Anthelmintic activity of some Homeopathic Mother Tinctures against Gastrointestinal Nematodes

Shah Muhammad[1], Khalil Ahmad[1*], Hafiz Muhammad Asif[1], Aasia Kalsoom[2], Maria Muddassir[2,] Tahira Shamim[1], Ghazala Shaheen[1],Sumair Raza[2], Faheem Hadi[1],Tahir Maqbool[2*]

[1]University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University Bahawalpur, Pakistan.
[2]Centre of Research in Molecular Medicine, Institute of Molecular Biology & Biotechnology, The University of Lahore, Lahore, Pakistan.

Correspondence: Dr. Khalil Ahmad, University College of Conventional medicine,Faculty of Medicine and Allied HealthSciences,The Islamia University of Bahawalpur, Bahawalpur, Pakistan
E-mail: khalil.ahmad@iub.edu.pk

Abstract

Objective: *In-vitro* anthelmintic activity of mother tincture of *Artemisia cina, Trachyspermum ammi* and *Punica granatum* were investigated on the eggs and adult nematodes of *Haemonchus contortus*.

Methodology:*In vitro,* eggs were exposed to different doses (75, 150, 300, 600, 1200, and 2400 µL) of mother tincture while adult *Haemonchus contortus* were exposed to different doses (250, 500, 1000, 2000, 4000, and 8000µL)following the standard procedures of egg hatch test and adult motility assay.Standard drug (Albendazole) 0.5mg/ml was used as a reference drug and normal saline as a control group.

Results: Percentage motility inhibition for *Artemisia cina, Trachyspermum ammi* and*Punica granatum* were observed on highest dose (8000 µL) as 81. 44%, 70.33% and 62.89% respectively whilepercentage hatch inhibition for *Artemisia cina, Trachyspermum ammi* and*Punica granatum* were also observed onhighest dose (2400 µL) as 76.85%, 70.48% and 69.82% respectively. The most potent homeopathic mother tincture of plants inhibiting egg hatching based on LC_{50} was *Artemisia cina* (LC_{50}=247.6281 µL) followed by *Punica granatum* (LC_{50}=364.8879µL) and *Trachyspermum ammi* (LC_{50}=610.942µL) while the most potent homeopathic mother tincture of plants inhibiting motility of adult worms based on LC_{50} was *Artemisia cina* (LC_{50}=1601.1052µL) followed by *Punica granatum* (LC_{50}= 2463.9619µL) and *Trachyspermum ammi* (LC_{50}= 3210.1180µL). The data of correlation of regression revealed the best dose-dependent effects on egg hatching with *Trachyspermum ammi* (R^2 = 0.9936) followed by *Punica granatum* (R^2 =0.9759) and *Artemisia cina* (R^2 = 0.9529) while on motility of worms with *Trachyspermum ammi* (R^2 = 0.973) followed by *Artemisia cina* (R^2 = 0.9970)and *Punica granatum* (R^2 =0.855).

Conclusion: The result therefore, showed that these homeopathic medicines(Cina)*Artemisia cina,* (*T. ammi*)*Trachyspermum ammi* and(Granatum)*Punica granatum* have therapeutic option to target adult worms and to prevent eggs from hatching, reduce the excretion and avoid environmental contamination. However, further experimentation is needed in this regard to decipher this activity.

Key Words: Anthelmintic activity, Mother tincture, Parasites, Helminthes

Introduction

Parasitic gastrointestinal infections are the major cause of relative incidence of disease and number of deaths in worldwide which are endemic for that particular type of infection(1).All over the world helminthes which are transmitted through the soil are much important infection causing groups which are accountable for retardation in physical and rational growth of children(2).Now a day the strategies which are being adopted worldwide to control the soil transmitted helminthes infectionsemphasizes episodic administration of anti-helminthic drugs to the populations which are at risk. However, this avenue is not successful to address the basic social and ecological sources of soil transmitted helminthes infections. For a long term control of this type of infections, it is recommended that there is need of improvement of hygienic, water and sanitation behavior (3).In one of study the pathogenicity of a parasite, *Haemonchus contortus*, compared with the pathogenicity of other nematodes. It was concluded that it is more prevalent in small ruminants like sheep and goats and it causes more fetal acute disease (Haemonchosis) and also induce high mortality as compared to the other nematodes. Characteristic of Haemonchosis is hemorrhagic anemia due to the blood loss which is attributed by the bloodsucking activities of *Haemonchus contortus* in the abomasum.For some decades all over the world, the use of commercial anthelmintics have been practiced to control the helminths infection.There is a need to explore alternative methods to control these types of infections because the parasites develop resistance against commercial anthelmintics. Some other options like, vaccine, traditional medicinal plants and biological control are being explored worldwide. The probable alternatives should be screened and evaluated properly (4). Modern synthetic medicines which now a day in practice for the treatment of various types of diseases are very effective in treating the diseases but also cause many side effects. The medicine prepare from various sources with homeopathic method of preparation also exerts anthelmintic effects against different helminthes groups of medical and veterinary importance in *ex-vivo* and *in-vivo* experimental models(5). Due to increasing trends of anthelmintic resistance there is need to search some alternate source of medicines which should be used as anthelmintics. In this regards homeopathic medicines from plant sources like Cina and Santoninum prepared from wormseed, granatum from pomegranate, *Trachyspermumammi* from Ajwain, Spigelia from pinkroot and Teucrium from cat-thyme as well as from mineral source like Calcareacarbonica and Naphthaline are claimed as Anthelmintic(6).

In homeopathic system of medicine the practitioners use various homeopathic medicines for the treatment of anthelmintic. Some of these drug used by practitioners are *Artemisia cina, Trachyspermum ammi* and *Punica granatum*commonly called as Cina, granatum and *T. ammi*. With this view, the *Artemisia cina, Trachyspermum ammi* and *Punica granatum* mother tincture was studied for their anthelmintic property.

Materials and Methods

Apparatus and instruments

Electric balance (Type AX200, No D432612501, SHIMADZU, JAPAN), Incubator (Model 14000, S. No. BPXOQ821260, 21502 Geesthacht/Germany), Stereo microscope (IM-850, IrmecoGmbh& Co, Germany), Inverted microscope(Type: 2002, Werk Nr. 0088995, Baujahr: 2009, Germany),

Micropipettes (Jencons, England, Cat No.480-083), Standard testing sieves (Royal Scientific Company, 36-Abkari Road, Lahore), 24-multiwell plates (), Glass slides, Cover slips, McMaster Slide, Syringe, Petri dishes.

Chemicals

Phosphate buffer saline (PROLABO, Ref # 331552S, batch 105643), Lugol's Iodine solution (Scharlab S.L, Spain. Ref # 32245502/0024), Ethanol, Distil water, Albensure(Albendazole) batch number L-556 BiogenPharma, Rawat, Pakistan, purchased from local market of Bahawalpur.

Plant Material

Fresh pomegranate fruit peel was collected from local area of Bahawalpur, Punjab, Pakistan. The pomegranate fruit peel was identified and authenticatedat department of Botany, The IslamiaUniversity of Bahawalpur, Pakistan.Dried seeds of *Trachyspermumammi*(*T. ammi*) were purchased from local market Bahawalpur Pakistan. The dried seeds of *Trachyspermumammi* were identified and authenticated at department of Botany, The Islamia University of Bahawalpur, Pakistan.

Preparation of mother tinctures

Fresh pomegranate fruit peel was collected from local area of Bahawalpur, Punjab, Pakistan. Peel was subjected to cut into small pieces with knife, washed under tap water thrice, rinsed by distil water and air dried under shade at room temperature. Dried seeds of *T. ammi* were cleared all the adulterants washed thoroughly with tap water, rinsed by distil water and shade dried at room temperature. Dried peel of pomegranate fruit and *T. ammi* were grinded by using domestic electric grinder machine to obtain the fine powder.

The homeopathic mother tincture by using powdered material of pomegranate peel and *T. ammi* seeds were prepared separately by cold maceration for 15 days[(7)].In standard temperature and pressure conditions, medicinal part of the target plant was made soft in a solvent for specified time period. 100g plant material in powder form was placed in a flask of 2 liter capacity for soaking in 900ml of 70% ethanol for 15 days. The flask was kept in dark cool place after sealing and shaken for 10 minutes daily. After that, the material was filtered by coarse filtration through multiple layers of muslin cloth and then filtered by Whatmann filter paper. The filtrate was taken in glass bottles, sealed and stored in dark cool place.[(7)]. Homeopathically prepared mother tincture of Cina Q (90% ethanol) Lot No. 1040211,Dr. Willmar Schwabe, Karlsuhe Germany was purchased from local market of Bahawalpur, Pakistan.

Collection of Parasite and Eggs

Abomasum of naturally infected sheep was obtained from local slaughter house of Bahawalpur and immediately transferred to the laboratory in University College of veterinary and animal sciences, The Islamia university of Bahawalpur. Adult female*Haemonchus contortus* were isolated from contents of abomasum. Worms were washed with distil water to remove the fecal contents and then crushed with thehelp of mortarand piston to liberate the eggs. The concentration of eggs were estimated in 50 µL samples and adjusted to 150–200 eggs ml-1.

Egg hatch assay

Egg hatch assay was performed by using World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) guidelines to check the anthelmintic resistance [(8)] with a little modification in experimentation that permitted the testing of natural products[(9)]. Briefly, the assay was carried out by using 24-multiwell plates. Dried extracts of Homeopathic mother tincture of experimental drugs were

dissolved and diluted in distil water. Albendazole was employed as a positive control. Untreated eggs served as negative control. Collected eggs were washed in Phosphate buffer saline to prepare eggs suspension, counted in 0.3ml of eggs suspension by using McMaster chambers slide and adjusted to approximately 150 to 200 eggs/ml. Six doses 2400, 1200, 600, 300, 150 and 75 µL of each homeopathic mother tincture and 0.5µg/ml concentration ofAlbendazole in distil water were placed in differentwells in triplicate. The plates were incubated at 28^{o}C for 48 hours, following which added one drop of Logul's Iodine solution to stop further hatching of eggs, hatched larvae (alive or dead) and eggs were counted by using inverted microscope.

Adult motility assay

Abomasum of naturally infected sheep was obtained from local slaughter house of Bahawalpur and immediately transferred to the laboratory in University College of veterinary and animal sciences, The Islamia university of Bahawalpur. Adult female *Haemonchus contortus* were isolated from contents of abomasum. Worms were washed with distil water to remove the fecal contents and placed in Petri dishes containing Phosphate buffer solution (PBS). Actively moving 10 adult female worms were placed in different Petri dishes.Six doses8000, 4000, 2000, 1000, 500 and 250 µL of each homeopathic mother tincture and 0.5mg/ml concentration of Albendazolein distil water were placed in different Petri dishes. Experiment was carried out in triplicate. Petri dishes were incubated at 28^{o}C for 24 hours.Following, worms were re-suspended in leuk warm PBS for 30 minute and then number of motile and immotile worms in each petri dish was recorded. This experiment was carried out in triplicate. The results are expressed as mean ± standard deviation. Motility inhibition percentage was calculated by comparing the number of immotile worms treated by test drug with motile worms in control.

Statistical analysis

For egg hatch test, probit transformation was performed to transform a typical sigmoid dose response curve to linear function. The extract concentration required to prevent 50%, i.e., lethal concentration 50 (LC_{50}) of hatching of eggs was calculated from this linear regression (for y = 0 on the probit scale). The data from adult motility assay was statistically analyzed using SPSS software version 20. The results are expressed as mean ± standard deviation (SD) of mean.

Results

The results of the present study revealed that percentage motility inhibition for *A. cina, T. ammi* and*P. granatum* were observed on highest concentration (8000 µL) as 81. 44%, 70.33% and 62.89% respectively **(Tables 1, 3, 5)** while percentage hatch inhibition for *A. cina, T. ammi* and*P. granatum* were observed on highest dose (2400 µl) as 76.85%, 70.48% and 69.82% respectively (**Tables 2,4,6)**. The most potent homeopathic mother tincture of plants inhibiting egg hatching based on LC_{50} was *A. cina* (LC_{50}= 0.8245 µl) followed by *P. granatum* (LC_{50}=1.222 µl) and *T. ammi* (LC_{50}=2.055 µl)**(Tables 7, 9)**. The data of correlation of regression revealed the best dose-dependent effects on egg hatching with *T. ammi* (R^2 = 0.993) followed by *P. granatum* (R^2 =0.975) and *Artemisia cina* (R^2 = 0.952)**(Tables 8, 10).**

Table 1: Adult motility inhibition activity of *T. ammi* against *H. contortus.*

Dosage (µL)	No. of Total Worms± SD	Mean No. of Immotile Worms± SD	Mean No. of Motile Worms ± SD	Motility Inhibition Percentage
8000 µL	10.00 ± 0.00	6.33± 1.15	3.66± 1.15	70.33%
4000 µL	10.00 ± 0.00	5.66± 0.57	4.33± 0.57	62.88%
2000 µL	10.00 ± 0.00	4.00± 0.00	6.00± 0.00	44.44%
1000 µL	10.00 ± 0.00	3.00± 0.00	7.00± 0.00	33.33%
500 µL	10.00 ± 0.00	2.66± 0.57	7.33± 0.57	29.55%
250 µL	10.00 ± 0.00	1.66 ± 0.57	8.33± 0.57	14.77%
Control	10.00 ± 0.00	1.00 ± 0.00	9.00± 0.00	11.11%
Standard	10.00 ± 0.00	8.33± 0.57	1.66± 0.57	92.55%

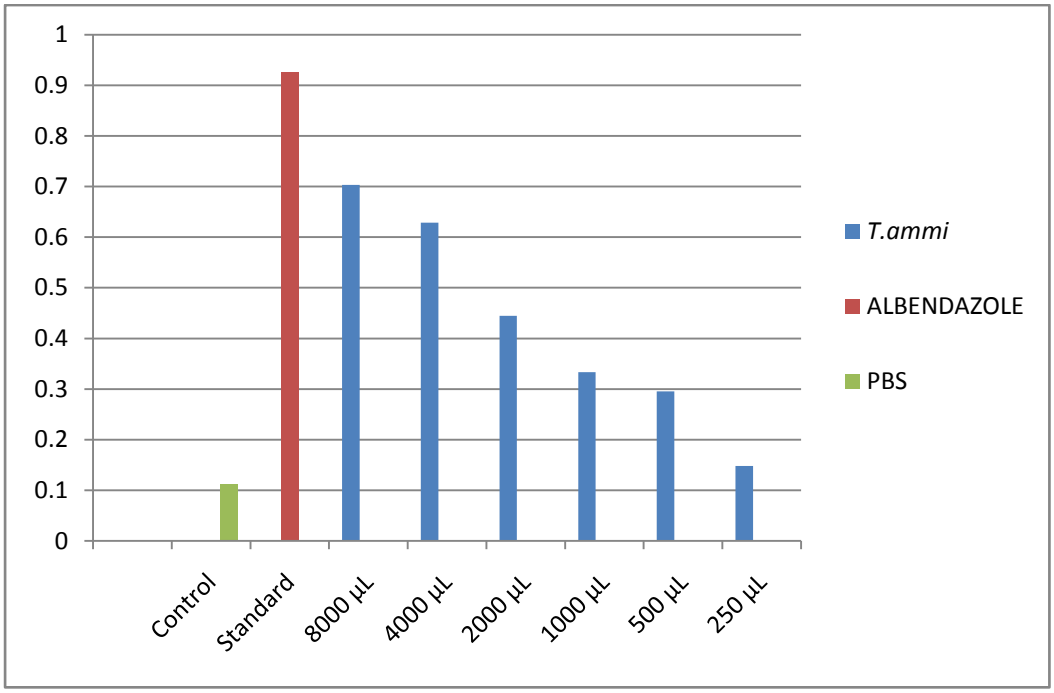

Figure 1: This figure shows the motility inhibition percentages of Trachyspermum ammi at doses of 8000, 4000, 2000, 1000, 500 and 250 uL, and motility inhibition percentage of control and standard. Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 2: Eggs hatch inhibition percentage of *T.ammi* extract.

Dosage (µL)	Total No. of Eggs	Percentage of Un-hatched Eggs	Percentage of Hatched Eggs	Hatching Inhibition Percentage
2400 µL	181.00	66.00	34.00	70.48%
1200 µL	184.33	61.00	39.00	65.14%
600 µL	165.00	50.00	50.00	53.39%
300 µL	187.67	41.00	59.00	43.78%
150 µL	192.67	32.00	68.00	34.17%
75 µL	166.66	23.00	77.00	24.56%
Control	173.00	6.36	93.64	6.79%
Standard	178.66	88.06	11.94	94.04%

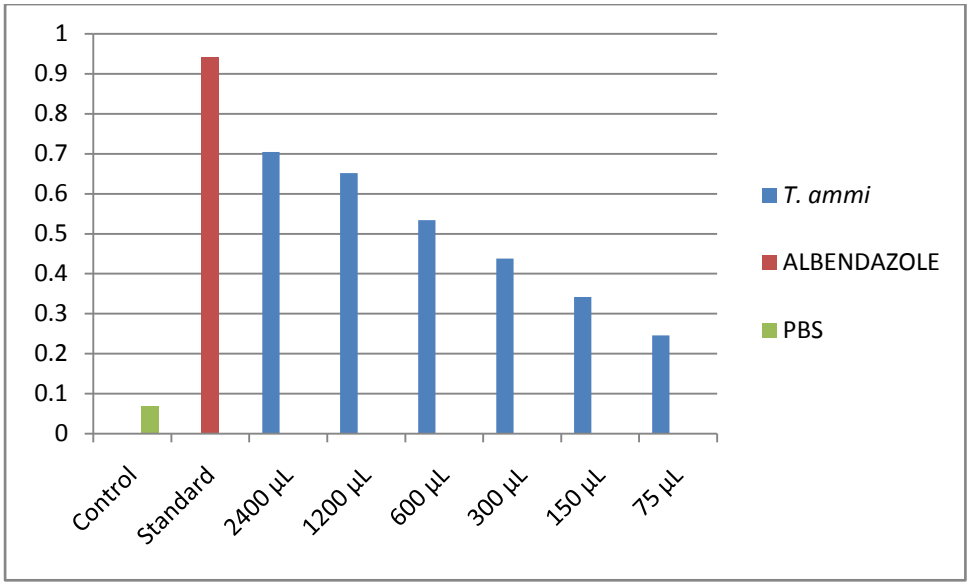

Figure 2: This figure shows the egg hatch inhibition percentages of Trachyspermum ammi at doses of 2400, 1200, 600, 300, 150 and 75 uL, and egg hatch inhibition percentage of control and standard. Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 3: Adult motility inhibition activity of *Artemisia cina* against *H. contortus*.

Dosage (µL)	No. of Total Worms±SD	Mean No. of Immotile Worms ±SD	Mean No. of Motile Worms ±SD	Motility Inhibition Percentage
8000 µL	10.00 ± 0.00	7.33 ± 0.57	2.66± 0.57	81.44%
4000 µL	10.00 ± 0.00	7.00 ± 0.00	3.00 ± 0.00	77.77%
2000 µL	10.00 ± 0.00	5.66 ± 0.57	4.33± 0.57	62.88%
1000 µL	10.00 ± 0.00	4.33 ± 0.57	5.66± 0.57	48.11%
500 µL	10.00 ± 0.00	2.33± 0.57	7.66± 0.57	25.88%
250 µL	10.00 ± 0.00	2.00 ± 0.00	8.00± 0.00	22.22%
Control	10.00 ± 0.00	1.00 ± 0.00	9.00± 0.00	11.11%
Standard	10.00 ± 0.00	8.33 ± 0.57	1.66± 0.57	92.55%

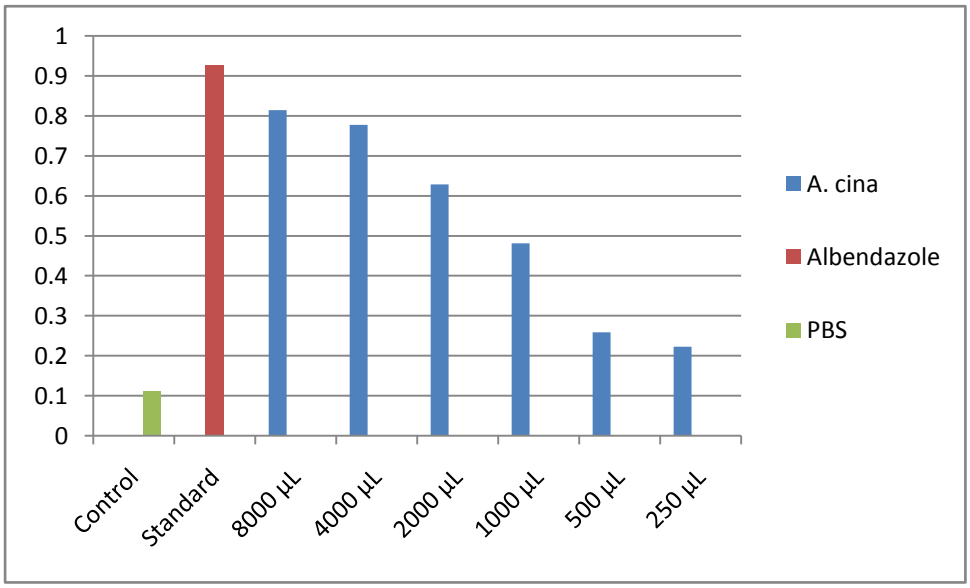

Figure 3: This figure shows the motility inhibition percentages of Artemisiacina at doses of 8000, 4000, 2000, 1000, 500 and 250 uL, and motility inhibition percentage of control and standard.

Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 4: Eggs hatch inhibition percentage of *Artemisia cina* extract.

Dosage (µL)	Total No. of Eggs	Percentage of Un-hatched Eggs	Percentage of Hatched Eggs	Hatching Inhibition Percentage
2400 µL	181	71.96%	28.04%	76.85%
1200 µL	184	63.51%	36.49%	67.82%
600 µL	165	53.48%	56.50%	57.11%
300 µL	187	52.02%	47.98%	55.55%
150 µL	194	47.95%	52.05%	51.21%
75 µL	166	38.77%	61.23%	41.40%
Control	173	6.36%	93.64%	6.79%
Standard	178	88.06%	11.94%	94.04%

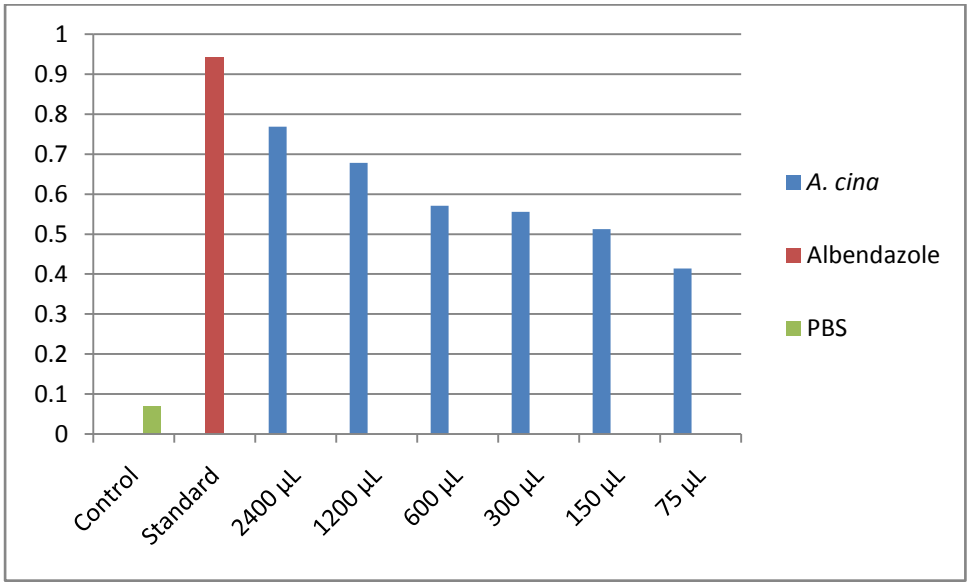

Figure 4: This figure shows the egg hatch inhibition percentages of Artemisiacina at doses of 2400, 1200, 600, 300, 150 and 75 uL, and egg hatch inhibition percentage of control and standard. Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 5: Adult motility inhibition activity of *P. granatum* against *H. contortus.*

Dosage(µL)	No. of Total Worms±SD	Mean No. of Immotile Worms±SD	Mean No. of Motile Worms ±SD	Motility Inhibition % age
8000 µL	10.00 ± 0.00	5.66 ± 0.57	4.33 ± 0.57	62.89%
4000 µL	10.00 ± 0.00	5.33± 0.57	4.66 ± 0.57	59.22%
2000 µL	10.00 ± 0.00	5.00 ± 00	5.00 ± 0.00	55.55%
1000 µL	10.00 ± 0.00	4.66 ± 0.57	5.33 ± 0.57	51.77%
500 µL	10.00 ± 0.00	4.00 ± 00	6.00 ± 0.00	44.44%
250 µL	10.00 ± 0.00	2.33 ± 0.57	7.66± 0.57	25.88%
Control	10.00 ± 0.00	1.0 ± 00	9.00 ± 0.00	11.11%
Standard	10.00 ± 0.00	8.33 ± 0.57	1.66 ± 0.57	92.55%

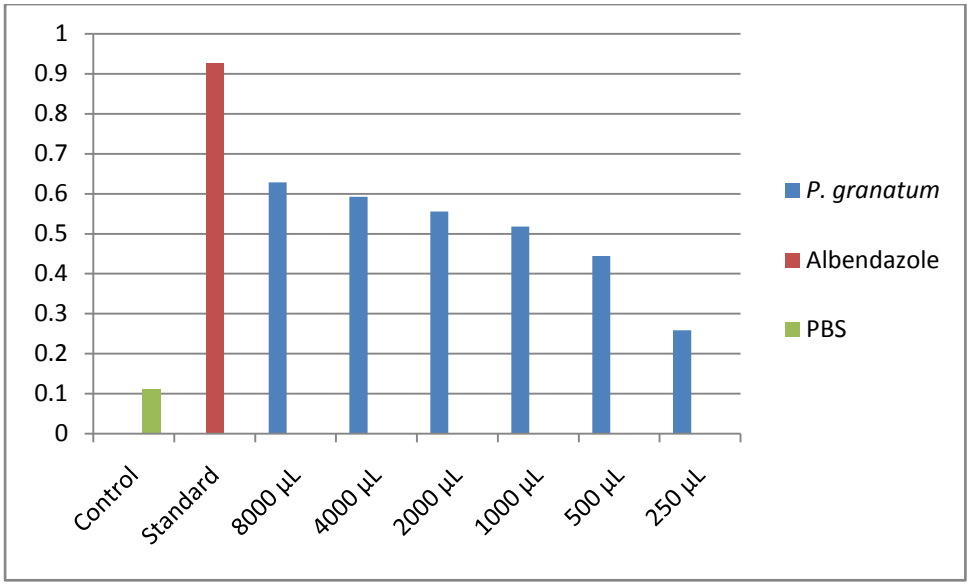

Figure 5: This figure shows the motility inhibition percentages of Punica granatum at doses of 8000, 4000, 2000, 1000, 500 and 250 uL, and motility inhibition percentage of control and standard. Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 6: Eggs hatch inhibition percentage of *Punica granatum* extract.

Dosage (µL)	Total No. of Eggs	Percentage of Un-hatched Eggs	Percentage of Hatched Eggs	Hatching Inhibition Percentage
2400 µL	182	65.38%	34.62%	69.82%
1200 µL	185	58.64%	41.36%	62.62%
600 µL	166	51.20%	48.80%	54.68%
300 µL	188	48.23%	51.77%	51.51%
150 µL	193	43.89%	56.11%	46.87%
75 µL	167	38.77%	61.23%	41.40%
Control	173	6.36%	93.64%	6.79%
Standard	178	88.06%	11.94%	94.04%

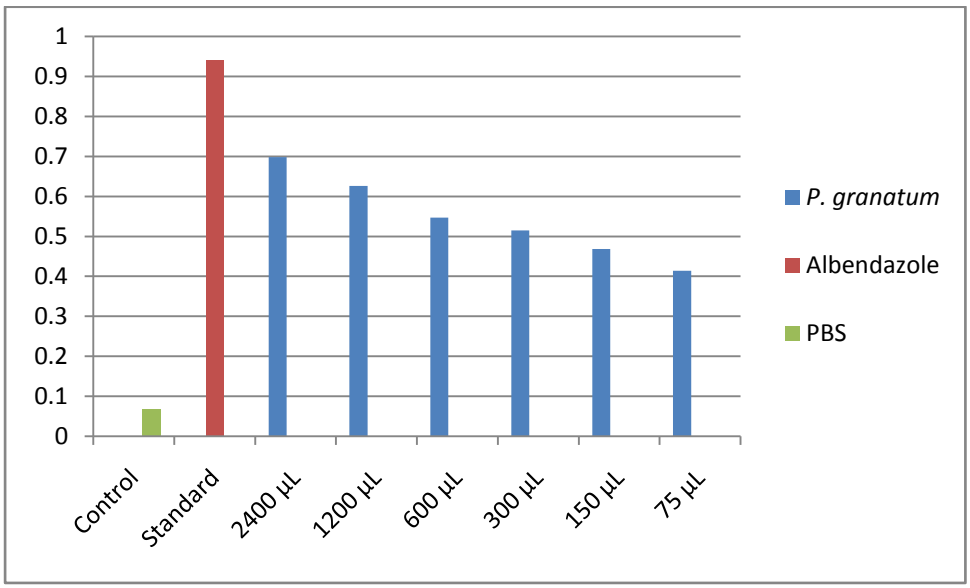

Figure 6: This figure shows the egg hatch inhibition percentages of Punicagranatum at doses of 2400, 1200, 600, 300, 150 and 75 µL, and egg hatch inhibition percentage of control and standard.

Phosphate buffer saline and Albendazole (0.5mg/ml) served as control and standard treatment respectively.

Table 7: Percent egg hatching inhibition and $LC_{50}$of different Homeopathic medicines.

Homeopathic mother tincture	Dosage of mother tincture applied (µL)						LC_{50} (µL)
	75	150	300	600	1200	2400	
Artemisia cina	61	52	48	47	36	28	247.6281
Trachyspermum ammi	77	68	59	50	39	34	610.942
Punica granatum	61	56	52	49	41	35	364.8879

Table 8: Regression values and correlation of regression of the effect of different plants on egg hatching.

Homeopathic mother tincture	LC_{50}	Regression values and correlation
Artemisia cina	0.8245	Y= - 0.5251 + 6.257, R^2 = 0.9529
Trachyspermum ammi	2.055	Y= - 0.7809x + 7.1756, R^2 = 0.9936
Punica granatum	1.222	Y= -0.4365x + 6.1184, R^2 = 0.9759

Table 9: Percent worm's motility and $LC_{50}$of different homeopathic medicines

Homeopathic mother tincture	Dosage of mother tincture applied (µl)						LC_{50} (µL)
	250	500	1000	2000	4000	8000	
Artemisia cina	80	76	56	43	30	26	1601.1052
Trachyspermum ammi	86	73	70	60	43	37	3210.1180
Punica granatum	76	60	53	50	46	43	2463.9619

Table 10: Regression values and correlation of regression of the effect of different plants on worm's motility.

Homeopathic mother tinctures	LC_{50}	Regression values and correlation
Artemisia cina	1.6011	Y= - 1.086x + 8.480, R^2 = 0.970
Trachyspermum ammi	3.2101	Y= - 0.920x + 8.226, R^2 = 0.973
Punica granatum	2.4639	Y= - 0.526x + 6.784, R^2 = 0.855

Discussion

Helminthes infections are important concern of medical field and have been considered a substantial problem for humans as well as for animals. Study conducted in Rawalpindi and Islamabad to determine the prevalence of Endoparasites in sheep and goats, the prevalence of *Haemonchus contortus* eggs in fecal samples was 80.64% in sheep and 75% in goats(9). Haemonchosis by blood loss results in low productivity due to loss of weight and wool production (10).Losses concerned with decreased wool and meat production in Faisalabad was 31.4 million per year(11,18).

Modern synthetic medicines which now a day in practice for the treatment of various types of diseases are very effective in treating the diseases but also cause many side effects(12). Parasitic gastrointestinal infections are the major cause of relative incidence of disease and number of deaths in countries which are endemic for that particular type of infection (1).

In this study, EHA was used for eggs of *Haemonchuscontortus* by using twofold dilutions of different homeopathic mothertinctures (2400 µl, 1200 µl, 600µl, 300µl, 150 µl and 75µl) to be tested along with Albendazole as a positive control.Egg hatch test was found useful in obtaining reliable data as evident from the varying efficacies (LC50) and dose-dependent effects of different homeopathic medicines screened in this study. Therefore, reliability of EHA as a drug/plant screening assay was in support of the earlier workers (13).

In this study, the homeopathic medicine prepared by using 70% aqueous ethanolic extract of *T.ammi*,*A.cina* and *P. granatum*possesses the anthelmintic properties and exerts effects in dose dependent pattern causing the inhibition of hatching of eggs and death of adult parasite. The results of a *T. ammi, A.cina, P. granatum* at different doseswhen compared to the results of control, the efficacy of *T. ammi*(70.33 %) and 70.48%),*A. cina* (81.44%and 76.84%) *P. granatum* (62.88 µl and 69.82%) was recorded as at highest dose of 8000 µlin adult motility inhibitions and 2400 µl in egg hatch inhibition experiments respectively. The lethal concentration for 50% inhibition of worm motility and inhibition of eggs hatching was *T. ammi*(LC_{50}= 3210.1180 µl and LC_{50}=610.9420 µl) A.*cina* (LC_{50}=1601.1052 µlandLC$_{50}$=247.6281 µl) and*P. granatum*(LC_{50}=2463.9619 µl and LC_{50}=364.8879 µl).

There are various studies on *T. ammi* which support its anthelmintic properties and explain its way of existing anthelmintic effects. Thymol has spasmodic effects on muscular system of helminthes and it is believed that it interfere with energy metabolism of helminthes through potentiation of ATPase activity that results in loss of energy reserves. Similarly, in numerous preliminary studies it was observed that Levant wormseed (*A. cina*) contains Thymol, tannins, santonin, Artemisinin and some other bioactive compounds (14)which are considered as potent anthelmintic agents against gastrointestinal parasites (15). Highest amount of condensed tannins is presents in pomegranate fruit peel and it is up to 28 %. This plant has a special value as anthelmintic for ruminants as well as for treatment of gastrointestinal parasite of human (16). Tannins (condensed and hydrolysable) are being investigated as a natural product to control gastrointestinal parasite in agricultural animals as well as to overcome the problem of developing resistance in gastrointestinal parasites (17).

We suggest that the homeopathic preparations of*Punicagranatum* Linn peel, *Trachyspermumammi* Linn seeds and *Artemisia cina* have interesting inclinationtoward the further study to inhibit gastrointestinal roundworm egg productions in huge number and to the other species in small ruminants and may be safely and economically used as an alternative anthelmintic drug forfarm management of agriculture animals. But there is need to evaluate these preparations in *in vivo* experiments.

Conclusion

A significant decrease in motility in adult parasites which were treated by the extracts of homeopathic medicines is an indication of potent efficacy. This showed that these homeopathic medicines are a therapeutic option to target adult worms and also to preventthe eggs from hatching, reduce theexcretion of eggs and avoid environmental contamination. However, further experimentation is needed in this regard to decipher this activity.

Acknowledgement

The authors would like to express sincere thanks to Dean and Chairman of University College of Conventional Medicine, Faculty of Pharmacy and alternative medicine providing support and necessary facilities for research.

References

1. Shiferaw, MB and Mengistu, A. D. Helminthiasis: Hookworm Infection Remains a Public Health Problem in Dera District, South Gondar, Ethiopia. PLoS One 2015; 10(12): e0144588.
2. Kaewpitoon SJ, Loyd RA. and Kaewpitoon N. Home Healthcare Program for Soil-Transmitted Helminthiasis in Schoolchildren along the Mekong River Basin. *J Med Assoc Thai*. 2015; 98(4): 1-8.
3. Steinmann P, Yap P, Utzinger J, Du ZW et al. Control of soil-transmitted helminthiasis in Yunnan province, People's Republic of China: experiences and lessons from a 5-year multi-intervention trial. *Acta Trop.* 2015; 141: 271-280.
4. Tadesse D, Eguale T, Giday M, Mussa A. Ovicidal and larvicidal activity of crude extracts of Maesa lanceolata and Plectranthus punctatus against Haemonchus contortus. *Journal of Ethnopharmacology*.2009; 122(2): 240-244.
5. Saxena RB. Entirely gone out useful plantâ€“. Artemisia cina 2015;648-663.
6. Boericke W. Pocket manual of homeopathic materia medica and repertory. Delhi: B: Jain Publishers, 2004.
7. Banerjee DD. Augmented Textbook of Homoeopathic Pharmacy: Jain Publishers (P) Ltd, 2004.
8. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller P J. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary parasitology*.1992; 44(1): 35-44.
9. Alawa CBI, Adamu AM, Gefu JO, Ajanusi OJ etal. In vitro screening of two Nigerian medicinal plants (Vernonia amygdalina and Annona senegalensis) for anthelmintic activity. *Veterinary parasitology.* 2003; 113(1): 73-81.
10. Asif M, Azeem S, Asif S. and Nazir S. Prevalence of gastrointestinal parasites of sheep and goats in and around Rawalpindi and Islamabad, Pakistan. *Journal ofVeterinary and Animal Sciences*.2008; 1: 114-17.
11. Iqbal Z, Akhtar M, Khan MN, Riaz M. Prevalence and economic significance of haemonchosis in sheep and goats slaughtered at Faisalabad abattoir. *Pak. J. Agri. sa*.1993; 30(1): 51-33.
12. Javed MS, Iqbal Z, Hayat B. Prevalence and economics of haemonchosis in sheep and goats. *Pak. Vet. J.*1992; 12(1): 36-38.
13. Molan AL, Waghorn GC, McNabb WC. Effect of condensed tannins on egg hatching and larval development of Trichostrongylus colubriformis in vitro. *The Veterinary Record*.2002;150(3): 65-69.
14. Gambelunghe C, Melai P. Absinthe: enjoying a new popularity among young people? *Forensic Science International*.2002; 130(2-3): 183-186.
15. Nguyen TM, Van Binh D. and Ã˜rskov ER. Effect of foliages containing condensed tannins and on gastrointestinal parasites. *Animal Feed Science and Technology*.2005; 121(1): 77-87.
16. Haque N, Sofi G, Ali W, Rashid M, Itrat M.A comprehensive review of phytochemical and pharmacological profile of Anar (*Punica granatum* Linn):

A heaven's fruit.*Journal of Ayurvedic and Herbal Medicine.* 2015; 1(1): 22-26.

17. Min BR, Hart SP. Tannins for suppression of internal parasites. *Journal of Animal Science.*2003; 81: 102-109.
18. Hadi Faheem *, Maqbool Tahir *, Khurshid Sameera , Nawaz Aisha , Aftab Saira , Tahir Sehrish , Awan J. Sana and Malik Arif , Anti-Fungal Activity of Cressa cretica, Leptadenia pyrotechnica and Pulicaria crispa, Indigenous Plants of Cholistan Desert, Pakistan, Anti-Infective Agents 2021; 19(3)