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Anthelmintic Potency and Curative Effect of Pomegranate Peels Ethanolic Extract against *Haemonchus contortus* Infection in Goats

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ABSTRACT

This study aimed to explore the *in vitro* and *in vivo* anthelmintic efficacy of pomegranate peels ethanolic extract (PEE) against *Haemonchus contortus* (*H. contortus*) infection among goats. Hemogram was also investigated. The direct effect of different concentrations of PEE at 50, 100, 200 and 400 mg/ml on adult worms and larvae in comparison with albendazole at 10µg/ml at various hours was assessed. The *in vitro* anthelmintic effect of PEE at 400 mg/ml was the most remarkable that caused 86% adult worm motility inhibition and 0.87 mortality index, prominent adult worms' cuticular microscopic deformities and induced 100% larval mortality. Fifteen parasite free Baladi Egyptian goats aged 6 to 10 months old, divided into five groups (G1 to GV) were utilized. Experimental infection of the goats with *H. contortus* was done except GI which was used as control uninfected untreated. GII was kept as infected control. GIII, GIV and GV were treated with PEE at a dose of 3 and 6 g/kg BW, and albendazole at 10 mg/kg BW, respectively. GIV displayed the maximum fecal egg count reduction of (90.55%), significant decrease of worm burden with efficacy of (96.39%), distinct elevation in Hb and HCT values and, retaining the normal total and differential WBC with improving animals' health condition. In conclusion, PEE had an interesting *in vitro* and *in vivo* anthelmintic activity against *H. contortus* and could be deemed as potent, safe and economic alternative anthelmintic against haemonchosis in goats.

Key words: *Haemonchus contortus*, *Punica granatum* peels, goats, *In vitro*, *In vivo*.

INTRODUCTION

Haemonchosis is one of the major global infectious parasitic diseases impeding the livestock productivity (Kamaraj and Abdul Rahuman 2011). *H. contortus* is the utmost predominant hematophagus abomasal nematode among small ruminants worldwide, including Egypt (Hassan *et al*. 2019a). Baladi goat (*Capra hircus*) is one of the most well known breeds reared by farmers in various rural areas throughout Egypt (Hassan *et al*. 2019b). The anthelmintic drug resistance had documented among parasites particularly *H. contortus* (Nega and Seyum 2017). *Punica granatum* L.; *P. granatum*, commonly known as pomegranate (Family *Punicaceae*) is a famous fruit in tropical and subtropical nations (Stover and Mercure 2007). Pomegranate is a substantial source of valuable phytochemical compounds of pharmacological benefits (Prakash and Prakash 2011). Pomegranate peels, which are used to be a waste, were found to have therapeutic features (Atta *et al* 2016). The major constituents of PEE are saponins, quinones, flavonoids, alkaloids, terpenoids, phenols, steroids and coumarins (Jayaprakash and Sangeetha, 2015). Several studies demonstrated the anti-parasitic potency of PPE against gastrointestinal nematodes (GINs) infection (Boonmasawai *et al*., 2013) and paramphistomosis (Lalhmingchhuanmawii *et al*. 2014). Additionally, Jabeen *et al*. (2015) proclaimed that fruit peel of *P. granatum* had an *in vitro* anthelmintic activity against *H. contortus* adult worms. Therefore, the current work is firstly conducted *in vitro*, to investigate the potency of the PEE against the vitality of adults and larvae. Then, the study is going to stand on the *in vivo* curative effect of this extract on goats experimentally infected with *H. contortus* and determining the most appropriate effective dose and its impact on hemogram.

MATERIALS AND METHODS

Ethics approval

This study was conducted according to the guidelines of the Institutional Animal Care and Use Committee at the National Research Centre, Giza, Egypt, Approval Protocol No. (16229).

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Plant

P. granatum fruits were brought from local markets in Giza Governorate, Egypt. Whole peels of pomegranate fruit were assembled using fine cutter, washed with distilled water. They were kept under shade until complete dryness and then they were coarsely powdered.

Pomegranate peels **ethanolic extract (PEE)**

The extract was prepared as method adopted by Harborne (1984). Each 500 g chopped plant material was soaked in 2.5 liter of ethanol 90% in dark place, for three days at room temperature with intermittent shaking. This suspension was filtered through Whatman No.1 filter paper. The soaking/filtration process was repeated three times. The combined filtrate was evaporated using a vacuum rotary evaporator under reduced pressure at 50°C to give the crude ethanolic extract.

Parasite

Adult worms

Adult *H. contortus* worms were assembled from abomasa of slaughtered sheep in El-Monieb abattoir at Giza Governorate. These worms were identified, washed thoroughly utilizing phosphate buffered saline according to Soulsby (1986).

Larvae

Firstly, Female adult worms were finely crushed for liberation of eggs, which used to achieve larval culture according to (Solusby 1986). The infective larvae were harvested. The number of the larvae was determined in 10 µl, five repetitions of counting under the light microscope (10×). Then, two worm free Baladi goat kids of 15-18 kg BW, were used as *H. contortus* larval donors. Each goat kid was given orally 350 infective *H. contortus* larvae per kg BW. Three weeks post infection, fecal samples were collected. Fecal culture was done to obtain the infective larvae to be utilized in the experimental infection of the animals.

***In vitro* study**

For the *in vitro*, a stock solution of PEE (800 mg/ml) was emulsified by adding 1% dimethyl sulphoxide (DMSO) and then suspended in distilled water. The appropriate volume of a PEE stock solution was diluted and added to give four different final concentrations (50, 100, 200 and 400 mg/ml). Albendazole (Evazole®- EVA Pharma, Cairo, Egypt) at a concentration of 10µg/ml was used as a reference anthelmintic drug dissolved in DMSO at 0.10%.

Adulticidal efficacy

Adult motility inhibition test

The anthelmintic effect of the PEE on adult *H. contortus* motility was tested as per the method (Jabeen *et al*. 2015). The negative control was DMSO (0.10%). Ten motile adult worms were subjected to each of the previously prepared concentrations (PEE and albendazole) at 37°C. Three replicates had been set for each concentration. Observation of worm motility and mortality was done at interval 0, 2, 4, 6 and 24 h. After 24 h, the treated worms were re-suspended in PBS for 30min to detect the worm motility revival. Assigning of percent worm motility inhibition (% WMI) was done according to Rabel *et al*. (1994) by

$$\text{WMI}\,\% = \frac{\text{Motile worm number in negative control} - \text{Motile worm number under treatment}}{\text{Motile worm number in negative control}} \times 100$$

The mortality index was estimated by the giving formula:

$$\text{Mortality index} = \frac{\text{Total number of immotile worm (dead)}}{\text{Total number of worm}}$$

Microscopy of *H. contortus* body wall

The effect of the PEE concentrations (100, 200 and 400 mg/ml) on adult worm body wall was studied compared with a control reference drug. The fresh *H. contortus* adult worms were added into culture medium under sterile condition and incubated for 24 h at 37°C in 5% CO_2 atmosphere, (Ibarra and Jenkins 1984). Normal control worms were undergone fixation rapidly just after washing. For each concentration, five worms were checked. After 24h exposure to various PEE concentrations, the worms were sliced into samples about 5µ, then added to 10% buffered formol saline for fixation (Bancroft and Stevens 1996). The alterations in the body wall of adult worms, were noted and viewed utilizing an Olympus CX41 microscope.

Larvicidal efficacy

The larval mortality assay was carried out according to Nasai *et al*. (2016) with brief modifications. About 25 active motile third stage larvae (L_3) were used for each PEE concentration. The negative control was DMSO (0.10%). Three replicates were set for each concentration. The petri dishes were subjected for gentle agitation, covered and kept at room temperature. Each petri dish was checked at 2 and 24 h post treated (PT). The motility of the larvae was examined, utilizing a light microscope at 4× and 10×. The larval mortality rate was estimated as follow:

$$\text{Mortality rate} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

***In vivo* study**

Animals and experiment design

A total of Fifteen Baladi Egyptian goats, aged 6 to 10 months old were utilized. They were free from parasitic infection. The animals were kept indoors and divided into five groups (GI, GII, GIII, GIV and GV). At 0 week of experiment, each animal had been exposed to experimental infection with 10.000 *H. contortus* larvae except goats in group GI which was maintained as a control uninfected. GII was kept as control infected. At the 4th week post infection (WPI), treatment was done for the goats as follows: GIII was treated with 3 g/kg of PEE (Boonmasawai *et al*. 2013). GIV was received 6 g/kg of PEE, divided in two equal doses with interval time of 3 days. GV was given albendazole at a single dose of 10 mg/kg according to Adediran and Uwalaka (2015).

Coprological examination

Fecal examination was done through a concentration floatation technique weekly till the end of the experiment (8th week). The McMaster's method was applied for counting eggs per gram as described by Urquhart *et al*. (1996). Fecal egg count reduction percent (FECR %) was estimated utilizing the following formula.

$$\text{EFCR \%} = \frac{\text{Pre-treatment fecal egg count /gram - Post-treatment fecal egg count /gram}}{\text{Pretreatment fecal egg count /gram}}$$

Worm burden

At the end of the experiment (8th week), the goats were humanely slaughtered. The count of *H. contortus* worms in each abomasums was recorded. The effect of the PPE on the worm burden was assigned utilizing the following formula:

$$\text{Efficacy \%} = \frac{\text{Mean count of wormin infected-untreated group- Mean count of wormin treated group}}{\text{Mean count of wormin infected-untreated group}} \times 100$$

Hemogram analyses

Blood samples were collected from the jugular vein on vacutainer tubes containing ethylenediaminetetraacetic acid tripotassium (EDTA-K3) as an anticoagulant at zero-time and then every 2 WPI until the end of the experiment. The red blood cell count (RBCs), packed cell volume (PCV), hemoglobin (Hb) concentration, [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total and differential white blood cells (WBC) counts (lymphocytes%, monocytes%, neutrophils%, eosinophils% and basophils%) were evaluated using a hematological analyzer (Exigo Vet, Sweden).

Statistical analysis

The obtained data were presented for the mean and standard deviation (SD). Statistical comparison between the means of different groups and different weeks was analyzed with SPSS program version 10.

RESULTS

Adulticidal efficacy

Worm motility inhibition test

The PEE had recorded a significant ($P \leq 0.05$) anthelmintic efficacy on *H. contortus* adult worms in term of non motility and death of worms, compared to the control (Table 1). The exposure of worms to different concentrations of PEE caused a gradual declining of the mean number of the motile worms. With increasing the concentration of the PEE, significant increment of the worms' death was noticed at different exposure hours (Table 1). It was found that PEE at 400mg/ml was the most potent and has a significant anthelmintic activity ($P \leq 0.05$). At this concentration, the mean number of the motile worms was (10±0.00) at 0 h , then after 4 h post exposure, it was decreased to (6.7±0.67) while it was reached (0.33±0.33) worms at 24h post exposure. No mortality was found in the untreated control worms. The albendazole treatment showed a faster effect than the PEE. Complete loss of worms' motility was observed at 6 h post albendazole exposure and no revival of the treated worms was detected (Table 1). The effect of PEE on the worms was statistically significant ($P \leq 0.05$) in comparison to albendazole. Table (2) illustrated the mean percent worm motility inhibition (%WMI) and the mean mortality index (MI) when worms were seen after 30 minutes post exposure to PBS after the different treatments. The WMI % and the MI at 400 mg /ml PEE was (86% and 0.87) and at 10 µg/ml albendazole was (100% and 1), respectively.

Microscopy of *H. contortus* body wall

Microscopical examination of the normal adult worms showed no alteration in the integrity of the body wall. Fig.(1a&b). After 24h incubation with 10µg/ml albendazole (standard drug), the cuticle of the worms appeared to be thinner and bright in contrast to the normal control. Little zones for connection loss between the cuticle and the muscle layer could be observed. In some areas of the cuticle, the underlying muscle cells exhibited degenerative changes (Fig.1c&d). Worms incubated in the presence of 100 mg/ml PEE for 24 h revealed distortion of the cuticle and appearance of large vacuoles in the cuticular musculature (Fig.1e&f). With concentration of 200 mg/ml extract, the cuticular distortion became so severe, accompanied with vacuolization of the muscle layer (Fig. 2a&b). Some specimens showed areas of the outer cuticular layer had been removed and severe damage was observed in the cuticular musculature (Fig.2c). The strongest extract effects were attained with concentration of 400 mg/ml where the cuticle lost its normal aspect showing severely wrinkled surface and extreme disruption of the muscle bundles (Fig.2d&e). Some specimens showed prominent wrinkles of the cuticle so that small areas of the cuticle were seen to be splitting (Fig.2f)

Larvicidal efficacy

Concerning larvicidal activity, the PEE showed an excellent effect on *H. contortus* larvae, registered as (23.3±0.88) and (25.0±0.00) mortality at 200 and 400 mg/ml, respectively, after 24h exposure (Table3). Contrary, no larval mortality was observed in the control throughout the experimental period. Normal larval motility was observed with mean mortality rate of 0.0±0.0%. Weak anti larval activity was observed at 50mg/ml where the mortality rate reached 40%. The

Table 1: Effect of the different concentrations of pomegranate peels extract and albendazol on adult *H. contortus* motility.

Treatments	Mean±SE of motile worms number post exposure to various extract concentrations					
	0h	2 h	4h	6h	24h	Fresh PBS*
PEE50 mg/ml	10^{aA}±0.00	10^{aA}±0.00	9^{abAB}±0.57	8.7^{bAB}±0.33	$8^{b\ A}$±0.57	$4.7^{\ c}$±0.88
PEE 100 mg/ml	10^{aA}±0.00	9.7^{aA}±0.33	9^{abAB}±0.57	8.0^{bB}±0.00	6.7^{cB}±0.33	$3.7^{\ d}$±0.88
PEE 200 mg/ml	10^{aA}±0.00	9.3^{abA}±0.33	8.3^{bB}±0.33	6.7^{cC}±0.33	3^{dC}±0.57	$2.6^{\ de}$±0.67
PEE 400 mg/ml	10^{aA}±0.00	9^{aAB}±0.57	6.7^{bC}±0.67	2.7^{cD}±0.33	0.33^{eD}±0.33	1.3^{d}±0.33
Albendazole10µg/ml	10^{aA}±0.00	$7.7^{\ bB}$±0.88	1.7^{cD}±0.33	0^{dE}±0.00	0^{dD}±0.00	0.0^{d}±0.00
Dmso 0.10%	10^{aA}±0.00	10^{aA}±0.00	10^{aA}±0.00	9.3^{bA}±0.33	9.0^{bA}±0.00	9^{b}±0.00

Different small letters indicate significance in the same raw. Different capital letters indicate significance in the same column ($P \leq 0.05$). S.E.: standard error of mean; *: indicates worms were subjected to PBS for 30 min following incubation in various treatments to confirm their mortality. DMSO (dimethyl sulphoxide) considers the negative control.

Table 2: Mean percentage of worm motility inhibition and mortality index induced by various PEE concentrations and albendazole

Treatment	Worm motility inhibition percent (WMI%)	Mortality index (MI)
PEE50 mg/ml	47.8	0.53
PEE 100 mg/ml	59	0.63
PEE 200 mg/ml	71.1	0.74
PEE 400 mg/ml	86	0.87
Albendazole10µg/ml	100	1

incubation of the larvae with 200mg/ml PEE for 24h caused significant increase of dead larvae (23.3±0.88) which was comparable to the reference drug effect; albendazol with 93.2% mortality rate. The most potent larvicidal effect was recorded at 400mg/ml with 100% mortality rate 24 h post exposure. The mortality of larvae demonstrated significant increased as PEE concentration increased as well as over the time ($P \leq 0.05$).

***In vivo* experiment**

The clinical investigation of the animals groups under experiment proved that the onset of infection with haemonchosis was three weeks post infection, compared with the uninfected untreated group. The animals were suffered from general weakness, pale mucous membranes, off-food and dullness. After then, the observed clinical signs gradually disappeared in the PEE treated groups (GIII and GIV) as well as albendazole treated goats (GV). At the end of the experiment, the clinical examination of these treated animals indicated that they had improved and looked like healthy in comparison with the control (GI).

Effect of PEE on fecal egg count (FEC)

Fecal examination exposed that all the goats under experiment were passing *H. contortus* eggs in feces after three weeks from infection. At the 5th week; one week post treatment (WPT), the mean FEC started to gradually declined, revealing ($P \leq 0.05$) significant decrease in the treated groups with PEE (GIII and GIV), and albendazole (GV), compared to the mean FEC of the control infected group (Table 4). Prominent decrease ($P \leq 0.05$) in the mean FEC of the groups GIV and GV was noted at (the 2nd and 3rd) WPT in comparison with the mean FEC of groups GIII and GII. Maximum FECR% was recorded at the 4th WPT for all the treated groups, whereas it was (77.47, 90.55 and 95.12) for PEE treated groups GIII and GIV, and albendazole treated one, respectively (Table 5). No egg could be found through fecal examination of the control uninfected untreated group till the end of experiment.

Worm burden

The mean worms count of the groups treated with PEE (GIII and GIV) and albendazole (GV) was ($P \leq 0.05$) significant and lower than that of the control infected group (GII) (Table 6). Meanwhile, the difference in reduction of worm burden was ($P > 0.05$) insignificantly recorded between all the treated goats. No worms were found in the uninfected control group (G1). The treatment with PEE (GIII and GIV) and albendazole (GV) resulted in a considerable decrease in worm count with the efficacy of (90.12, 96.39 and 96.9%), respectively.

Hematological Findings

Figures (3 & 4) illustrated the changes in blood pictures in different experimental groups of goats infected with *H. contortus* and treated with PEE. All hematological parameters of uninfected-untreated goats (GI) oscillated within the normal values during the experimental period.

Erythrogram

The data of the RBCs count showed that no significant changes were recorded within or/and among all experimental groups. There were significant decreases ($P \leq 0.05$) in HCT values and Hb concentrations in the infected goats (GII) which reach its maximal reduction at 8th and 6th WPI, respectively while, their values increased

Table 3: The mean number of dead larvae±standard errors (SE) and mortality% following exposure of *H. conortus* larvae to different concentrations of PEE and albendazol

Treatment	Mean number of dead larvae±SE			Mortality %	
	0h	2 h	24h	2 h	24h
PEE50 mg/ml	0.00 ^{a}±0.00	0.33^{a}±0.33	10.0 ^{c}±1.00	1.32	40
PEE 100 mg/ml	0.00 ^{a}±0.00	0.67 ^{a}±0.67	18.3 $^{b±}$3.5	2.68	73.2
PEE 200 mg/ml	0.00 ^{a}±0.00	1.67 ^{a}±0.88	23.3 ^{ab}±0.88	6.68	93.2
PEE 400 mg/ml	0.00 ^{a}±0.00	2.00 ^{a}±1.15	25.0 ^{a}±0.00	8	100
Albendazole10µg/ml	0.00 ^{a}±0.00	1.33 ^{a}±0.67	23.3 ^{ab}±0.88	5.32	93.2
Dmso 0.10%	0.00 ^{a}±0.00	0.00 ^{a}±0.00	0.00 ^{d}±0.00	0.00	0.00

Small different letters in the same column are significant ($P \leq 0.05$).

Table 4: Mean fecal egg count in goat infected with *H. contortus* and treated with pomegranate ethanolic extract and albendazol

Weeks	Mean of eggs per gram of feces ×100±S.E. in different goats groups			
	GII	GIII	GIV	GV
0 *	0.00^{C}	0.00^{E}	0.00^{E}	0.00^{D}
3	65.00±15.27^{aAB}	58.50±8.97 ^{aB}	62.66±8.22 ^{aB}	61.33±3.16 ^{aB}
4 **	92.83±6.86 ^{aA}	89.50±11.55 ^{aA}	84.66±6.82 ^{aA}	85.33±10.17 ^{aA}
5	85.16±10.13 ^{aAB}	47.33±11.05^{bBC}	28.16±4.10^{bC}	29.00±4.80 ^{bC}
6	59.66±8.19 ^{aB}	32.50±4.19 ^{bCD}	15.50±1.15^{cCD}	10.83±2.12^{cD}
7	82.50±10.10 ^{aAB}	25.66±0.66 ^{bCD}	9.00±1.04 ^{cDE}	5.83±0.88 ^{cD}
8	62.16±9.01 ^{aAB}	20.16±2.74 ^{bDE}	8.00±0.76 ^{bDE}	4.16±0.60 ^{bD}

GII; control infected group, GIII; 3 g/kg PEE treated group, GIV;6 g/kg PEE treated group, GV; albendazole treated group. (*) 0 week of infection, (**) 0week of treatment. Different small letters in the same raw means significant. Different capital letters in the same column means significant ($P \leq 0.05$).

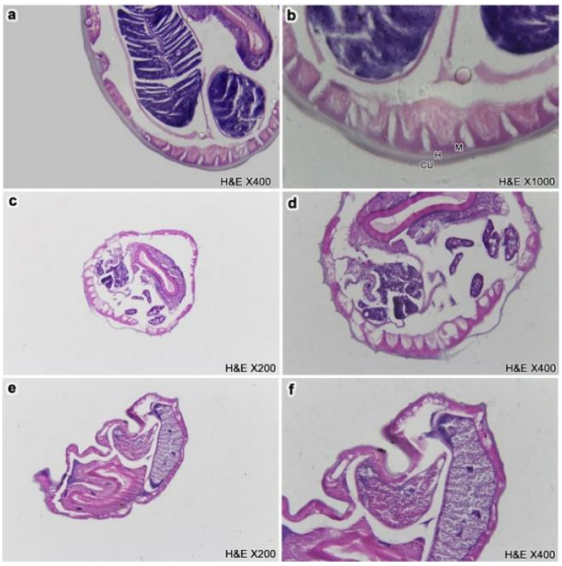

Fig. 1: Light micrographs of the body wall cross section of adult *H. contortus*. **a, b** Normal fresh worm. **c, d** Following 24 h incubation *in vitro* with 10 µg/ml albendazole (reference drug). Note the little zone for connection loss between the cuticle and the muscle layer. **e, f** After 24 h incubation *in vitro* with 100 mg/ml PEE. Note distortion of the cuticle and appearance of large vacuoles in the cuticular musculature. *CU* cuticle, *H* hypodermis, *M* muscle layer.

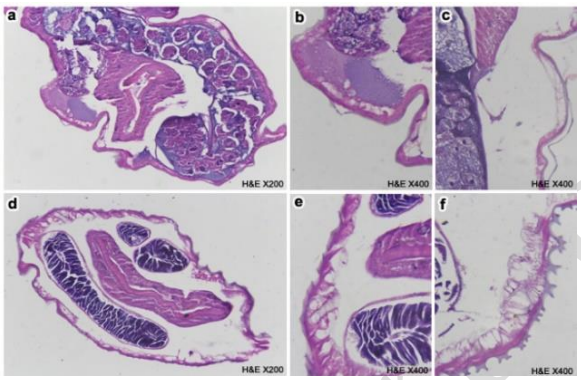

Fig. 2: Light micrographs of the body wall section of adult *H. contortus* following 24 h incubation with PEE. **a – c** With 200 mg/ml PEE. Note vacuolization of the muscle layer. **c** Light micrograph of the body wall longitudinal section shows removal of the outer cuticular layer. **d – f** With 400 mg/ml PEE. The cuticle shows wrinkled surface and extreme disruption of the muscle bundles. **f** Light micrograph of the body wall longitudinal section shows prominent wrinkles of the cuticle.

Table 5: Fecal egg count reduction percentage in pomegranate ethanolic extract and albendazole treated groups

Week post treatment	FECR%		
	GIII	GIV	GV
1	47.11	66.73	66.0
2	63.68	81.69	87.30
3	71.32	89.36	93.16
4	77.47	90.55	95.12

GII; control infected group, GIII; 3 g/kg PEE treated group, GIV; 6 g/kg PEE treated group, GV; albendazole treated group.

significantly (P≤0.05) after PEE (GIII and GIV) and albendazole treatment (GV) at 6th and 8th WPI compared to the infected group. With respect to red blood cell indices, the present data declared that marked decreased in MCV and MCHC values resulted in microcytic hypochromic anemia was observed in the infected goats (GII). Also, microcytic hypochromic anemia was noticed in goats of GIII, GIV and GV at 4th WPI. After PEE (GIII and GIV) and albendazole (GV) treatment, red blood cell indices increased significantly (P≤0.05) and returned into their normal values at 6th and 8th WPI (Figure 3).

Table 6: Comparative worm count in goat infected with *H. contortus* and treated with pomegranate ethanolic extract and albendazole

Animals groups	Mean worm count±SE	Efficacy %
GII	573.67 ^{a}±31.51	0.00
GIII	56.67^{b}±6.01	90.12
GIV	20.67 ^{b}±6.93	96.39
GV	17.67 ^{b}±2.84	96.9

GII; control infected group, GIII; 3 g/kg PEE treated group, GIV; 6 g/kg PEE treated group, GV; albendazole treated group.

Leukogram

Figure (4) illustrated the results of leukogram parameters in different experimental groups of goats infected with *H. contortus* and treated with PEE. The leukogram parameters of uninfected- untreated goats (GI) were within normal values during the experiment. The present study showed that there were significant (P≤0.05) increases in total WBCs counts in the infected goats (GII) during the experiment as compared to normal control (GI). However, significant (P≤0.05) decrease was observed in total WBCs counts after PEE (GIII and GIV) and albendazole (GV) treatment at 6th and 8th WPI compared with infected group (GII). Also, lymphocytes, monocytes and eosinophils count percentages were significantly (P≤0.05) elevated in the infected goats (GII). But, neutrophils were significantly decreased. After PEE (GIII and GIV) and albendazole treatment (GV), there was significant (P≤0.05) decrease in eosinophils count percentages at 6th and 8th WPI without any significant alteration in the lymphocytes and monocytes counts while, the neutrophils count percentages significantly increased at 8th WPI as compared with infected group (GII).

DISCUSSION

The current *in vitro* study revealed that the PEE had a potent adulticidal impacts on the adult *H. contortus* worms. These go parallel with the findings obtained by Jabeen *et al*. (2015) who found that the pomegranate fruit peels methanolic extract caused mortality of *H. contortus* worms. Yones *et al*. (2016) reported that the ethanolic extracts of leaves and stem bark of either dried edible or ornamental pomegranate had anthelmintic effect on adult worm *Schistosoma mansoni*. They found that the ethanolic extract of edible pomegranate had induced post exposure tegumental changes. The current PEE promising adulticidal effects might be attributed to its beneficial bioactive compounds, especially saponins, which induce damages for the parasite's membrane and causing vacuolization and tegument disintegration (Wang *et al*. 2010).

This study declared that the PEE possessed a strong *H. contortus* larvicidal activity. This result agreed with the results obtained by Anjos *et al*. (2016) who reported that aqueous extract of pomegranate stem resulted in a remarkable reduction of *Haemonchus* spp larvae count per gram feces.

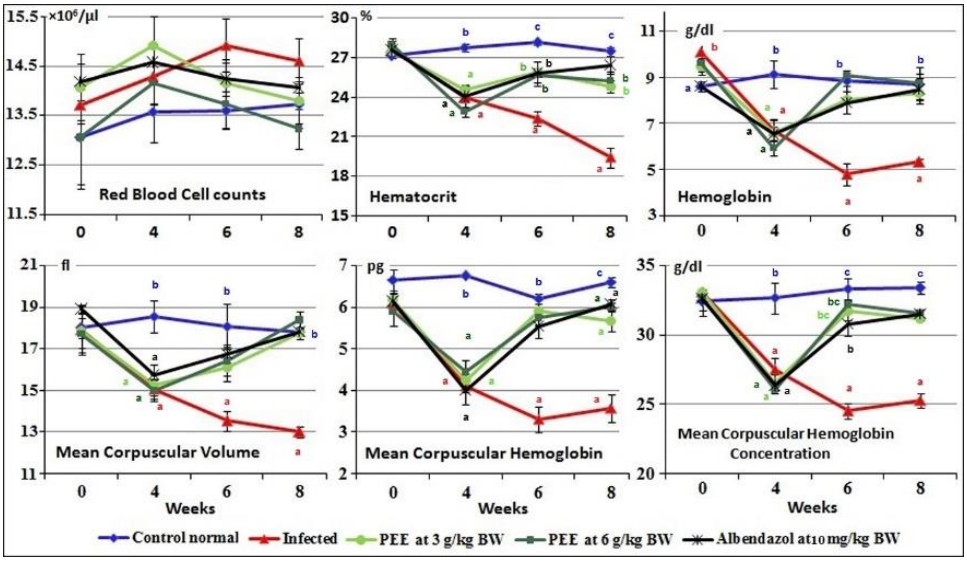

Fig. 3: Erythrogram in different experimental groups of goats infected with *H. contortus* and treated with pomegranate ethanolic extract (PEE) and albendazol-as standard drug. (Mean ± SE). Means with different letters in the same period are significantly different at P<0.05.

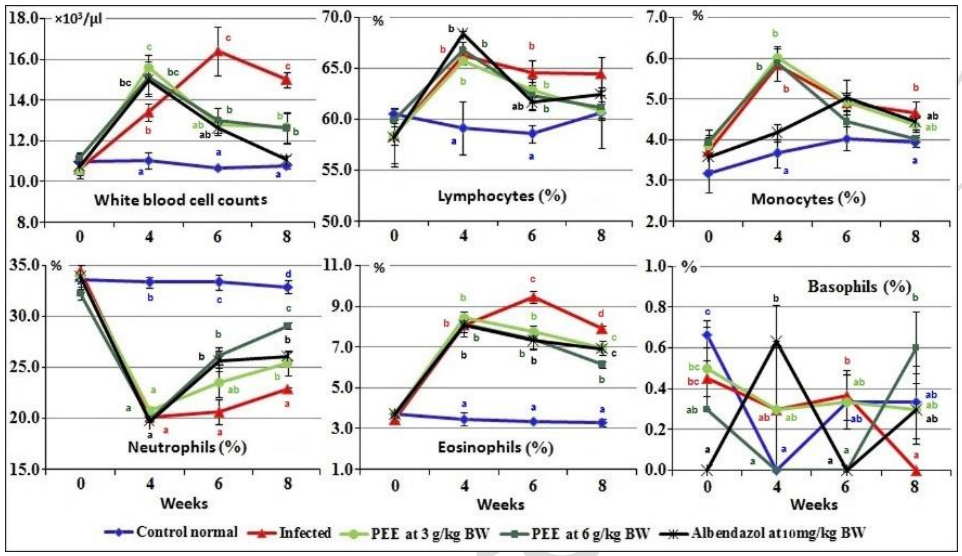

Fig. 4: Leukogram in different experimental groups of goats infected with *H. contortus* and treated with pomegranate ethanolic extract (PEE) and albendazol-as standard drug. (Mean ± SE). Means with different letters in the same period are significantly different at P<0.05.

The potency of the PEE was of direct correlation with increasing the concentration, whereas PEE at 400mg/ml was the most effective against adult *H. contortus* worms and larvae. This was in accordance with the previously recorded by Anjos *et al*. (2016). The clinical investigation of the infected groups at the 4th week of the experiment revealed that the animals were suffered from haemonchosis as described by Iliev *et al*. (2017) This might be resulted from the using of high infecting dose of larvae that could cause the severe form of the disease (soulsby 1986).

The PEE proved impressive *in vivo* anthelmintic effect through the gradual decrease of FEC, FECR% and lowering of worm burden of the treated groups compared to the infected control and albendazole treated groups. The current study revealed that anthelmintic activity of PEE was of dose-dependent. These are in accordance with Boonmasawai *et al*. (2013) who used the PEE at 300 mg/kg, to treat female goats naturally infected with GINs and revealed significant FECR (55, 43 and 36 %) at 1, 3 and 7 days post treatment, successively. Moreover, Lalhmingchhuanmawii *et al*. (2014) who evaluated the anthelmintic activity of PEE against paramphistomiasis among sheep and recorded a maximum FECR (97.95 %) three WPT using 50 mg/ml concentration. This study declared that PEE had diminished the worm burden in the treated groups in contrast to the infected untreated one. Similar findings were obtained by Jaheed *et al*. (2019) who recorded a significant lowering of the *H. contortus* worms' count as a result of using *Balanites aegyptiaca* ethanolic extract at 9 g/kg in goats. This distinct efficacy of the PEE might be returned to therapeutic features of peels of pomegranate (Qnais *et al*. 2007; Atta *et al* 2016).

In this study, goats infected with *H. contortus* exhibited decreases the values of Hb, HCT, MCV and MCHC which caused microcytic hypochromic anemia without any significant changes in RBCs counts. The non-significant changes in RBCs counts might be due to the erythropoietic activation of host to compensate the blood loss (Bordoloi *et al*. 2012). After PEE and albendazole treatment, the Hb and HCT values increased, probably due to their therapeutic efficacy which caused the reduction of worm burden. This might return to the anti parasitic effect of the extract as reported by Lalhming-chhuanmawii *et al*. (2014), Jabeen *et al*. (2015) and Yones *et al*. (2016). Besides the possible therapeutic potential of PEE, pomegranate enhanced the level of Hb and RBCs counts (Manthou *et al*. 2017). On the other hand, significant increases in WBCs counts, lymphocytes, monocytes, eosinophils and basophils percentages were

recorded in infected goats which reinforced previous findings of Jaheed *et al*. (2019). Moreover, Artis and Grencis (2008) stated that peripheral eosinophilia and basophillia was reported to be one of the most immmunopathological changes in the nematodes infection. After PEE treatment, the decreased counts of WBCs, lymphocytes and eosinophils could be attributed to the anti-inflammatory activity of pomegranate and its major components (Danesi *et al*. 2017). Hassan *et al*. (2013) revealed that the infected goat received 10 mg/kg BW of albendazol as a single dose showed a significant decrease on RBC and MCHC on day 15 and PCV % on day 30 PT. So the PEE had efficiently improved the health conditions of the treated goats relieved the consequence of the induced infection that possibly could explained as pomegranate is a rich source of polyphenols such as ellagitannins, anthocyanins and ellagic acid which provides health benefits relating to its anti-oxidants and anti-inflammatory properties as reported by Qnais *et al*. (2007).

Conclusions

It was deduced that PEE exhibited a potent *in vitro* anthelmintic activity through the adult worm motility inhibition, the larval mortality and the deterioration of the adult worm's body wall integrity. The PEE caused significant decrease to the FEC, diminution of the worm burden and improvement of animal health through returning of the normal blood picture among the treated goats. This obvious consistency between the current *in vitro* and *in vivo* studies on *H. contortus* have confirmed the superiority of the PEE that could be utilized as safe, efficient and economical alternative anthelmintic and as a general health enhancer of natural origin.

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