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CONTROL OF HYDATIDOSIS THROUGH LOCAL PLANTS: HAEMATOLOGICAL STUDIES

AKHTAR TANVEER

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Abstract: Out of thirty-eight healthy rabbits acclimatized to the optimal laboratory conditions, six were kept as normal control and rest were inoculated intraperitoneally and subcutaneously with 45,000 protoscoleces of *Echinococcus granulosus* of sheep origin. They were randomly divided into four groups *i.e.*, one infected control (n=8) and three treated groups (having 8 rabbits in each group). These treated rabbits were individually given (2g/day) leaves of *Aloe vera* or ovary part of *Punica granatum* or fruit of *Azadirachta indica* along with ordinary green fodder. Rabbits were individually weighed and their blood samples were pooled for haematological analyses up to 90 days with an interval of 10 days. The body weight (g) in the experimental control rabbits decreased (<0.001) and in treated rabbits body weight initially decreased but later on improved gradually ($P<0.001$). Among haematological parameters total erythrocyte counts (TEC) (10^6 mm^3), haemoglobin (g/dl), packed cell volume (% ml); total leukocyte counts (TLC) (thousands/mm^3), and erythrocyte sedimentation rate (mm/hr) fluctuated in the rabbits inoculated with protoscoleces of sheep origin. However, these values improved gradually in the rabbits treated with different plants/parts of plants. The improvement was significant in the rabbits treated with leaves of *A. vera* and fruit of *A. indica* as compared to those given ovary part of *P. granatum*. Their respective haematological indices *i.e.*, mean corpuscular volume (femtolitre), mean corpuscular haemoglobin (picogram) and mean corpuscular haemoglobin concentration (g/dl) also reflected the changes faced by haemoglobin and packed cell volume. The above results showed that all the three plants play important role in curing the haematological parameter in experimentally induced hydatidosis. These plants may have some chemical that can antagonise the effects of protoscoleces.

Key words: Hydatidosis, sheep, protoscoleces, rabbits, haematology, herbal control.

INTRODUCTION

Hydatidosis is medically and economically one of the most important parasitic diseases and both unilocular (*Echinococcus granulosus*) and multilocular (*E. multilocularis*) of the disease are widely and actively expanding their range into areas previously considered to be free of this infection (Schwabe, 1986). It is also an important zoonosis and the chances of its high infection in man are more in those areas where sheep and cattle grazing is aided by the dogs (FAO Report, 1981) as in Pakistan. Its wide prevalence in the livestock of Pakistan has been given consideration by Munir (1980), Khan and Haseeb (1984), Iqbal *et al.* (1986), Pal and Jamil (1986), Hussain (1987) and Iqbal *et al.* (1989). Its seriousness in human beings is also far

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greater than what the published work shows and its incidence is much higher than what is generally believed to be (Chaudhry *et al.*, 1992; Naveed *et al.*, 1993; Junejo *et al.*, 1995).

The suitable climate, poor hygienic conditions and unawareness about this disease have made the conditions favourabe for the growth and completion of the life cycle of *E. granulosus*. That is why livestock in Pakistan is constantly at risk of hydatidosis that has direct effect on the economy of the country and results in loss of million of rupees each year. It has also worsen the protein deficiency for human consumption in terms of condemned organs and lowered products of infected animals. Keeping in view their medical and veterinary importance it was considered desirable to find out some remedy. Although mebendazole and albendazole are now routinely used in cystic echinococcosis (Smyth, 1994). Besides them many plant drugs have also been in practice since ancient times for the treatment of parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al.*, 1956; Said, 1969). Akhtar (1987) tested the anthelmintic activity of several plants (in powdered and extract form) in goats, buffaloes and calves and found number of plant drugs and their extracts that possess interesting potent and safe anthelmintic principle for veterinary usage. Anwar *et al.* (1997, 1997a) successfully used indigenous plants to control hydatidosis in experimentally infected rabbits. In the present investigation 3 plants (*Aloe vera, Punica granatum* and *Azadirachta indica*) were orally given to experimentally infected rabbits with common fodder and their effects were studied in terms of changes/ improvements they induce in their haematology.

MATERIALS AND METHODS

Thirty-eight healthy rabbits maintained under optimal laboratory conditions were fed twice a day on seasonal green fodder. They were individually weighed in the beginning of experiment and then before each blood sampling.

Hydatid cyst fluid (HCF)

Hydatid cyst fluid (HCF) was aspirated from the cyst present in liver, lungs and spleen of infected sheeps at local slaughter house and only viable protoscoleces were selected for inoculation. HCF was centrifuged at 500 rpm for 5 minutes and the pallet thus formed was washed with saline solution, containing the Penicillin and Streptomycin sulfate (Ohnishi, 1985). The protoscoleces were counted in the chamber of white blood cells of haemocytometer. Two inoculums each having 22500 protoscoleces were injected sub-cutaneously and intra-peritoneally in the left flank of rabbit after taking all antiseptic precautions.

Grouping of rabbits

Rabbits were divided into 5 groups. For each plant treatment 3 treated (eight rabbits each) and one experimental control (n=8) along with one normal control (n=6) were maintained. Following plants and their parts were used:

	Name of Plant	*Local Name*	*Parts of Plants*
1.	*Aloe vera*	Kwargandal	Leaves
2.	*Punica granatum*	Anar	Ovary part

3. *Azadirachta indica* Neem Fruit

Preparation of plant/herbal extracts

The dried plants and their parts (Anar, Neem) were ground to fine powder, 2 g of each powdered plant was mixed with 25 ml of tap water and then given to rabbits. The fresh leaves of kwargandal were mashed and 2 g mixed with 2.5 ml of water was given to rabbits. Three treated groups were orally given 2 g/day of *A. vera* or *P. granatum* or *A. indica* upto 90 days. The experimental control (without herbal treatment) and normal control were also maintained.

Blood sampling and haematological studies

After every 10 days 4.0 ml blood was pooled from the marginal vein of each rabbit and mixed with EDTA (anticoagulant). This blood was used for the estimation of haemoglobin (Hb) contents according to Van-Kampan and Zijistra (1961), Packed cell volume (PCV; haematocrit) according to microhaematocrit method of Strumia *et al.*, 1954), total erythrocyte counts (TEC) and total leukocyte counts (TLC) were made according to Dacie and Lewis (1991). The data thus obtained was utilized for calculating different haematological indices, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) according to Dacie and Lewis (1991). For the determination of erythrocyte sedimentation rate (ESR) Westergren's method reported by Swarup *et al.* (1986) was followed.

RESULTS

Body weight

The initial body weight (g) in the normal control, experimental control and treated rabbits was 1134.3 ± 36.4, 1104.5 ± 58.2, 1122.0 ± 34.2, 1108.3 ± 36.2, 1121.4 ± 48.3. Body weight (g) gradually increased in the normal control rabbits and reached up to 41.5% after 90 days. However, in the experimental control the body weight (g) increased only 18.05%. In the rabbits treated with *A. vera, P. granatum* and *A. indica* the body weight increased 24.98, 28.2 and 28.43% respectively ($P < 0.001$) (Fig.1).

Treatment with A. vera

TEC ($10^6 m^3$) in the beginning of experiment was 6.3 ± 9.0, 4.8 ± 0.9 and 6.4 ± 0.8 in the normal control and experimental rabbits. In the normal control group these values remained almost constant throughout the experiment with slight decrease (5.6%) in the end. However, the similar values decreased constantly in the rabbits inoculated with protoscoles of sheep origin except on the 40th day of experiment. In the end they faced an overall decline (27.08%). However, in the treated rabbits TEC number also decreased in 20th, 30th and 40th day but later on improved positively and in the end decrease was only 6.25% as compared to the 27.08% in the infected control rabbits (Fig.2A).

In the normal control group the haemoglobin (g/dl) contents slightly fluctuated and finally decreased (6.83%). However, these values declined (72.9%) in the infected

control rabbits while improved in the rabbits treated with *A. vera*. Although, decline of 6.96% was also noted in this group ($P<0.01$; Fig.2B).

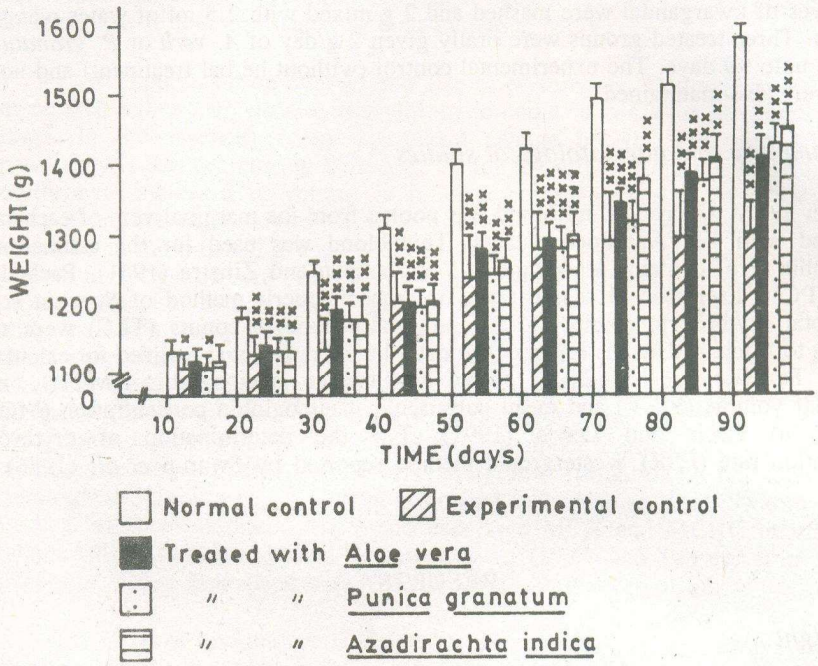

Fig. 1: Normal control, Experimental control, Treated with *Aloe vera*, Treated with *Punica granatum*, Treated with *Azadirachta indica*.

Packed cell volume (PCV) (%/ml) remained almost unaltered in the normal control group with a slight decrease (3.3%) in the end. These values increased (58.9% and 8.02%) in the experimental control and rabbits treated with *A. vera* and *A. indica* (Fig.2C). Their haematological indices (MCV, MCH, MCHC) computed from the results of TEC, Hb and PCV also reflected the similar changes. After an initial alterations their values reached near control values ($P<0.001$) except in the rabbits of experimental control group (Fig.2D, E, F).

TLC (thousands/mm^3) showed slight fluctuations and in the end increased up to 3.06% in the normal control rabbits. However, in the infected controls these values increased up to 65.6%. The treated rabbits showed increase in the 20th and 30th day but later on they reached near the normal values and increased up to 4.08% only ($P<0.01$; Fig.2G).

Erythrocyte sedimentation rate (ESR) (mm/hr) increased 74.86, 215.01 and 58.3% in the normal control, infected control and treated rabbits ($P<0.01$; Fig.2H).

Treatment with P. granatum

In this group the TEC remained almost unaltered in the normal control rabbits. However, slight decrease (5.1-6%) was noted in the end. These values drastically decreased in the infected control rabbits up to 27.08 percent. However, in the rabbits treated with *P. granatum* TEC decreased continuously and reached up to 33.33% in the end ($P<0.01$; Fig.3A). In this case no improvement due to *P. granatum* was noted Hb contents showed slight variations but in the 90th day they showed 6.32% decrease. However, *P. granatum* treated rabbits showed initial decrease but after 40th day of treatment they improved and reached 8.4 ± 0.8 (g/dl) when compared with the normal control values 8.8 ± 0.8 (g/dl) ($P<0.05$; Fig.3B).

PCV values in the control rabbits showed slight variations and in the end decreased up to 3.3% as compared to the infected control rabbits which showed 58.9% increase ($P<0.001$). However, PCV values in the rabbits given 2 g/day of *A. granatum* showed slight improvement and in the end they increased up to 10.2% ($P<0.05$; Fig.3C). As far as their haematological indices are concerned MCV, MCH and MCHC showed fluctuation as compared with the normal control values but ultimately showed improvements. While in the experimental controls these values changed drastically ($P<0.001$; Fig.3D, E, F).

TLC of normal control rabbits showed slight alterations in 50th day of experiment when they dropped to 7.9 ± 1.3 and afterwards they again improved and reached the normal values. Similar values in their corresponding experimental controls increased upto 65.6% in the end ($P<0.001$; Fig.3G). ESR values increased in the normal control, experimental control and treated control rabbits up to 74.8, 218.5 and 35.5% respectively ($P<0.001$; Fig.3H).

Treatment with A. indica

TEC also showed improvement (3.7%) in the rabbits given (2 g/day) *A. indica* as compared to the decrease of 27.08% and 5.6% faced by the infected control and normal control rabbits respectively ($P<0.01$; Fig.4A).

Hb contents showed slight changes in the normal control rabbits except in the end with an overall reduction of 6.38%. Infected control rabbits faced a gradual decline that reached up to 72.9% in the end ($P<0.01$; Fig.4B) as compared to the slight improvements (2.83%) faced by the treated rabbits.

PCV also showed little fluctuations throughout the course of experiment with an overall decrease of 3.3% in normal control rabbits and 0.30% in infected controls as compared to 58.9% increase in the infected controls ($P<0.001$; Fig.4C).

The haematological indices like MCV increased and MCH and MCHC decreased ($P<0.001$; Fig.4D, E). Overall increase noted in the TLC of normal control and infected control was 3.07 and 65.6% respectively. Increased TLC was also noted in treated groups but after 50th day of treatment they improved and in the end increase was only up to 13.18% ($P<0.001$; Fig.4G). ESR increased up to 74.8, 218.5 and 35.5% in the normal control, infected control and treated rabbits respectively ($P<0.001$; Fig.4H).

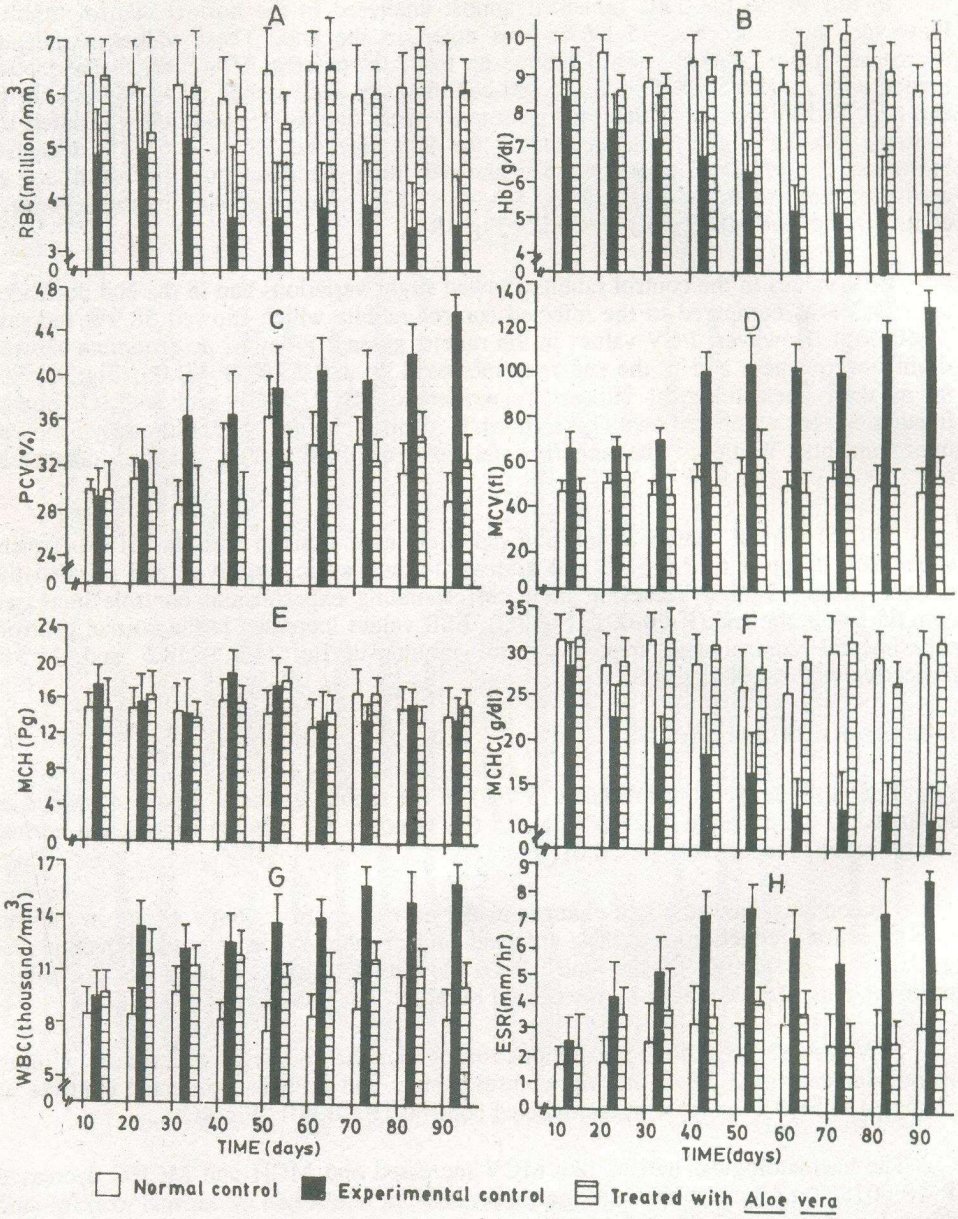

Fig. 2: Normal control, Experimental control, Treated with *Aloe vera*.

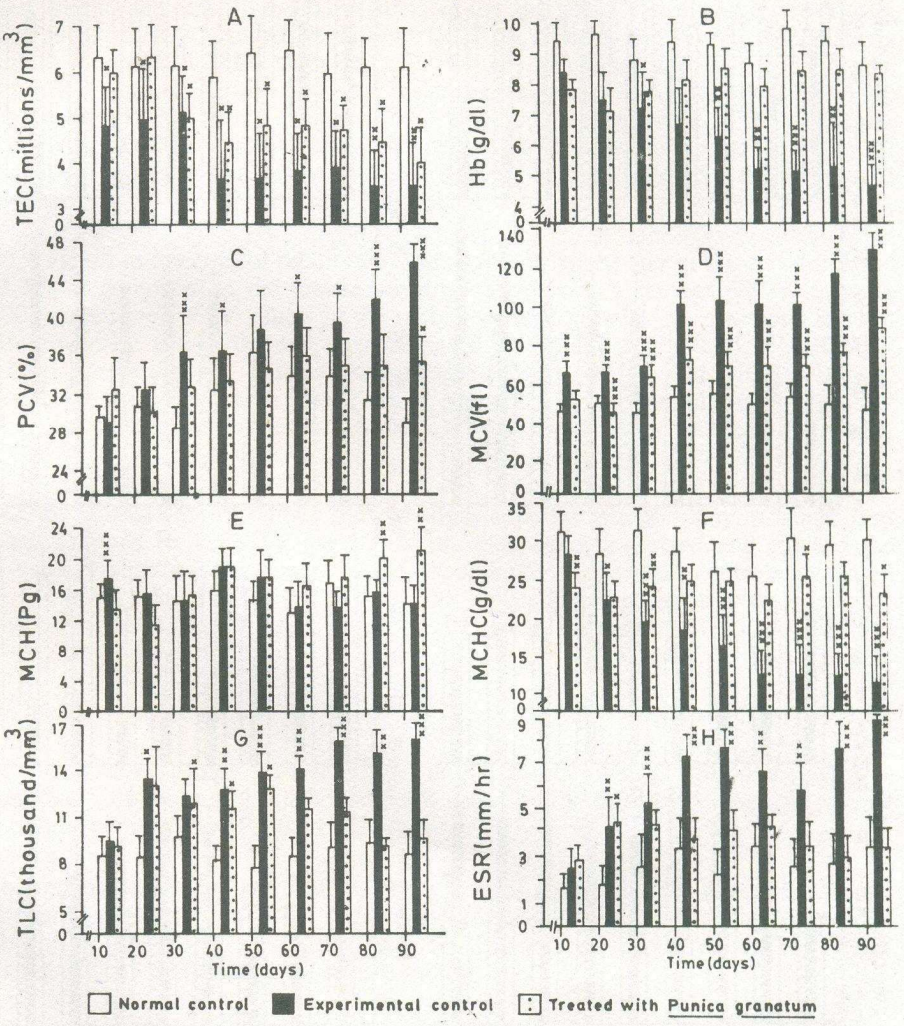

Fig. 3: Normal control, Experimental control, Treated with *Punica granatum*.

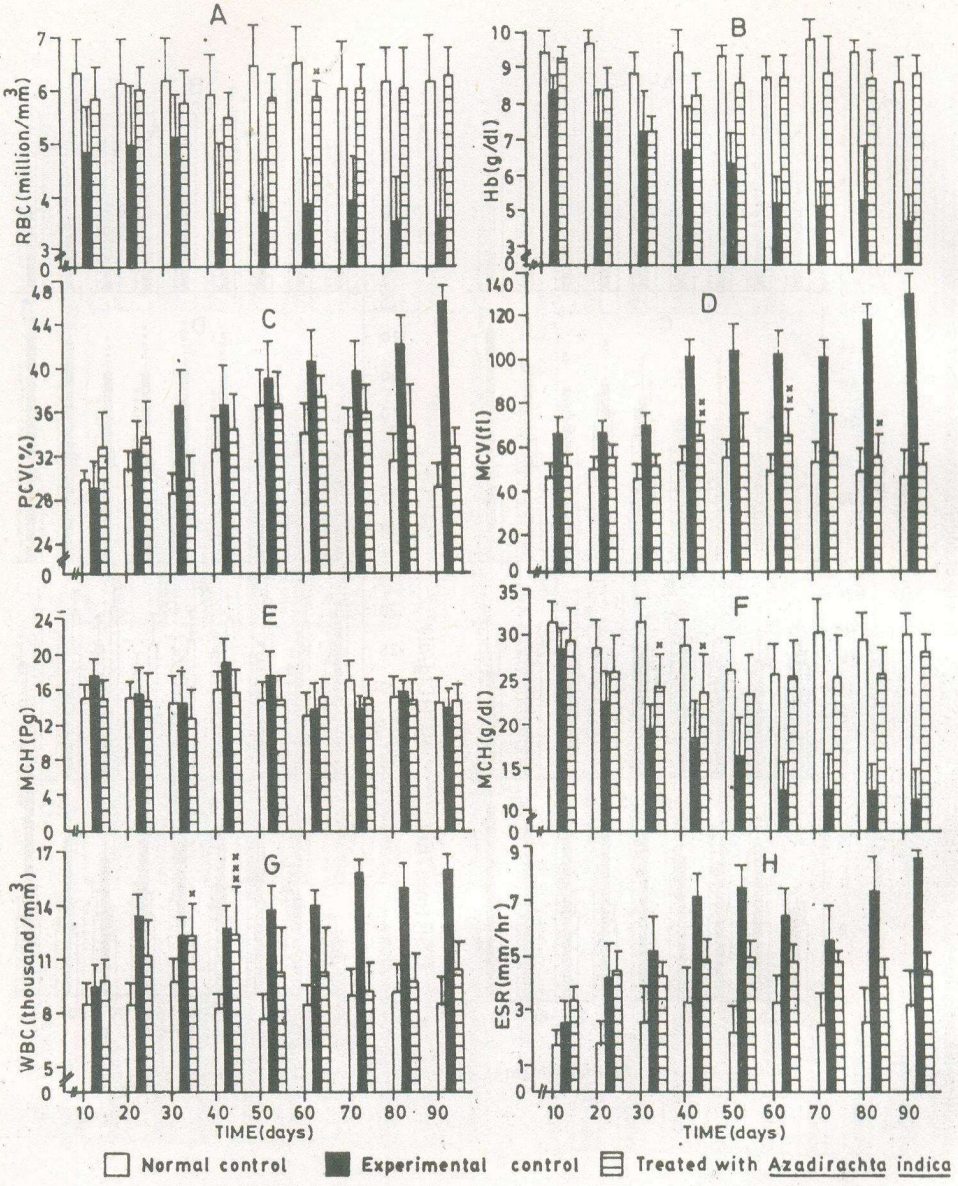

Fig. 4: Normal control, Experimental control, Treated with *Azadirachta indica*.

DISCUSSION

Rabbits maintained as infected control and those orally given *A. vera, P. granatum* and *A. indica* initially showed sharp decline in their body weight (g) when compared with their respective control ones. It is suggested that this decrease in body weight may be due to the combined effect of some toxicity present in the inoculated protoscoleces. Decreased body weight due to hydatidosis have also been reported by Pandey (1971) who studied its effects on liver, lungs and spleen of goats and reported gross changes in these tissues. He suggested that toxicity of hydatid cyst fluid may have changed the surrounding cells thereby resulting in organ condemnation which leads to weight loss. It also leads to economic losses through low quality and reduced yield of milk, meat and retarded growth (FAO, 1981; Schwabe, 1986). Animal suffering from hydatidosis also showed protein deficiency that ultimately lead in weight reduction (Iqbal *et al.*, 1989). Toxic effect induced by the incoming protoscoleces also have affected some of their physiological phenomenon which have made them, lethargic, inactive and minimized their food intake that ultimately aided in their weight reduction.

Present results showed that body weight of treated rabbits showed more improvement as compared to the experimental control rabbits. Although this increase in weight was less as compared to the normal controls. Most probably this was due to the development of some resistance/ immunity or some inhibitory effect of plants against the incoming protoscoleces. Almost similar findings have been reported by Anwar *et al.* (1997) and Tanveer *et al.* (1997, 1998, 1998a).

Erythrocytes play a very important role in the transport of haemoglobin which carries oxygen to different parts of the body by converting it to metahaemoglobin. A nice balance kept between normal and destroyed erythrocytes in normal conditions was disturbed by the inoculation of protoscoleces that have decreased or destroyed them. Similar findings have been reported by Aminzhanov (1977) who noted decreased TEC in sheep naturally infected with *E. granulosus*. Decreased TEC due to alveolar hydatidosis in mice (Hinz and Gehring, 1987) and due to the inoculation of hydatid cyst fluid have been reported in rabbits (Anwar *et al.*, 1997; Tanveer *et al.*, 1997, 1998, 1998a). The decrease thus produced have caused anaemia. Anaemic condition noted in the present investigation was probably produced by the increased blood cell destruction. Damaged erythrocytes and reduced erythropoiesis are also related to decreased TEC that ultimately led to anaemia. Anaemia can also be induced by the reduced TEC (due to destruction), PCV and Hb contents, etc. However, an initial decrease in TEC was followed by a gradual improvement in the rabbits treated with *A. vera, P. granatum* and *A. indica*. Among the three plants tested, more promising results were achieved by *A. vera* and *A. indica*. However, the normal control rabbits showed minor fluctuations in their TEC. Improvement in the TEC may be due to some chemical present in the plants that have antagonized the harmful impact of incoming protoscoleces.

Hb transport oxygen to all parts of the body and bring carbon dioxide back. During present studies the Hb in normal control rabbits fluctuated but remained within normal range, while in infected control groups it decreased continuously. The rabbits, orally given *A. vera, P. granatum* and *A. indica*, also showed decreased Hb in the beginning, that later on improved with the passage of time. This improvement reached near normal values in case of *P. granatum* and *A. indica*, while in case of *A. vera* this value increased more than the control values. Reduced TEC in the infected control rabbits and treated rabbits (in the beginning) was mainly attributed to haemolysis that leads to binding of haemoglobin to plasma haemoglobin. During this process the extra

haemoglobin is excreted through urine (Gottstein *et al.*, 1993). Hb is partly converted to methaemoglobin prior to conversion to bilirubin. This haemolysis factor probably have increased haemoglobin in each RBC (Charles and Norman, 1966; Eastham, 1986; Dacie and Lewis, 1991). Gradual decline in the Hb level have also been reported in rabbits after administration of hydatid cyst fluid of sheep origin (Anwar *et al.*, 1997). However, improved Hb contents in treated rabbits may be due to some chemical in the plants that may have antagonise/ produced resistance against the toxicity of protoscoleces. This increase was further confirmed by their increased MCV.

PCV depends up on number and size of erythrocyte. Decrease in PCV is normally associated either with decreased erythrocytic count or MCV or increased blood plasma level. In the absence of any significant change in Hb, TEC and MCV the only possibility is that decrease in PCV may be due to increased plasma level. In this case the erythrocyte increased in volume in infected control group so their volume increased more than the erythrocytes of rabbits orally given plants. Here the MCV values increased in the infected groups while the MCH and MCHC decreased. However, in the present investigation size of erythrocyte may have increased due to some toxic effect of protoscoleces and not due to haemoglobin formation. This increase may be associated with increased bone marrow activity, anemia, haemolysis and some deficiency of haemopoetic factor (Benjamin, 1985). MCHC in normal control rabbits was 30.1 g/dl which is close to the maximum value of Hb that RBC can hold (Sachdew, 1991). Increase MCV in infected rabbits showed macrocytic normochromic anemia (Benjamin, 1985; Sachdew, 1991). If MCH reached the upper normal limit it showed that the erythrocytes are of bigger size and therefore, contain/hold maximum amount of haemoglobin. Such a cell is always associated with macrocytosis (Swarup *et al.*, 1986). General trend of increased MCV and decreased MCH and MCHC in infected control rabbits have also been reported by Anwar *et al.* (1997) and Tanveer *et al.* (1997, 1998, 1998a) in rabbits inoculated with hydatid cyst fluid of sheep origin.

The role of leukocytes is defensive in the body. They always require certain foreign stimulus for their increase. Hydatid cyst fluid have reported to stimulate the leukocytosis (Tanveer *et al.*, 1997, 1998, 1998a). In the present work TLC sharply increased in rabbits administered with protoscoleces but later on the pace of increase slowed down in the treated rabbits. This increase in infected rabbits may be due to some resistance develop through the plants. Increase in TLC in the initial stage may be attributed to tissue damage (Eastham, 1985). Similar findings have earlier been reported by Hinz and Gehring (1987) and Alkarmi and Khan (1989) in mice infected with *E. multilocularis*. The phagocytic activity of blood monocytes in mice infected with *E. granulosus* and increase in WBC due to monocyte increase was noticed by Alkarmi and Behbehani (1989) and Wangoo *et al.* (1989). The effect of plant treatment (*Prosopis glandulosa* and *Embelia ribes*) with some curing effects have been reported by Anwar *et al.* (1997).

Inoculation of protoscoleces of sheep origin have also altered the ESR of rabbits. ESR depends on the rouleaux formation, which in turn depends on the concentration of fibrinogens and globulins (Frayha and Haddad, 1980) so their administration in the rabbits is responsible for the increased ESR values. However, these values, gradually came down in the rabbits orally given *A. vera, P. granatum* and *A. indica*.

In the present investigation, different haematological parameters of rabbits have been investigated after inoculating 45000 protoscoleces of sheep origin. The alterations shown in various parameters were found improving only in those rabbits which were

given (2 g/day) *A. vera* (leaves), *P. granatum* (ovary) and *A. indica* (fruit). The results clearly showed that these plants have some potential in antagonising the harmful/toxic effects of protoscoleces. Further studies in this connection are needed.

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