Combined Use of Herb Extract as Anthelmintic for Controlling Gastro-Intestinal Parasites and Hemoto-Biochemical Effect on Sheep

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Abstract

This study was conducted on sheep for the evaluation of anthelmintic efficacy of some selected indigenous medicinal plants comparison with synthetic anthelmintic of ivermectin (0.1

Index terms— gastrointestinal parasites, medicinal plants, pharmacokinetics, extracts, ivermectin, GIT, EPG.

1 Introduction

elminthosis is a parasitic disease of animal that are major problems of livestock production throughout the world, particularly in tropical and subtropical areas (Hussain et al., 2010). Bangladesh is an agro-based developing country of South Asia which has huge livestock population. Livestock is an important sector which plays important contribution to solve unemployment, poverty alleviation, promote human health by supplying animal protein sources with high calorie value in the forms of meat and milk and help to achieve the sustainable development goals (SDGs). But parasites hinder the growth of livestock production and it has been identified as one of the important limiting factors in small ruminant specially in sheep farming (Hussain et al., 2010). It is estimated over 90% of the endoparasitism cases in small ruminants are due to such as Haemonchus contortus and Trichostrongylus axei whose are found in the abomasums of small ruminants (Sani et al., 1990). Other most common gastrointestinal parasites are Paramphistomus spp, Gastrothillus spp, Cooperia spp in sheep **??**Eysker and Ploege, 2000). Clinically it is manifested by reduced weight, roughness hair, anaemic condition and lowered meat and milk production (Githigia et al., 2005). For controlling of helminthes a lot of chemicals have been used in most of the part of the world. Frequently use to livestock development which grow resistance against chemical anthelmintics (Papadopoulos et al., 2012). This view has renewed the interested to study of medicinal plants for the development of novel anthelmintics. Plants have been used for human benefit from time immemorial (Koehn and Carter, 2005). According to the World Health Organization **??**WHO, 2008), almost 80% of Asia's population has incorporated into their primary modality of health care by using traditional medicine, which has compounds derived from medicinal plants (Hossain et al., 2003). The use of plants as medicine is slowly increasing day by day in the world because they have minor or no side effects (Jordan et al., 2010). Bangladesh is endowed with vast resources of medicinal plants. About 5000 plant species have been estimated to be present in this country and most of them are reported be used in traditional medicines for the health care of the millions of people of this country **??**Rahman et al., 2010). Neem (Azadirachta indica) is a tropical evergreen tree native to Indian sub-continent (Girish and Bhat, 2008). The various parts of neem such as fruits, seeds, leaves, bark and roots are used as antiseptic, anthelmintic, antibacterial, antiviral, antiulcer and antifungal, insecticides, pesticides and agrochemicals (Brahmachari, 2004). It has been recommended for using against gastro-intestinal nematodes and related problems in many parts of the world (Biswas et al., 2002;Subapriya and Nagini, 2005). Bitter gourd (Momordica charantia) is a traditional medicine of India sub-continent are used to relieve diabetes, as a stomachic, laxative, emetic, anthelmintic agent, for the treatment of cough, respiratory diseases, hyperglycemia, increasing milk flow, intestinal parasites, jaundice, kidney stones (Sampath and Bhowmik, 2010).

Clove (Eugeniu caryophyllus) used as carminative and to increase hydrochloric acid in the stomach that improve peristalsis (Chaieb et al., 2007). Clove has been used a natural anthelmintic digestive stimulant (Patil et al.,

2014). A large number of chemical anthelmintics are now available but most of them are expensive, anthelmintic resistance, high price value and adverse effects (Hannan et al., 2003). The multiple drug resistance not only increases morbidity and mortality but also increase expenditure and prevention and control of parasitic diseases are becoming very difficult day by day. In Bangladesh very limited research works have been conducted on the use of medicinal plants as anthelmintic. This present study was considered with the following objectives i) To evaluate the in vivo anthelmintic efficacy from Azadirachta indica, Momordica charantia and Eugeniu caryophyllus against GIT parasites in sheep. ii) To find out the combine in vivo efficacy at different concentration from methanol and aqueous treated extract. iii) To evaluate the effects of herb extracts on animal body by analysis the heamatological (Hb, PVC, ESR, TEC TLC and DLC) and biochemical (AST, ALT and creatinine) parameters.

2 II.

3 Materials and Methods

4 a) Study area, study period and study design

The study area was included the sheep farm, a small gabble type farm housing during July to December 2015. An intervention study was conducted on in-vivo screening of herbs extract by using the three indigenous medicinal plants (Neem, Bitter gourd and clove) against gastrointestinal parasites in sheep.

5 b) Collection and processing of plant materials

Fresh leaves of neem (Azadirchta indica) Bitter gourd (Momordica charantia) fruits and dry clove (Eugeniu caryophyllus) were collected from the local area. Neem and bitter gourd washed thoroughly into running tap water to ensure removing of extraneous dusts materials **??**Sujan et al., 2008). Then cut into small pieces and taken a plastic jar. Then perform air-dried and finally sun dried for 3 days on the roof by covering a piece of cloth as prevention oxidation such as antioxidants and others chemical components (Amin et al., 2009). Clove was cleaned and be prepared for use.

Dust was prepared from the dried leaves by using blender, mortar and pestle. Dried bitter gourd and clove dust was prepared with the help of a blender (Sujan et al., 2008). A 25-mesh diameter seize was prepared to obtain fine dust and were preserved them into air-tight plastic container until being used (Amin et al., 2009).

6 c) Preparation of Crude methanol extract (CME)

Crude methanol extract (CME) was prepared from the selected three medicinal herbs according to the standard herb extraction methods (Gilani et al., 2004). Ten (10) gm of each category of dusts were taken into a 500ml beaker and separately mixed with 100ml 70% aqueous methanol. Then the mixtures were stirred for 30 minute by a magnetic stirrer (6000 rpm) and left as such for next 24 hrs (Amin et al., 2009). The extracts were filtered through a fine cloth and final filtration was done through filter paper (Whatman No. 1) (Hussain et al., 2010). Evaporation of water from filtrate by using a vacuum rotary evaporator at 50 0 C till it reached the final volume of 10 ml (Amin et al., 2009).

Stored in a refrigerator in air tightly corked-labeled bottle at 4 0 C temperature until use (Hussain et al., 2010).

7 d) Preparation of Crude aqueous extract (CAE)

Crude aqueous extract (CAE) was prepared by using the selected herbs according to the standard herb extraction methods (Gilani et al., 2004). Half kilogram (kg) of each two category (neem and bitter gourd) plants parts and 250 gm of clove were taken separately and washed thoroughly in the running tape water. Each sample was dried in room temperature at 30 minutes and then bitter gourd was cut into small pieces. Then 50 gm of neem leaves was taken in blender's plastic pocket and mixed with 300 ml distilled water and prepared juice **??**Anonymous, 1996). Then the juice was filtered through a fine piece of porous cloth and final filtration was done by using the filter paper (Whatman No. 1) (Amin et al., 2009). The juice performed evaporation by using evaporator at 50 0 C till it reached the final volume of 10 ml as condense form. Stored in air tightly corked-labeled bottle at 4 0 C temperature in a refrigerator until use (Hussain et al., 2010). f) Herbal anthelmintic dose Herbal anthelmintic dose was prepared for in vivo screening by adding required amount of distilled water after weighting stock solution (Amin et al., 2009). For in vivo screening combine herbal anthelmintic dose was given 1 ml/kg (100mg/ml) body weight for this study.

8 g) Sampling Strategy

A total number of 33 sheep of both sexes (male and female) and different age (6-24 month) were selected by taking interview with the help of prepared questionnaire. Highly infected (>840 EPG) sixteen (16) sheep were used for this present study. The sheep were divided into four (4) groups; each group was consisted of four (4) populations with the mean EPG are 947.5, 918.7, 923.7 and 911.5 for group A, B, C and D, respectively. Group A was represented as infected control group and B, C and D were treated groups.

9 h) Treatment intervention, Dose and Dosing

This study was investigated the herbs extracts dose was 1 ml/kg body weight at the concentration of 100 mg/ml (Amin et al., 2009). Ivermectin (1%) was used at 0.2 mg/kg body weight at sub cuteneous route in group B. 1 ml/ kg (100mg/ml) body weight was used as herbal anthelmintic doses in group C and D on day 0 and 7.

10 i) In vivo screening of plant extracts for anthelmintic efficacy

Oral administration of crude aqueous extract (CAE) and crude methanol extract (CME) at 1 mg/kg were performed and compared with ivermectin (Acimec ® -ACI Pharmaceuticals Ltd.) on day 0, 7, 14, 21 and 28 by McMaster egg counting technique. The efficacy of different treatment was determined by faecal egg count reduction test. The effect of herbs extracts on animal body specially circulatory and visceral organs effects were determined by analysising the haematobiochemical parameters.

11 j) Collection, preservation and transportation of samples

Faecal and blood samples were collected from each sheep at day 0, 7, 14, 21 and 28 of the pre and post treatment period. Fresh eight gm fecal samples were collected from rectum in the morning before they are fed and then put the samples immediately into a sterile container containing six ml formalin. Blood samples were collected from jugular vein of each sheep and four ml blood placed into vacutainer tube, containing ethylene diamine tetra-acetic acid (EDTA) and four (4) ml placed in another vacutainer tube without containing EDTA. Samples were then being immediately transferred by transport media to laboratory through ice eskie and stored temporarily in refrigerator before laboratory evaluation.

12 k) Examination of fecal samples for parasitic egg count

In each case, three gm of fresh faeces was accurately weighed and mixed in 42ml of saturated salt solution (Sodium chloride-400gm, water-10000ml; specific gravity-1.2) while the number of eggs per gram of faeces was obtained by multiplying the total number of eggs counted in the two squares of the counting chambers of the McMaster slide by the dilution factor of 50. Externeous particles were removed and residue was left pass through. Homogenus distribution was performed by well stirring. McMaster slide was filled by using a Pasteur pipette and remove the bubbles. Then second counting chamber was filled in the same way. Then egg floated up and sticks to the cover glass. Characteristics of eggs were identified using standard parasitological criteria described by Soulsby (1986). Then egg was counted by using microscope at low magnification.

13 l) Determination of the drug efficacy

During the pre and post-treatment period EPG and clinical performance were monitored. Faeces were examined on day 0, 7, 14, 21 and 28 of post-treatment period. Efficacy of the drug was calculated as per described formula by Moskey and Harwood (1941). EDTA containing blood samples were used to determine the haematological parameters such as Hb, TEC, TLC and DLC with the help of microscope at day 0, 7, 14 and 28 during the treatment period.

14 n) Evaluation of biochemical parameters

The activities of biochemical parameters like as AST, ALT and creatinine concentration were determined at day 0 and 7, 14 and 28 of post treatment. Serum was separated by centrifugation at 3000 rpm for 15 minurtes. The separated serum was used for the estimation of biochemical parameters. AST and ALT activity was determined according to the method described by Reitman and Frankel **??**1957). Creatinine was determined by the method described by Husdan and Rapoport (1968). The efficacy was observed and compared with the control group A (non-treated) and group B with C and D groups. The efficacy of group C and D was determined at the concentration of 100 mg/ml. Efficacy of ivermectin and herbs extract was considered based on declination of EPG count. The average EPG loads per gm faeces sample were 947.5, 918.7, 923.7 and 911.5 in the group A, B, C and D, respectively on day 0 of the pre-treatment. The EPG load were reduced in different post-treatment period and reached 109 (89 % reduction), 130.7 (86 % reduction) and 84 (90.7 % reduction) for group B, C and D, respectively on day 28, compared to the results obtained at day 0. Highly significant differences (p?0.01) were observed among the treated groups. The highest reduction of EPG was observed on day 28 irrespective of treatment groups (Table-1). Conversely, in the control group, the EPG load sharply increased, ranging from 947.5 at day 0 to 1572.5 at day 28 but the differences were not significantly differed (p?0.05). Each group consists of four sheep. SE= Standard error; * = significant differences (p?0.05); **= highly significant differences (p?0.01)

The maximum reduction rate was observed in Crude methanol extract (90.7 % reduction where the ivermectin treated group (89 % reduction) and crude aqueous extracts (86 % reduction).

15 b) Effects on haematological parameters

The Hb (gm/dl) in untreated control group it decreased from 8.4 at day 0 to 6.1 at day 28 posttreatment. The Hb contents were increased from 7.8 at day 0 to 8.6 at day 28, 7.9 at day 0 to 8.4 at day 28 and 8.2 at day 0 to 9.2 at day 28 in ivermectin, CAE and CME treated groups, respectively. The PCV contents were increased

from 28.2 at day 0 to 34.2 at day 28, 29.2 at day 0 to 36.6 at day 28 and 28.6 at day 0 to 35.2 at day 28 in ivermectin, CAE and CME treated groups, respectively. The PCV of the untreated control group reduced significantly (p?0.01) in different interval of the post-treatment, compared to 32.8 at the day 0, 23.2 at the day 28 . The mean values of ESR (mm/1st hr) were 0.4, 0.7, 0.5 and 0.5 for group A, B, C and D, respectively at day 0. TEC levels increased among the anthelmintic treated groups and reached from 6.8 at day 0 to 11.4 at day 28, 6.2 at day 0 to 9.2 at day 28 and 7.2 at day 0 to 10.8 at day 28, across the study period in ivermectin, CAE and CME treated groups (Table-2), correspondingly but the variation was not significant (p?0.05). The mean value of TLC content decreased from 7.3 at day 0 to 5.4 at day 28. The TEC levels increased among the treated groups and reached from 6.2 at day 0 to 10.1 at day 28, 7.4 at day 0 to 9.6 at day 28 and 6.2 in day 0 to 8.5 at day 28 in ivermectin, CAE and CME treated groups, respectively.

16 c) Effects on differential lymphocyte count

The mean values of lymphocyte (%) were reduced in different post-treatment period and reached from 66.2, 65.2 and 63.2 at the day 0 to 51.7, 52.7 and 52.5 for group B, C and D, respectively on day 28 of post-treatment (Table 3). The average values of neutrophil (%) of sheep were 36.5, 36.7 and 36 at the day 0 and reached 26.75, 28.5 and 29.2 on day 28 of post-treatment of group B, C and D (Table 3). Highly significant differences (p?0.05) were observed among treated groups. The average values of monocyte (%) of sheep were 1.5, 1.2 and 1.5 at the day 0 and reached 2.5, 2.2 and 2.5 on day 28 of post-treatment of group B, C and D (Table 3). Highly significant differences (p?0.05) were observed among treated groups across the study period, compared to day 0. Conversely, in control group, the values of monocyte increased, ranging from 2.2 at day 0 to 0.2 at day 28. The eosinophil contents were decreased from 7 at day 0 to 5.7 at day 28, 6.7 at day 0 to 6 at day 28 and 7.2 at day 0 to 6.25 at day 28 in ivermectin, CAE and CME treated groups, respectively (Table 3). The eosinophil percentage of untreated control group increased significantly (p?0.05) 8.2 at day 28, compared to 6.2day 0. The basophil contents were decreased from 0.5 at day 0 to 0.2 at day 28, 0.5 at day 0 to 0.2 at day 28 and 0.5 at day 0 to 0.2 at day 28 in the ivermectin, CAE and CME treated groups. The basophil of the untreated control group declined from 0.7 to 0 on day 28.

17 d) Effects on biochemical parameters

The AST (U/L), ALT (U/L) and creatinine (mg/dl) values were differentiated among the treated and control groups. The levels of AST, ALT and creatinine varied significantly (p?0.01) at different observational periods within the ivermectin, CAE and CAME treated groups. The result recommended that the AST, ALT and creatinine level decreased significantly in ivermectin, CAE and CAME treated groups on days 7, 14, 21 and 28 compared to day 0 (Table 4). The levels of AST, ALT and creatinine also varied significantly (p?0.01) among the groups on days 7, 14, 21 and 28. The values of AST, ALT and creatinine were significantly lower in the treatment groups than in the untreated group across the study period.

18 Discussion

Efficacy was founded on the basis of reduction of EPG count in comparison with the control and ivermectin treated group with other group on the day o to 28 day. The efficacy of neem, Bitter gourd and clove at the form of crude aqueous and methanol treated Extract against parasitic infestation in sheep was satisfactory level which was determinated by iv vitro and in vivo anthelmintic activity. The present study showed higher efficacy at the concentration of 100 mg/ml than the concentration of 25 mg/ml and 50 mg/ml. The anthelmintic efficacy was compared with corresponded studies Bhalke et al., (2011); Sujon et **??**011), after oral administration were observed and compared with the ivermectin. Efficacies of drug and herbs extract were considered based on declination of EPG count. In the study the maximum reduction level of ivermectin treated group (89 %) efficacy was observed which was close to the following author activites. The result is also consistent with Sujon et al., (2008) and Jaiswal et al., (2013) who found efficacy of Ivermectin and neem 94% and 81%, respectively. The maximum EPG reduction rate was observed in aqueous treated extracts 86 % reduction at the concentration of 100 mg/ml by oral administration of 1 ml per kg body weight. The control group A where the EPG count increased from 947.5 at the day 0 to 1572.5 at the day 28. On the other hands the maximum EPG reduction rate was observed in methanol treated extracts 88.6 % reduction at the concentration 100 mg/ml by oral administration of 1 ml per kg body weight. Costa et al., (2008) The hematological parameters were analyzed on the comparison with control and treated groups (Table 2). The following investigated blood parameters such as PCV, Hb, TEC and TLC were improved significantly in parasites affected sheep with the anthelmintic (ivermectin) and selected herbs extracts treatment. Due to reduction of blood-sucking parasites (Haemonchus spp) and other gastrointestinal parasites infections in sheep the blood parameters such as Hb, PCV, TEC and TLC increasing day by day. The ESR percentages were decreased in control group due to blood cell destruction by comparison on the day 0 to day 28 (Table 2). The effectiveness of herbs extracts both aqueous and methanol treated indicated the stimulatory effect on the hemopoietic system. The rise in mean PCV after treatment might be associated with the increase of Hb%, as these parameters are closely interrelated with each other. The improvement of blood PCV, Hb, TEC and TLC levels in the treated sheep might be due to the elimination of external and internal parasites, which was expected. Similar kinds of improvement of these blood parameters after anthelmintic treatment have been

previously reported in ??2013). The effects of herbs extracts as anthelmintic in animal body were indicated that the Eosinophil, basophil were decresed on the day 0 to on the day 28. On the other hand the monocytes count was increased day by day. In this study has showed the eosinophil and basophil were decresed and monocyte levels were increased which indiacates that the herbs extracts have effectiveness against gastrointestinal parasites in sheep. The percentages of eosinophil, basophil were decresed and monocytes were increased after post-treatment in parasitic infections reported by (Aruwayo et al., 2011). Similarly, Biu et al., (2009) reported that the mean values for monocytes, basophils and eosinophils increased significantly with increasing dose of anthelmintics while mean values for lymphocytes and neutrophil decreased significantly.

19 b) Effects on biochemical parameters

Effect of herbs extracts on animal body in the levels of AST, ALT and creatinine in anthelmintic treated groups decreased, which indicates the removal of parasites from the affected sheep. Furthermore, it indicates that treatment with ivermectin, aqueous and methanol treated extracts are not toxic to the liver and kidney. By external palpation of liver and kidney in sheep are normal in size and shape. No debilitating lesion was founded on the liver. These results are in near similar with earlier reports (Sokumbi and Egbunike, 2000;Gupta et al.,2005).

V.

20 Conclusion

Efficacy of herb extracts and drugs were measured in vitro and in vivo after the preparation and use of various concentration viz. 25 mg/ml, 50 mg/ml and 100 mg/ml of crude aqueous extract (CAE) and crude methanol extract (CME). In vitro screening the anthelmintic efficacy (96.6%) of methanol extract at the concentration of 100 mg/ml was higher than the aqueous extracts (86.6%). Highly parasitic infested sheep (16) age between 6 to 24 months were selected based on EPG count (>840 EPG) indicating anemic condition. In vivo screening of aqueous extracts and methanol extract at the concentration of 100mg/ml were reasonably effective 86 % and 88.6 % reduction of EPG. By hemato-biochemical parameters analysis the percentages of eosinophil and basophil were decrease which indicates reduction of endoparasites and correction of anemia. Therefore, these herbs can be used as alternatives to conventional anthelmintic and this could reduce the unnecessary use of conventional drugs which make parasites more resistant to the drugs. [1] [2] [3] [4] [5]

1

Group	Treatment	Pre-treatment		Post-treatment		
		Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day (Mean±SE)
A	Control	947.5±26.2	990.5±19.8	1162.5±33	1442±26.8	1572.5±22.1
B	Ivermectin	918.7±27	586.5±20.5 (36 %)	294.2±26.1* (66.8 %)	157±15.7** (82.8 %)	109±11.6** (89 %)
C	Crude aqueous extracts (CAE)	923.7±18.8	505±26.4 (45.2 %)	306.5±17.2 (66.8 %)	236.7±34** (74.4 %)	130.7±17.1* (86 %)
D	Crude methanol extract (CME)	911.5±29.3	454.5±24.3 (50.1 %)	279.5±27.5** (69.3 %)	157±19.1** (82.7%)	84±6.9** (90.7 %)

Figure 1: Table 1 :

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[4] Combined Use of Herb Extract as Anthelmintic for Controlling Gastro-Intestinal Parasites and Hemoto-Biochemical Effect on Sheep

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		ESR, TEC and TLC				
		Pre-treatment		Post -treatment		
Treatment	Parameters	Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean
	Hb	8.4±0.7	8.14±0.5**	8.06±0.4**	7.84±0.3*	6.11±0
	PCV	32.4±01.2	29.8±0.67**	27.4±0.5 **	25.2±0.6**	23.2±0
Control	ESR	0.7±0.2	0.1± 0.1*	0.1±0.2*	0.1±0.1*	0±0*
	TEC	7.90±0.3	7.45±0.3**	6.84±0.1**	6.67±0.3**	6.24±0
	TLC	7.29±0.5	6.95±0.5**	6.44±0.5**	5.72±0.5**	5.34±0
	Hb	7.8±0.7	7.78±0.6	7.8±0.5	8.2±0.3	8.6±0.
	PCV	28.2±01.4	29±1.6	30.8±1.2	33.2±1.4	34.2±1
Ivermectin	ESR	0.7±0.2	0.1± 0.1*	0.1±0.1*	0.1±0.2*	0±0*
	TEC	6.82±0.6	7.87±0.7	8.97±0.7	9.84±0.6	11.41±
	TLC	6.22±0.6	7.16±0.6	8.28±0.7	9.02±0.6	10.07±
	Hb	7.9±0.2	7.48±0.3	7.34±0.2	7.9±0.2	8.4±0.
Crude aqueous	PCV	29.2±01.2	29.8±1.1	31.2±1.2	33.4±0.9	36.6±0
extracts (CAE)	ESR	0.5±0.2	0.3±0.2	0.2±0.1*	0.1± 0.1*	0±0*
	TEC	6.17±0.3	6.75±0.3	7.57±0.4	8.31±0.5	9.20±0
	TLC	7.4±0.3	7.94±0.3	8.57±0.3	9.11±0.3	9.64±0
	Hb	8.4±0.7	8.5±0.7	8.6±0.6	8.9±0.4	9.2±0.
Crude	PCV	28.6±1.5	30.2±1.2	32.6±1.3	34.4±01.1	35.4±1
methanol	ESR	0.5±0.2	0.2±0.1*	0.2±0.1*	0.1± 0.1*	0±0*
extract	TEC	7.16±0.5	7.88±0.4	8.92±0.4	9.97±0.2	10.84±
(CME)	TLC	6.23±0.7	6.8±0.7	7.53±0.7	8.01±0.6	8.53±0

Each group consists of four sheep.
SE= Standard error; * = significant differences (p?0.05); **= highly significant differences (p?0.01)

Figure 2: Table 2 :

3

Treatment	Parameters	Pre-treatment Day 0 (Mean±SE)	Day 7 (Mean±SE)	Post -treatment Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean±SE)
	Lymphocyte	63±1.4	63.7±0.9	65.7±0.9	68.7±0.9	70.5±1.2
	Neutrophil	34.5±1.9	36±1.4	38.7±1.5	41.5±2.2	44.2±1.7
Control	Monocyte	2.25±0.5	1.5±0.5*	0.7±0.5**	0.5±0.5**	0.2±0.5**
	Eosinophil	6.2±0.9	6.5±0.5	6.5±0.5	7±0.8**	8.2±0.5**
	Basophil	0.7±0.6	0.5±0.5	0.2±0.5	0±0	0±0
	Lymphocyte	66.2±2.0	63.2±0.9*	61.2±0.9**	56.2±1.7**	51.7±1.7**
	Neutrophil	36.5±1.2	34.7±0.9	33.75±0.5**	30±1.6**	26.7±0.9**
Ivermectin	Monocyte	1.5±0.5	0.7±0.5	1±0.8	1.5±0.5	2.5±0.5
	Eosinophil	7±1.1	6.7±0.9	6.5±0.5	6±0.8	5.7±0.9
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0.5±0.5	0.2±0.5
Crude	Lymphocyte	65.2±2.7	63.7±1.7	60.7±1.8*	55±2.5*	52.7±.95**
aque-	Neutrophil	36.7±1.7	35.2±.55*	33.5±.57**	31.7±1.5**	28.5±2.6**
ous	Monocyte	1.25±0.5	0.8±0.5	1±0.8 6±1.1	1.7±0.5	2.2±0.5
ex-	Eosinophil	6.7±0.9	6.5±0.5		6.25±1.2	6±0.8
tracts (CAE)						
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0±0	0.2±0.5
	Lymphocyte	63.2±.9	62.5±1.7	60.1±1.9	56±1.6	52.5±1.2
Crude	Neutrophil	36±1.6	34.5±1.2	33±1.1	31±1.5*	29.2±0.9*
methanol	Monocyte	1.5±0.5	0.7±0.9**	1±0.8**	1.5±1.1**	2.5±0.5**
extract	Eosinophil	7.2±0.9	6.7±0.1	6.5±1.7	6.25±1.2	6.25±0.9
(CME)						
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0±0	0.2±0.5

Each group consists of four sheep.

*[Note: SE= Standard error; * = significant differences (p?0.05); **= highly significant differences (p?0.01)]*

Figure 3: Table 3 :

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	Treatment Control	Parameters	Pre-treatment	Day 7 (Mean±SE)	Post -Day 14 (Mean±SE)	99.5±6.1 24
		AST	Day 0	95.7±5.4		
		ALT	(Mean±SE)	22.9±1.4		
		Creatinine	92.9±5.2	1.7±0.1		
		AST	21.7±1.5	96.6±6.4**		
			1.6±0.1			
			99.4±6.7			
	Ivermectin	ALT	24.5±2.1	22.1±2.0**	19.8±1.7**	17.6±1.6**
		Creatinine	1.7±0.1	1.3±0.1**	1±0.1**	0.8±0.1**
	Crude aqueous extracts (CAE)	AST	90.9±3.1	87.4±2.7*	84.4±3.0*	80.6±2.7**
		ALT	24.5±1.4	23.3±1.3**	21.1±1.0**	19.8±0.8**
		Creatinine	1.8±0.1	1.5±0.2**	1.2±0.1**	1.0±0.2**
	Crude methanol extract (CME)	AST	95.7±9.4	92.7±9.5**	89.5±8.8**	86.5±8.4**
		ALT	23.7±2.9	21.7±2.5**	19.5±2.2**	18.3±2.2**
		Creatinine	1.8±0.0	1.6±0.1*	1.4±0.1**	1.1±0.1**

Each group consists of four sheep.
SE= Standard error; * = significant differences (p?0.05); **= highly significant differences (p?0.01)

[Note: GIV.]

Figure 4: Table 4 :

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