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Effect of Garlic (*allium sativum*) on Gastrointestinal Nematodes of Sheep in Sodo Zuria District, Southern Ethiopia

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Abstract

Garlic is a naturally health promoter. It has been traditionally used as animal and human phyto-thearapeutics since ancient time. This study was aimed at investigating the effectiveness of garlic against the gastrointestinal nematodes of sheep and assesing adverse garlic reactions in treated animals. Thirty sheep, naturally infested by parasites were randomly selected and allocated into three treatment groups. Each group consisted ten sheep. Group A animals were treated with albendazole 300 mg bolus (Chengdu Qiankun Veterinary Pharmaceuticals co., Ltd. China) as positive control. Group B animals were treated with nine gram garlic powder suspended in 100 ml tap water each for four consecutive days and Group C animals were given 100 ml tap water only for four consecutive days as negative control. T-test was employed to compare pre-and post-treatment results. Fecal egg count (FEC) and fecal egg count reduction (FECR) test were done to determine efficacy of garlic against gastrointestinal nematodes of sheep. Further pilot experiment clinical examination, blood parameters and enzyme analyses were carried out to assess adverse effect of garlic in the treated animals. The respiratory rate of all groups of animals as well as pre-and post-treatment was normal (20 to 31 beats /minute). However, the mucus membranes were changed to normal after treatment. The FEC in sheep of group A was significantly reduced ($p = 0.000$) after albendazole administration (1850.00 ± 350.397 versus 122.22 ± 44.096). Similarly, the FEC of sheep in group B treated with garlic was significantly decreased ($p = 0.000$) at 14^{th} day after treatment (1810.00 ± 360.401 versus 128.57± 48.795). In contrary FEC of the negative control sheep in group C did not show an improvement during the study period ($p = 0.564$) (1890.00 ± 430.633 versus 1950.00 ± 527.573). The FECR in sheep treated with albendazole was found to be 94% while in sheep treated with garlic was 95%. There was a significant increase ($p = 0.000$) of RBC, Hgb and HCT at 14^{th} day after treatment with garlic (12.730 ± 2.370 $x10^{12}$/L versus 6.441 ± 0.946 $x10^{12}$/L, 14.580 ± 3.591 mg/dL versus 8.160 ± 0.583 mg/dL, 42.110 ± 6.882% versus 22.870 ± 1.574%, respectively). Whereas the mean WBC count was significantly higher ($p = 0.000$) before treatment than post-treatment (15.240 ± 2.250 $x10^{9}$/L versus 10.510 ± 1.038 $x10^{9}$/L). Blood levels of liver enzymes AST, ALT and ALP were significantly reduced ($p = 0.000$) at 14^{th} day post-treatment with garlic. Therefore, aqueous suspension of garlic powder was effective in the treatment of gastrointestinal nematodes of sheep. Garlic has also no adverse effect on the health of recipient sheep at the given dose and frequency.

Keywords: Effect; Egg count; Feces; Garlic; Gastrointestinal nematode; Sheep

Introduction

Sheep are integral to the livestock production systems in the highlands where crop-livestock mixed agriculture is practiced and in the pastoral and agro-pastoral livestock production in Ethiopia. They have enormous share in economy of the country as they provide a major source of income especially to economically marginal farmers. However, according to [1, 2, 3, 4], benefits obtained from sheep to date do not match their tremendous potential. Significant losses result each year from the decreased production and death of sheep as a result of mainly health problems. Several studies in different parts of Ethiopia have shown that gastro-intestinal parasites are major problems in small ruminants by causing morbidity, mortality and production losses [5, 6]. Dabasa *et al*. (2017) [7], recently confirmed that gastrointestinal (GIT) nematodes still belong among the most important diseases of sheep.

Infestation with GIT nematodes is complex and produces an inflammatory enteropathy which results in mal-absorption and impaired intestinal motility and microcirculation [8, 9, 10, 11]. They also expose their hosts to sever diseases like septicemia and endotoxemia. The animals suffer from loss of appetite, weight loss, intermittent diarrhea, lethargy, deterioration of condition and anemia [8, 12]. They can cause mechanical damage and inflammation in the liver, pancreas and peritoneal cavity. The a fore mentioned and other effects of GIT nematodes prevent sheep from playing their significant socioeconomic role in mitigating food insecurity and poverty in households and their broad community. The diverse agro-climatic conditions, backward animal husbandry practices, communal grazing system, environmental and socio-economic conditions of the country are highly conducive for the development, maintenance and continuous transmission of helminth infestation throughout the year. Furthermore, sheep graze closer to the ground. This further increases their susceptibility to the gastrointestinal parasites more than other type of livestock [13].

Sheep should be in excellent health status for high performance. But, it is barely possible for grazing sheep to be parasite free unless strategic control measures are developed and accordingly implemented. Nematodes are strategically controlled with the help of chemical anthelmintics. However, the widespread dependence and frequent use of chemical anthelmintics has led to the development of anthelmintic resistance [14]. Increased levels of resistance to benzimidazoles and pyrantel being reported worldwide [15]. In addition, a recent multinational study performed in the United Kingdom, Germany and Italy reported ivermectin resistance on 3% of studied farms with one farm having apparent resistance to all three drug classes [16]. Further, synthetic anthelmintics tend to leave residues in meat and milk. They are also expensive. The expensiveness of anthelmentic drugs for the strategic control of parasites, development of resistance and as the result uncertainty of their future perspective have stimulated the search for alternative anthelmintics, for which medicinal plants have been chosen [17, 18, 19].

Usage of medicinal plants to treat animal ailments has a long history in Ethiopia. About eighty percent of Ethiopians depend on medicinal plants for primary health care [20, 21]. Although the contribution of traditional medicinal plants in animal health care of the poor animal keeping society who live mainly in the rural area is very high, very little research has examined the medicinal plants as remedies. However, it is evident that the traditional veterinary medicinal knowledge of animal health has been playing significant role in the husbandry of livestock on pastoral, agro pastoral and small holder animal farms [22].

The various climatic and topographic conditions of Ethiopia contribute to a rich biological diversity. Ethiopia is believed to be home for about more than 6500 species of higher medicinal plants with approximately 12 % endemism [22]. Many rural animal farmers including some of the urban animal keepers in the country are still relying on traditional animal health care system to

address myriad animal ailments. World Health Organization (WHO) (2013) [23], confirmed that traditional and complementary medicine continues to be widely used in most countries, and these practices are increasing in prevalence in others. It is estimated that nearly 80% of the earth's inhabitants still rely on ethnomedicine [24], and 80-90% of humans were thought to rely on ethnoveterinary medicine for livestock health care [17].

According to Mathias *et al*. (1996), Inhorn and Wentzell (2012), and Witeska-Mynarczyk (2015) [17, 18, 19], the soaring prices of modern veterinary drugs and technologies and development of resistance against anthelmintics have led to greater interest in continuous acceptance of medicinal plants. Other additional reasons for sustainable acceptance of medicinal plants are that medicinal plant remedies are practical in use, effective and cheap. They rely on easily accessible local plants and reflect centuries of experience of application [25]. Also, the advent of organic farming in the developed countries of the world and the resultant push for the use of more environmental friendly and humane methods of raising animals gives a lot of hope for the growing utilization of traditional knowledge and medicinal plants for the treatment of animal diseases [26]. The larger traditional treatments based on medicinal plants prepared and administered according to time-tested prescriptions and regimes tend to be environmentally more benign than their synthetic commercial equivalents. They pose less danger of seriously polluting local water supplies, lands, or animal based food stuffs [17].

On the other hand, traditional use of medicinal plants has drawbacks like; imprecise dosages, unhygienic preparation and administration methods. The degree of their effectiveness against various pathogenic micro-organisms is not also experimentally confirmed. Thus, scientific based evidences on the efficacy and safety of traditional medicinal plants before using them for therapeutic purposes must be provided by research results. This is what the modern health professionals and some of the consumers ask for. Research on medicinal plants should be directed towards considering their degree of effectiveness, quality control and examining active herbal constitute for efficacy and toxicity of the herbs [27].

Livestock keepers utilize a variety of medicinal plants to treat or prevent animal illness on the farm. Many of these medicines are also utilized in humans for similar conditions; however some therapies appear to be used exclusively and/or uniquely in animals. Very little information was available on the effect of garlic (*Allium sativum*) on GIT nematodes of sheep except that it has been used as anthelmintics in humans for decades [28, 29]. Therefore, the objective of this study was:

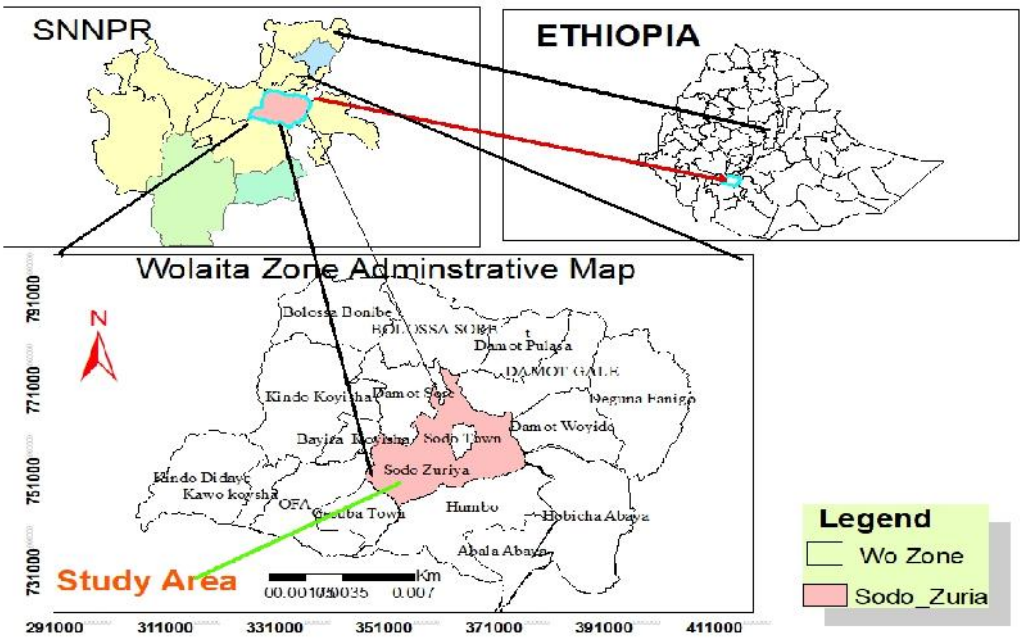
- To investigate the effectiveness of garlic against gastrointestinal nematodes of sheep
- To assess adverse effects of garlic on treated sheep

Materials and Methods

Study Area

The study area is Sodo zuria district. It is one of the 12 districts in the Wolaita zone, Southern Nations, Nationalities, and Peoples' Region of Ethiopia. It is bordered on the Southwest by Offa, on the West by Kindo Koysha, on the Northwest by Damot Sore, on the North by Boloso Sore, on the Northeast by Damot Gale, on the East by Damot Weyde, and on the Southeast by Humbo. It is located at 156 km South west of the Hawassa town which is the capital of Southern Regional State and 330 km from Addis Ababa the capital city in the Southern direction, through Hosanna road. Soddo zuria district is located between 6.72 to 6.99 longitudes to the East and 37.59 to 37.86 latitudes to the North. The district covers 38,040.8 hectare of land and it has two agro ecological zones, namely highland *Dega* 10%, *Woynadega* 90%, characterized by 81% plain, 13% undulated and 6% mountainous topography. Its altitude ranges from 1500-2950 meters above sea level. The nature of rainfall is bimodal with mean annual rain fall of 1201-1600 mm. The short rain ('*Belg*') starts from February to April and the long

rainy season (*Meher*) is from June to September. The mean annual temperature and humidity on average range from 17°C to 19°C and 50% to 60%, respectively [30]. The livestock species include cattle, sheep, goats, horse, mule, donkey, and poultry. The total population of sheep in Sodo zuria district is 35, 873. The vegetation in the area is mainly wooded bush land 31].



Source: WZFEDD, (2018) [32]

Figure 2: Map of the study area

Study Design

This study was continuation of the cross-sectional study conducted to estimate prevalence of gastrointestinal helminths of sheep in selected districts of Wolaita zone, Ethiopia. A longitudinal experimental study was performed on sheep naturally infested by gastrointestinal parasites to investigate the effect of garlic (*Allium sativum*) on the gastrointestinal nematodes of sheep.

Sampling Method and Sample Size

Thirty sheep were selected purposively based on their treatment history and fecal egg count criteria from the sheep on which the cross-sectional study was conducted to assess prevalence of gastrointestinal nematodes then simple random selection method was used for this experiment to divide thirty sheep into three experimental groups. The experimental animals were selected from the sheep fulfilling the following criteria: fecal egg count above 1000, willingness of the farmers to involve their sheep in the experiment (The owners were informed of the nature of the experiment and the possible negative impact on their animal due to the experiment), sheep that had not received any anthelmintic in the previous 12 weeks, and living in the same area under similar management and nutrition.

Experimental Animals and Treatment Groups

The experimental sheep were kept under extensive farming system and maintained on communal grazing land with access to the same watering points. The animals were fed crop

residues like teff, barley and wheat straw, in addition to communal grazing. Ages of the study animals were determined either by asking the owner or based on dentition by eruption pattern as per the method described by Steele (1996). The study animals were between two and four years of age and their body weight was estimated to be between 25 and 30 kg. They were randomly allocated into three groups, GA, GB, and GC. Each group contained 10 animals. Animals in group A (GA) were given 300 mg albendazole bolus (Chengdu Qiankun Veterinary Pharmaceuticals co., Ltd. China) with bowling gun at the dose of 7.5mg /kg. Each animal in group B (GB) was drenched with nine gram of garlic powder mixed in 100 ml tap water with drenching gun for four consecutive days. Animals in group C (GC) were control group and were drenched with only 100 ml tap water for four consecutive days. The animals were painted with different permanent ink and accordingly given code numbers to identify them individually and their groups.

Preparation of garlic for the treatment

Hard-neck silver skin type of garlic was bought from Sodo town market. After removing the bulb wrapper of each clove, the garlic was allowed to dry on a clean dry utensil in the laboratory at room temperature for 14 days. Then, it was ground by a small mill (Nima, Model: NM-8300 electric grinder, Japan) bought for this purpose. The powder of dried garlic was kept in a dry clean firmly closed glass container at room temperature until it is used for the experimental treatment.

Clinical examination of the experimental animals

Clinical examination of the experimental animals was performed in order to investigate whether the experimental animals show signs of toxicity of garlic or not. History taking and physical examination of the experimental animals was carried out before the treatment and after the treatment just before sample collection. After history taking, the animals were inspected from a distance to evaluate their behavior, posture, gate, and presence of diarrhea, salivation, nasal and/or ocular discharges. Then, they were physically examined to determine their respiratory rate by auscultating over the trachea, body temperature by taking rectal temperature with digital thermometer, pulse rate by palpating femoral artery, visible mucus membrane, capillary refill time, and rumen motility just before the start of medication and at the 7^{th}, 10^{th},and 14^{th} days after treatment. The results were recorded in a format prepared for this purpose.

Fecal sample collection and transportation

About 30 g of fecal sample was collected with surgical glove directly from the rectum of each study animal. To prevent cross contamination, one glove was used only for one animal at each fecal collection. The collected fecal sample was placed in a clean and dry screw cupped universal bottle. Each specimen was labeled with the code numbers given to the animal and group, date of collection, owner's name, and place of collection with permanent marker. Then, the samples were transported to the laboratory of School of Veterinary Medicine, Wolaita Sodo University in an ice box at about a temperature of $+4^{o}$C. Fecal samples were examined either on the day of collection or stored in a refrigerator at $+4^{o}$C and processed in the next day. The burden and type of gastrointestinal parasites in the experimental sheep were determined by fecal egg count and identifying larvae from the fecal culture at genus level, respectively under the microscope.

Fecal examination

Centrifugal flotation procedure

The observation of helminth parasites eggs in the feces of the study animals was evaluated by using the coprological flotation techniques [33, 34, 35]. The fecal samples were subjected to saturated sodium chloride floatation technique to isolate the eggs of various helminths and examine under the microscope. The specific gravity of the saturated

sodium chloride solution was 1.20. Two grams of feces from each fecal sample was taken and placed in a mortar. About 30 ml of flotation solution was added to each sample, and by using a pestle, emulsion was made by thoroughly mixing the solution with the feces until no large pieces of feces remain. The emulsion was strained through the cheese cloth into a labeled centrifuge tube. Then the tubes were centrifuged for five minutes at 1500 revolutions per minutes. After, the tubes were removed from the centrifuge and gently placed in a test tube rack. A wire loop bent at a 90° angle to the handle was used to touch to the surface of the liquid of each centrifuged sample and the drop of fluid contained in the loop was transferred to a microscope slide. Then, each sample was covered with the cover slip and examined under the microscope at 10x. Any eggs seen were recorded.

Sedimentation procedure

Two grams of feces from each sample mixed thoroughly with tap water in mortar using a pestle. The mixture was strained through cheese cloth into a labeled centrifuge tube. Then, the samples were centrifuged at about 1500 rpm for five minutes. The supernatant was poured off without disturbing the sediment at the bottom. Then using the pipette and bulb, a small amount of the top layer of the sediment was transferred to a microscope slide. When the drop was too thick, it was diluted with a drop of water. A cover slip was applied on the drop. Then, the fecal samples were examined under the microscope [33, 35].

Fecal egg count

Positive samples were examined by modified McMaster egg counting technique to estimate the shedding of eggs per gram of feces by infected sheep [35]. The ratio of feces to flotation solution was 1:30 [35]. Then, the filtrate was homogenized by stirring before a small amount was taken to fill the chambers of the McMaster slide with a Pasteur pipette. The slide was left for a few minutes to allow eggs to float up, before being examined microscopically to count the eggs present. The number of eggs counted was multiplied by hundred to determine egg per gram (EPG) [36, 37, 38].

Fecal culture

About 20 g of sheep fecal sample from each experimental animal was thoroughly crumbled to a depth of about five cm in a petridish. The culture was moistened sufficiently to ensure that it did not dry out whilst being incubated, but without it becoming water-logged. Thereafter, the petridish was incubated in the dark at room temperature for 14 days, during which time it was checked periodically and moistened when necessary. After the 14^{th} day, the incubated fecal cultures inside the petridishes were sprayed lightly with 30^{o}C warm water. The warm water stimulated the L_3 larvae to separate from the feces into the water. Then, the warm water with the larvae was collected into labeled separate screw cupped bottle. Sample was taken from the suspension of larvae with pipette and a drop of the larvae suspension was placed on a glass slide and a drop of iodine was added into it and observed under the microscope at 40x magnification. Larvae were identified at genus level by examining their caudal and cranial extremities [39, 40].

Fecal egg count reduction test

A modified McMaster technique with a sensitivity of fifty eggs per gram was used for the counting of nematode eggs in the fecal samples collected just before the beginning of the treatment and at the 14^{th} day after treatment [41]. Then the fecal egg count reduction percent (FECR) was calculated as previously used by Sivajothi and Sudhakara [42]:

$$\text{FECR (percent)} = \frac{[(\text{Pretreatment FEC} - \text{Posttreatment FEC})}{\text{Pretreatment FEC}] \times 100$$

According to the recommendations of the World Association for the Advancement of Veterinary Parasitology [41, 43], garlic was considered effective when the FECR was ≥ 90 %.

Blood Samples Collection and Analysis

Blood laboratory examination was carried out in order to investigate toxic effect of garlic, if there is, on the experimental animals. Blood sample was withdrawn from each study animal by vein puncture from the jugular vein into labeled test tubes coated with EDTA and heparin just before treatment and at 14th day after treatment. The test tubes were slowly inverted to mix the blood with EDTA and heparin. Then, the blood samples were transported to Wolaita Sodo University, Otona Referral and Teaching Hospital Medical Laboratory in an ice box at the temperature of about +4^{o}C. Blood in test tubes coated with EDTA was used to determine number of leukocytes, red blood cells; hemoglobin concentration, and packed cell volume by BC-3000 plus auto hematology analyzer (Schenzhen Mindray Bio-medical electronics co., Ltd.China) (**Figure 3)**. Whereas, plasma from heparinised test tubes was used to determine plasma levels of Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Total protein, Glucose, and Creatinine by BS-200 chemistry analyzer (Schenzhen Mindray Bio-medical electronics co., Ltd.China).

Figure 3: BC-3000 plus hematology analyzer (A); BS-200 blood chemistry analyzer (B)

Data Management and Statistical Analysis

Differences between fecal egg count reduction change in between days 0 and 14th for gastrointestinal nematodes for naturally infested sheep in three groups (GA, GB and GC) were determined using Statistical Package for Social Science (SPSS) Version 20. The T-test was used to compare the effect of treatments on the fecal egg count of the study animals, the effect of garlic on the blood parameters of sheep and on the liver enzymes, glucose and protien before and after treatment of experimental animals in group B (GB). For statistical analysis, a confidence level of 95% and P-values less than 5% were judged as significant.

Preliminary Experiment

Before conducting the above experiment, a preliminary experiment (pilot) was performed using Veterinary School`s two sheep. The purposes of the pilot experiment were:

- To estimate the necessary frequency of garlic administration
- To compare the effectiveness of fresh crushed garlic mixed with tap water and dried garlic powder mixed with tap water
- To estimate the doses and concentration of the garlic water suspension for the treatment of gastrointestinal helminthes of sheep
- To evaluate the toxicity of the estimated dose and concentration of garlic powder water suspension on sheep
- To fine-tune various sample handling and procedural details

During the preliminary experiment, the procedures indicated in the protocol were followed in two concentrations (9 grams of dried garlic powder mixed with 100 ml tap water and 9 grams of fresh crushed garlic mixed with 100 ml tap water). However, dried garlic powder mixed with tap water has shown higher efficacy than fresh crushed garlic mixed with tap water on the same doses (9 grams), frequency (four consecutive days) and concentrations (mixing with 100 ml of tap water).

Results

Clinical Examination and Signs of Toxicity

The clinical examinations of experimental groups were indicated in Table 1. The respiratory rate of all groups of animals as well as pre and post-treatment was normal. No ptyalism, nasal and ocular discharges were observed in the study animals before and after treatment. There were also no behavioral, posture and gate changes observed. Pale mucus membranes were recorded in the study animals before treatment. However, the mucus membranes of group A and B animals were changed to normal appearance after treatment while mucus membranes of the negative control animals remained pale throughout the study.

Table 1: Clinical examination results of experimental animals before and after treatment

Grps.	Exd.	Behavior	Posture	Gait	RR/m	RT(°C)	PR/m	Crt/s	RM/m	MM
GA	0	Normal	Normal	Normal	24-29	38.6-39.5	74-88	1-3	1-2	Pale
	7th	Normal	Normal	Normal	22-31	38.7-40	76-90	1-2	1-3	Pale
	10th	Normal	Normal	Normal	20-28	38.8-39.8	72-86	1-2	1-2	pink
	14th	Normal	Normal	Normal	24-30	38.9-39.8	74-90	1-2	1-2	pink
GB	0	Normal	Normal	Normal	24-28	38.7-39.5	70-84	1-3	1-3	pale
	7th	Normal	Normal	Normal	26-30	38.9-40	76-90	1-2	1-2	pale
	10th	Normal	Normal	Normal	22-31	38.6-39.7	74-88	1-2	1-2	pink
	14th	Normal	Normal	Normal	24-30	38.8-39.9	72-86	1-2	1-2	pink
GC	0	Normal	Normal	Normal	24-28	38.8-41	70-84	1-3	1-2	Pale
	7th	Normal	Normal	Normal	22-28	38.7-39.6	76-82	1-2	1-2	pale
	10th	Normal	Normal	Normal	24-28	38.7-40	72-86	1-3	1-3	pale
	14th	Normal	Normal	Normal	22-30	38.7-39.4	70-82	1-3	1-2	pale

Grps = groups (GA = group A, GB = group B, GC = group C), Exd = examination days, RR/m = respiratory rate per minute, RT = rectal temperature, PR/m = pulse rate minute, Crt = capillary refill time, RM = rumen motility per minute, MM = mucus membrane Fecal Egg Count

The fecal egg count in sheep before treatment and at 14th day after treatment is presented in Table 2. The fecal egg count in sheep of group A was significantly reduced (p=0.000) after administration of albendazole 300 mg bolus (Chengdu Qiankun Veterinary Pharmaceuticals co., Ltd. China) (1850.00 ± 350.397 versus 122.22 ± 44.096). Similarly, the fecal egg count of sheep in group B, treated with garlic powder suspended in 100 ml tap water was significantly decreased (p= 0.000) at 14th day after treatment (1810.00 ± 360.401 versus 128.57± 48.795). In contrary, fecal egg count of the negative control sheep in group C did not show any improvement during the study period (1890.00 ± 430.633 versus 1950.00 ± 527.573) (Table 2).

Table 2: Effect of treatment on the fecal egg count of the study animals

Groups of the study animals	FEC of samples collected		95% CI		Sig.
	Before treatment (Mean±SD)	At the 14th day after treatment (Mean±SD)	Lower	Upper	
Group A	1850.00 ± 350.397	122.22 ± 44.096	1599.34	2100.66	0.000
Group B	1810.00 ± 360.401	128.57± 48.795	1552.18	2067.82	0.000
Group C	1890.00 ± 430.633	1950.00 ± 527.573	-286.718	166.718	0.564

Fecal Egg Count Reduction Test

The fecal egg count reduction test was calculated from the fecal samples collected just before treatment and at the 14th day after treatment (Table 3). The fecal egg count reduction in sheep of group A, treated with albendazole was found to be 94%, whereas the fecal egg count reduction in sheep of group B treated with garlic was 95%. The fecal egg count reduction in sheep treated with garlic was relatively high compared to in sheep treated with albendazole (95% versus 94%).

Table 3: Results of fecal egg count reduction test

Groups of the study animals	Total egg count		FECR(%)
	Before treatment	At 14th day after treatment	
Group A	18500	1100	94
Group B	18100	900	95
Group C	18900	19500	-3.2

Group A= Albendazole (Chengdu Qiankun Veterinary Pharmaceuticals co., Ltd. China), Group B= Garlic, Group C= Control (tap water), FECR=Fecal egg count reduction

The floatation and sedimentation techniques were conducted to examine and isolate the eggs of various helminthes under the microscope but under sedimentation technique no eggs of helminthes were found (negative results seen). Fecal culture were performed on the samples collected before treatment (on the day 0) and after treatment at the 14th day. Larvae from the fecal culture identified on examination of its caudal and cranial extremities as described by Van Wyk and Mayhew (2013) [40] before the treatment were predominantly *Haemonchus contortus* about (56%), *Trichostrongylus* about (28.57%), and *Oesophagostomum* about (21.42%) while after treatment very rare larvae of these genuses survived were observed (Table 4).

Table 4: Larvae identified at genus level before and after treatment

Treatment groups	Before treatment(at 0 day)			After treatment (at 14th day)		
	Esophagostomum	Haemonchus	Trichostrongylus	Esophagostomum	Haemonchus	Trichostrongylus
A-01	-	√	√	-	√	√
A-06	-	√	-	-	-	-
A-11	-	√	√	-	-	-
A-17	√	√	-	-	-	-
A-21	-	√	-	-	-	-
A-27	√	√	√	-	-	-
A-32	-	√	√	-	-	-
A-38	-	√	-	-	-	-
A-46	√	√	√	√	√	√
A-58	-	√	-	-	-	-
B-61	-	√	-	-	-	-
B-66	-	√	-	-	-	-
B-72	√	√	-	-	-	-

B-79	-	√	-	-	-	-
B-84	-	√	√	-	-	-
B-95	-	-	√	-	-	-
B-101	-	√	-	-	-	-
B-108	√	√	-	√	-	-
B-114	√	-	√	-	-	-
B-119	-	√	-	-	√	-

A = Group A treated with albendazole, B = Group B treated with garlic, (√) = presence, (-) = absence of larvae

Blood Parameter Analysis

The blood parameter values before and after treatment were shown in Table 5. The mean number of erythrocytes, hemoglobin concentration and packed cell volume in samples collected from sheep just before treatment with garlic was below the normal value (6.441 ± 0.946 $x10^{12}$/L versus 9.00 $x10^{12}$/L, 8.160 ± 0.583 mg/dL versus 9.00 mg/dL, 22.870 ± 1.574% versus 42.110 ± 6.882%, respectively). There was a significant increase (p= 0.000) in RBC, Hgb and HCT values of the study animal at the 14th day after treatment with garlic compared with values of pre-treatment. These values however, were within the normal range. The mean white blood cell count was significantly higher (p = 0.000) before treatment than post-treatment (15.240 ± 2.250 $x10^9$/L versus 10.510 ± 1.038 $x10^9$/L), the post-treatment values were within the normal range (12.730 ± 2.370 $x10^{12}$/L versus 6.441 ± 0.946 $x10^{12}$/L, 14.580 ± 3.591 mg/dL versus 8.160 ± 0.583 mg/dL, 42.110 ± 6.882% versus 22.870 ± 1.574%, respectively).

Table 5: Effect of garlic treatment on the blood parameters of sheep

Blood	*Normal ranges	Blood parameters of samples collected (Mean±SD)		95% CI		Sig
		Before treatment	At14th day after treatment	Lower	Upper	
WBC ($x10^9$/L)	4 - 12	15.240 ± 2.250	10.510 ± 1.038	2.847	6.612	0.000
RBC ($x10^{12}$/L)	9-15.8	6.441 ± 0.946	12.730 ± 2.370	-7.807	-4.770	0.000
Hgb (mg/dL)	9 - 15	8.160 ± 0.583	14.580 ± 3.591	-9.079	-3.760	0.000
HCT/PCV(%)	27 - 45	22.870 ±1.574	42.110 ± 6.882	-24.889	-13.590	0.000
MCV (fL)	28 - 40	27.930 ± 3.875	29.180 ± 5.181	-5.487	2.987	0.521
MCH (pg)	8 - 12	8.710 ± .912	10.680 ± 1.236	-2.821	-1.118	0.001
MCHC (g/dL)	31 - 34	27.010 ± 2.471	31.280 ± 1.098	-5.624	-2.915	0.000
LYMP ($x10^9$/L)	2-9	7.790 ± 1.332	9.520 ± 2.729	-4.032	.572	0.123
NEU ($x10^9$/L)	0.7-6	12.010 ± 2.437	6.090 ± 2.232	3.153	8.686	0.001

Mg/dL = milligram per deciliters, fL = femtolitre, pg = picogram, g/dL = gram per deciliters
*Normal ranges adapted from: (Research Animal Resources, 2009).

Blood Enzyme Analysis

Blood levels of liver enzymes AST, ALT and ALP before garlic treatment were higher than the normal ranges (Table 6). These values however, were significantly reduced (p = 0.000) at 14th day post-treatment with garlic (287.50 ± 5.148 U/L versus 144.70 ± 44.202 U/L, 41.90 ± 4.012 U/L versus 28.00 ± 6.037 U/L, 395.20 ± 5.138 U/L versus 255.60 ± 87.400 U/L, respectively). Although the post treatment blood levels of liver enzymes were reduced, they were within the

normal range (Table 6). The renal enzyme, creatinine was also relatively reduced post-treatment with garlic (1.519 ± 0.254 mg/dL versus 1.628 ± 0.414 mg/dL). But both pre-and post-treatment results of creatinine were within the normal range. On the other hand the total protein and glucose plasma levels were significantly elevated (p = 0.000) at 14th day post-treatment with garlic (5.41 ± 0.757 g/dL versus 7.130 ± 0.623 g/dL, 39.900 ± 5.896 mg/dL versus 69.10 ± 9.243 mg/dL, respectively). These results however were within the normal range.

Table 6: Effect of garlic treatment on liver and kidney enzymes, glucose and protein

Blood Enzymes	*Normal ranges	Blood enzyme parameters of samples collected (Mean±SD)		95% CI		Sig.
		Before treatment	At 14th day after treatment	Lower	Upper	
AST(U/L)	60-280	287.50 ± 5.148	144.70 ± 44.202	111.748	173.851	0.000
ALT (U/L)	22-38	41.90 ± 4.012	28.00 ± 6.037	8.133	19.666	0.000
ALP (U/L)	70-390	395.20 ± 5.138	255.60 ± 87.400	79.097	200.102	0.001
Total protein g/dL)	6-7.9	5.41 ± 0.757	7.130 ± 0.623	-2.328	-1.111	0.000
Glucose (mg/dL)	50-80	39.900 ± 5.896	69.10 ± 9.243	-37.375	-21.024	0.000
Creatinine (mg/dL)	1.2-1.9	1.628 ± 0.414	1.519 ± 0.254	-0.243	0.461	0.502

*Normal ranges of sheep biochemistry reference values adapted from (Jackson and Cockcroft, 2002) [45].

Discussion

Garlic (*Allium sativum*) is one of the most important bulb crop in Ethiopia. It is the oldest cultivated herb [46]. Allicin is its chemically and therapeutically active constituent [46, 47]. It is released only by crushing or chewing or cutting raw garlic. It cannot however be formed from cooked garlic [47, 48]. Because of its therapeutically active ingredient, garlic is a naturally health promoter [49]. It has been used as animal and human traditional phyto-therapuetics since ancient time. However, there are conflicting or inconsistent conclusions on its efficacy against diseases. According to Ried *et al*. [50], garlic can be employed for management of blood pressure. Similarly, Durak *et al*. [51] recommended garlic for the treatment of atherosclerosis and high plasma cholesterol level. Alder *et al*., [52] also reported that garlic can be used for the treatment of heart attack and coronary heart disease. In addition, Fleischauer and Arab., [53] announced garlic is effective in the treatment of lung, prostate, heart, stomach and colorectal cancers. Sethi *et al*., [54] declared that the juice and milk of garlic can be used as a vermifuge. Further, Anthony *et al*., [55] reported that garlic has parasiticide property. On the other hand Rohner [56] in his result of clinical research to determine the possible effects of consuming garlic on high blood pressure have found no clear effect. Additionally, Sahebkar *et al*., [57] in their meta-analysis indicated there was no effect of garlic consumption in blood levels of lipoprotein a biomarker of atherosclerosis. Chiavarni *et al*., [58] also in their meta-analysis found no effect of garlic on colorectal cancer.

The results of this study however, showed garlic has anti-gastrointestinal nematodes effect in sheep by significantly (p = 0.000) reducing the mean fecal egg count. Its 95% fecal egg count reduction test further demonstrated its anti-parasite therapeutic efficacy. According to the recommendation of the World Association for the Advancement of Veterinary Parasitology [43], anti-parasite drug is considered effective when the fecal reduction is $\geq$ 90%. Garlic is rich in protein, enzymes, carbohydrate, flavonoids, vitamins and minerals. These bioactive compounds probably played important role by supplementing anti-nematode effect of active ingredients of garlic like

allicin and saponin through strengthening the body defense mechanism of the treated animals. The side effects of garlic as a therapeutic are largely unknown. Possible side effects include gastrointestinal discomfort, depression, allergic reactions and bleeding. The liver is susceptible to the toxicity from *Alluim sativum* [59]. Previous experimental work has also revealed significant increases ($P<0.05$) in ALT, AST and ALP on higher dosage of aqueous *Allium sativum* (400mg and 550mg/kg) which can induce liver damage on rat [60]. As with all medicinal products, safety of garlic treatment was an important component of this experiment.

The clinical examination of the study animals before treatment and at the seventh, tenth and fourteenth day post-treatment was intended to assess adverse gastro intestinal, neuromuscular, respiratory and circulatory effects of garlic. The clinical outcome of this study indicated no difference between before treatment and post-treatment as well as between treated and control animals. Absence of ptyalism, ocular and nasal discharges, diarrhea, behavioral, postural and gate changes after treatment with garlic might have revealed non-toxic effect of garlic in the study sheep. The pale mucus membrane before treatment and similarly, the decreased value of erythrocyte number, hemoglobin concentration and packed cell volume revealed the presence of anemia in the study sheep before treatment (Table 5). The anemia might be caused by sucking of RBC, hemorrhage in the GIT and consuming nutrients that are essential for among others erythropoiesis by the strongyle parasites. On the other hand the significant increase ($p = 0.000$) in RBC, Hgb and HCT values and the change of mucus membrane from pale to normal after the treatment of the study animals with garlic might be additional evidence to the efficacy of garlic against GIT nematodes. The blood parameters have been shown to be important indices of the physiological, pathological and nutritional status of an animal and change in the constituent compounds of blood when compared to normal values could be used to interpret the health status of an animal [61]. Decreased hematological values before treatment may be attributed to effect of gastrointestinal parasite in decreasing the life span of RBCs and suppression of hematopoietic system [62], and may be due to acute loss of blood by sucking activity and hemorrhages caused by various gastrointestinal parasites [63]. While there was a significant increase in total white blood cells count before treatment as compared with after treatment, which might be caused by stimulation of lymphoid tissues and stem cells in the bone marrow by the parasites and their toxins [64]. In addition the number of eosinophils increases in animals infested by parasites. This might have also contributed for the rise of white blood cells numbers in the study animals before treatment [65]. Therefore, the blood parameter results obtained after garlic treatment were neither above nor below the normal ranges probably indicating non- toxic effect of garlic as phyto-therapuetic at the given dose and frequency on the treated sheep.

Many toxic substances are metabolized and /or detoxified and removed by liver and kidneys where they or their metabolites can cause hepatic and renal damage. Because liver contains high amount of different enzymes, the liver tissue damage caused by any toxin accompanied by higher blood levels of liver enzymes that leaked or released from affected hepatocytes. Liver enzymes tests are relatively specific and sensitive. Minimum tissue damage is ensued by elevated serum enzyme activities which are observed long before other clinical symptoms become apparent. Alanine aminotransferase is liver specific enzyme while aspartate aminotransferase and alkaline phosphatase are multi tissue enzymes [66].

The significantly increased ($p = 0.000$) blood level of liver enzymes AST, ALT and ALP before treatment in this study might be due to the damage of liver parenchyma caused by the migrating nematode larvae and / or toxin produced by GIT nematodes. At 14^{th} day post-garlic treatment, however blood levels of the liver enzymes AST, ALT and ALP decreased to the normal levels (287.50 ± 5.148U/L versus 144.70 ± 44.202U/L, 41.90 ± 4.012 U/L versus 28.00 ± 6.037 U/L, 395.20 ± 5.138 U/L versus 255.60 ± 87.400 U/L, respectively). Similarly, the renal

enzyme, creatinine was relatively higher pre-treatment than post- treatment. But both results of creatinine were within the normal range. Blood chemistry analysis before treatment in sheep naturally infested with gastrointestinal nematodes showed a significant reduction (p = 0.000) in values of total protein than after treatment and this may be attributed to increased plasma leakage through the injured gut caused by the parasites [67]. Further this loss is caused by selective loss of albumin which having smaller size and osmotic sensitivity to fluid movement. The damage caused to the gastrointestinal tissue by gastrointestinal helminthes also leads to hemorrhage and subsequent protein leakage into intestine. Maldigestion and malabsorption of nutrients through injured gut was additional cause of reduction of plasma proteins and other blood components [67, 68]. Serum level of glucose was significantly decreased (p = 0.000) before treatment than after treatment (Table 6). According to Radostits *et al*., [67] the decreased plasma level of glucose was due to rapid absorption and utilization of soluble carbohydrate by the parasites and also impaired absorption of glucose from the gut. These results are in agreement with finding of Uppal and Rai [69], Maiti *et al.*, [70] Dhanlakshmi *et al.*, [71] and Purohit *et al.*. [72] Changes in the plasma levels of blood constituents when compared to normal values could be used to interpret health status of an animal [73]. Taking into consideration the post-treatment results of clinical examination, blood parameter and chemistry, it is reasonable to conclude that garlic as phyto-therapuetic at the given dose and frequency had no toxic effect in the treated sheep.

Conclusion and Recommendations

The result of this study revealed that aqueous suspension of garlic powder is effective in the treatment of gastrointestinal nematodes of sheep. Further, it has no adverse effect on the health of recipient sheep at the given dose and frequency. Therefore, garlic can be used as an alternative anthelminthic for the treatment of gastrointestinal nematodes.

Based on above conclusion, the following recommendations were forwarded:

-The efficacy of garlic on mono-specific species of parasites under confined experimental setting should be studied.

-Further detailed in vitro study should be conducted to understand how the product affects the parasites.

-The post mortem examinations should be conducted to demonstrate worm burden.

-Active ingredients of garlic should be extracted and tasted.

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