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SOME METABOLIC ALTERATIONS INDUCED BY HIGH DOSES OF UNILOCULAR FILTERED HYDATID CYST FLUID ON RABBIT LIVER

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Abstract: Out of 46 rabbits acclimatized for 2 weeks to the optimal conditions of animal house, 23 were inoculated upto 7 weeks with different doses (0.50, 0.75, 1.0, 1.25, 1.50, 1.50, 1.50, 1.50 ml) of filtered hydatid cyst fluid (FHCF) of sheep origin. Each dose was daily inoculated for one week and then calculated according to their body weight. Control rabbits were given distilled water with similar protocol. Three treated and three control rabbits were weekly slaughtered and biochemical analyses of liver tissue were made. It was noted that body weight increased gradually in both control and experimental rabbits but when both groups were compared, a significant reduction was noted for all the rabbits treated with FHCF except the last three doses which were kept constant. Their respective specific growth rate also showed increasing trend but with lot of fluctuations. In the liver tissues of treated rabbits increase in total protein, soluble protein ($P<0.001$), glutamate oxaloacetate transaminase ($P<0.001$), glutamate pyruvate transaminase ($P<0.01$), bilirubin ($P<0.05$), alkaline phosphatase ($P<0.001$), acid phosphatase, ribonucleic acid, deoxyribonucleic acid and free amino acid was found statistically significant when compared with their respective controls, while cholesterol level decreased in all the treated rabbits.

Key words: *Echinococcus granulosus*, rabbit, liver tissue, biochemical analyses.

INTRODUCTION

Hydatidosis or echinococcosis is of zoonotic importance, infecting not only man but also a wide variety of domestic and wild animal. It is characterized by the formation of cysts of variable size in various body organs (Thompson, 1986). The casual agent *Echinococcus granulosus* is a cestode belonging to the family Taeniidae and genus *Echinococcus* (Rudolphi, 1801). Man gets infection by ingesting *E. granulosus* eggs in food contaminated with dog faeces or from hands contaminated while handling the infected dogs (Cheesbrough, 1987).

Hydatidosis has been considered as the most important threat to the public health (Schantz, 1982; Feng and Zho, 1986). Its economic effects also arise from public health aspects, hospitalization, associated with control programmes, losses from condemnaton of affected organs from live stock at slaughters and affects animal reproduction and meat quality (Schawabe, 1986; Iqbal *et al.*, 1989).

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In Pakistan, livestock is constantly exposed to this disease because the poor hygienic conditions and climate are best suited for its growth and completion of life cycle. Although pathology and symptoms are generally not very dramatic hence it has given less importance. Most of the work reported from Pakistan is not only fragmentary but are also theoretical revision of already known facts about its transmission, epidemiology and prevalence (Anwar and Munir, 1980; Khan, 1982; Bilquees, 1984; Islam, 1985; Pal and Jamil, 1986; Hussain, 1987; Iqbal *et al.*, 1989).

At present no quantitative data exist for the biochemical alterations produced in naturally or experimentally infected animals. So the main objective of the present investigation is to determine the level of different biochemical alterations in the liver of rabbits parenterally administered with high doses of filtered hydatid cyst fluid of sheep origin. For this purpose, rabbits as model are given consideration because they are common in those localities where sheep, goat and cattle are raised and they can be easily infected parenterally by injecting scolex (Cameron and Webster, 1959). They are also reported susceptible to secondary echinococcosis of sheep origin (Sweatman and William, 1963) hence we used hydatid cyst fluid of sheep origin.

MATERIALS AND METHODS

Experimental animals and their maintenance

Forty six adult healthy rabbits (*Oryctologus cuniculus*) were acclimatized for 2 weeks prior to experimentation in the Animal House of the Zoology Department, Punjab University, Quaid-e-Azam Campus, Lahore. They were supplied with green fodder and tapwater *ad libitum* along with few crystals of $KMNO_4$. Whenever, required, Gentamicin injections were given to save them from different infections. Electric heater were used to maintain the room temperature at $25 \pm 2°C$. Rabbits were weighed weekly.

Collection of hydatid cyst fluid

With the help of B.D. syringe and veterinary needle (18 G), hydatid cyst fluid was aspirated from the cysts located in liver, lungs and spleen of sheeps in the local slaughter house. The fluid was transferred into sterile vials and was placed in the ice boxes containing water at 4°C. It was brought to the laboratory and filtered through Whatman's filter paper and stored in the deep freezer at -20°C for further use.

Dose administration

For inoculation, hair from the left ear's vein were removed with the help of hair removing cream and different doses (0.5, 0.75, 1.0, 1.25, 1.5, 1.5, 1.5 ml) of filtered hydatid cyst fluid (FHCF) were inoculated upto 7.0 weeks. Each dose was daily administered upto one week and then calculated according to their body weight noted in the end of each week. Control rabbits were given distilled water with similar protocol.

Three treated and three control rabbits were slaughtered weekly and their liver was

processed for biochemical analyses.

Tissue processing for biochemical analysis

Saline extract was prepared by homogenizing weighed piece of liver tissue in 6.0 ml of 0.98% NaCl solution in a motor driven high speed tissue homogenizer at 4°C. The homogenate was centrifuged at 35000 for 15 minutes and the clear supernatant was further used for the estimations.

For the estimation of some enzyme activities *i.e.*, alkaline phosphatase (AP) and acid phosphatase (AcP) methods recommended by Deutsche Graseleschaft fur Klinische Chemie (1972) modified from Bessey *et al.* (1946) was followed. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) was estimated according to Reitman and Frankel (1957). The estimation of bilirubin and cholesterol was made according to Jendrassik and Grof (1938) and Richmond (1973). Total proteins and soluble proteins were estimated according to Biuret method (Henry *et al.*, 1974, modified by Gowenlock *et al.*, 1988). All these estimations were made by using diagnostic kits of Randox (U.K.).

Some tissue metabolites like total free amino acids (FAA) were measured according to Goodwin (1968). Nucleic acids (DNA and RNA) were extracted as mentioned by Shakoori and Ahmad (1973) and estimated according to Schneider (1957).

Statistical analysis

All the data was analysed by Student 't' test (Steel and Torri, 1981) and specific growth rate was calculated according to Odum (1971).

RESULTS

Body weight changes and some biochemical alterations induced by different doses of filtered hydatid cyst fluid of sheep origin are mentioned in Figs.1 & 2 as mean ± S.D. and percent variations from control groups.

Figure 1 shows slow but gradual increase in the body weight (mg) of both control and treated rabbits. When the treated rabbits were compared with their respective control groups they showed reduced body weight (-2.42%, -6.70%, -1.49% and -1.05%) for 0.50, 0.75, 1.0 and 1.25 ml of FHCF. However, when the dose was kept constant a significant increase (5.48%, 14.46%, 17.03%) was noted. In the control groups specific growth rate (SGR) per week increased with varied values (1.13%, 1.09%, 1.77%, 1.4%, 1.38%, 3.58%) while in experimental rabbits, SGR showed a decrease in 0.75ml dose only. Other treatments showed an increase with varied values.

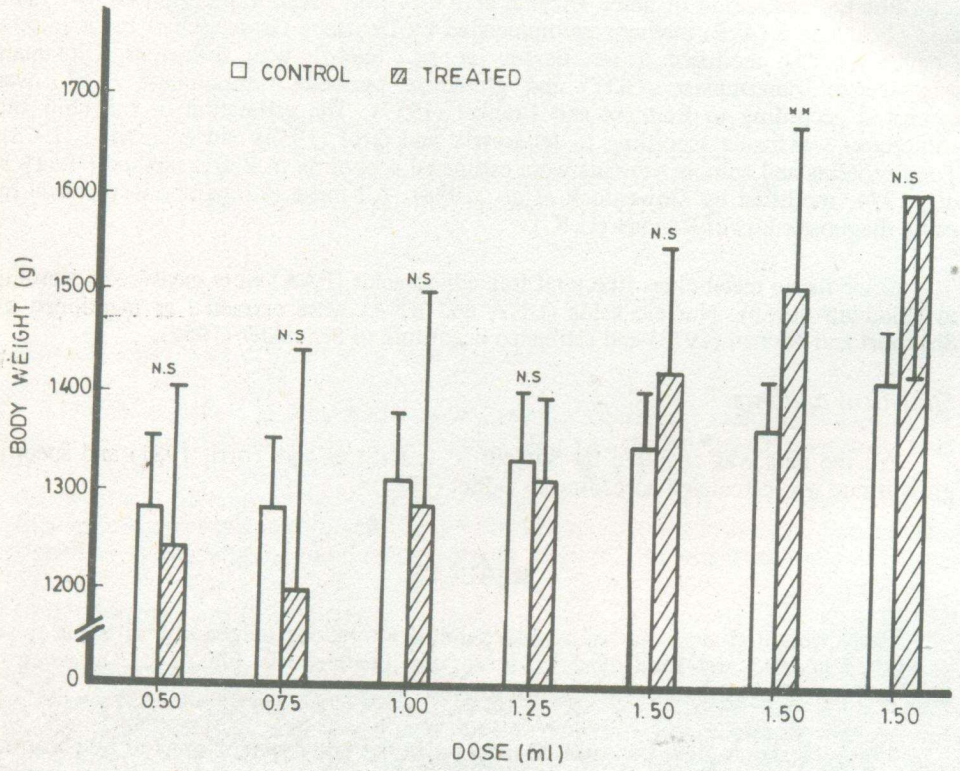

Fig. 1: **Live body weight changes in rabbits due to parenteral administration of high doses of filtered hydatid cyst fluid (FHCF). The statistical significance has been determined by Student's 't' test and the probability represented by stars *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$.**

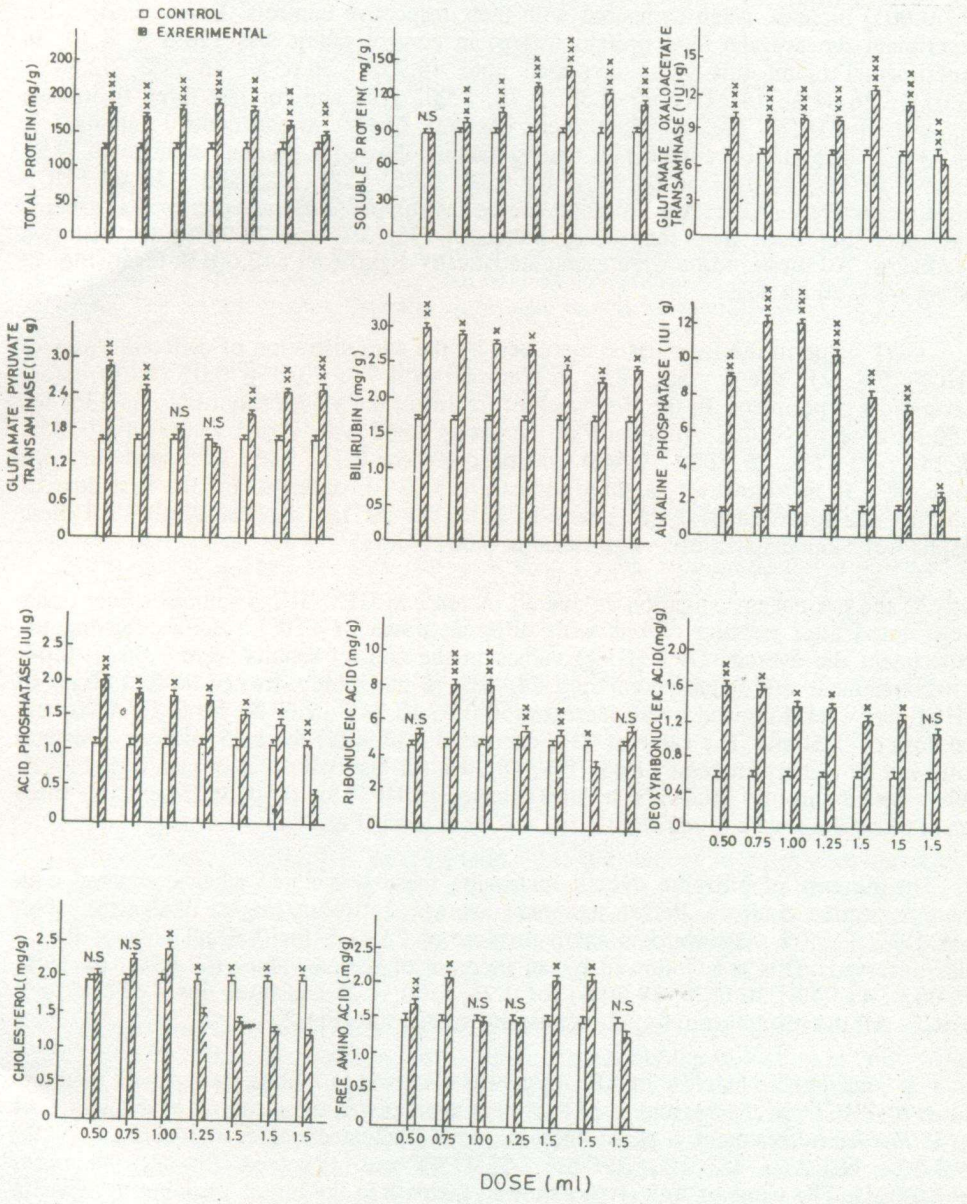

Fig. 2: **Some biochemical changes in liver of rabbit due to high doses of filtered hydatid cyst fluid (FHCF). The statistical significance has been determined by Student's 't' test and the probability represented by stars *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$.**

Total protein contents (mg/g) in the liver tissue of rabbits showed a significant ($P<0.001$) increase when compared with their respective controls. Before starting the experiment the average total protein (mg/g) in control rabbit was 126.3 ± 4.10. In experimental rabbit this value increased upto 46.95%, 36.65%, 46.55%, 52.88%, 43.07%, 26.84%, 16.23%, for 0.50, 0.75, 1.00, 1.25 and for last three treatments (1.5 ml) of FHCF. The soluble protein contents (mg/g) in the control rabbits was 89.3±0.1. The similar contents in treated rabbits showed a gradual increase (0.11%, 9.96%, 19.3%, 44.0% and 59.35%) for 0.50, 0.75, 1.00, 1.25 and 1.50 ml FHCF doses respectively. Afterward a slight decrease (as compared to the experimental groups) was noted but even then the values increased (36.73% and 25.75%) for last two treatments. All these results were found statistically significant at 0.001% level (Fig. 2) except for 0.50 ml dose.

GOT value in the liver tissue increased by the administration of different doses of FHCF (Fig. 2). The average GOT in control rabbit was 7.02 ± 0.01 (IU/g) before starting the experiment. In the first week of treatment the values shoot upto 53.13% for 0.50 ml dose of FHCF. Afterward an increasing trend with varied values of 47.15%, 48.14%, 43.87%, 75.92%, 59.54% was observed for 0.75, 1.00, 1.25, and first two doses of 1.50 ml. However, a sharp increase (75.92%), observed for the first dose of 1.50 ml was followed by a decrease (-14.24%) for the last dose of FHCF. All these results were found statistically significant at 0.001% level.

In the present investigation an overall increase in GPT (IU/g) values of liver tissue were noted after treating rabbits with different doses of FHCF. Before starting the experiment the average GPT (IU/g) values in the control rabbits were 1.66 ± 0.04. After treatment this value shoot upto 74.49% in the rabbits treated with 0.50 ml of FHCF per week followed by an increase (50.0%, 10.24%, 9.63%) for 0.75, 1.00, and 1st dose of 1.50 ml. The value of GPT decreased (-25.90%) for 1.25 ml dose. This was followed by a sharp increase of 45.78%, 48.79% for last two doses of (1.5 ml), FHCF. When tested through Student's 't' test changes in GPT for 0.50, 0.75 and last three doses of 1.50 ml were found significant at 0.01% level (Fig.2).

In the case of bilirubin overall increasing trend was noted when compared with their respective controls. Before treatment average, bilirubin (mg/g) of control rabbit was 1.73 ± 0.05. Afterwards a sharp increase of 73.41% for 0.50 ml dose of FHCF was observed. This was followed by an increase of varied values (67.63%, 64.16%, 56.06%, 41.04%, 30.05%, 39.30%) for 0.75, 1.00, 1.25 and three doses of (1.50 ml) FHCF. All the results were found statistically significant (Fig.2)

A considerable increase in AP (IU/g) was observed in rabbits treated with different doses of FHCF. In the beginning of treatment average value for control rabbit was 1.44 ± 0.01. After treatment a sharp increase with fluctuated values (526.3%, 747.2%, 740.2%, 601.3%, 452.0%, 396.5%, 53.47%) was observed for all the doses ($P<0.001$). The value of AcP (IU/g) showed increase in the initial stage but decrease in last stage. In the beginning the average AcP (IU/g) value of control rabbit was 1.15 ± 0.01. Afterward an increase with gradually decreasing values (78.26%, 60.86%, 57.39%, 53.04%, 34.78%, 25.21%) was observed for 0.50, 0.75, 1.00, 1.25 and Ist two doses of 1.50 ml of FHCF. This was again followed by a sudden decrease of

-64.34% for last dose of 1.50 ml of FHCF. The results were found statistically significant when analysed by Student 't' test (Fig.1).

In the first week of treatment the values for RNA contents were found closer to their respective controls (Fig.2) alongwith 15.33% increase. Later on this increase gradually reached upto 70.79% and 70.37% for 0.75 and 1.0 ml doses. Afterwards the process of increase in the RNA contents slowed down through 56.72, 52.10, 28.15 and 16.38% for 1.25 and last three doses of 1.50 ml of FHCF. All the results were found statistically significant except that of 0.50 ml and last dose (1.50 ml).

Maximum increase in the DNA contents was observed after the inoculation of first dose (0.50 ml). This value later on gradually declined upto 83.8% till the last dose of 1.50 ml. The results were statistically significant except for the last dose (Fig.2).

Cholesterol (mg/g) in control rabbits before starting the experiment was 2.04 $\pm$ 0.02. After treatment this value increased upto 5.88%, 12.74% and 20.09% for 0.50, 0.75, and 1.00 ml doses of FHCF. Then decrease of -24.01%, -29.90%, -37.7%, -38.23% was observed for 1.25 ml and last three doses of 1.50 ml ($P<0.05$).

In the beginning the average values for plasma free amino acids (mg/g) in control rabbits was 1.53 $\pm$ 0.05. After significant increase (13.7% and 42.48%) for 0.50 and 0.75 ml a slight decrease (-0.65% and -1.30%) for 1.00, 1.25 ml dose was observed. In the end a significant increase (39.21%, 42.48%) was noted for the first two doses of 1.50 ml followed by a decrease of -14.37% for the last dose of FHCF (Fig.2).

The results were found statistically significant for 0.50, 0.75 and first two doses of 1.50 ml (Fig.2).

DISCUSSION

Biochemical effects of filtered hydatid cyst fluid on rabbit liver were statistically significant when compared with their respective controls. The results showed that high doses of FHCF have not only altered the biochemical parameters but also the general appearance of rabbits that became pale and weak with the passage of time. They further showed loss of appetite, agility and shedding of hair.

In the present investigation live body weight showed decreasing trend in the first four stages, and then increased in the last three stages. Similar reduction in body weight has also been reported by Pandey (1971) who studied the effect of hydatidosis in liver, lungs and spleen of goats in Patna (India) and reported that gross changes in these tissues were due to fluid which seeped out of the hydatid cyst and damage the surrounding cells thereby causing condemnation of the organs that leads to the reduction in weight. Economic losses due to hydatidosis through low quality and reduced yield of milk, meat and retarded growth have also been reported on the same basis by Anonymous (1985), Schwabe (1986) and Iqbal *et al*. (1989).

It has been an established fact that decrease in body weight is associated with liver enlargement which may be either due to accumulation of triglycerides in the liver (Kohli *et al.*, 1975) or due to increased energy requirements for the induction of body's defence mechanism to detoxify that chemical (Zhou *et al.*, 1985). However, in the last three doses, the increase in the body weight can be attributed to some resistance which the animals have attained against filtered hydatid cyst fluid (FHCF).

Liver is not only the primary site for biotransformation of foreign compounds but also the centre of drug metabolism which makes it greatly vulnerable to toxic substances. The role of liver in metabolic conversion of foreign compound is even more important in its susceptibility to biochemical injury (Zimmerman, 1974).

Since, HCF contained different kind of enzymes that are liable to alter the hepatic physiology and its metabolites. In most of the cases these enzymes leaked out from the necrotic hepatocytes into the blood stream in abnormal amounts. Several of these soluble enzymes have been considered as indicators of liver function and damage (Kulkarni and Hodgson, 1980).

Frayha and Haddad (1980) noted the presence of protein contents, enzymatic protein (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, phosphates) and dehydrogenases in HCF obtained from different intermediate host. It has earlier been reported that out of ninteen protein components isolated from HCF, 10 were antigens of parasitic origin (Biguet *et al.*, 1962; Capron *et al.*, 1962; Chordi and Kagan 1965; Castagnari and Pozzuoli, 1969). Host immunoglobulins have also been reported in the cyst wall, fluid and on the surface of protoscoleces (Kassis and Tanner, 1977). *In vitro*, studies have also proved that hydatid fluid of the cyst took proteins from their surroundings (Coltortic and Varela-Dias, 1975; Hustead and William, 1977). Although proteins are large molecules and their size have biological significance which prevents them from leaking out of cells through the plasma membrane (Conn and Stumpt, 1976). In the present work an over-all increase in the total and soluble proteins was estimated in the liver tissue of rabbits. This was probably due to the fact that protein synthesis was increased in the liver to over come the toxic effects of FHCF or utilization of free amino acid contents for energy needs under stress conditions, or in the absence of glucose oxidation (Abdel Salam *et al.*, 1982).

Transaminases are enzymes involved in the metabolism of amino acids and are responsible for conversion of amino acids to α-Ketoacids which later on enter into Krebs cycle and are metabolized to produce energy for different metabolic processes (Meena *et al.*, 1978).

The present investigation showed an increasing trend of GOT in the liver tissues of experimental rabbits. In the start this increase may be due to the presence of GOT in FHCF that was inoculated to the experimental rabbits (Frayha and Haddad, 1980) and this may be further attributed to the pathological response of the hepatocytes of liver (Sanchez and Sanchez, 1971). Moreover, a decrease in hepatic GOT in the 7th week may be due to the blockage at transcription or translation level (Meena *et al.*, 1978) or some disfunctioning of liver (Ramaligam and Reddy, 1981) or cell damage (Hendrickson and Bowden, 1976; Meany and Pocker, 1979) or development of some resistance against

the incoming toxins present in the FHCF.

Tissue GPT activity is generally required for transamination purpose. In the present investigation the rise in the activity of this enzyme is an indication of disturbed liver function, necrosis of hepatic cells which favour cellular damage or gluconeogenesis through which amino acids may be transmitted and utilized for energy requirements or due to efficient conversion of alanine to pyruvate which enters into TCA cycle to compensate for energy requirements. Another important reason for increased hepatic GPT is that some GPT was already present in that FHCF which was administered to the rabbits (Frayha and Haddad, 1980).

In the present investigation the increased bilirubin contents may be attributed to some bilirubin already present in HCF (Frayha and Haddad, 1980) that was given to the rabbits, and increased break down of haemoglobin (hemolysis). About 85% of bilirubin is formed from haemoglobin liberated from senescent erythrocytes destroyed by reticulo endothelial cells in the spleen. The remaining 15% is derived from other hemoproteins which are mainly cytochromes (Benjamin, 1985).

Alkaline phosphatase is a membrane bound enzyme found at the bile pole of hepatocytes, pinocytic vesicles and golgi complex. It catalyzes transphosphorylation reactions and involved in the hydrolysis of phosphate monomers at alkaline pH (9.0). They play important role in the transport of sugar and phosphate in liver and other tissues (Benjamin, 1985). In the present investigation sharp increase in the AP activity at different doses of FHCF was probably due to increased active transport process for supply of nutrients that can be used in energy generation to counter the toxic effects of FHCF.

Acid phosphatase (AcP) is a hydrolytic enzyme found in the lysosomal fraction of the cell (deDuve, 1955). There are also extralysosomal acid phosphatases which are found in the endoplasmic reticulum and cytoplasm and used to estimate the interference with catabolic and autophagic processes in the liver. In the present investigation its increased activity may be attributed to the increased breakdown activities of waste cellular components produced as a result of toxic effects of FHCF. Another reason for increased AcP activity may be due to increased biosynthesis of enzyme protein to fulfill the elevated demand in the cell. Thus, it can be safely suggested that in the present investigation AP and AcP activities increased to meet the stress condition produced by incoming FHCF.

In this study it was further investigated that among the nucleic acids, RNA contents gradually increased but later on decreased with the increase of dose and time. While DNA showed an overall increasing trend for all the doses of FHCF. This can be explained that under stress condition the DNA level increased to produce large amount of RNA which then further take part in protein synthesis (this is also confirmed by increased protein contents in the present investigation). Increased level of DNA is also due to the presence of DNA in the incoming HCF (McManus and Smyth, 1982). However, inhibitory effects of FHCF on RNA biosynthesis is another important consideration. It is also important to note that under stress condition the animal minimize the food uptake and due to decrease in dietry proteins and energy, RNA in the

liver cells also decreased (Waterlow and Hauge, 1978).

In the present study cholesterol content initially increased and latter on decreased. As cholesterol is precursor of steroid hormones so possibly it is used in the synthesis also. In the present investigation its elevated level for 0.50, 0.75, 1.00 ml doses indicates that it is not being used in the biosynthesis of steroid hormone. Afterward a decrease in cholesterol content was observed for 1.25 ml and last three doses (1.5 ml) of FHCF. This can be explained that under stress condition the animal minimize the food uptake and under starved conditions the cholesterol is metabolized to meet the energy requirements of the animals. Another reason for decreased level of cholesterol in liver is that incoming HCF has inhibitory effect on cholesterol synthesis. Under disease condition the liver cholesterol ester decrease due to decreased quantity of liver enzyme that influence esterification (Benjamin, 1985). In the present study the decreased cholesterol level in the treated rabbits can be attributed to the liver damage due to inhibitory effect of FHCF.

Among other biochemical components free amino acids fluctuated a lot. Amino acids are used in protein synthesis. Their increase in the initial three stages showed that during this period glucose was not available for energy generation, rather amino acids, available in surplus/higher amounts were being utilized for various activities of liver tissue. Another reason for increased FAA may be due to the release of amino acids from liver, as a result of proteolysis in the liver tissue. Consequently the restoration of plasma free amino acid contents to initial pre-treatment level was also due to their utilization in gluconeogenesis (Mommsen, 1986).

From the results of present study it can be concluded that different doses of FHCF injected, parenterally into rabbits were high enough to induce significant alterations in the liver enzymes and other metabolites.

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