

SOME HAEMATOLOGICAL CHANGES PRODUCED IN RABBITS AFTER INTRAVENOUS INOCULATION OF UNILOCLAR HYDATID CYST FLUID OF SHEEP ORIGIN

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Abstract: Experimental rabbits were inoculated with hydatid cyst fluid (HCF) of sheep origin to determine its toxicity by hematological changes in rabbits (*Oryctolagus cuniculus*). Five groups of animals were administered with crude low dose (CLD), filtered low dose (FLD), crude medium dose (CMD), filtered medium dose (FMD) and high dose (HD) of HCF and one control group given distilled water with same protocol. Blood samples were collected after every 14 days and different haematological analyses were made. An increasing trend of WBC count in all the groups was noted except for HD group in which decrease was noted. It was noted that RBC count shows initial increase in low dose groups but ultimately decreased resulting in anemic condition. Under both low dose treatments, PCV increased but decreased in medium and high dose groups. Haemoglobin (Hb) also decreased with the corresponding increase in the dose of HCF. MCV increased in low dose groups but decreased in medium dose and HD groups. There was general decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin content. ESR however, showed increasing trend with the increase in HCF dose. The interesting feature was the development of tolerance against HCF in almost all the cases. This is probably responsible for this disease to be of non dramatic type as for as symptoms are concerned in natural infection. It was further noted that the low dose groups reflected macrocytic hypochromic anemia where as medium dose and HD group reflected microcytic hypochromic anemia.

Key words: Experimental hydatidosis, haematological changes, rabbit.

INTRODUCTION

Bidirectional movement of different materials through the hydatid cyst has been given consideration by a number of workers. *In vitro* permeability studies on hydatid cysts of *Echinococcus granulosus*, obtained from rodents by experimental secondary infection, have shown protein uptake by cysts (Coltorti and Varela-Diaz, 1975). Hustead and Williams (1977) stated the occurrence of cyclic uptake activity. The permeability of secondary *E. granulosus* hydatid cysts to water, sodium and chloride ions has been determined *in vitro* under steady state conditions by Rotunno *et al.* (1974). The HCF have some altering effects on the hematology of experimental animals. There was increased leukocyte count, decreased erythrocytic count and haemoglobin content as reported by Amnizhanov (1977) in sheep experimentally infected with *E. granulosus*. Hinz and Gehring (1985) studied the mice

infected with *E. multilocularis* and found an increase in lactation, red blood cell count, haemoglobin content, packed cell volume and neutrophils. In contrast, *Meriones unguiculatus* (Mongolian gerbils) when intraperitoneally inoculated with *E. multilocularis* showed a decrease in packed cell volume after 2 and 14 week of inoculation (Kroeze and Tanner, 1986). Similarly a decrease in packed cell volume, erythrocyte number and haemoglobin content was noted after two week in mice experimentally infected with *E. multilocularis* (Hinz and Gehring, 1987). Wangoo *et al.*, (1989) observed a significant increase in phagocytic activity of blood monocytes in albino mice in later stages of infection with *E. granulosus*. El-Gindy *et al.* (1990) intraperitoneally infected five groups of 60 Swiss albino mice with 2000 aseptic normal (control group) and gamma-irradiated (experimental group) *E. granulosus* larvae. Hematological studies showed a marked leukocytosis, a progressive increase in the average percentage of eosinophils as well as a large successive decrease in haemoglobin concentration throughout the time of infection in controls, compared to the experimental groups. Alterations in various haematological parameters due to hydatid cyst fluid have also been reported by Tanveer *et al.* (1998, 1998a) in rabbits. Present work deals with some hematological changes in rabbits after inoculating intravenous hydatid cyst fluid both in filtered and crude forms.

MATERIALS AND METHOD

Rabbits (*Oryctolagus cuniculus*) maintained in the optional conditions of animal house were fed on seasonal green fodder and tap water *ad libitum*. The feeders and drinkers were daily washed, with concentrated KMnO_4 solution to disinfect them. Rabbits were acclimatized for fifteen days to the conditions of animal house prior to experimentation. They were divided into five experimental groups depending upon the type of treatment and one control group ($n=3$) given distilled water with same protocols was run for each experimental group like (a) crude low dose (CLD), (b) filtered low dose (FLD), (c) crude medium dose (CMD), (d) filtered medium dose (FMD) and (e) high dose (HD) groups. Dose of HCF to each group was given through intravenous injections according to following schedule.

Hydatid cyst fluid (HCF) aspirated from infected sheep harboring cysts was transferred into air-tight sterile glass vials and placed at 4°C . Required quantity of fluid was filtered through Whatmann filter paper No.1. As slaughtering is legally prohibited in Pakistan on every Tuesday and Wednesday, during these days refrigerated HCF was administered to the rabbits. Flame cell activity of the protoscoleces was always observed before giving fresh and stored HCF. Blood samples were fortnightly pooled from control and experimental groups. About 2 ml of blood was taken from each rabbit in the test tubes containing 4 mg of anticoagulant, disodium ethylene diamine tetra acetic acid (EDTA) (E. Merck Germany). The tubes were gently rotated for about five minutes to mix EDTA with blood. Different hematological parameters like, white blood cell counts (WBC), red blood cell counts (RBC) both according to Dacie and Lewis (1991), packed cell volume (Strumia *et al.*, 1954), haemoglobin content (VanKampen and Zijlstra 1961) and ESR were determined by Westergren's method (Swarup *et al.*, 1986) and hematological indices like, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were

calculated according to Dacie and Lewis (1991). The data was analyzed by "Students 't" test. according to Steel and Torrie (1981).

Duration (days)	GROUPS			
	CLD	CMD	HD	
	FLD (ml/day)	FMD (ml/day)	(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

Footnote: In each group, n=7.

For abbreviations, see Figure 1.

RESULTS AND DISCUSSIONS

Fig.1 showed that in control group WBC counts remained almost unchanged through out the study period. While they increased in the CLD and CMD group non-significantly. An increase ($P < 0.05$) in WBC count of FLD group was noted (Fig.1). The increase in WBC count of CMD group was found significant ($P < 0.05$) after 70, 84 and 98 days of FHCF administration. HD group showed decreasing trend in WBC count. The decrease in WBC count in HD group is not in accordance with the other groups. The decrease was however, statistically significant ($P < 0.05$) after 70 days of FHCF administration. White blood cells or leukocytes constitute a very important mechanism in the animal body in combating different infections and other invading toxic agents like trauma, germs and various foreign particles. They destroy invading agents either by the process of phagocytosis or they form antibodies and sensitized lymphocytes which may destroy the invader (Gusto, 1991). Subsequent to the tissue damage, either caused by bacteria, trauma, chemicals, heat or any other phenomenon (like inoculation of HCF), multiple substances are released by the injured cells. That results due to the migration of large number of granulocytes and monocytes into the tissue with an ultimate increased number of white blood cells in the experimental groups (Boyd, 1970). During present investigation elevated WBC count in CLD, FLD, CMD and FMD groups is probably due to the increase of phagocytes and/or eosinophils to combat with incoming foreign proteins and allergic threat evoked by crude and filtered HCF. WBC increase was more pronounced in both of the crude groups than the filtered. That was probably due to the presence of insoluble protein sub-units like degraded protoscoleces, brood capsules, fragmented germinal membrane etc., in crude hydatid cyst fluid that have been removed from FHCF during filtration. Increase in WBC counts can also be

attributed to tissue damage (Eastham, 1985) which may be caused by the proteolytic enzymes and/or toxins present in HCF. Present results are in accordance with Aminzhanov (1977), who observed an increase in leukocytic count in infected sheep with *E. granulosus*. Similar increase in phagocytic activity of monocytes was also studied by Wangoo *et al.* (1989) and Alkarmi and Behbehani (1989) in albino mice infected with *E. granulosus*. Increased WBC counts in mice infected with *E. multilocularis* was also noted by Hinz and Gehring (1987) and Alkarmi and Ali Khan (1989). In the blood tissue granulocytes and agranulocytes are most important elements for defense against invading micro-organisms. Their power to attack foreign bodies depends mostly upon their motility and desire for ingestion of solid particles. The latter action is termed as phagocytosis. These two types of WBC are free lancing among the body cells (Charles and Norman, 1966). It was also noted during present studies that the toxic and pathological impact of HCF upon blood and tissue was parallel in many of the case to the poisonous properties of insecticides, pesticides, herbicides, and with exo and endotoxins. Because HCF and insecticides disturbed the blood biochemistry and physiology in almost similar way, hence the toxicity of HCF has been compared to such compounds. On the other hand leukocytic count in HD group showed constant decrease as compared to elevated WBC count of other four groups. This may be due to higher concentration of toxins in HCF administered under High Dose dose, which may inhibit the synthesis or increase the destruction of WBCs due to the presence of enzymes (McManus and Smyth, 1982) and toxins (Wangoo *et al.*, 1987). Results of all the experimental groups except High Dose group showed normal values of WBC count probably due to the tolerance developed by the animal against toxins and/or antigens.

In normal body conditions the population of red cells and the concentration of haemoglobin in the blood are kept at normal level by nice balance between the population of newly formed and the old erythrocytes to be destroyed (Gusto, 1991). Both CLD and FLD groups showed increase in RBC count up to 28 days of HCF administration. This trend indicated that HCF may trigger RBC and haemoglobin synthesis under low doses and that may be due to interference of HCF in the binding of oxygen to haemoglobin. During present investigation it was noted that increase in RBC count and haemoglobin content did not persist but decreased after 42 days of HCF administration. This is probably due to inhabitation of RBC biosynthesis and/or destruction at high or prolonged dose of HCF which significantly decreased haemoglobin content after 70 days of HCF administration in both CLD ($P < 0.01$) and FLD ($P < 0.05$) groups. CMD, FMD and HD groups showed reduction in the total erythrocyte count from the start of HCF administration probably due to damage or inhibition of erythrocyte synthesis. However, in the final stages this reduction was prominent in all the experimental groups. This RBC deficiency syndrome is called anemia, which results by rapid loss or slow production of red blood cells. In the present study ultimate reduction noted in RBC count is similar to the findings reported by Aminzhanov (1977) in sheep, infected with *E. granulosus*. Increased RBC breakdown is also responsible for the increased plasma bilirubin (Charles and Norman, 1966). This loss may be due to the direct effect of toxicant on the blood cell or indirectly through its effect on bone marrow, the blood forming tissue (Rajini *et al.*, 1987). It was observed that in many organs, these incoming toxicants are responsible for the development of necrosis (Chaitow *et al.*, 1975) and in such cases histamine was found in these areas (Billewicz-Stankiewicz, 1955) which damage the capillary cell wall and fine veins leading to the blood extravasation (Cown, 1974).

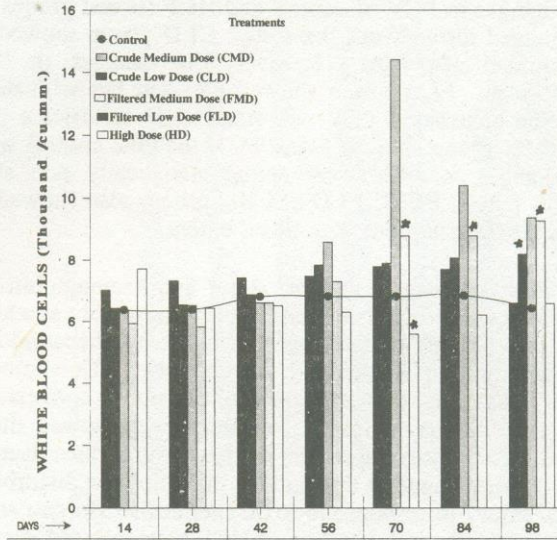


Fig. 1: Changes in WBC count of rabbit after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. *P<0.05; **P<0.01; ***P<0.01.

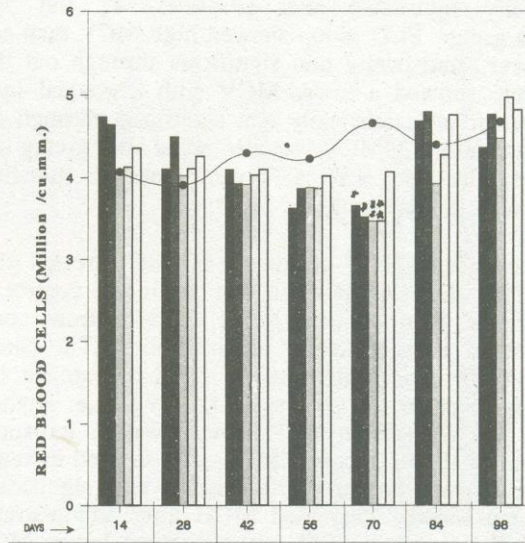


Fig. 2: Changes in RBC count of rabbit after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

Fig. 3 showed changes in PCV of control and HCF treated groups. In control group PCV remained unchanged through out the study. CLD group showed increase in PCV initially which decreased afterward. However, the changes in PCV were found statistically non significant. FLD group showed elevated but with decreasing trend in PCV. CLD group. The increase in PCV was statistically significant ($P < 0.05$) after 28 days of treatment. CMD group showed lower PCV than the control with corresponding increase. All the changes in PCV were found statistically non significantly when analyzed by Student's 't' test. FMD, FLD and HD groups also showed a non-significant decrease in PCV like CMD group but with lesser extent.

In the present study, reduction in RBC count and haemoglobin content in all the experimental groups was either due to inhibition of haemoglobin synthesizing enzyme or haemolysis that leads to binding of haemoglobin to plasma hepatoglobins. During this process excessive haemoglobin is excreted through urine (Gowenlock *et al.*, 1988). Haemoglobin is partly converted to metahaemoglobin prior to conversion in to bilirubin. In the present investigation the increased bilirubin level showed the enhanced break down of haemoglobin. The ability of certain chemicals to induce haemolytic disease by generation of autoantibodies suggests that similar immunologic disturbances could be the basis of bone marrow injury in response to toxic chemicals (Morgan *et al.*, 1980). In the present investigation repeated administration of HCF have induced anemia in rabbits.

Fig. 4 showed changes in MCV of control and HCF treated groups. It was noted that the values for MCV remained unchanged through out the study in control group. The CLD group showed an increased MCV as compared to their control group, which is highly elevated even from the beginning (14 days) of CHCF treatment. The increase in MCV was statistically significant after ($P < 0.05$) 42 and 56 days of CHCF administration in this group. FLD group showed high MCV than control. The changes in MCV were however, statistically non significant through out the treatment in this group. In CMD group showed a lower MCV with a gradual increasing trend. The changes in MCV were found statistically non significant through out the treatment in this group. Decreasing pattern in MCV was also noted after giving intravenous FHCF to FMD and HD groups. The changes in MCV were however, statistically non significant through out the treatment in this group.

Fig. 5 showed changes in MCH of control and HCF treated groups. IT was noted that the values of MCH showed minor fluctuations in the control group. CLD group showed initially declined then elevated MCH contents from control Fig. 5 showed maximum MCH after 70 days of CHCF administration. The changes in MCH were however, statistically non significant through out the treatment in this group. FLD group followed the same pattern as that of CLD group, i.e. slight elevation in MCH than that of control. The changes in MCH were however, statistically non significant through out the treatment in this group. CMD group showed increasing trend of MCH. The changes in MCH were however, statistically non significant through out the treatment. FMD group showed decreased MCH after administration of FHCF with statistically non significant values. HD group showed lower MCH through out the period of treatment. The decrease in MCH were however, statistically significant ($P < 0.01$) after 70 days of treatment.

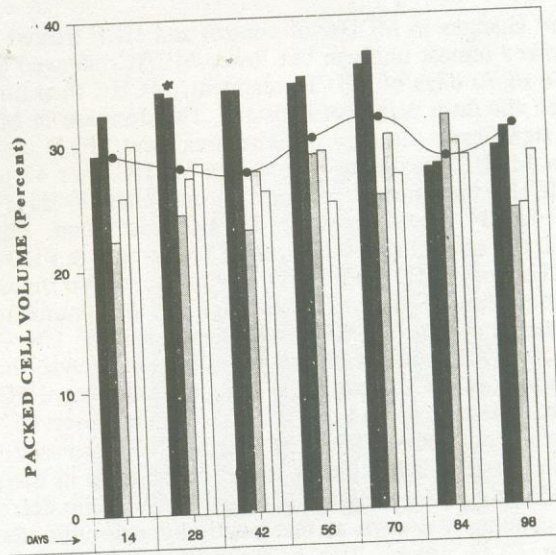


Fig. 3: Changes in packed cell volume of rabbit after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

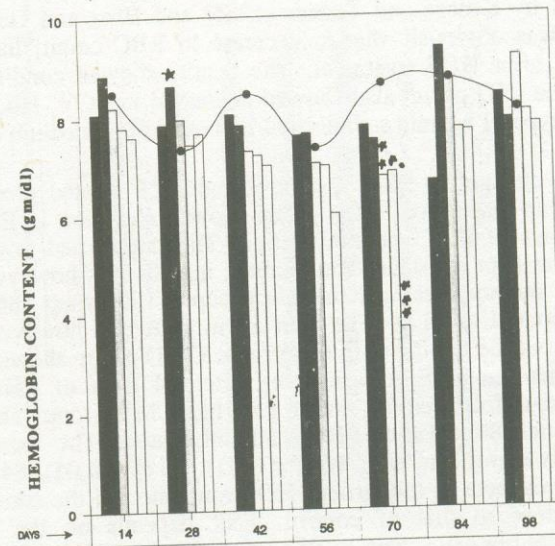


Fig. 4: Changes in haemoglobin content of rabbit after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

Fig.6 showed changes in MCHC of control and HCF treated groups. In the CLD group MCHC showed almost uniform but lower MCHC, showed a maximum decrease in MCHC after 56 to 70 days of CHCF treatment. MCHC then turned towards normal after 70 days when the dose was kept constant. The decrease in MCHC was however, statistically non significant through out the treatment. FLD group showed almost constant but lower MCHC maximum decrease in MCHC after 42 to 56 days of FHCF administration. MCHC then turned towards normal after 70 days at consecutive three constant doses of FHCF. The decrease in MCHC was found, statistically significant only after 42 ($P < 0.01$) and 70 ($P < 0.05$) days of treatment in FLD group. Fluctuations noted in the MCHC of CMD and FMD group were also found statistically non-significant. HD group showed low MCHC through out the administration of FHCF. The MCHC raised when the dose of HCF was kept constant after 70 days of FHCF administration ($P < 0.01$). Results of present investigation showed that at very low doses of HCF administration (as in CLD and FLD groups) an increased PCV was noted which is in accordance with Hinz and Gehring (1985). It was observed that in FLD group haemoglobin content increased while MCH and MCHC decreased. This showed RBC deficit haemoglobin most probably due to some disturbance in the permeability of the cell membrane. FLD group also showed increased MCV while decreased MCHC. Such type of anemia is commonly known as macrocytic hypochromic. Same type of anemia was also noted in CLD group. The enlargement of erythrocyte may be due to accumulation of fluids after disturbance in cell membrane permeability (Sood, 1992). The situation remained as such in latter stages but ultimately RBC and haemoglobin content decreased in both CLD group and FLD group. This was probably due to the break down of RBC and haemoglobin which resulted into increased bilirubin content (Anwar and Tanveer, 1997). In contrast to both low dose groups the medium dose group showed non-significant decrease in PCV after HCF administration. Similar findings have also been reported by Kroeze and Tanner (1986) and Hinz and Gehring (1987). This decrease in PCV was obviously due to decrease in RBC count, haemoglobin, MCH, MCV and MCHC after HCF treatment. The results showed conditions of microcytic hypochromic anemia. HD group also showed decreased in PCV, Hb, MCV and MCHC representing same type of anemia as indicated by both of the medium dose groups.

Fig.7 showed changes in ESR of control and HCF treated groups. No change in ESR of control group was noted. CLD group showed elevation in ESR with maximum increase after 70 days of CHCF administration. The rate turned towards normal when the dose of HCF was kept constant. The increase in ESR was however, statistically non significant through out the treatment in this group. FLD group showed slightly high ESR values as compared to CLD. The increase in ESR was however, statistically non significant through out the treatment in this group. CMD group showed rapid increase in ESR. Maximum increase in ESR was noted after 84 days of CHCF administration ($P < 0.05$). FMD group followed the similar path like CMD group. There was maximum increase in ESR after 84 days of FHCF administration. The increase in ESR was however, statistically significant after 42 ($P < 0.01$), 56 ($P < 0.05$), 84 ($P < 0.01$) and 98 ($P < 0.05$) days of treatment in this group. HD group showed the constant but increased ESR which was close to that of control. ESR depends on the concentrations of fibrinogen and globulins (Eastham, 1985 and Sood, 1992). Since HCF contain fibrinogen and globulins (Sanchez and Sanchez, 1971) therefore its administration in the blood of experimental animals is responsible for the increased value of ESR. Comparatively less pronounced increased ESR was noted for CLD group as compared to FLD group. Similarly CMD group showed initially less prominent ESR than FMD

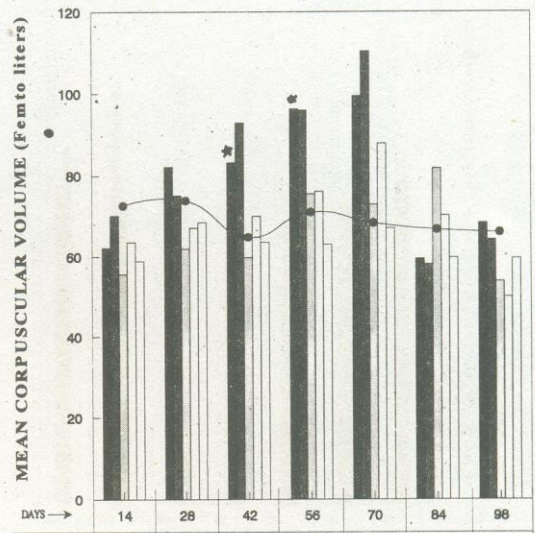


Fig. 5: Changes in mean corpuscular volume of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

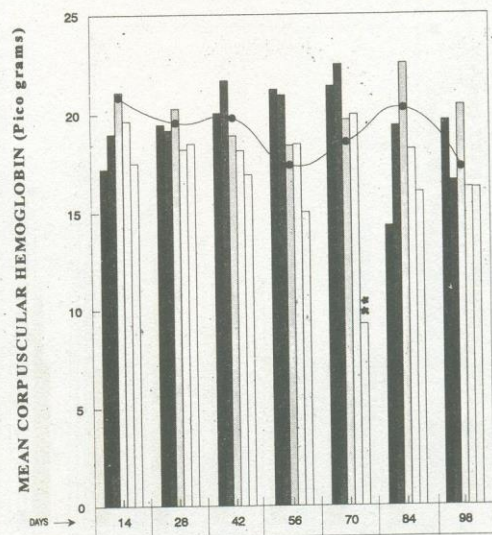


Fig. 6: Changes in mean corpuscular hemoglobin of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

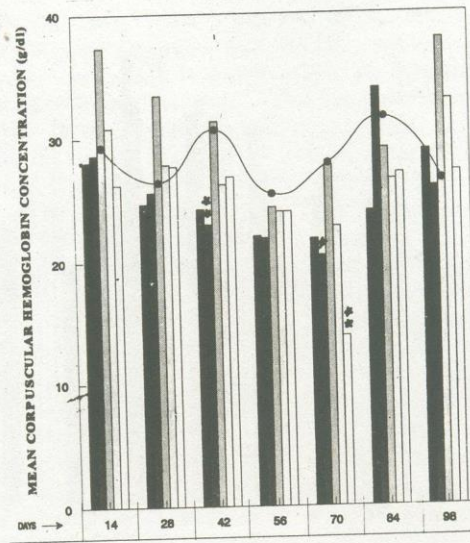


Fig. 7: Changes in mean corpuscular hemoglobin concentration of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

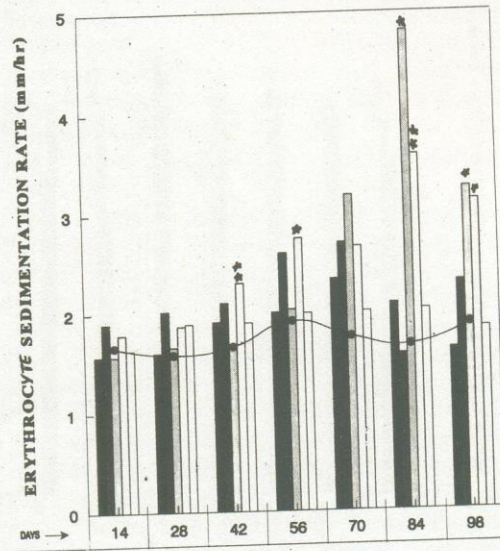


Fig. 8: Changes in erythrocyte sedimentation rate of rabbit blood after administrating different doses of sheep originated unilocular hydatid cyst fluid in various forms and doses. For abbreviations and key to graph, see Fig.1.

group is probably due to the interference of insoluble proteins, brood capsules, protoscoleces and or their metabolites, which interfere in the rouleaux formation, hence decreasing the ESR value in crude groups. However, after 70 days of continuous administration of CHCF to CMD group, these values increased most probably due to the activity of protoscoleces which were in the course of cyst formation and were absent in FMD groups.

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