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## Biochemical changes associated with gastrointestinal nematode infection in sheep

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### Abstract

Present study was conducted to evaluate biochemical changes in sheep of Kashmir valley suffering from gastrointestinal nematode infection. Thirty (N=30) animals infected with gastrointestinal nematodes were divided into five groups comprising of 6 animals each and treated with different anthelmintic drugs. An additional group comprising of six healthy animals apparently free from any infection was taken as healthy control. Different biochemical parameters like as blood glucose, total protein and serum albumin were significantly ( $p<0.05$ ) low in clinically affected animals at the 0<sup>th</sup> day of study compared to healthy control. Following treatment with different anthelmintics there was significant ( $p<0.05$ ) improvement in biochemical parameters at the end of study. A significant ( $p<0.05$ ) increase in the blood glucose levels in Ivermectin treated group recorded on 7<sup>th</sup> day post treatment were  $58.50 \pm 0.56$  mg/dl which were significantly ( $p<0.05$ ) higher than infected control group ( $43.50 \pm 1.91$  mg/dl) but comparable to healthy control ( $56.13 \pm 0.52$  mg/dl). For Artemisia group, the values recorded also showed a significant ( $p<0.05$ ) increase in blood glucose levels ( $55.62 \pm 0.58$  mg/dl) on day 7 post-treatment which was significantly ( $p<0.05$ ) higher as compared to untreated infected control group ( $43.50 \pm 1.91$  mg/dl) but comparable to healthy control ( $56.13 \pm 0.52$  mg/dl). In Ivermectin treated group, on day 7 post-treatment the values recorded were  $6.47 \pm 0.08$  g/dl which showed significant ( $p<0.05$ ) increase in total protein than infected control group ( $5.65 \pm 0.18$  g/dl) but comparable to healthy control ( $7.05 \pm 0.52$  g/dl). In group artemesia, a significant ( $p<0.05$ ) increase in serum albumin level was recorded by day 7 and values were  $4.73 \pm 0.09$  g/dl as compared to infected control ( $3.70 \pm 0.19$  g/dl) improvement in biochemical parameters was also observed in other groups. However, the results were comparatively better for Ivermectin and Artemisia treated groups.

**Keywords:** biochemical changes, gastrointestinal nematode infection, sheep

### Introduction

A primary constraint and a major threat to productivity of sheep, their health and welfare is gastrointestinal nematodes infection that occurs worldwide (Larsen *et al.* 1995<sup>[13]</sup>; Campos *et al.* 2009<sup>[4]</sup>). However, the scenario is severe in developing countries like India (Dhar *et al.*, 1982)<sup>[5]</sup>. Some of the important gastrointestinal nematodes that infect ruminants belong to the family Trichostrongylidae including *Haemonchus* spp., *Trichostrongylus* spp., *Bunostomum* spp., *Cooperia* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Chabertia* spp., *Trichuris* spp. *Haemonchus contortus* among all the GI nematodes affecting sheep, is prevalent in tropical, subtropical and temperate regions, especially under warm and wet conditions (Jabbar *et al.* 2008<sup>[9]</sup>; Paraud *et al.* 2010<sup>[15]</sup>; Khan *et al.* 2010<sup>[10]</sup>). Presence of *Haemonchus contortus* appears to interfere with the digestion and absorption of proteins, calcium and phosphorus (Sood, 1981)<sup>[16]</sup>. In case of *Haemonchus*, *Fasciola*, and *Dictyocaulus* species, the total serum protein and serum albumin gets decreased while serum globulin contents are increased (Chakraborty and Lodh (1994)<sup>[3]</sup>. As per reports of Kumar *et al.*, 2010<sup>[12]</sup>, sheep infected with fenbendazole resistant as well as susceptible strain of *Haemonchus contortus* showed depressed levels of serum protein and iron values. In sheep infected with GI nematode parasites, there is marked decrease in albumin, where as alpha globulin and beta globulin contents increase especially at peak of infection (Ahmad *et al.*, 1989)<sup>[11]</sup>. In clinical haemonchosis, disturbance in the levels of various haematological and biochemical parameters have been reported as drop in hemoglobin, haematocrit, TEC, serum protein and albumin and appears to be more pronounced in infected animals as compared to healthy ones (Ijaz *et al.*, 2009)<sup>[6]</sup>. Because of the increasing anthelmintic resistance and demand for clean, green, residue free animal products, there is necessary requirement for alternative techniques to combat GI infections like as haemonchosis. As per Iqbal *et al.*, 2007<sup>[7]</sup>, under integrated pest management programmes, biological control by use of medicinal plants like Artemisia can be used for GI nematodosis and thereby improving its biochemical parameters and help in controlling the infection.

## Materials and Methods

The animals suffering from gastrointestinal nematodosis (N=30), were selected and allotted to 5 different groups, comprising of 6 animal each and were treated with different anthelmintic drugs as presented in Table 1. An additional group comprising of 6 animals apparently free from any disease was selected and kept as healthy control or negative control group.

### Blood sample collection and serum extraction

For biochemical studies the blood was collected from each animal using sterilized gloves in a clean EDTA vial. The samples were collected at the beginning of the study i.e., day zero and followed on day 7, 14, 21 and 28<sup>th</sup> post-treatment. Serum was separated simply by keeping blood undisturbed in centrifuge tubes in slanting position for about an hour at room temperature. Once the blood clot was formed, it was broken and centrifuged at 2000 rpm for 30 minutes to yield clot free clean serum. Later this serum was used for estimation of glucose, total protein, albumin and globulin by following procedures.

### I. Glucose

Glucose was estimated by God/Pod Method (1972), using reagents supplied by Coral- Clinical System India and the serum concentration of glucose was determined by spectrophotometer using diagnostic kits as per the kit manufacturer's instructions.

### II. Total Protein, Albumin and Globulin

To determine total protein, albumin and globulin, Doumas method (1978) using kits supplied by Coral- Clinical System, India was used.

### III. Globulin

For estimation of serum globulin, the values of albumin determined by using diagnostic kits were subtracted from the total protein values and hence, the values so obtained were expressed in gm%. This was followed by comparison of serum concentration of globulin among different groups of animals.

**Table 1:** Treatment dosage and route of administration schedule for the study

Group	No. of animals	Dosage	Route	No. of treatments
Fenbendazole	06	5mg/kg body weight	Oral	One
Ivermectin	06	0.2 mg/kg body weight	S/C	One
Closantel	06	10mg/kg body weight	Oral	One
Artemisia	06	10g/animal	Oral	One
Untreated infected control	06	-	-	-
Healthy control	06	-	-	-

## Results and Discussion

### I. Blood glucose

At different stages of the experimental trial the blood glucose values were recorded and are presented in Table 2. The (Mean±SE) values of blood glucose on day '0' varied from 40.01±2.39 to 66.00±3.27 mg/dl and was non-significantly ( $p>0.05$ ) higher than the healthy control group (56.13±0.57 mg/dl). In fenbendazole treated group, there was non-significant ( $p>0.05$ ) increase in blood glucose levels within the group along the course of the experimental trial from 7 to 28 days post-treatment and the values recorded on 28<sup>th</sup> day were 75.67±3.04 mg/dl which were significantly ( $p<0.05$ ) higher than infected control group as well as healthy control group (41.83±1.45 mg/dl and 56.13±0.57 mg/dl respectively). Similar observations were recorded by Bhat *et al.*, 2004<sup>[2]</sup>. Thus the decline in the blood glucose levels in sheep suffering from GI nematode infection can be attributed to the fact of

impaired glucose absorption and assimilation due to gastrointestinal tract damage caused by these nematode parasites. There was significant ( $p<0.05$ ) increase in the blood glucose levels in Ivermectin treated group. The values recorded on 7<sup>th</sup> day post treatment were 58.50± 0.56 mg/dl which were significantly ( $p<0.05$ ) higher than infected control group (43.50±1.91 mg/dl) but comparable to healthy control (56.13±0.52 mg/dl). In case of Closantel treated group the blood glucose levels on 28<sup>th</sup> day were significantly ( $p<0.05$ ) higher than untreated infected control group but significantly ( $p<0.05$ ) lower than healthy control group (41.83±1.70 mg/dl and 56.08±0.50 mg/dl respectively). For Artemisia group, a significant ( $p<0.05$ ) increase in blood glucose levels (55.62±0.58 mg/dl) on day 7 post-treatment was observed which was significantly ( $p<0.05$ ) higher as compared to untreated infected control group (43.50±1.91 mg/dl) but comparable to healthy control (56.13±0.52 mg/dl).

**Table 2:** Mean± SE blood glucose (mg/dl) in sheep infected with GI nematodes

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Healthy control	56.13±0.57 <sup>Aa</sup>	56.13±0.52 <sup>Aa</sup>	56.13±0.53 <sup>Aa</sup>	56.15±0.53 <sup>Aa</sup>	56.08±0.50 <sup>Aa</sup>
Infected control	40.83±1.45 <sup>Ab</sup>	43.50±1.91 <sup>Ab</sup>	42.50±2.12 <sup>Ab</sup>	40.83±2.15 <sup>Ab</sup>	41.83±1.70 <sup>Ab</sup>
Fenbendazole	66.00±3.27 <sup>Ac</sup>	68.67±3.61 <sup>Ac</sup>	70.75±3.47 <sup>Ac</sup>	73.00±3.18 <sup>Ac</sup>	75.67±3.04 <sup>Ac</sup>
Ivermectin	55.83±0.70 <sup>Aa</sup>	58.50±0.56 <sup>Ba</sup>	61.33±0.56 <sup>Ca</sup>	62.83±0.54 <sup>Cd</sup>	64.83±0.63 <sup>Dd</sup>
Closantel	40.01±2.39 <sup>Ab</sup>	42.43±2.40 <sup>ABb</sup>	44.40±2.13 <sup>ABCab</sup>	46.98±1.12 <sup>CBe</sup>	49.74±0.71 <sup>Ce</sup>
Artemisia	54.22±0.68 <sup>Aa</sup>	55.62±0.58 <sup>ABa</sup>	56.72±0.71 <sup>BCa</sup>	57.25±0.76 <sup>BCa</sup>	58.72±0.91 <sup>Ca</sup>

Values with same superscript in a column (small letters) and same superscript in rows (capital letters) do not vary significantly ( $p>0.05$ )

### II. Total protein

Results for total protein at various stages of experimental trial in the infected groups & healthy control group are presented in Table 3. The total protein values (g/dl) (Mean±SE) on day '0' varied from 4.62±0.06 to 6.37±0.10 g/dl and were significantly ( $p<0.05$ ) lower than the healthy control group

(7.00±0.60 g/dl). Similar observations have been reported by Bhat *et al.*; 2004<sup>[2]</sup>; Mir *et al.*, 2007<sup>[14]</sup> and Kumar *et al.*, 2010<sup>[12]</sup>. Protein loss in gastrointestinal nematode infection predominantly occurs due to selective loss of smaller sized albumin and its osmotic sensitivity to fluid movement. In group treated with fenbendazole, there was non-significant

( $P>0.05$ ) increase in the serum protein levels along the course of experimental trial except for day 28 when the values reached  $5.54\pm 0.82$  gm/dl which were significantly ( $p<0.05$ ) lower than healthy control ( $7.33\pm 0.32$  g/dl) while comparable to infected control group ( $5.50\pm 0.18$  g/dl). In case of ivermectin treated group, by the day 7<sup>th</sup> post-treatment the values reached  $6.47\pm 0.08$  g/dl which were significantly ( $p<0.05$ ) higher than infected control group ( $5.65\pm 0.18$  g/dl) but comparable to healthy control ( $7.05\pm 0.52$  g/dl). In group closantel group, there was non-significant ( $p>0.05$ ) increase in the serum protein levels within the group along the passage of therapeutic trial. by day 28 post-treatment the serum values recorded were  $5.05\pm 0.35$  g/dl which were observed to be

significantly ( $p<0.05$ ) lower than healthy control ( $7.33\pm 0.32$  g/dl). In case of Artemisia treated group artemisia treated group, there was non-significant ( $p>0.05$ ) increase in protein levels till day 14. The values on day 14 reached  $6.23\pm 0.22$  g/dl which were significantly ( $p<0.05$ ) higher than infected control group ( $5.48\pm 0.24$  g/dl) but comparable to that of healthy control ( $7.18\pm 0.49$  g/dl). The return of total serum protein towards normal could be due to destruction of parasitic stages by anthelmintics and thereby leading to decrease in protein consumption by these parasites as well as increase in fresh absorption of dietary proteins as also reported by Kishan and Gupta, 2004<sup>[11]</sup>.

**Table 3:** Mean $\pm$  SE total protein(g/dl) in sheep infected with GI nematodes

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Healthy control	$7.00\pm 0.60^{Ab}$	$7.05\pm 0.52^{Ab}$	$7.18\pm 0.49^{Ac}$	$6.84\pm 0.09^{Ab}$	$7.33\pm 0.32^{Ac}$
Infected control	$5.83\pm 0.15^{Aa}$	$5.65\pm 0.18^{Aa}$	$5.48\pm 0.24^{Aab}$	$5.55\pm 0.21^{Aa}$	$5.50\pm 0.18^{Aa}$
Fenbendazole	$5.59\pm 0.07^{Aa}$	$5.59\pm 0.06^{Aa}$	$5.60\pm 0.07^{Aab}$	$5.49\pm 0.12^{Aa}$	$5.54\pm 0.82^{Aa}$
Ivermectin	$6.37\pm 0.10^{Abc}$	$6.47\pm 0.08^{Abc}$	$6.58\pm 0.12^{ABcd}$	$6.77\pm 0.07^{BCb}$	$6.91\pm 0.07^{Ccd}$
Closantel	$4.62\pm 0.06^{Ac}$	$4.70\pm 0.13^{Ad}$	$4.87\pm 0.24^{Aa}$	$4.96\pm 0.30^{Ac}$	$5.05\pm 0.35^{Aa}$
Artemesia	$5.91\pm 0.16^{Aa}$	$6.09\pm 0.23^{Ac}$	$6.23\pm 0.22^{Abd}$	$6.56\pm 0.17^{Ab}$	$6.52\pm 0.19^{Ad}$

Values with same superscript in a column (small letters) and same superscript in rows (capital letters) do not vary significantly ( $p>0.05$ )

### III. Serum albumin

Results of serum albumin (g/dl) in all the treatment groups are presented in Table 4 and are in accordance with the findings of Chakraborty and Lodh (1994)<sup>[3]</sup>; Bhat *et al.*, (2004)<sup>[2]</sup> and Jas *et al.* (2008)<sup>[8]</sup>. In group fenbendazole, a non-significant ( $p>0.05$ ) increase in the serum albumin levels was observed along the course of the experiment. The serum albumin values recorded on day 28 post-treatment in fenbendazole treated group were  $2.64\pm 0.12$  g/dl and were significantly ( $p<0.05$ ) lower than group infected control and healthy control ( $3.62\pm 0.14$  g/dl and  $5.23\pm 0.22$  g/dl respectively). In group ivermectin group by day 14, the serum albumin levels recorded were  $5.33\pm 0.09$  g/dl which were significantly higher

( $p<0.05$ ) than infected control ( $3.57\pm 0.20$  g/dl) comparable to healthy control ( $4.86\pm 0.31$  g/dl). For group closantel, by day 14 post-treatment, the values recorded were  $4.52\pm 0.19$  g/dl which came to be significantly higher than infected control ( $3.75\pm 0.20$  g/dl) and also comparable to healthy control ( $4.86\pm 0.31$  g/dl). In group artemesia, a significant ( $p<0.05$ ) increase in serum albumin level was recorded by day 7 and values were  $4.73\pm 0.09$  g/dl as compared to infected control ( $3.70\pm 0.19$  g/dl). Hypoalbuminaemia as observed in present study get aggravated due to increased catabolism of albumin and protein malabsorption through damaged mucosa of intestines as reported by Tanwar and Mishra, 2001<sup>[17]</sup>.

**Table 4:** Mean $\pm$ SE albumin (g/dl) in sheep infected with GI nematodes

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Healthy control	$4.70\pm 0.43^{Ac}$	$4.72\pm 0.30^{Ab}$	$4.86\pm 0.31^{Acd}$	$5.03\pm 0.18^{Acd}$	$5.23\pm 0.22^{Ad}$
Infected control	$3.78\pm 0.19^{Ab}$	$3.70\pm 0.19^{Ab}$	$3.57\pm 0.20^{Ab}$	$3.55\pm 0.17^{Ab}$	$3.62\pm 0.14^{Ab}$
Fenbendazole	$2.66\pm 0.11^{Aa}$	$2.82\pm 0.17^{Aa}$	$2.66\pm 0.11^{Aa}$	$2.66\pm 0.11^{Aa}$	$2.64\pm 0.12^{Aa}$
Ivermectin	$5.24\pm 0.08^{Ac}$	$5.34\pm 0.83^{Ad}$	$5.33\pm 0.09^{Ac}$	$5.31\pm 0.13^{Ac}$	$5.30\pm 0.37^{Ac}$
Closantel	$4.72\pm 0.18^{Ac}$	$4.58\pm 0.19^{Ac}$	$4.52\pm 0.19^{Ac}$	$4.56\pm 0.23^{Ac}$	$4.57\pm 0.28^{Ac}$
Artemesia	$4.57\pm 0.89^{Ac}$	$4.73\pm 0.09^{Abc}$	$4.80\pm 0.12^{ABcd}$	$4.95\pm 0.14^{Bcd}$	$5.02\pm 0.12^{Bcd}$

Values with same superscript in a column (small letters) and same superscript in rows (capital letters) do not vary significantly ( $p>0.05$ ).

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