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**A. TAHIR, R. AKHTAR, M. LATEEF, F. SATTAR, M.A. HAFEEZ, M.O. OMER, B. ZAHID, A. JAVED**

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## Βιβλιογραφική αναφορά:

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## ***In-vitro efficacy of *Arachis hypogaea* (Peanut) peels extract against *Haemonchus contortus****

**Tahir A.<sup>1</sup>, Akhtar R.<sup>2\*</sup>, Lateef M.<sup>1</sup>, Sattar F.<sup>1</sup>, Hafeez M. A.<sup>1</sup>, Omer M. O.<sup>3</sup>, Zahid B.<sup>4</sup>, Javed A.<sup>2</sup>**

<sup>1</sup>Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, 54000. Pakistan

<sup>2</sup>Department of Pathology, University of Veterinary and Animal Sciences, Lahore, 54000. Pakistan

<sup>3</sup>Department of Pharmacology, University of Veterinary and Animal Sciences, Lahore, 54000. Pakistan

<sup>4</sup>Department of Pathology, College of Veterinary and Animal Sciences, Narowal, 51600. Pakistan

**ABSTRACT:** Synthetic anthelmintics are becoming ineffective due to development of parasitic resistance. For this reason, traditional medicinal plants are being explored for their anthelmintic potential. The aim of this study was to evaluate the *in-vitro* anthelmintic activity of *Arachis hypogaea* L. (peanut) peels against *Haemonchus contortus*. To assess the anthelmintic effect of *Arachis hypogaea* L. on 3 life cycles of *Haemonchus contortus*, egg hatch assay (EHA), larval developmental assay (LDA) and adult motility assay (AMA) were conducted. In these tests, methanol and n-hexane extracts of the plant were used in three concentrations of 10, 15 and 20 mg/ml. Levamisole and PBS were used as positive and negative control groups respectively. Results of these tests showed that methanol extract of *Arachis hypogaea* L. had higher anthelmintic effect than that of n-hexane extract. Overall both extracts exhibited a significant ( $p<0.05$ ) dose and a time dependent anthelmintic effect. At 20 mg/ml, methanol extract and n-hexane extract showed 87% and 80% egg hatching inhibition respectively. Methanol and n-hexane extracts at 20 mg/ml showed 83.3% and 76.6% larval mortality respectively. Adult motility test with both extracts showed maximum immobilization of worms after 6 hours of treatment at 20 mg/ml concentration. It is concluded that peels of *Arachis hypogaea* possess significant anthelmintic potential against nematodes. It may be suggested that the plant can be used further to investigate the *in-vivo* activity.

**Keywords:** *Arachis hypogaea*, *Haemonchus contortus*, Anthelmintic, Egg inhibition, Larval mortality

*Corresponding Author:*

Raheela Akhtar, Department of Pathology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan  
E-mail address: raheela.akhtar@uvas.edu.pk

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## INTRODUCTION

Livestock sector plays a vital role in Pakistan economy. It is the second most important sub-sector in agriculture of Pakistan (Amin *et al.*, 2010) and High prevalence of gastrointestinal parasites in small ruminants have been reported in Pakistan (Gadahi *et al.*, 2009; Bilal *et al.*, 2009). Gastrointestinal parasites are the major problem worldwide that can lead to weight reduction, lowered milk and meat production (Githigia *et al.* 2005). The severe clinical signs include diarrhea, anorexia, oedema and anaemia that are further related with poor performance and mortality particularly in young, aged and in those animals that lack immunity (Eysker and Pleoher 2003).

*Haemonchus contortus*, commonly known as the *barber's pole worm* is a highly pathogenic, blood-feeding nematode of small ruminants, and is a major constraint on ruminant health and production worldwide (Sargison, 2012). It has a remarkably high propensity to develop resistance to anthelmintic drugs, making control increasingly difficult. The expected increase in prevalence and severity of resistances in field isolates of this species to the available drugs will make it difficult for producers to control *H. contortus* on a worldwide scale (Kaplan, 2004).

Due to development of parasitic resistance against synthetic anthelmintics, veterinarians are now exploring anthelmintic potential of traditional medicinal plants. Peanut is one of the most important crops in the world, both as oil and as a protein source. It contains flavonoids, carbohydrate, mineral and vitamins. The former studies suggest that peanut can exhibit potential activity against microbes and parasites. It has also endocrine, relaxing effects and reduces inflammation (Al-Snafi 2014). Some studies have showed inhibitory activity of legumes against larvae and adult motility of *H. contortus* (Naumann *et al.*, 2014; Von Son-de Fernex *et al.*, 2012). Current study has been planned to test the anthelmintic efficacy of *Arachis hypogaea* (peanut) peels against the eggs, larvae and adult forms of *Haemonchus contortus*.

## MATERIALS AND METHODS

*Arachis hypogaea* peels were purchased from local market of Lahore. It was identified from Botany Department in Govt. College University Lahore with the batch number GCU-HERB-BOT-4001A. The peels of the plant were subjected to grinding to form fine powder. Powder was kept in air tight jar at 4°C until use. *H. contortus* infected sheep was taken from

Department of Parasitology, UVAS, Lahore for eggs and worm collection. This study was approved from the ethical committee of UVAS, Lahore and all efforts were taken to minimize pain and discomfort to the animal while conducting these experiments.

## Preparation of extracts

Soxhlet apparatus (Iqbal *et al.* 2006) was used to prepare the methanol and n-hexane extracts of *Arachis hypogaea*. Briefly, 50 gm of plant powder, wrapped in Brazil filter paper was introduced in into the Soxhlet extractor for extraction against 800ml methanol solvent to get crude methanol extract or N-hexane solvent for crude N-hexane extract. The extraction was preceded until the thimble of plant powder became almost colorless. The semi-solid extracts were used to formulate different concentrations (10 mg/ml, 15 mg/ml and 20 mg/ml) using PBS. The extract yield (% w/w) from the plant material was recorded. The parasites were collected from the abomasum of sheep with the help of forceps. Three *in vitro* assays were performed to check the efficacy of methanolic and n-hexane extracts against adults, larvae and eggs of *H. contortus*. EHA and LDA were conducted in triplicate and % egg inhibition and % larval mortality were then calculated as mean±SD.

## Egg Hatch Assay (EHA)

Microscopic examination of fecal samples of infected sheep was done through floatation method and observed under microscope for eggs identification of *Haemonchus contortus*. Egg hatch assay was carried out according to the typical method pronounced by Coles *et al.*, (1992) to check the efficacy of methanol and n-hexane extracts of *Arachis hypogaea* peels on *Haemonchus contortus* eggs. Methanol extract was applied on eggs (150 eggs/well) at different concentrations of 10, 15 and 20 mg/ml in a microtiter plate. The activity of levamisole drug at the concentration of 0.55 mg/ml as positive control and PBS as negative control was also determined. Same procedure was done for n-hexane extract. The observations were recorded after examination of samples under microscope.

## Larval Developmental Assay (LDA)

Eggs were incubated to develop into larvae. Larvae were then separated by Bearmann technique (Mehlhorn, 2008). Methanol extract was applied on larvae at different concentrations of 10, 15 and 20 mg/ml in a microtiter plate. The activity of levamisole drug at the concentration of 0.55 mg/ml as positive control and PBS as negative control was also determined.

Same procedure was repeated for n-hexane extract. The observations were recorded after examination of samples under microscope.

#### Adult Motility Assay (AMA)

*H. contortus* worms were collected from abomasum of the infected sheep after slaughtering it. These parasites were washed and confirmed by microscopy, then kept in PBS. Moving worms were placed in Petri dishes (10 worms in each dish) with 10, 15 and 20 mg/ml of the Methanolic extract of *Arachis hypogaea* and in petri dishes with same concentrations of n-hexane extract. PBS was used as negative control. Levamisole diluted in PBS at the concentrations of 0.55 mg/ml was used as a positive control. After 24hrs, the extracts were washed away and the parasites were suspended in PBS for 30min for possible recovery of the parasitic motility. Under dissecting microscope, the number of alive and dead worms was calculated and recorded for each concentration. The motility of worms was checked at intervals of 0, 1, 2, 3 and 6 hours. A mortality index was calculated as the number of departed or paralyzed worms divided by the total number of worms per petri dish. Mortality percentage was then calculated from mortality index.

$$\text{Mortality Index} = \frac{\text{Dead parasites}}{\text{Total number of parasites}}$$

#### STATISTICAL ANALYSIS

Statistical Program SPSS VERSION 16 was used to apply the technique ANOVA for assessing the significance of the anthelmintic activity of extracts in comparison with negative control.

#### RESULTS

The physical features and percentage yield of plant extracts are given in the table 1. The plant powder yielded 12% methanol and 10% n-hexane extracts.

The maximum egg inhibition and larval mortality was seen at high concentration of 20mg/ml with both extracts. Methanol and n-hexane extract showed 87% and 80% egg inhibition respectively at 20mg/ml concentration. At 20 mg/ml, methanol and n-hexane extract showed 83.3% and 76.6% larval mortality respectively. Results revealed significant dose dependent inhibitory activity ( $p<0.05$ ) for both extracts (methanol and n-hexane) as well as for levamisole (positive control) in both tests when compared with the negative control. The ovicidal and larvicidal activity of both extracts is given in table 2.

Efficacy of *Arachis hypogaea* peels methanol extract against adult worms of *H. contortus* is represented in figure 1 while that of n-hexane extract is represented in figure 2. In both cases, number of motile worms decreased significantly ( $p<0.05$ ) during 6-hour period. The maximum mortality was seen at high concentration of 20mg/ml after 6 hours. Methanol and n-hexane extract showed 77% and 60% mortality rate at 20mg/ml concentration after 6 hours of treatment respectively. Positive control (Levamisole) and negative control showed 100% and 1% mortality after 6 hours of treatment. Result of adult motility assay showed dose and time dependent anthelmintic activity of extracts of *Arachis hypogaea* peels.

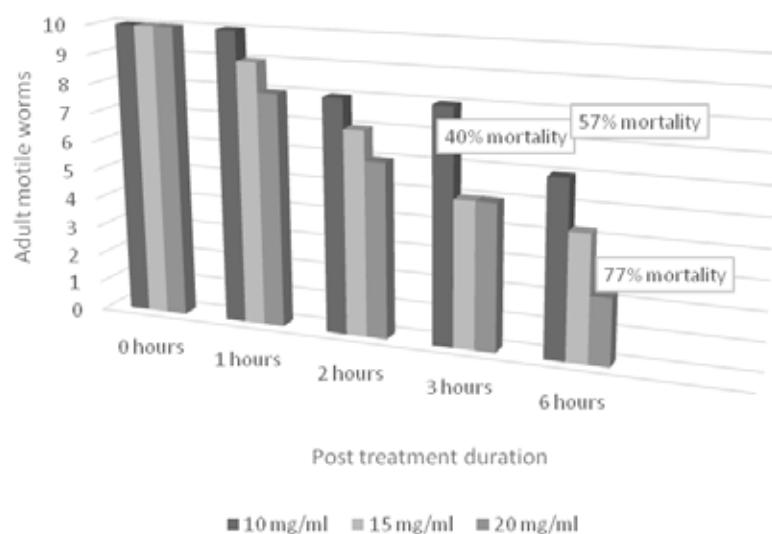
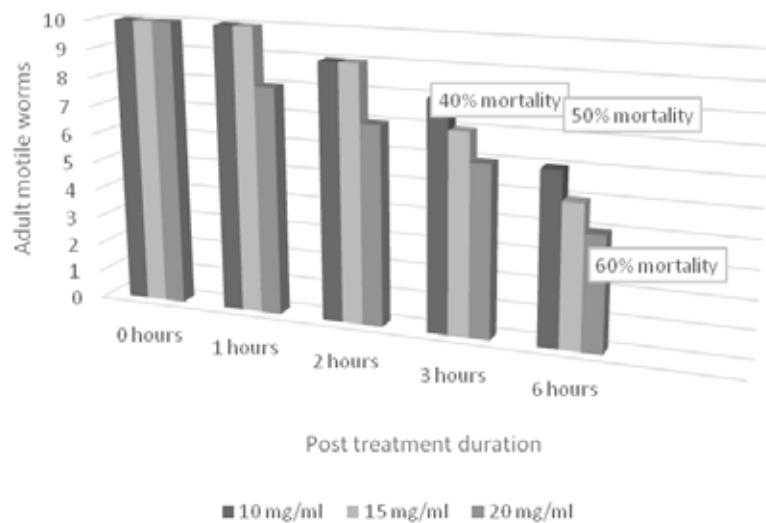


Figure 1. Efficacy of *Arachis hypogaea* methanol extract against adult worms of *H. contortus*



**Figure 2.** Efficacy of *Arachis hypogaea* n-hexane extract against adult worms of *H. contortus*

**Table 1.** Physical characteristic features of plant extracts

| Wt. of dry powder (g) | Wt. of extract (g) | Yield (%) | Extract color | Extract consistency | Solvent used |
|-----------------------|--------------------|-----------|---------------|---------------------|--------------|
| 50                    | 12                 | 24        | Dark brown    | Semisolid           | Methanol     |
| 50                    | 5                  | 10        | Yellow        | Semiliquid          | n-hexane     |

**Table 2.** Dose based ovicidal and larvicidal efficacy of methanol and n-hexane extracts

|                    | Dose      | % Egg inhibition<br>(Mean±SD) | % larval mortality<br>(Mean±SD) |
|--------------------|-----------|-------------------------------|---------------------------------|
| Methanolic extract | 10mg/ml   | 66.0±2.0                      | 53.3±5.7                        |
|                    | 15mg/ml   | 79.0±1.0                      | 70±10.0                         |
|                    | 20mg/ml   | 87.0±3.4                      | 83.3±5.7                        |
| N-hexane extract   | 10mg/ml   | 62.0±1.0                      | 53.3±5.7                        |
|                    | 15mg/ml   | 71.0±1.0                      | 66.6±5.7                        |
|                    | 20mg/ml   | 80.0±1.0                      | 76.6±5.7                        |
| Positive control   | 0.55mg/ml | 97.3±0.5                      | 96±5.7                          |
| Negative control   | -         | 0.0±0.0                       | 0.0±0.0                         |

## DISCUSSION

Traditional medicinal plants have been reported to have anthelmintic potential (Satyavati *et al.* 1976; Lewis, 1977). Scientists are exploring anthelmintic potential of traditional plants due to the increasing in-effectiveness of synthetic anthelmintics against parasites. As a part of this exploration, we aimed to assess the *in vitro* efficacy of *Arachis hypogaea* (Peanut) Peels extract on eggs, larvae and adults of

*Haemonchus contortus*. The major benefit of using *in vitro* methods is to assess the antiparasitic properties present in plants and their extract including low expenditure as well as quick yield (Tiwari *et al.*, 2011). In our study, maximum egg inhibition and larval mortality by methanol and n-hexane extracts of *Arachis hypogaea* was seen at higher dose of 20 mg/ml. Significant time-dependent inhibition in motility of adult worms was seen during 6 h of treatment with

different concentrations of extracts. Overall, all tests showed significant dose-dependent anthelmintic effect by both methanol and n-hexane extracts. These results suggest that *Arachis hypogaea* peels contain possible anthelmintic compounds which can be effective against other parasites in addition to *Haemonchus contortus*.

Hounzangbe-Adote *et al.* (2005) screened extracts of four tropical plants (*Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya*) *in vitro* for potential anthelmintic against eggs, larvae and adult *Haemonchus contortus*. Their results showed significant anthelmintic activity by extracts of all four plants. Egg hatching inhibition was dose dependant but larvaicidal effect and adult mortality was not found dose dependant in their results. In a similar kind of study, Ferreira *et al.* (2013), evaluated the *in-vitro* anthelmintic effect of *A. muricata* aqueous leaf extract against eggs, infective larvae and adult forms of parasitic nematode *H. contortus*. In their study, *A. muricata* extract at higher doses, showed 84.91% and 89.08% of efficacy in egg hatch test and larval motility test, respectively. In the adult worm motility test, worms were completely immobilized after 6–8 h of treatment with different concentrations of extract. On the base of phytochemical analysis, they suggested that phenolic compounds in the extract may be responsible for anthelmintic activity.

Kamaraj and Rahuman, (2012) tested ovicidal and larvicidal activities of methanol extracts of five medicinal plants (*Annona squamosa*, *Eclipta prostrata*, *Solanum torvum*, *Terminalia chebula*, and *Catharanthus roseus*) on *Haemonchus contortus*. They used extracts in different concentrations. Overall results of their study suggest that the tested plants contain anthelmintic compounds. Marie-Magdeleine *et al.* (2010) in their study also showed similar kind of results.

Results of our study are in concordance with the study conducted by Adama *et al.* (2009), who examined the anthelmintic effects *in-vitro* of *Anogeissus leiocarpus* leaf and *Daniellia oliveri* stem bark extracts on eggs, first stage larvae and adults of *Haemonchus contortus*. They used different concentrations of the extracts. PBS and levamisole (at 0.125 µg/ml in PBS) were used as negative and positive control groups, respectively. Their results showed that both plant extracts induced significant anthelmintic effects on the three life-cycle stages of *H. contortus*. Moreover, the effect was dose-dependent on egg hatching and first stage larvae but unlike the results of our study, the effect was not dose dependent on adult worms. This can be due to difference in the nature and properties of anthelmintic compounds of different plants

## CONCLUSION

This study concludes that both extracts (methanol and n-hexane) of *Arachis hypogaea* peels show significant anthelmintic activity against eggs, larvae and adult motility of *Haemonchus contortus*. Both extracts displayed significant ( $P<0.05$ ) dose and time dependent anthelmintic activity. The maximum activity was recorded at high dose concentration of 20mg/ml. At low concentrations, the extracts revealed no significant activity. These results suggest that *Arachis hypogaea* can be used against *Haemonchus contortus* as an alternative for synthetic anthelmintic drugs. Further research for evaluation of *in-vivo* efficacy of *Arachis hypogaea* against *Haemonchus contortus* in sheep is recommended.

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