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BIOCHEMICAL CHANGES IN SHEEP AFTER ORAL ADMINISTRATION OF *ECHINOCOCCUS GRANULOSUS* EGG AND ITS CONTROL BY *ALOE VERA*

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Abstract: Three dogs (age 7.0 ± 0.33; weight 12-14 kg) were maintained on optimal conditions and fed 300g of infected sheep liver having 2-3 cm fertile hydatid cyst for five days early in the morning. They were kept for 3 months and their freshly voided faeces were examined for gravid segment of *Echincoccus granulosus*.

Nine healthy sheep of (age 4.0 ± 1.25 months) were maintained under optimal conditions and acclimatized for two weeks prior to experimentation. 3 of them were retained as control group and 6 as experimental group. Twenty fresh gravid segments collected from faecal sample of experimental dog, were fed to the sheep in experimental group for 5 days and then blood sample was monthly-pooled up to six months. In the 7th month three of them were considered as treated group and orally given mashed *Aloe vera* (2g/kg body wt. /day) till the termination of experiment. Their blood samples were pooled for further two months to evaluate the effects of *A. vera*.Infected group showed an overall decrease in glucose ($P<0.01$), protein ($P<0.01$) & cholesterol ($P<0.001$) and non significant increase in bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT),alkaline phosphatase (ALP) and acid phosphatase (ACP) in the infected group. After treatment with *A. vera* there was noted a gradual increase in glucose, protein ($P<0.01$) & cholesterol ($P<0.01$) and non significant decrease in ASAT, ALAT, alkaline phosphatase and acid phosphatase.

Key words: hydatidosis, sheep, biochemical changes, *Aloe vera*

INTRODUCTION

Hydatidosis is a recognized zoonosis affecting both man and his livestock. It is caused by metacestode stage of the dog tapeworm *Echinococcus granulosus* (Dowling *et al.*, 2000; Taherkhani and Rogen, 2000). Cystic larval forms occur in sheep and other grazing animals as part of their normal life cycle (Schantz *et al.*, 1995; Bush *et al.*, 2001) and results in loss of millions of rupees per year due to reduced quality and yield of milk, meat or wool, retarded growth, decreased fertility and condemnation of infected organs (Iqbal *et al.*, 1989). Munir (1980), Khan and Haseeb (1984), Iqbal *et al.* (1986), Pal and Jamil (1986) and Chaudary *et al.* (1992) have given its high prevalence in the local livestock.

Bresson-Handi *et al.* (1989)., Aceti *et al.* (1990), Anwar and Tanveer *et al.* (1997), Tanveer *et al.* (1997 a, 1998), Anwar and Tanveer (2000) have reported

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haematological changes due to experimental hydatidosis. Although mebendazol and albendazol have been used in cystic echinococcosis with conflicting result (Smyth, 1994).

Besides them many plant drugs have also been in use since ancient times for the treatment of parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al.,* 1956; Said, 1969). Akhtar (1986, 1987) has investigated the antithelmintic efficacy of some indigenous plants and herbs. Pakistan is very rich in herbal wealth, a variety of medicinal plants grow widely in different parts and especially in northern hilly areas. Large number of such medicinal plants has been listed by Ikram and Hussain (1978).

Anwar *et al.* (1997, 1997a) and Tanveer (1999) controlled the hydatidosis through native plants in rabbits experimentally induced with protescoleces of *E. granulosus* of sheep origin. They then orally have given dried powdered leaves and fruits of *Prosopis glandulosa* and *Embelia ribes* respectively up to three months. Their findings showed that both the plants were equally effective against hydatidosis.

The present study is aimed to work out the effect of *E. granulosus* on the blood biochemistry of sheep as a mammalian model with emphasis on total proteins, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), glucose, bilirubin, alkaline phosphatase (ALP), acid Phosphatase (AcP) and cholesterol. So this study will help in the diagnosis of hydatidosis through serum based biochemical tests and its control by local plant *Aloe vera.*

MATERIALS AND METHODS

Nine sheep (age 4.0 ± 1.25 months; weight 6.83 ± 0.12 Kg) were kept at Agriculture Farm, Kalakhatai Road, Lahore 31km from the University of the Punjab in an enclave of 9 x 9m. They were fed thrice a day on seasonal fodder like Bursim (*Trifolium alexendrianun*) and Jai (*Avena sativa*) *ad libitum.* To minimize the chance of bacterial and protozoal infections few crystals of $KMnO_4$ were added into their drinking water. These animals were acclimatized for 14 days prior to experimentation.

3 dogs (age 7.00 ± 0.33 months; weight 8.5 ± 0.08 Kg) were maintained at the University of Veterinary and Animal Science, Lahore and were fed milk and bread twice a day. The infected sheep liver having unilocular hydatid cyst was collected from slaughterhouses and placed in icebox for transportation. 300 g of infected liver having 2-3 cm hydatid cyst was cut into 2 to 3 pieces and were fed to each experimental dog daily for five days early in the morning.

9 sheep were randomly divided into 3 groups as control (n=3), experimental control (n=3) and treated group (n=3). Freshly voided faeces of infected dog were examined macroscopically for the presence of gravid segments 2 month after introducing infection. The gravid segments collected from the faeces were washed in saline solution &

fed to all the sheep of experimental control and treated groups up to five days. The sheep were maintained up to 24 weeks.

After 24 weeks out of 6 infected sheep, 3 were considered as experimental control and 3 treated group. The freshly mashed *A. vera* leaves were given orally to the treated sheep (2 g/kg body weight/day) for further 8 weeks.

The blood samples were pooled at 4 weeks interval upto 32 weeks and were subjected to biochemical analysis i.e. serum enzyme activities i.e. aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) (Reitman and Frankel, 1957), glucose (Braham and Trinder, 1972), total protein (Henry *et al.,* 1974), modified by Gowenlock *et al.,* 1988), bilirubin (Jendrassik and Grof, 1938), acid phosphatese and alkaline phosphatase (Bessey *et al.*, 1946), cholesterol (Roeschlau *et al.,* 1974).

Results were statistically analysed by Student's't' test (Steel and Torrie, 1981). The magnitude of difference in between the groups was expressed as percent increase / decrease in comparisons.

RESULTS

Present finding showed that there was an overall decrease in the glucose level (g/dl) in experimental sheep as compared to the control sheep. The average initial glucose value in control sheep was 85.54± 5.11. The average glucose value decreased from initial 84.55 ± 6.05 to 35.41 ± 2.14 (8.59%) at the end of experiment ($P<0.01$) as shown in (Fig.1A). Infected sheep treated with *A. vera* showed a gradual increase in glucose (g/dl). After one month's treatment glucose amount significantly increased from 44.77 ± 4.41 to 56.83 ± 4.78 (7.58%)($P<0.01$). In the last month of experiment glucose amount increased 67.54 ± 5.04 showing a non-significant change.

Initial normal value of protein was 8.04 ± 0.04 (g/dl) in control group. Protein contents in the serum of infected sheep showed significant decrease when infected by *E. granulosus*. The average protein (g/dl) value decreased from initial 8.31 ± 0.115 to 3.11± 0.92 at the end of experiment with some fluctuation. Gradual increase in the protein contents was observed in treated group. The change in protein (g/dl) value changed after two month's treatment by *A. vera* was 5.72 ± 1.76 and 6.58 ± 0.49 in the treated group, hence significant result ($P<0.001$) (Fig.1B).

Oral administration of *E. granulosus* showed an increased trend in bilirubin (g/dl) from 0.20 ± 0.02 to 0.81 ± 4.59 in experimental group. The normal value in the control group was noted as 0.28 ± 0.02. After treatment with *A. vera* bilirubin showed gradual decline (g/dl) in treated group from 0.48 ± 2.05 to 0.36 ± 0.22 (Fig.1C).

Initial cholesterol level was decreased from 119.21 ± 6.18 to 72.16 ± 1.63 in experimental group ($P<0.001$). However, infected sheep treated with *A. vera* showed

gradual increase in cholesterol level. Before the treatment average cholesterol value (g/dl) was 72.16 ± 1.03. After one month's treatment cholesterol value increased up to 91.43 ± 2.01 this value further improved to 98.31 ± 2.98 ($P<0.01$) (Fig.1D).

Infection produced by *E. granulosus* showed an overall increase in the aspartate aminotransferase (ASAT) of experimental group. The average ASAT in the control group was 63.42 ± 1.59 in the start of the experiment. Prominent increase in ASAT value from 61.7 ± 2.66 to 121.28 ± 16.34 in response to the *E. granulosus* was observed. After oral administration of mashed *A. vera* leaves (2 g/kg/day) along with fodder decreased ASAT activity (81.42 ± 5.64) in treated group. ASAT further decreased (78.17 ± 4.84) at the end of 2nd month of treatment. Changes were found to be non-significant when analyzed by Student't' test ($P<0.05$) (Fig.1E).

The change observed in alanine aminotransferase (ALAT) activity was almost similar to that of ASAT. Normal value of ALAT was 53.14 ± 4.21. In experimental group gradual increase in the ALAT value from 53.37 ± 5.06 to 89.70 ± 12.36 was observed. After treatment the ALAT value prominently decreased from62.37 ± 8.41 to 58.63 ± 7.64 in treated group. Results were found statistically non-significant (Fig.1F).

A considerable increase in alkaline phosphatase was observed for the experimental group infected by *E. granulosus*. Alkaline phosphatase value in the control group was 80.21 ± 2.35. In experimental group significant increase (107.22 ± 15.03) was observed after infection. However, when treated with *A. vera* gradual and non-significant decrease from 93.20 ± 7.89 to 89.34 ± 6.42 was noted at the end of the experiment (Fig.1G).

Acid phosphatase in the serum of control group was 10.48 ± 0.44(IU/L). Experimental group showed gradual increase from10.7 ± 0.44 to 25.19 ± 7.68. Before the treatment average value of acid phosphatase was 25.19 ± 7.68(IU/L). After treatment with *A. vera* AcP decreased from 19.43 ± 4.35 to 17.21 ± 3.29 and non-significant trend was noted **(Fig.1H).**

DISCUSSION

In the present study a significant decrease in glucose level was noted. In infected sheep, however, in the 7th and 8th month there was a gradual increase in glucose contents after treatment with *A. vera*. This increase was due to the effect of chemicals present in the plant. Gradually low level of glucose in the experimental sheep showed stress condition due to the infection of *E. granulosus*. Under such circumstances glucose is used for energy production to cope with stress. McManus and Smyth (1982) measured substrates and enzymes of glycolysis associated enzymes both in *E. granulosus* and *E. multilocularis*. Decrease in glucose level after injecting crude cystic hydatid fluid (CHCF) in rabbits have also been reported by Tanveer *et al.* (1997, 1998, 1998a) and Anwar and Tanveer

(2000).Glucose elevation in blood during the last stages of present experiment may be due to the treatment by *A. vera* or development of resistance in experimental sheep against the foreign antigen.

Anonymous (1982) and Schwabe (1986) observed the economic losses due to hydatidosis in domestic livestock, through reduced quality and yield of milk, meat and retarded growth. Iqbal *et al.,* (1989) studied that due to hydatidosis protein deficiency is noted in infected animals. Low protein values showed direct proteolytic effect of hydatid cyst fluid as it contains many lytic enzymes (Frayha and Haddad, 1980), while increased protein level may be due to the formation of antibodies against the antigen present in hydatid cyst fluid. Frayha and Haddad (1980) further noticed the presence of albumin, globulin, many enzymatic proteins, lactate dehydrogenase phosphatase, ASAT and ALAT.

In the present findings, decrease in protein content in experimental group and increase in the treated group orally provided with *A. vera* leaves were noted. Reduction of serum protein by inducing hydatid cyst fluid have been reported by Abidi *et al.,* (1989), Tanveer *et al.,* (1997, 1998, 1998a) and Anwar & Tanveer (2000). Nineteen protein components have been isolated from HCF of which ten were antigen of parasite origin (Biguet *et al.,* 1962; Capron *et al.,* 1962). Host immunoglobulins have also been reported in cyst wall fluid and on the surface of the protoscoleces (Kassis and Tanner, 1977).

Bilirubin level in the serum is increased due to blood loss and haemolysis and its level in serum is directly related with the breakdown of haemoglobin (Cotran *et al.,* 1999). In the present study, infection produced drastic effect on blood bilirubin level in experimental group. Increasing trend was noted before the treatment. After treatment with *A. vera* during 7th and 8th month, a gradual decrease in bilirubin level was observed. It showed that although production of haemoglobin in this group increased but the chemicals of *A. vera* have inhibited the breakdown of haemoglobin. Increased bilirubin levels by the toxic effect of HCF in rabbits have also been reported by Anwar and Tanveer (2000).

Cholesterol is steroid alcohol with lipid like solubility and almost every cell in the body produces it. Besides the production of cholesterol, the liver also esterifies it, chiefly with linoleic acid and liver converts a portion of it to alcoholic acid and also secretes it in the bile (Cotran, *et al.,* 1999) .In the present study decrease in cholesterol level was observed after oral administration of *E. granulosus*. It is metabolized to meet the energy requirements of the animals. Severe liver disease decreases cholesterol ester due to a decreased release of liver enzymes that influence esterification. In the present result gradual increase of cholesterol level in the treated group showed the effect of certain chemicals present in *A. vera* with curing effects.

Aspartate aminotransferase (ASAT) occurs in the cytoplasm and mitochondria of the cell and is important in the metabolism of glutamate and alpha-ketoglutamate. The present investigation showed an overall increasing trend in ASAT. Initially ASAT

increased in the serum of infected sheep and this may be attributed to the pathological response of the hepatocytes of liver. Decrease in ASAT was also observed during 7th and 8th month after treatment with *A. vera*.

Increase in ASAT activity was also noted by Anwar and Tanveer (2000) in rabbits is the toxic effects of hydatid cyst fluid of sheep origin. Alanine aminotransferase (ALAT) mainly found in the cytoplasm of liver cells. Since detoxification of pathogen antibiotics, antigens happened in liver that is why in present study ALAT values were examined to observe the influence of *E. granulosus* in the liver of sheep. ALAT catalyzes the transfer of alpha-amino group alanine to alpha-keto-glutaric acid resulting in the formation of pyruvic acid and glutamic acid. In the present study ALAT (IU/L) showed significant increase in the experimental animals (after inoculation of *E. granulosus*) thereby indicating interruption in the liver function. Increased ASAT and ALAT in the liver tissues resulted increased serum ASAT and ALAT. This may be due to increased permeability of hepatocellular tissues for ASAT and ALAT. An increased amount of ASAT and ALAT is an indication of liver damage, necrosis of hepatic cells, which favour cellular damage or gluconeogenesis through amino acid. Increased ASAT and ALAT in animals infected with *E. multilocularis* have been reported by Kroeze *et al.* (1985). Tanveer *et al.* (1998) and Anwar and Tanveer (2000) reported that ALAT increased after high doses of crude hydatid cyst fluid in rabbits.

The alkaline phosphatase constitutes a group of enzymes that are involved in the hydrolysis of phosphate monoesterase at about an alkaline pH (about 9). Alkaline phosphatases are important in the transport of sugar and phosphate in the liver, intestinal mucosa, kidney lobule, bone and placenta. All the cells in the body utilizing glucose for energy contain phosphate (Benjamin, 1985). Alkaline phosphatase is present in the linings of bile canaliculi of the liver. Acid phosphatase is a lysosomal enzyme and mostly increases in the blood and tissue at the time of tissue or cell damage to hydrolyse or remove the dead tissue or cells.

In the present investigation both alkaline phosphatase and acid phosphatase increased in the infected group, phosphatases are involved in the dephosphorylation and their increased activity may be attributed to induction of enzymes after infection produced by *E. granulosus*. This may be necessary for increased energy requirement, which results to overcome the toxic stress of *E. granulosus*. In the present investigation, increase of alkaline phosphatase and acid phosphatase activities were probably to meet the stress condition of incoming infection by *E. granulosus*. Present findings showed that different biochemical values (in infected groups) either decreased or fluctuated as compared to their respective control values. However, when treatment with *A. vera* was given these values showed alterations approaching normal values. Further studies in this connection are needed.

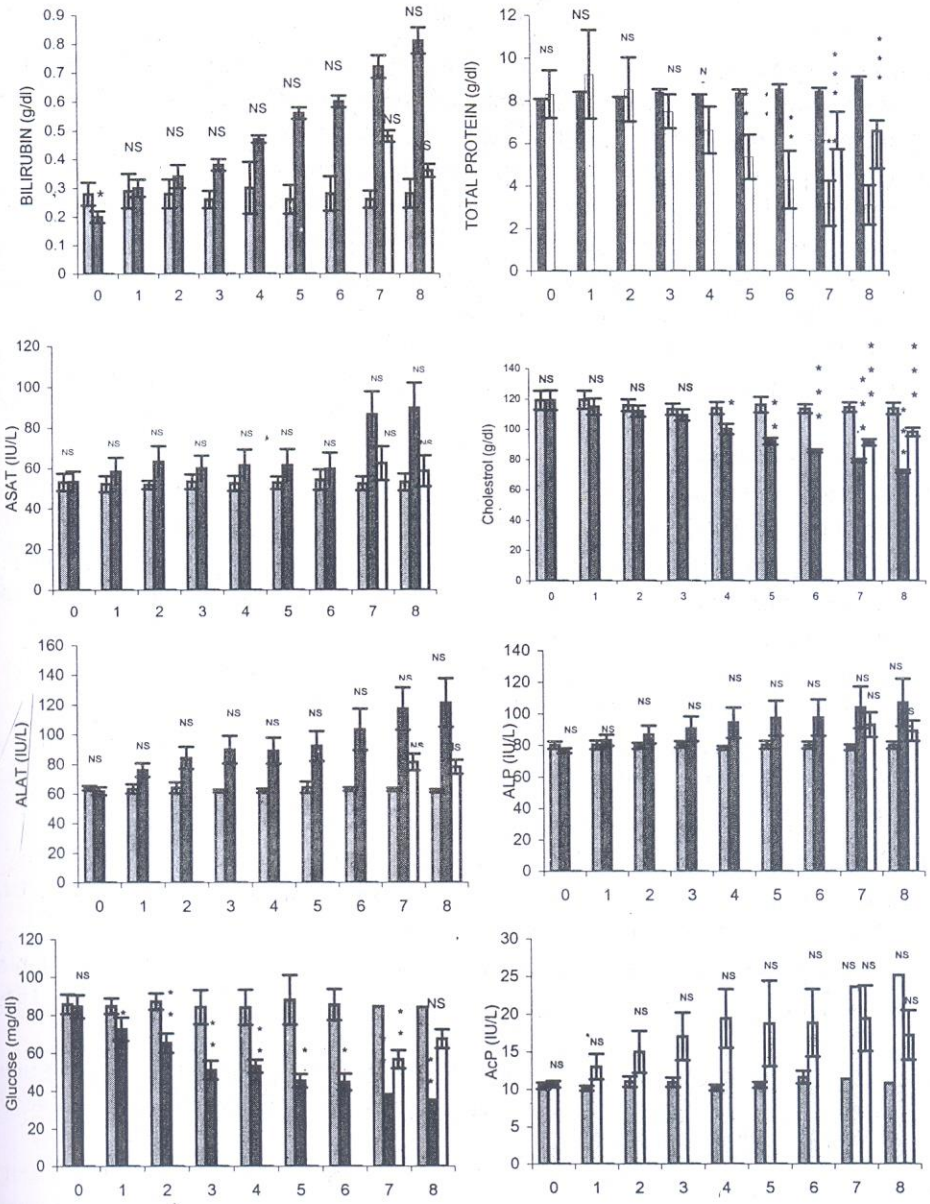

Fig.1: Biochemical changes observed in serum of sheep after oral administration of *Echinococcus granulosus* eggs and control by *Aloe vera.* The statistical significance has been determined by student's "t" test and probability represented by stars, * P<0.01, ***P<0.0001.
(ASAT: Aspartate Aminotransferase, ALAT: Alanine Aminotransferase,
ALP: Alkaline Phosphatase,
AcP: Acid Phosphatase)

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