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Preliminary using oil extract and nano emulsion of Artemisia herba alba compared with albendazole in treatment of artificially infected sheep with Trichostrongylidae

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ABSTRACT

The objective of this study is to evaluate the efficacy of Artemisia herba alba oil extract and its nano-emulsion in treatment of artificially infected lambs by Trichostrongylids. A total of 15 lambs were allocated into five groups. On 0 day, the animals of the first four groups were inoculated with 3000 Trichostrongylids L3 and 5th group were left as control negative. On the 2nd DPI, G1, G2 and G3 were treated with 100 mg/kg b. w. A. herba alba oil extract, 100 mg/kg b. w. of A. herba alba nano-emulsion and 7.5 mg/kg b. w. albendazole respectively. Animals of group 4 were kept as control positive group. The shedding of eggs began from 15th day in G1 and G4 only. There was significant decrease ($P<0.05$) in RBCs count and non-significant decrease in Hemoglobin and PCV on 22nd DPI compared to 0 DPI and control positive group. There was significant increase in S. GOT in all treated group except nano-emulsion group. The adulticide effect of nano-emulsion of A. herba alba was evaluated by using the same dose in treatment of highly infected lambs (Group 4) at 37th DPI. The egg count reduction percent was 97.28%. The results at 43th DPI revealed that Monocyte and blood platelet counts were only significantly decreased. We could conclude that the nano-emulsion of A. herba alba oil which used for the first time in treatment of Trichostrongylids in lambs had an excellent and safe effect for protection and treatment.

1. INTRODUCTION

Sheep production is considered a major sector of meat supply for human consumption in Egypt as they provide more than 30% of local meat consumption and contributes to development of the rural areas (Statistics of Live Stocks, 2011).

Gastrointestinal nematodes are known to invade the digestive tract and are the worst parasites for livestock production worldwide (Diehl et al., 2004). Beside some species of nematodes such as the Haemonchus spp. and Trichostrongylus are blood sucking parasites results in anemia (Hendrix, 1998).

The chemical drugs may not have been effective enough to totally cure the infection but at least they reduced the aggressiveness of the disease (Eguale et al., 2011). The high cost and side effects of chemical anthelmintics forced the researcher to use medicinal plants as they are accessible, affordable, and environmentally friendly (Adelmola and Elaff, 2011).

Artemisia herba alba essential oil has antimicrobial (Juteau et al., 2003) and antifungal activity (Saban et al., 2005). Chemical analysis of Artemisia has shown that its volatile oil is rich in thujone (a and b) which has been earlier reported as an anthelmintic (Meschler and Howlett, 1999).

Recently nanotechnology has attracted considerable interest of researchers all over the world. The nano emulsions are characterized by low surface tension plus good wettability which increase surface adhesion (Mostafa and Hussein 2020).

So, the objective of this study aimed to evaluate the anthelmintic efficacy of A. heba alba oil extract and its nano emulsion in comparing with chemical medicinal Albendazole in experimentally infected sheep with Trchostringylids.

2. MATERIAL AND METHODS

The study is divided into two experiments.

2.1. Experiment 1: In vivo effect of Artemisia herba alba on gastrointestinal nematodes of sheep

2.1.1. Artemisia herba alba plant

2.1.1.1. Oil extract of A. herba alba

It was obtained from oil extraction unit at National Research Center.

2.1.1.2. Nano emulsion of A. herba alba

Method of preparation of O/W nano emulsion of the plants (Tubesha et al., 2013) with some modifications:

The nano-emulsion was prepared by adding the surfactant 0.5% Tween 80 (non-ionic surfactant) to 12 gm of the given oil and mixed gently, distilled water is then added to complete the total volume to 100 ml. The mixture was stirred vigorously using high speed mechanical blender for 5 successive speed cycles within 15 to 20 mins. The obtained nanodroplets of the oil which were dispersed in the aqueous phase were obtained with concentration 12 % w/v in the final formulation, then the formulated nano emulsion was examined for shelf stability. All Steps were performed at

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room temp. 25◦C. The nano emulsion was stored at 4 ℃ for storage.

The characterization of nano-emulsion, measuring of the particle size and zeta potential were done using particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom) at fixed angle of 173° at 25° C. Samples were analyzed in triplicate. The same equipment was used for the determination of zeta potential. Particle size was recorded as 240.6 ± 2.2 nm and Zeta potential: -14.3 ± 0.5 mV. Transmission electron microscopy (TEM) micrograph shows that the nanodroplets size ranges from 5-15nm with average size about 10 nm. They are nearly spherical in size with uniform distribution and some aggregation (Fig 1).

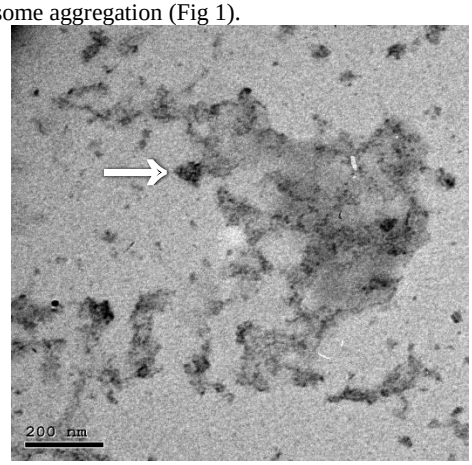

Fig 1 TEM micrograph→ of Artemisia Nano droplets

2.1.2. Collection of infective third larvae

The eggs of Trichostrongylidae were collected from naturally infected sheep and subjected to fecal cultures according to Georgi and Georgi (1990). The hatched larvae were collected using a modified Bearmans technique after Thomas et al. (1970).

2.1.3. Preparation of the infective dose of larvae (inoculum)

Third larval stages of Trichostronylids were collected from cultured fecal samples, identified, and counted per ml of culture material. The inoculum consists of 66% Trichostrongylus, 35% Haemonchus and 5% Chabertia ovina. The single dose was prepared in to be 3000 larvae / ml and kept in an Eppendorf tube at 4 c till use.

2.1.4. Experimental animals

A total of 15 of Baladi breed lambs (aged 4-5 months) weighed 23-26 kg were randomly allocated into 5 groups; each of three lambs and housed in separate clean, disinfected pens in the Lab Animals Center of Faculty of Veterinary medicine, Benha university. They fed on corn and dry hay with a free access of water. Parasitological examination of lamb feces was done for two weeks before the start of the experiment and fasted overnight before experiment.

2.1.5. Experimental design

On zero day, all animals in the first four group (G1, G2, G3 and G4) were inoculated orally with an average of 3000 3rd larval stages of mixed trichostrongylid spp. by using tuberculin syringe at mouth commissure (Wood et al., 1995). Animals of G5was kept free as control negative. At 2nd DPI the animals in group 1 were treated with 100 mg/kg A. herba alba oil extract, G2 were treated with 100 mg/kg Artemisia oil Nano emulsion and G3 were treated with 7.5 mg/kg albendazole, while Group 4 was not treated and kept as control positive group.

2.1.6. Sample collection from experimental lambs

Fecal samples were collected daily from 15th DPI for parasitological examination till the end of the experiment (36th DPI). Moreover, blood samples were collected from jugular vein from all experimental lambs on zero day and 22nd DPI. for determination of hematological and biochemical parameters (Coles et al., 1992).

2.1.7. Counting of eggs per gram of feces:

The shedding eggs were counted from fecal sample of each lamb in all groups using Mc master technique according to soulsby (1986) as follows:

$$EPG = \frac{total\ nomber\ of\ eggs}{total\ nomber\ of\ counting\ chamber} \times 100$$

2.2. Experiment 2:

The aim of this experiment is to evaluate the effect of A. herba alba nano-emulsion at dose of 100 mg\Kg on the patent period of Trichostrongylids in the highly infected lambs.

Three highly infected lambs (mean egg count was 3066 epg) were treated at 36th DPI with 100 mg/kg B. W. Artemisia nano emulsion, the animals were housed in separate clean, disinfected pens in the Lab Animals Center of Faculty of Veterinary medicine, Benha university. They fed on corn and dry hay with a free access of water. Fecal samples were collected daily for one week as well as blood samples for hematological and biochemical parameters were taken at 36th day (beginning of experiment) till 43rd day (end of experiment).

Fecal egg count reduction percent was calculated as follow:

$$\text{Egg coun reduction \%} = \frac{\text{egg\gram pre treatment} - \text{egg\gram post treatment}}{\text{egg\gram pre treatment}} \times 100$$

2.3. Statistical analysis

It was done utilizing ANOVA with two factors under significance level of 0.05 for the entire result using SPSS (ver. 25). (Steel et al., 1997) data were dealt with as a complete randomization outlines and different examinations were done applying LSD.

3. RESULTS

The results of experiment 1, denoted that the fecal examination of treated animals with A. herba alba nano emulsion and that with albendazole at 2nd day post infection showed no egg shedding at 15th day PI till the end of the experiment (22nd day), thus revealed that both treatments achieved 100% protection compared to control positive group that start shedding of Trichostrongylidae eggs from 15th DpI (4766.67±145.30) till the end of the experiment at 22nd day (4933.33±133.33). Concerning A. herba alba oil extract group, it gave good result as it showed very low shedding of Trichostrongylidae eggs from 15th DPI and till 22nd DPI and the highest significant egg count ($P<0.05$) was on 15th DPI (283.33±33.33) and reached to 150.00±44.10 at 22nd day. The lowest significant egg output of Trichostrongylid eggs was recorded at 36th DPI (66.00±57.74) (Table 1).

Regarding live body weight of experimented animals, there was non-significant difference in animal weight between 0 day and 22nd day in comparing with control negative group (G5) (Table 2)

Table 1 Counting of gastrointestinal Trichostrongylidae egg output in A. herba alba oil extract group and control positive group in experiment.

	G 1 Artemisia oil extract	G 4 Control Positive
Day15	283.33±33.33 ^{Ba}	4766.67±145.30 ^{Aa}
Day16	250.00±50.00 ^{Bab}	4666.67±158.99 ^{Aa}
Day17	150.00±50.00 ^{Babcd}	4633.33±109.29 ^{Aa}
Day18	216.67±44.10 ^{Babcd}	4700.00±152.75 ^{Aa}
Day19	243.33±33.33 ^{Babcd}	4650.00±76.38 ^{Aa}
Day20	233.33±16.67 ^{Babc}	4666.67±120.19 ^{Aa}
Day21	216.67±60.09 ^{Babcd}	4733.33±116.67 ^{Aa}
Day22	150.00±28.87 ^{Babcd}	4933.33±133.33 ^{Aa}
Day23	116.67±44.10 ^{Bbcd}	5000.00±150.00 ^{Aa}
Day24	150.00±50.00 ^{Babcd}	5000.00±144.34 ^{Aa}
Day25	133.33±33.33 ^{Babcd}	5100.00±208.17 ^{Aa}
Day26	183.33±72.65 ^{Babcd}	5016.67±176.38 ^{Aa}
Day27	166.67±60.09 ^{Babcd}	4983.33±220.48 ^{Aa}
Day28	133.33±60.09 ^{Babcd}	5133.33±185.59 ^{Aa}
Day29	116.67±44.10 ^{Bbcd}	5033.33±208.83 ^{Aa}
Day30	116.67± 44.10 ^{Bbcd}	5100.00±202.07 ^{Aa}
Day31	116.67±60.09 ^{Bbcd}	5116.67±216.67 ^{Aa}
Day32	116.67±44.10 ^{Bbcd}	4383.33±192.21 ^{Aab}
Day33	100.00±50.00 ^{Babcd}	3866.67±466.67 ^{Abc}
Day34	83.33±33.33 ^{Bcd}	3333.33±440.96 ^{Acd}
Day35	66.67±16.67 ^{Bd}	3166.67±300.46 ^{Ad}
Day36	66.00±57.74 ^{Bbcd}	3066.67±233.33 ^{Ad}

Artemisia Nano emulsion group, albendazole group and control negative group recorded no shedding of egg i.e., zero egg counting.

Concerning hematological examination, the results in experiment I, showed that a significant decrease (P<0.05) in RBCs counts in different groups on 22nd DPI (2.70±0.12, 2.60±0.06, 2.50±0.10 and 2.38±0.18) for G1, G2, G3 and G4 respectively as compared with zero day of the experiment and control negative group (3.13±0.09) (Table 4). Hemoglobin value showed no significant difference in different groups, except for control positive which showed a significant decrease (P< 0.05) in hemoglobin (HB) and PCV values in comparing zero day and 22th DPI. The values of MCV, MCH and MCHC were displayed no significant change in all groups in comparing 0 day and 22nd DPI. Inversely, Only Group 1 showed as significant increase in WBCs count on 22nd DPI as compared with zero day (11.20±1.60 and 7.80±1.04; respectively) (but this group was significantly within the normal range of the other groups and even the control negative group). Moreover, platelets, neutrophils, lymphocytes, basophils, eosinophils, and monocytes counts were showed non-significant variations upon comparing 0 day and 22nd DPI in different groups (Table 3).

The biochemical assessment of liver and kidney function (table 4) showed no significant alteration in serum Creatinine, S. Urea and S. GPT at 0 day and 22nd DPI in different groups. While there was significant increase in S. GOT in all treated group except nano-emulsion group between day 0 and day 22 and in comparison, to control negative group, GOT and GPT were significantly increased in animals treated with albendazole. Albumin were relatively decreased in all groups.

The results of experiment 2 (Table 5) showed that the egg shedding of the treated control positive group on 36th DPI with 100 mg/kg Artemisia nano emulsion led to a gradual decrease in egg count and greatly declined at 41st DPI (233.33±16.67) till reached to (83.33±16.67) at 43rd day of the experiment. The egg count reduction percent was 97.28%.

Table 2 Comparison of body weight of animals between 0 day and 22nd day in experiment 1.

	G 1 Artemisia oil extract	G 2 Artemisia Nano emulsion	G 3 Albendazole group	G 4 Control Positive	G 5 Control Negative group
Day0	24.67±1.17^{Aa}	23.33±0.44^{Aa}	23.33±0.60^{Aa}	26.17±1.59^{Aa}	23.87±0.47 ^{Aa}
Day22	22.67±1.67^{Aa}	21.00±0.58^{Aa}	20.33±0.33^{Aa}	21.67±0.88^{Ab}	22.17±0.38^{Aa}

Table 3 Hematological results of different experimental groups in experiment 1

	Group 1 Artemisia oil extract		Group 2 Artemisia Nano emulsion		Group 3 Albendazole group		Group 4 Control positive		Group 5 Control negative	
	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22
RBCs	2.93±0.09^{Aa}	2.70±0.12^{Ba}	2.97±0.23^{Aa}	2.60±0.06^{Ba}	3.15±0.35^{Aa}	2.50±0.10^{Ba}	2.81±0.31^{Aa}	2.38±0.18^{Ba}	3.14±0.11 ^{Aa}	3.13±0.09^{Aa}
Hemoglobin	9.14±0.99^{Aa}	7.30±0.40^{ABa}	8.67±0.65^{Aa}	7.69±0.23^{ABa}	9.00±0.29^{Aa}	7.93±0.33^{ABa}	8.27±0.91^{Aa}	6.93±0.58^{Ba}	8.60±0.32^{Aa}	8.60±0.32^{Aa}
PCV	26.50±2.80^{Aa}	21.50±0.80^{ABa}	25.03±1.87^{Aa}	22.27±0.61^{ABa}	24.13±0.84^{Aa}	22.90±0.89^{ABa}	23.83±2.63^{Aa}	20.17±1.61^{Ba}	24.80±0.85^{Aa}	24.80±0.85^{Aa}
MCV	84.37±0.72^{Aa}	84.95±0.85^{Aa}	84.53±0.64^{Aa}	85.67±0.35^{Aa}	84.50±0.49^{Aa}	84.97±0.72^{Aa}	84.87±0.64^{Aa}	84.84±0.61^{Aa}	86.47±3.01^{Aa}	86.47±3.01^{Aa}
MCH	29.14±0.24^{Aa}	29.15±0.45^{Aa}	29.20±0.21^{Aa}	29.57±0.20^{Aa}	28.70±0.20^{Aa}	29.33±0.30^{Aa}	29.00±0.32^{Aa}	29.17±0.26^{Aa}	29.23±1.00^{Aa}	29.23±1.00^{Aa}
MCHC	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	34.60±0.00Aa	35.00±1.23^{Aa}	35.00±1.23^{Aa}
WBCs	7.80±1.04^{Ba}	11.20±1.60^{Aa}	10.40±2.66^{ABa}	13.53±1.50^{Aa}	13.60±0.69^{Aa}	13.80±0.35^{Aa}	10.43±1.07^{ABa}	10.97±1.31^{Aa}	13.73±0.47^{Aa}	13.73±0.47^{Aa}
Platelets	923.33±73.56^{Aa}	1273.00±114.00^{Aa}	945.00±44.44^{Ab}	1271.00±55.76^{Aa}	858.33±24.55^{Aa}	1232.33±63.39^{Aa}	991.67±69.66^{Ab}	1193.00±10.44^{Aa}	829.33±28.67^{Aa}	829.33±28.67^{Ba}
Neutrophils	9.33±0.88^{Aa}	11.33±0.88^{Aa}	10.00±0.58^{Aa}	10.00±1.15^{ABa}	8.00±0.58^{Aa}	8.00±0.00^{Ba}	8.00±0.00^{Ba}	10.00±0.58^{ABa}	9.33±0.33^{Aa}	9.33±0.33^{ABa}
Lymphocytes	80.00±1.53^{Aa}	79.33±1.20^{Aa}	82.67±0.33^{Aa}	82.67±1.20^{Aa}	84.00±1.15^{Aa}	84.00±0.00^{Aa}	82.67±0.88^{Aa}	80.33±1.20^{Aa}	82.67±2.91^{Aa}	82.67±2.91^{Aa}
Monocytes	7.00±0.58^{Aa}	6.33±0.33^{Aa}	5.67±0.33^{Aa}	6.00±0.00^{Aa}	5.67±0.33^{Aa}	6.50±0.50^{Aa}	6.00±0.58^{Aa}	6.67±0.33^{Aa}	6.00±0.00^{Aa}	6.00±0.00^{Aa}
Eosinophils	2.67±0.33^{Aa}	2.00±0.00^{ABa}	1.67±0.33^{Aa}	1.33±0.33^{Ba}	1.67±0.33^{Aa}	1.50±0.50^{Aba}	2.33±0.33^{Aa}	2.33±0.33^{Aa}	2.00±0.00^{Aa}	2.00±0.00^{ABa}
Basophils	1.00±0.00^{Aa}	1.00±0.00^{Aa}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.67±0.33^{Aa}	0.00±0.00^{Ba}	1.00±0.00^{Aa}	1.00±0.00^{Aa}	1.00±0.00^{Aa}	1.00±0.00^{Aa}

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Table 4 Clinical chemistry reports of different groups in experiment 1

	Group 1 Artemisia oil extract		Group 2 Artemisia Nano emulsion		Group 3 Albendazole group		Group 4 Control positive		Group 5 Control negative	
	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22	Day 0	Day 22
S. Creatinine (mg|dl)	1.38±0.06^{Aa}	1.67±0.15^{Aa}	1.35±0.03^{Aa}	1.53±0.10^{Aa}	1.27±0.04^{Aa}	1.60±0.20^{Aa}	1.46±0.27^{Aa}	1.61±0.20^{Aa}	1.34±0.05^{Aa}	1.34±0.05^{Aa}
S. Urea (mg|dl)	41.63±2.13^{Aa}	39.27±1.18^{Aa}	41.20±1.85^{Aa}	37.90±1.65^{Aa}	42.38±1.20^{Aa}	41.63±1.43^{Aa}	40.25±2.12^{Aa}	40.23±2.40^{Aa}	41.97±1.46^{Aa}	42.07±1.46^{Aa}
S. GOT (U|L)	36.00±2.89^{ABa}	50.67±5.24^{Aa}	31.33±2.60^{Ba}	40.00±11.93^{ABa}	54.00±11.72^{Ba}	70.50±23.50^{Aa}	37.67±1.67^{ABa}	48.33±7.54^{Aa}	41.33±1.45^{ABa}	41.33±1.45^{Aa}
S. GPT (U|L)	12.00±0.00^{Aa}	12.67±1.76^{Aa}	9.00±1.00^{Ab}	15.67±2.03^{Aa}	13.67±2.73^{Aa}	19.50±5.50^{Aa}	12.00±2.31^{Aa}	14.00±2.65^{Aa}	14.00±0.58^{Aa}	14.00±0.58^{Aa}
S. Albumin (g|dl)	3.17±0.03^{Ba}	2.80±0.10^{Bb}	3.18±0.11^{Ba}	2.63±0.18^{Bb}	3.14±0.08^{Ba}	2.75±0.05^{Ba}	3.17±0.03^{Ba}	2.80±0.10^{Bb}	3.47±0.12^{Aa}	3.47±0.12^{Aa}

Table 5 Egg output of treated highly infected animals by 100 mg/kg b. wt. Artemisia oil nano emulsion for one week post treatment.

Days post infection	No. of egg per gram
Day36	3066.67±233.33
Day37	2666.67±166.67
Day38	2333.33±333.33
Day39	2333.33±333.33
Day40	2166.67±166.67
Day41	233.33±16.67
Day42	83.33±16.67
Day43	83.33±16.67

This result indicated that, Artemisia nano emulsion had adulticide effect on Trichostrongylid spp.

Regarding the hematological examination of the treated control positive lambs, the result showed a non-significant change in RBCs, PCV, Hemoglobin, WBCs, MCV, MCH and MCHC before and after treatment, the blood platelets and monocyte counts showed a significant decrease. While there was significant decrease in RBCS, hemoglobin, PCV, There was significant decrease in S. GOT and S. albumin between treated and control negative group (Table 7).

WBCS, blood platelets and monocyte count in comparing treated group to control negative group. (Table 6).

The biochemical assessment of liver and kidney function showed that there were non-significant changes in creatinine, urea, GPT and albumin before and after treatment. While there was significant decrease in S. GOT before and after treatment (48.33±7.54a and 37.67±1.67b).

Table 6 Hematological result of treated highly infected animals by 100 mg/kg b. wt. Artemisia oil nano emulsion before and after treatment in comparison to control negative group in experiment.

	Control Positive (before treatment)	Control Positive (after treatment)	Control Negative (47th day)
RBCs	2.38±0.18^{b}	2.01±0.29^{b}	3.14±0.11^{a}
Hemoglobin	6.93±0.58^{ab}	5.83±0.84^{b}	8.60±0.32^{a}
PCV	20.17±1.61^{ab}	16.93±2.42^{b}	24.80±0.85^{a}
MCV	84.84±0.61^{a}	84.34±0.38^{a}	86.47±3.01^{a}
MCH	29.17±0.26^{a}	29.00±0.25^{a}	29.23±1.00^{a}
MCHC	34.60±0.00^{a}	34.60±0.00^{a}	35.00±1.23^{a}
WBCs	10.97±1.31^{ab}	8.80±0.99^{b}	13.73±0.47^{a}
Platelets	1193.00±10.44^{a}	301.67±13.64^{b}	829.33±28.67^{c}
Neutrophils	10.00±0.58^{a}	19.67±5.93^{a}	9.33±0.33^{a}
Lymphocytes	80.33±1.20^{a}	73.33±7.06^{a}	82.67±2.91^{a}
Monocytes	6.67±0.33^{a}	4.33±0.33^{b}	6.00±0.00^{a}
Eosinophils	2.33±0.33^{a}	2.00±0.58^{a}	2.00±0.00^{a}
Basophils	1.00±0.00^{a}	0.67±0.33^{a}	1.00±0.00^{a}

Table 7 Clinical chemistry reports of treated highly infected animals by 100 mg/kg b. wt. Artemisia oil nano emulsion before and after treatment in comparison to control negative group in experiment.

	Control Positive (before treatment)	Control Positive (after treatment)	Control Negative (47th day)
Creat	1.61±0.20^{a}	1.18±0.10^{a}	1.34±0.05^{a}
Urea	40.23±2.40^{a}	38.40±1.65^{a}	41.97±1.46^{a}
GOT	48.33±7.54^{a}	37.67±1.67^{b}	41.33±1.45^{a}
GPT	14.00±2.65^{a}	13.00±1.73^{a}	14.00±0.58^{a}
Albumin	2.80±0.10^{b}	2.80±0.06^{b}	3.47±0.12^{a}

4. DISCUSSION

The present study aimed to compare the effect herbal treatment (Artemisia oil extract and its nano-emulsion) with chemical treatment (albendazole). The results showed that administration of Artemisia herba alba nano emulsion and albendazole on 2nd DPI to artificially infected lambs with Trichostrongylids caused 100% protection effect, while Artemisia oil extract caused significant reduction in egg output after 15th day of infection in comparison to control positive group. In this respect, Rogia Osman (2010) found that treated sheep with aqueous extract of A. herba alba before infection, showed slight decrease in egg count (EPG) after 14th day of treatment. Also, Hassan et al., (2021) recorded that crude ethanolic extract of A. herba alba had good efficacy in treatment of Haemonchus contortus infection in sheep.

Regarding live body weight of experimented animals, the present study showed that, there was no significant difference on weight of animals before and after treatment and in comparing to untreated control negative group. Similarly, Rogia Osman (2010) recorded that the treated lambs with aqueous extract of A. herba alba before infection showed no significant changes in body weight compared with the untreated control. Also, Komáromyová et al., (2021) and Lima et al., (2016) recorded that there were no significant changes in body weight before and after treatment with Herbmix, Selplex in lambs infected with Haemonchus contortus and aqueous extract of C. mollis in goats naturally infected with gastrointestinal nematodes, respectively.

The present results of hematological examination showed that there was a significant decrease ($P<0.05$) in RBCs counts and no significant changes in Hemoglobin and PCV value in all groups on 22nd DPI in comparing with control positive group. These decreases might be attributable to the blood loss that resulted partly from sucking activity of Trichostrongylidae both larval and adult stages associated with the damaged epithelium of the abomasi (Rahman and Colins, 1990). Similarly, were met those of Rogia Osman (2010) who recorded that animals which treated with water extract of A. herba alba before infection didn't show any significant difference in PCV values. On other hands Hassan et al., (2021) recorded that there was improving in RBCs count, hemoglobin and PCV after 30 days of treatment with alcoholic extract of Artemisia.

The biochemical assessment of liver and kidney function showed no significant alteration in S. Creatinine and S. Urea while, S. GOT and S. GPT were significantly increased in animal treated with albendazole. The animals treated with nano emulsion of Artemisia showed non-significant increase in S. GOT and significant increase in S. GPT. Similar results were recorded by Rogia Osman (2010).

These results indicated that the treatment with A. herba alba nano-emulsion is safe and have no effect on liver and kidney function in comparing with albendazole.

The results of experiment 2 denoted that using of 100 mg\kg B. w. of nano emulsion of Artemisia oil extract in treatment of highly infected lambs led to significant egg count reduction percent which was 97.28% after one week of treatment, this result higher than that of Irum et al. (2017) who treated experimentally infected sheep with different doses of aqueous extract of Artemisia spp. (A. siversiana and A. parviflora) and found that maximum parentage reduction in EPG was 77.0 % for A. sieversiana and 73.6 % for A. parviflora. Lower percent was recorded by Rogia Osman (2010) who recorded that A. herba alba showed anthelmintic efficacy of 62.5% in epg of feces, Moreover, Iqbal et al. (2004) recorded maximum reduction (67.2%) in eggs per gram (EPG) of feces was recorded on day 14 post treatment in sheep treated with A. brevifolia CAE (crude aqueous extract). Shamaila et al. (2015) recorded that there was a significant decrease in fecal egg count (FEC) after post-treatment period for both plants (Artemisia Vestita and

A. maritime), where the highest fecal egg count reduction for A. vestita was 87.2%, while for A. maritima was 84.5% on day 28 post-treatment.

The using of Artemisia nano-emulsion in treatment of heavy infected lambs give excellent results so, it could be used as protection and adulticide for Trichostrongylidae in sheep.

Concerning live body weight there is no significant differences in weight gain before and after treatment Similarly Komaromyova et al. (2021) and Rogia Osman (2010) were recorded that Treatments did not affect the final live weight of sheep.

Regarding hematological results of the treated control infected lambs with Artemisia nano-emulsion, the result showed a significant decrease in blood platelets and monocyte counts. In this respect Katiki et al. (2019) evaluated encapsulated anethole and carvone in lambs infected with Haemonchus contortus and recorded that there was significant decrease in RBCs, hemoglobin, PCV and leukocytes' count. On other hands Wasso et al. (2020) recorded anthelmintic efficacy of chitosan encapsulated bromelain against gastrointestinal Strongyles in small East African goats in Kenya and found that the PCV did not show any significant difference. This difference may relate to method of extraction of plant, type of extract, doses as well as method of testing.

The results of biochemical assessment of liver and kidney function in treated animals showed non-significant changes in creatinine, urea, GPT and albumin before and after treatment. While there was significant decrease in S. GOT. There was significant decrease in S. GOT and S. albumin between treated and control negative group.in this respect Wasso et al. (2020) recorded that no significant differences were observed between the urea and creatinine levels of treated and the control (non-treated) goats.

5. CONCLUSION

It was concluded that the using of A. herba alba nano emulsion in protection of Trichostrongylid infection is better than Artemisia oil extract. There was no significant variation in hematological values or alteration in biochemical tests of liver and kidney function. As well, Artemisia nano emulsion treatment could decrease Trichostrongylid egg count in highly infected lambs without alteration of hematological and biochemical parameters.

6. REFERENCES

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