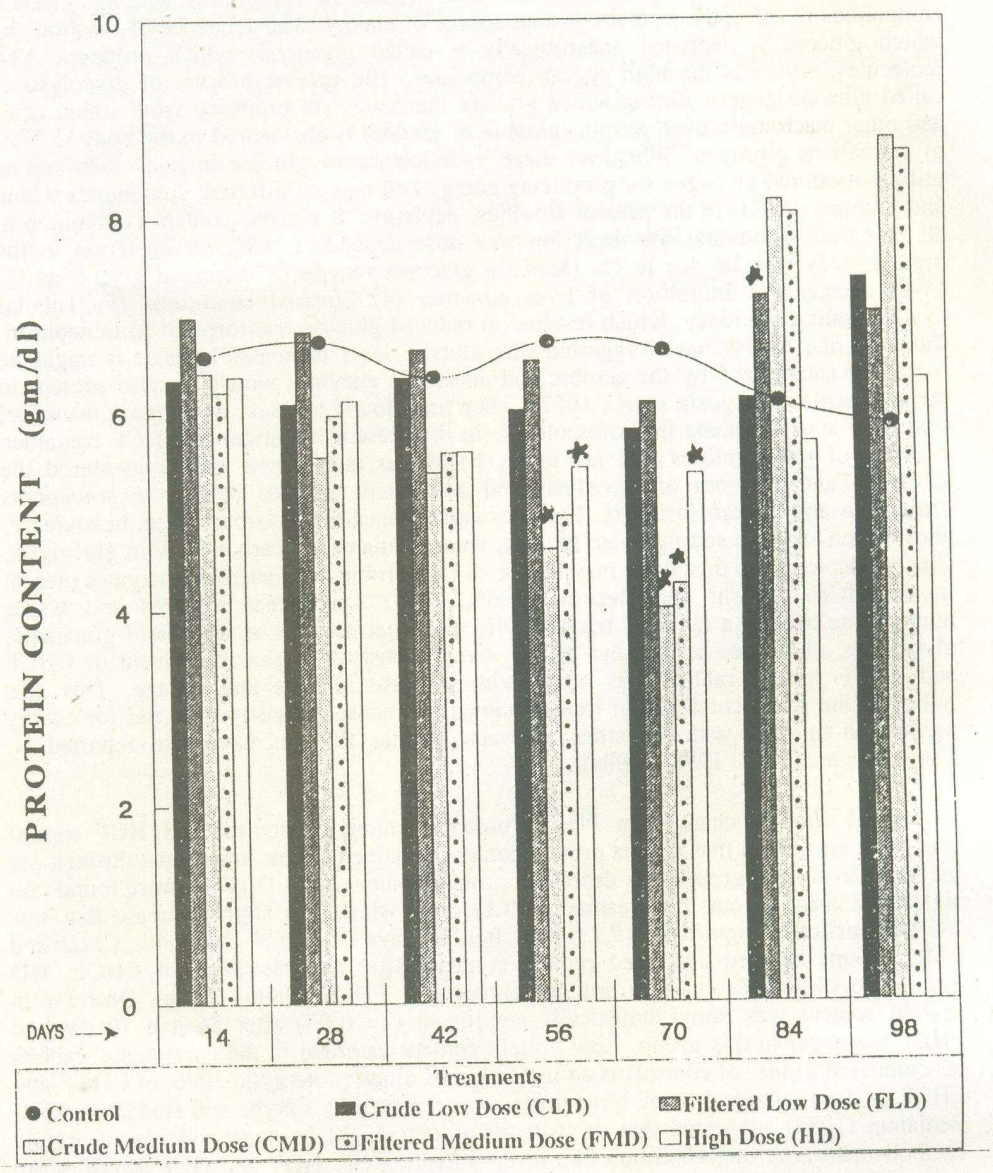

Fig. 5: Changes in Protein Content (mg/dl) of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses like crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) (please see materials and methods). Statistical significance has been determined by student's "t" test and the probability represented by stars; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

in all the treated groups. Nineteen protein components have been isolated from HCF of which ten were antigen of parasite origin (Biguet *et al.*, 1962; Capron *et al.*, 1962; Chordi and Kagan, 1965; Castagnari and Pozzuoli, 1969). Host immunoglobulins have also been reported in cyst wall, fluid and on the surface of the protoscoleces (Kassis and Tanner, 1977).

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