



Research Article

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Comparative studies on the efficacy of methanolic extracts of (Punicagranatum) and triclabendazoal on Haemonchusspp by using scanning electron microscope (SEM) examination.

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ABSTRACT

Comparative studies on the efficacy of methanolic extracts of (Punicagranatum) and triclabendazoal on Haemonchusspp by using scanning electron microscope (SEM) revealed that, Punicagranatum and ACBZ showed significant effect on adult worms of Haemonchus contortus after 24h in vitro cultivation, the worms started to stop motion animation and paralysis leading 100 % to death of worms.

Adult H. contortus: Scanning electron microscopy (SEM) of normal fresh worms showed, the mouth of the normal fresh worm was hexagonal with six semicircular rudimentary lips, lateral amphids, papillae and dorsal buccal lancet. The latter was a specialized cuticular structure

arising from the cuticular lining of the dorsal wall of the buccal cavity. The lancet's anterior tip and lateral edges were slightly rounded. A pair of cervical papillae was prominent and spine-like. The cuticle was transversally striated and with lateral ridges.

SEM of Treated Worms H. contortus after 24 h incubation with 50 mg/ml ethanolic extracts of Punicagranatum peel showed the buccal capsule which presented a smooth surface in control worm, lost their normal aspect and showed distortion with severe blebbing of the lips.

The cuticle including the lips appeared to be more swollen than normal. The transverse striations became less pronounced and lost their normal aspect showing longitudinal wrinkles. The treated worms showed distortion of both buccal capsule and cuticle.

The lips were deformed and the cuticular surface had a wrinkled, corrugated appearance. Besides, longitudinal thickening and wrinkling of the cuticular ridges were observed.

Keywords: *Punicagranatum, H. contortus, ACBZ, SEM, haemonchosis, helminthes, triclabendazole and goat.*

INTRODUCTION

The control of *H. contortus* infection is largely based on pasture management and the use of anthelmintics. However, clean pasture is not readily available under intensive grazing conditions and there is an increasing occurrence of parasites resistant to anthelmintics [1] [2]. Therefore increasing attention had been given to the development of alternative control method, such as the use of vaccines [3] or used of various traditional and herbal medicine. *Punicagranatum* is described for its medicinal properties and is used in traditional medicine for the treatment of various diseases. Extravagant in the use of chemotherapy in the treatment of parasites leads to the emergence of resistance to these worms (drug resistant), environmental pollution and the side effects on the health of

the animal. As well as the use of chemotherapy to treat parasites harmful to human health as a result of the accumulation of the drug residue.

The use of medicinal plants has gained much attention in the last decade, and among those plants commonly used as medication in folk medicine, various extracts have been the subject of many pharmacological studies. Pomegranate (*Punicagranatum*) is a fruit with rich ethno medical applications, has been also introduced as potential anti-microbial, anti-helminthic agent, possesses strong antioxidant and anti-inflammatory properties [4], observed the antiprotozoal chemotherapeutic effect of *P. granatum* against *E. histolytica* & *G. lamblia* when used for treatment of dysentery in Mexican traditional medicine. Experiments were carried out to show antiparasitic activity of *Punicagranatum* (Pomegranate, fruit-rind) in the treatment of various helminthes. Niaz et al. (2012) [5] showed that *Punicagranatum* may be a good herbal medicine to treat animals infected with schistosomiasis with no side effect and the cheapest one.

The present study was designed to:-

Comparative studies on the efficacy of methanolic extracts of (*Punicagranatum*) on *Haemonchus* spp by using scanning electron microscope (SEM) examination.

Concerning the therapeutic efficacy of *P. granatum* extracts:

Akhtar and Aslam (1988) [6] in Faisalabad studied anticestodal efficacies of total alkaloids and glycosides isolated from fruit-rinds of *punicagranatum* (Anar) in goats infected naturally with cestode. Both these isolates were administered separately at the dose rates of 75, 150 and 225 mg/kg body weight orally respectively. A standard synthetic tapeworm remedy, Niizan (Levamisole, 1.5% + Oxytoclozanide, 3%) was also given to a group. The percentage EPG reduction at dose rates of 150 mg and 225 mg/kg were statistically similar to that of the control drug, Niizan. Therefore, it is conceivable that alkaloids of *punicagranatum* are as potent as Niizan against the cestode infection in goats and these data suggested that they could be at least in part responsible for the anticestodal action of this indigenous plant drug.

Javed and Akhtar (1990) [7] in Faisalabad (Pakistan) showed that the antinematodal activity of a mixed prescription of veronica anthelminca seed (kali zeeri) and Embelia vibes was evaluated in goats. Their study seems to support the use of this cheap combined herbal therapy containing equal parts of *Veroniaanthelmintica* (seeds), and *Embelia vibes* (fruit) for the treatment of goats suffering from gastrointestinal nematode infection.

Amorin et al. (2003) [8] observed Promising antischistosomal effect in the murine treated with *Punicagranatum* fruit. Albrecht et al. (2004) [9] recorded the dietary antioxidants of *P. granatum* peel extract were nature's gift molecules endowed with preventive and therapeutic properties against prostate cancer.

Calzada et al. (2006) [4] observed the antiprotozoal chemotherapeutic effect of *P. granatum* against *E. histolytica* & *G. lamblia* when used for treatment of dysentery in Mexican traditional medicine.

Adhami and Mukhtar (2007) [10] defined the usefulness of *P. granatum* as dietary antioxidants for chemoprevention of cancer besides the antiparasitic effect.

Sujon et al. (2008) [11] in Bangladesh performed detailed investigation with the aim to find out the indigenous medicinal plants having anthelmintic action. Ten (10) indigenous medicinal plants were primarily selected and the ethanol extracts were prepared for anthelmintic trials and determination of anthelmintic properties in vitro and in vivo against the GINs in goat during the period from July 2006 to December 2006. The results obtained showed that ethanol extract of Labanga, Neem, Karolla and Pineapple at the dose of 100 mg/kg showed a significant and potent antinematodal effect.

Niaz et al. (2012) [5] in Punjab (Pakistan) studied the antiparasitic activity of *punicagranatum* (pomegranate, fruit rind) among buffaloes infected with schistosomiasis at different doses. To compare their efficiency another group of animals were treated with praziquantel at dose level 10 mg/Kg body weight. They showed that *Punicagranatum* may

be a good herbal medicine to treat animals infected with schistosomiasis with no side effect. The efficacy (%) of drugs was calculated on the basis of reduction in egg count after treatment.

Murthy et al. (2004)[12] reported that the anti-oxidative effects of *P. granatum* on serum and macrophages could contribute to attenuation of atherosclerosis development in diabetic patients.

Wang et al. (2004)[13] enumerated 4 compounds isolated from *P. granatum* that exhibited anti-oxidant activity, and the later was evaluated by measurement of low-density lipoprotein susceptibility to oxidation.

Malik et al. (2005)[14] supported that these dietary antioxidants of *P. granatum* peel extract were nature's gift molecules endowed with preventive and therapeutic properties against prostate cancer.

Rozenberg et al. (2005)[15] concluded that the antioxidants and anti atherogenic effects of *P. granatum* could be due to the presence of unique complex sugars and phenolic sugars.

Toklu et al. (2007)[16] reported that administration of *P. granatum* peel extract alleviated oxidative injury of the liver and improved the hepatic structure and function.

Adhami and Mukhtar (2007)[10] defined the usefulness of *P. granatum* as dietary antioxidants for chemoprevention of cancer besides the antiparasitic effect .

Sujon et al.(2008)[11]in Bangladesh performed detailed investigation with the aim to find out the indigenous medicinal plants having anthelmintic action. Ten (10) indigenous medicinal plants were primarily selected and the ethanol extracts were prepared for anthelmintic trials and determination of anthelmintic properties in vitro and in vivo against the GINs in goat during the period from July 2006 to December 2006. The results obtained showed that ethanol extract of LabangaNeem, Karolla and Pineapple at the dose of 100 mg/kg showed a significant and potent antinematodal effect.

Niaz et al. (2012)[5]in Punjab (Pakistan) studied the antiparasitic activity of punicagranatum (pomegranate, fruit rind) among buffaloes infected with schistosomiasis at different doses . To compare their efficiency another group of animals were treated with praziquantel at dose level 10 mg/Kg body weight. They showed that Punicagranatum may be a good herbal medicine to treat animals infected with schistosomiasis with no side effect. The efficacy (%) of drugs was calculated on the basis of reduction in egg count after treatment.

MATERIALS AND METHODS

In vitro, determination of the anthelmintic efficacy of Punicagranatum and TCBZ on adult Haemonchusspp.

Adult worm of *Haemonchusspp* collected from the abomasa of goats slaughtered in Taif abattoir. Under sterile conditions in a laminar flow cabinet, worms were washed in several changes of warm (37.8 C), sterile RPMI 1640 culture medium containing antibiotic (penicillin, 50IU/ml; steromycin, 50 mg l ml). The worm were subsequently transferred to fresh culture medium containing 50% (v/v) heat denatured rabbit serum, 2% (v/v) rabbit red blood cell; as recommended by Ibarra and Jenkins (1984) [17]. and plant extract of (*Punicagranatum*) at five different concentration 10,20,30,40 and 50 mg/ml were added.

Dilutions were made from a stock solution of plant extract at 10 mg/ml, prepared with 70% (v/v) ethanol. The worm incubated for 24 hour at 37.8 C in an atmosphere of 5% CO₂. A positive control group was prepared by incubating worm for 24 hour in RPMI culture medium containing 20 mg/ml TCBZ-SX. This level corresponded to maximum blood levels in vivo [18]. The TCBZ was initially prepared as a stock solution in Dimethyl Sulphoxide (DMSO) and added to the culture medium to give a maximum solvent concentration of 0.1% (v/v). Solvent control worms incubated for 24 hrs in RBMI 1640 culture medium containing 0.1% (v/v) DMSO. Normal control worm fixed immediately following the initial washing. One worm were examined for each concentration.

Preparation of methanolic extracts of Punic a granatum:

The alcoholic extract from p. granatum were prepared at the laboratory of Medicinal and Aromatic Plant Research Department, National Research Center according to the method of (Tariq et al.2009)[19] and (El-Menshawi, 2003)[20].

Preparation of stock solution:

Stock solutions of plant extracts were prepared by diluting the condensed extracts with water. Different concentrations of each category of plant extracts were prepared by dissolving them in the water prior to anthelmintic screening.

Determination of Effective Doses for in Vitro Assay.

Active extracts of peels was bioassayed at ascending concentrations (10, 20, 30, 40 and 50/ml) to evaluate viability of *Heamonchusspp* after 24 hours incubation. The results were used to calculate the death rate following 24 hours incubation (Yousif et al., 2007)[21]. Control group: adult *Heamonchusspp* were suspended in DMSO added to media and incubated for 24 hours.

In vitro screening of plant extracts for anthelmintic activity:

Screening of water extracts of plant at various concentration viz. 10, 20,30,40 mg/ml and 500 mg/ml were performed in the Petri-dishes containing adult live stomach worms of goats collected from slaughter house in Phosphate buffer saline (PBS). PBS (100 ml) containing 10 adult worms (female) were pipetted in 5 Petri-dishes at ratio of the recommended dose were then added, respectively. The drug-parasite Petri-dishes were incubated for three hours at room temperature and the efficacy was observed by counting the dead parasites and expressed in percentages (%).

Morphological changes were observed with plant extracts (*Punicagranatum*) at five different concentration 10, 20, 30, 40 and 50 mg/ml and TCBZ at 20 mg/ml after 24 hrs incubation

Scanning electron microscope (SEM):

Following incubation, the adult worms were fixed intact for 12 hrs in a 3:1 mixture of 4% (w/v) glutaraldehyde in 0.12 M-Millonig buffer, PH 7.4 and 1% aqueous osmium tetroxide. The specimen washed repeatedly in double-distilled water, dehydrated through acetone, critical point dried in carbon dioxide, fixed to aluminum stubs and coated with gold-palladium. The specimen viewed in a Jeol scanning electron microscope (Jeol Corp., Mitaka, Japan) operated at 15 k V. Measurements of the worm and the group of treated worms were made according to method proposed by Valero et al. (1996)[22], using a computer image analysis system (ELICA QW in 500, Cambridge, England).

RESULTS

The present study was designed for, in vitro, comparative study on efficacy of methanolic extracts of *Punicagranatum* peel and triclabendazole (TCBZ) on adult flukes, on treatment of haemonchosis in goats in Taif, through investigate the parasite morphological changes by using Scanning Electron Microscopy (SEM).

Table (1): In vitro effect of *P. granatum* methanol extracts on adult worms, after 24 hours incubation (Death rate of adult worms).

Concentration of <i>p.granatum</i>	Worm suspended in <i>p.granatum</i> methanolic extract	Percentage
10 mg/ml	All worms dead	100
20 mg/ml	All worms dead	100
30 mg/ml	All worms dead	100
40 mg/ml	All worms dead	100
50 mg/ml	All worms dead	100
control (DMSO added to media)	All live for 2- 3 days	-----

Table 2: In vitro effect of TCBZ on adult worms, after 24 hours incubation (Death rate of adult worms) .

Concentration of TCBZ	Worm suspended in TCBZ.	Percentage
10 mg/ml	All worms dead	100
20 mg/ml	All worms dead	100
30 mg/ml	All worms dead	100
40 mg/ml	All worms dead	100
50 mg/ml	All worms dead	100
control (DMSO added to media)	All live for 2- 3 days	-----

In Vitro study: *Punicagranatum* and TCBZ Table (1) and (2) showed significant effect on adult worms of *Haemonchus contortus* after 24 h in vitro cultivation revealed, 100% of worms were dead with peels extracts of *P.granatum* and TCBZ. The results showed that the different concentrations of Pomegranate peel and TCBZ have an impact outside the host body and worms started to stop motion animation worms and paralysis leading to death.

Scanning electron microscopy (SEM) of normal fresh adult *Haemonchus* spp worms:

Scanning electron microscopy (SEM) of normal fresh of *Haemonchus* spp. showing the narrow, coiled anterior part of the body Fig(1) .S.E. Micrograph of anterior extremity of *Haemonchus* spp showing the beginning of the longitudinal cuticular ridges short distance behind the interior extremity of the worm Fig(2) . S.E. Micrograph of enlarged body surface of *Haemonchus* spp showing longitudinal cuticular ridges, and transverse striations Fig (3). The anterior extremity of *Haemonchus* spp. showing: 1- mouth , 2- cervical papilla Fig(4). SEMs of normal fresh worm showing the mouth with six semicircular rudimentary lips and a pair of spine-like cervical papillae (arrows, and a dorsal buccal tooth (5).

SEM of treated worms show changes in *Haemonchus* spp .adult worms after 24 h incubation with 30 mg/ml TCBZ. The buccal capsule showed distortion with severe blebbing of the lips. No obvious differences were detected between the treated and untreated worms concerning their cuticle Fig(6- 7-8). After 24 h incubation with 50 mg/ml, the cuticle including the lips appeared to be more swollen than normal so that the transverse striations became less pronounced and lost their normal aspect showing longitudinal wrinkles Fig (9-10).

Concerning Scanning electron microscopy (SEM) of treated adult *Haemonchus* spp worms with methanolic extracts of *Punicagranatum* peel

of naturally infected with haemonchosis in goats in Taif with a dose (30 mg/ml). SEM Micrograph of anterior extremity of *Haemonchus* spp. showing: irregularly corrugated cuticular surface of the worm ,distorted cephalic plate and mouth opening , irregularly rounded mouth opening , and bulging cephalic papillae.

S.E. Micrograph of en-face view of *Haemonchusspp.* treated with methanolic extracts of *Punicagranatum* peel, showing: distorted cephalic plate, irregularly rounded mouth opening, and bulging cephalic papillae Fig (11-12) .

The strongest effects were observed after 24 h incubation with drug (50 mg/ml) methanolic extracts of *Punicagranatum* peel, where the treated worms showed distortion of both buccal capsule and cuticle and bulging of the most terminal extremity of the cervical papilla (Fig.13,14).

The longitudinal thickening and wrinkling of the cuticular ridges were observed (Fig.15)..

The cuticle including the lips appeared to be more swollen than normal so that the transverse striations became less pronounced and lost their normal aspect showing longitudinal wrinkles (Fig.16).

SEM of experiment nontreated Control *Haemonchus spp.*

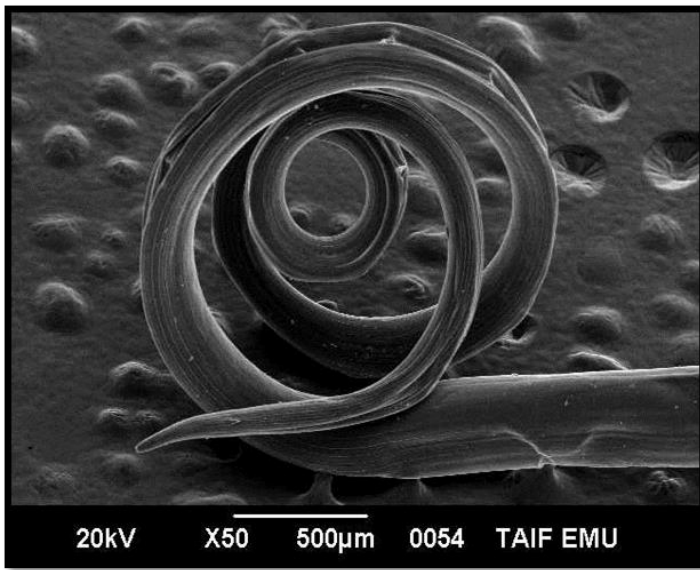
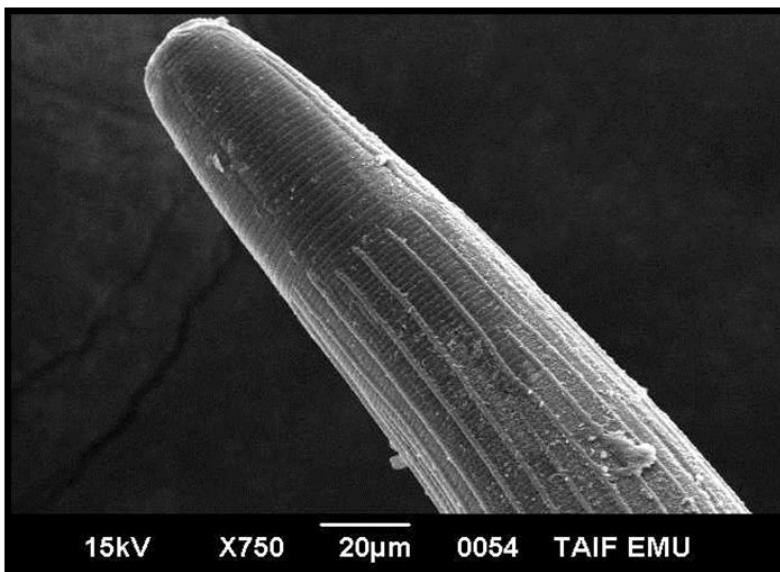


Fig.1: S.E. Micrograph of *Haemonchusspp* showing the narrow, coiled



Adult worm.

Anterior part of the *Haemonchusspp.*

Fig 2: S. E. Micrograph of anterior extremity of *Haemonchusspp* showing the beginning of the longitudinal cuticular ridges short distance behind the interior extremity of the worm.

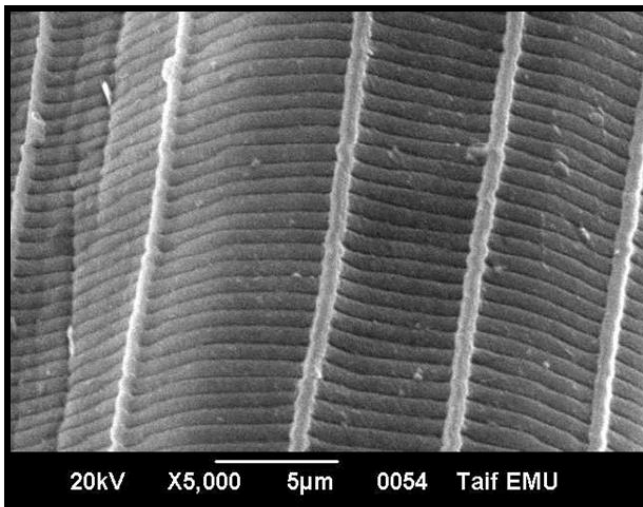


Fig.3: S.E. Micrograph of enlarged body surface of *Haemonchusspp* showing:- longitudinal cuticular ridges, and transverse striations.

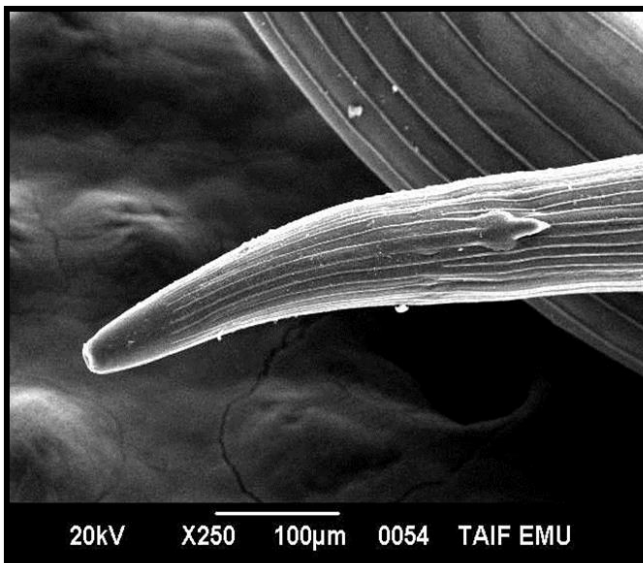


Fig. 4 : S.E. Micrograph of anterior extremity of *Haemonchusspp* showing: 1-mouth , 2- cervical papilla

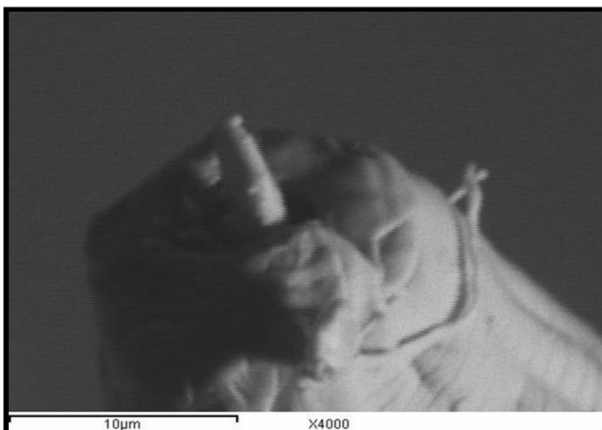


Fig .5:Scanning electron micrographs (SEMs) of the anterior end of adult *Haemonchusspp.* SEMs of normal fresh worm showing the mouth with six semicircular rudimentary lips and a pair of spine-like cervical papillae (arrows, and a dorsal buccal tooth.

SEM of Treated *Haemonchusspp.* with Triclabendazole

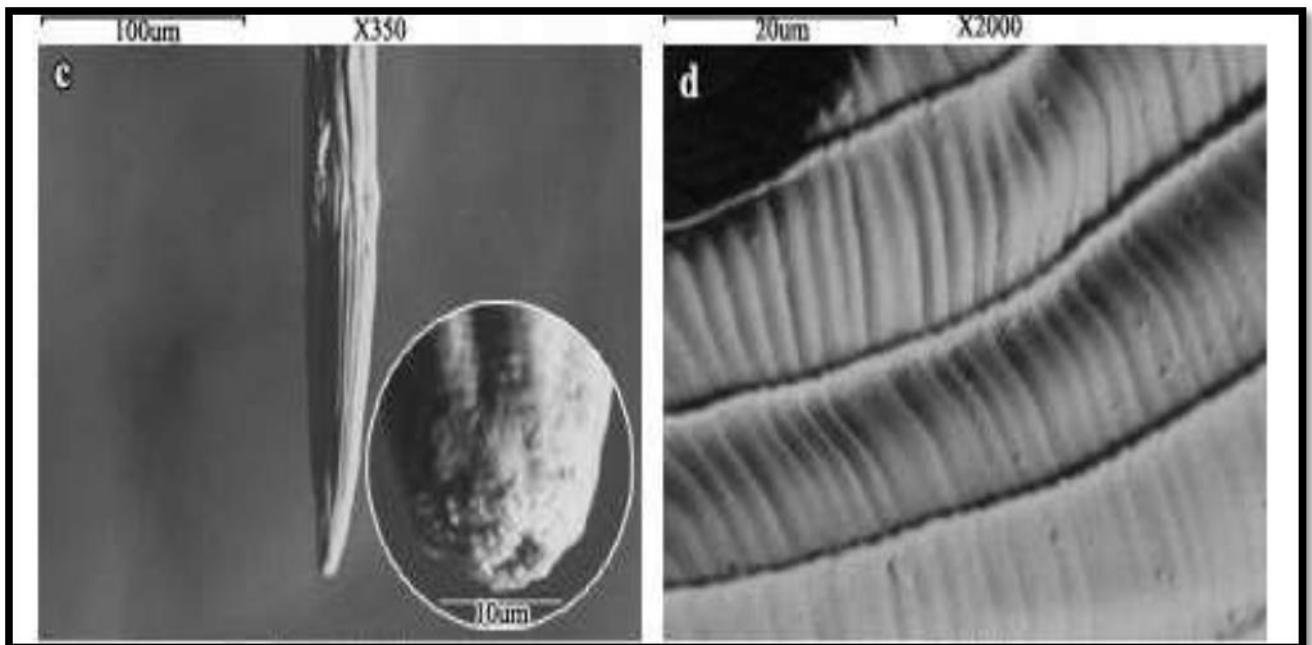
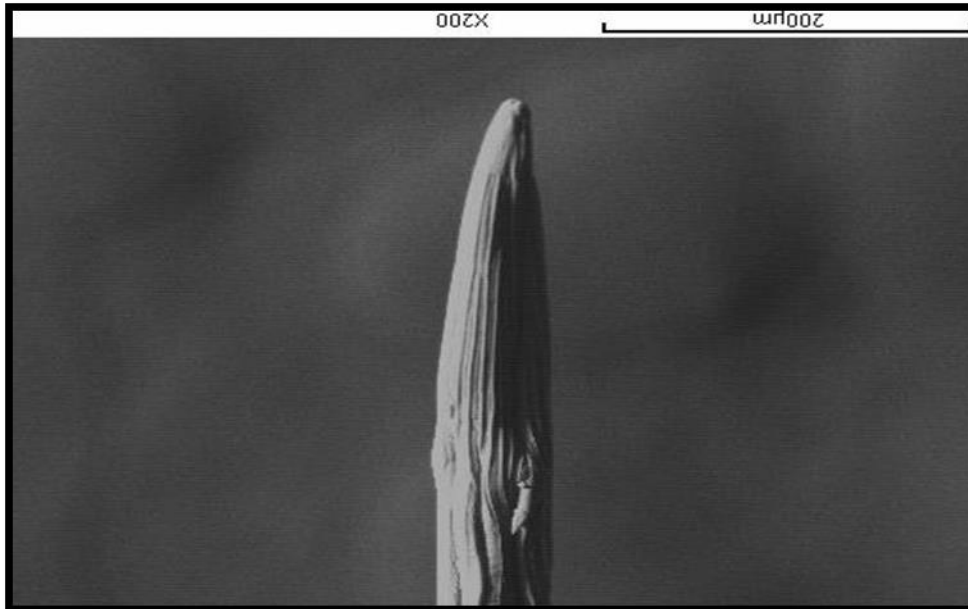


Fig.6-7-8: SEM of treated worms: The changes in *H. contortus* adult worms after 24 h incubation with 30 ng/ml TCBZ. The buccal capsule showed distortion with severe blebbing of the lips. No obvious differences were detected between the treated and untreated worms concerning their cuticle.

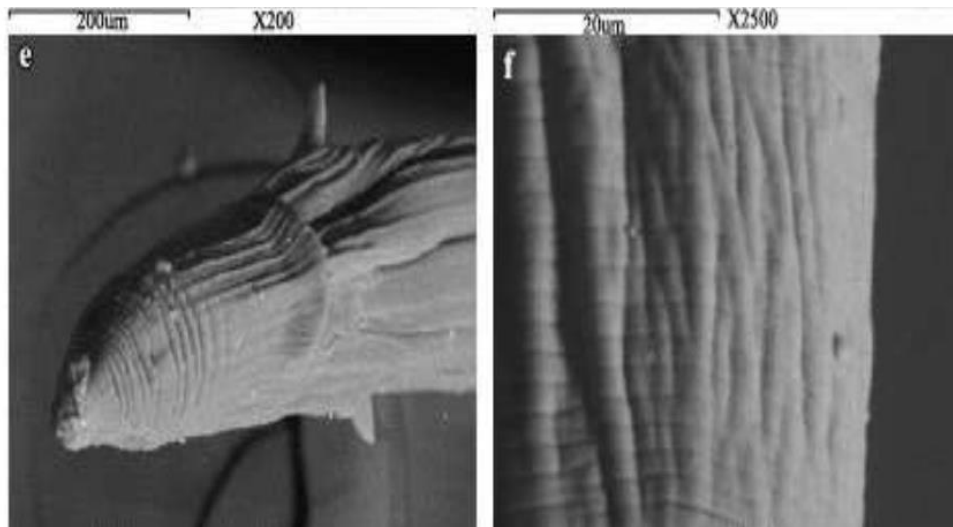


Fig.9-10 :After 24 h incubation with 50 mg/ml, the cuticle including the lips appeared to be more swollen than normal so that the transverse striations became less pronounced and lost their normal aspect showing longitudinal wrinkles.

SEM of treated *Haemonchusspp* with methanolic extracts of *Punicagranatum* peel.

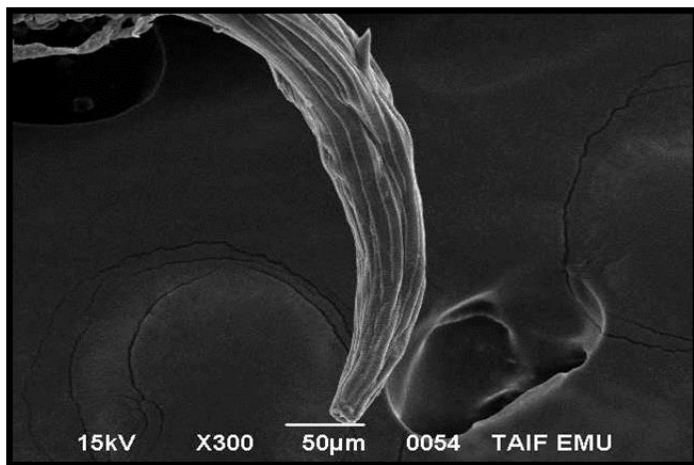


Fig.11 : SEM Micrograph of anterior extremity of *Haemonchusspp* treated with methanolic extracts of *Punicagranatum* peel, showing: irregularly corrugated cuticular surface of the worm.

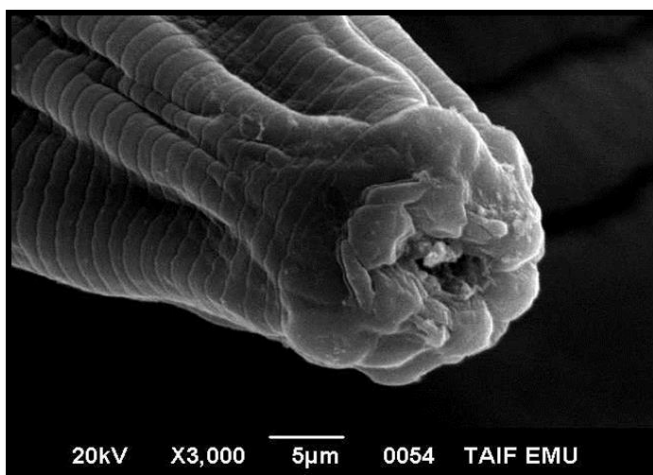


Fig.12: S.E. Micrograph of en-face view of *Haemonchusspp.* treated with methanolic extracts of *Punicagranatum* peel , showing: distorted cephalic plate, irregularly rounded mouth opening , and bulging cephalic papillae.

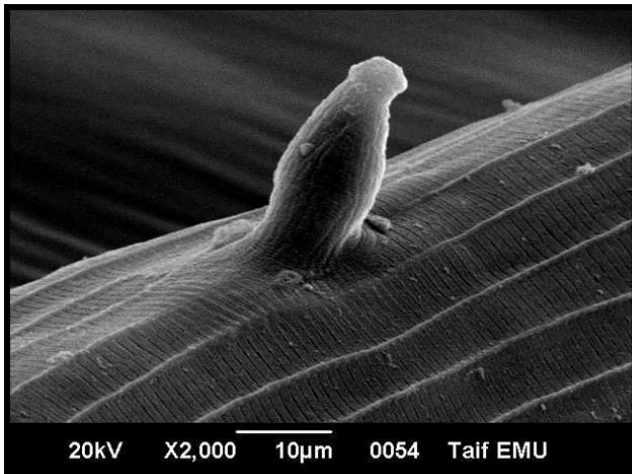


Fig.13 : S.E. Micrograph of *Haemonchusspp.* treated with methanolic extracts of *Punicagranatum* peel , showing bulging of the most terminal extremity of the cervical papillae .

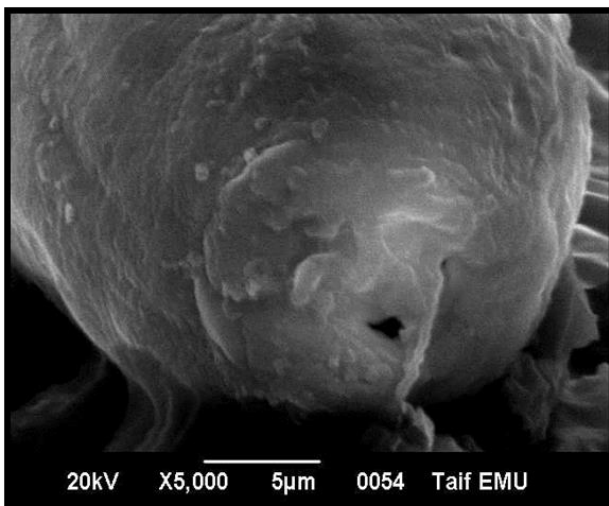


Fig.14 : S.E. Micrograph of *Haemonchusspp.* treated with methanolic extracts of *Punicagranatum* peel , showing distorted mouth opening and papillae.

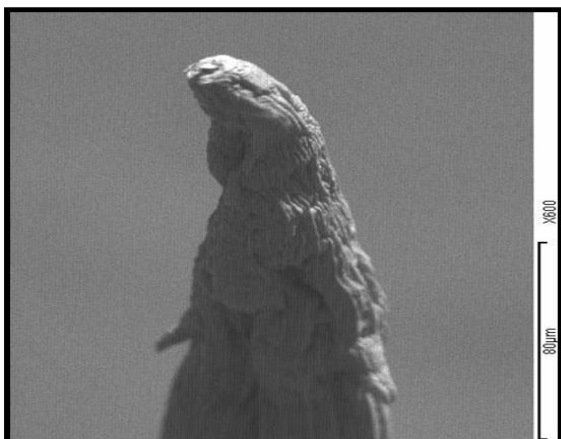


Fig.15:After 24 h incubation with methanolic extracts of *Punicagranatum* peel (50 mg/ml), where the treated worms showed distortion of both buccal capsule and cuticle .The longitudinal thickening and wrinkling of the cuticular ridges were observed.

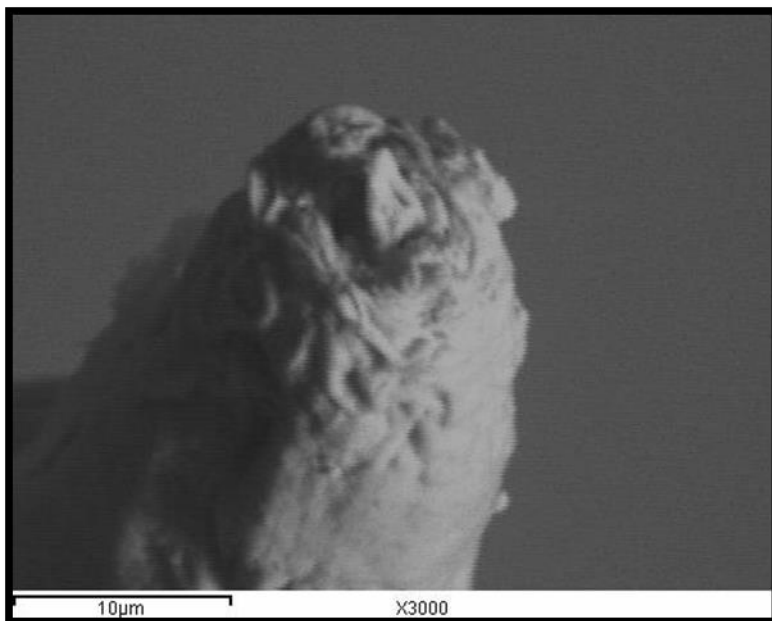


Fig.16:After 24 h incubation with methanolic extracts of *Punicagranatum* peel (50 mg/ml), the lips were deformed and the cuticular surface had a wrinkled, corrugated appearance .

DISCUSSION

Punicagranatum and TCBZ showed significant effect on adult worms of *Haemonchus* spp after 24h in vitro cultivation, 100% of worms were dead with peels methanolic extracts of *P. granatum* and TCBZ. The results showed that the different concentrations of *punicagranatum* peel and TCBZ have an impact outside the host body and worms started to stop motion animation worms and paralysis leading to death.

Scanning electron microscopy (SEM) of normal fresh worms showed, the mouth of the normal fresh worm was hexagonal with six semicircular rudimentary lips, lateral amphids, papillae and dorsal buccal lancet The later was a specialized cuticular structure arising from the cuticular lining of the dorsal wall of the buccal cavity. The lancet's anterior tip and lateral edges were slightly rounded. A pair of cervical papillae was prominent and spine-like The cuticle was transversally striated and with lateral ridges.

The changes in *Haemonchus* spp adult worms 24 h incubation with 30 mg/ml methanolic extracts of *Punicagranatum* peel concerned, showed the buccal capsule which presented lost their normal aspect and showed distortion with severe blebbing of the lips while, at dose rate of 40 mg/ml methanolic extracts of *Punicagranatum* peel, the cuticle including the lips appeared to be more swollen than normal so that the transverse striations became less pronounced and lost their normal aspect showing longitudinal wrinkles .The strongest effects were observed after 24 h incubation with drug (50 mg/ml methanolic extracts of *Punicagranatum* peel), where the treated worms showed distortion of both buccal capsule and cuticle. The lips were deformed and the cuticular surface had a wrinkled, corrugated appearance. Besides, longitudinal thickening and wrinkling of the cuticular ridges were observed. The recorded results which obtained concluded that *P. granatum* had highly efficacy in treatment of *Haemonchus* spp in Taif governorate.

The same results were observed by Scifo et al, (2004) [23], (Ajaikumar et al., 2005) [24] and (Calzada et al., 2006) [4]. The promising antihelminthes properties of *P. granatum* reported in this study could be added to its known potency in traditional folk medicine.

Effect of methanolic extracts of *Punicagranatum* peel and triclabendazole (TCBZ) using SEM examination:

Helminth infections are amongst the most common parasitic infections of animals worldwide now well recognized as an important veterinary problem, both in developing and in developed countries (Sultan et al. 2010) [25].

The sheep and goats industry still relies heavily on the use of anthelmintics to alleviate the infections of gastrointestinal nematodes, cestodes and liver flukes that are the most important. At a time, anthelmintic resistance has become a serious problem in veterinary medicine (Wolstenholme et al (2004) and Rahman et al. , 2011) [26] [27].

The present study demonstrated the comparative morphological effects of methanolic extracts of *Punicagranatum* peel and triclabendazole against *Haemonchus* SEM. The results of *Punicagranatum* peel were more severely affected with distortion of both buccal capsule and cuticle against distortion of buccal capsule in the latter group. The cuticle of nematodes is metabolically active and morphologically specialized for selective absorption of nutrients and osmoregulation. Thus, passive diffusion of anthelmintics through the cuticle [28] would probably be responsible for destructive changes and deformation of the nematode body surface [29] [30]. Similar to the present observations, the surface cuticle or tegument was found to be a principal target site for different synthetic drugs diseases and natural anthelmintic products as proved by veterinary histomorphological and ultrastructural studies [31] [32]. In general, the cuticle of nematodes and the teguments of cestodes and trematodes were known to be the basic entry route and primary site of activity of anthelmintic drugs [33] [29].

The recorded results which obtained in this study were agreement with results reported by Amorin et al (2003) [8], Zeinab Fahmy et al. (2009) [34], Zaman et al (2012) [35] and Murthy, et al (2004) [13] who studied potential antiparasitic activity of *punicagranatum* extracts against *Shistosomules* and mature worms of *Schistosomamansonii*: in vitro and in vivo. They recorded that the morphology of schistosomes recovered from host animals after administration of *P. granatum* leaves and peels extracts indicated marked ultrastructural alterations, manifested by degeneration in tegument with completely implanted or lost spines in addition to alterations in genital system of male worms and marked disruption in sub-tegumental musculature. Electron microscopic examination of perused adult worms, confirmed the parasitological results and revealed the effect of the methanolic extracts of *P. granatum* in inducing major ultrastructural alterations in the tegument and the male genital systems of the worms that lead to their death. The tested peels and leaves extracts of *punicagranatum* were found to possess antischistosomal activity at 100 mg /ml, 300mg/l and 500 mg /ml and the death rates of the adult worms reached 100 % after 24 hours for all 3 concentrations.

This effect was dependent on the duration of exposure, and the concentration level where the highest concentration revealed 100% death rate within 10-12 hours. The recorded results which obtained in this study was agreement with results reported by Arigomer et al. (2007) [36] in Jeddah, Saudi Arabia who studied in vitro potential antiparasitic activity of Pomegranate extracts and Piperazine against *Ascaridiagalli*. They concluded that *P. granatum* had highly efficacy as antiparasitic against *Ascaridiagalli*.

CONCLUSION

The aim was to study, *in vitro*, antiparasitic activity of methanolic extracts of *Punicagranatum* peel, in the treatment of heamonchosis. The leaves and peel extracts of *Punicagranatum* could represent promising bioactive natural agents that deserve further investigation, with the aim of introducing novel anti-helminthic agent. The marked changes in most parasitological and morphological structure encountered in this study was verified by the transmission electron microscopic results, introducing this natural compound as a drug had high efficacy in the treatment of heamonchosis in goats. Since the active constituents of many of these products are poorly known, there is also a strong need to focus future studies on phytochemical examination of these efficacious plants.

Further, the possible mode of actions of these products needs to be well established, so as to also exploit them from commercial point of view.

Pomegranate (*Punicagranatum*) a fruit with rich ethnomedical applications, has been also introduced as potential anti-microbial and anti-helminthic agent.

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