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Research Article

Abutilon theophrasti from Kashmiri Himalayas: A Life Savior for Livestock

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Abstract

Haemonchus contortus, the causative agent of haemonchosis, is an economically important parasite in ruminant populations. The parasite has a strong ability towards drug resistance, so chemotherapy is not effective for its control. There has been a continuous exploration for alternative ways of its treatment particularly from plant sources. The present study intended to evaluate the antihelminthic activity of crude methanolic and hexane extracts of *Abutilon theophrasti* fruitbody against *H. contortus*. The extraction was done by simple maceration. Methanol and hexane extracts were used for the analysis. The egg hatch test and larval motility test were employed to evaluate the potential of the extract against *H. contortus*. Concentrations of 500, 250, 125, and 62.5 mg/ml were made. Levamisole and distilled water served as control, respectively. Anthelmintic activity was initially tested on *Pheretima posthuma* due to its morphological resemblance with other gastro-intestinal nematodes. All selected extracts displayed concentration-dependent inhibition. At higher concentration (500 mg/ml), methanol and hexane extracts showed 90.68% and 80.65% inhibition in EHT, respectively. Meanwhile, inhibition of larval motility was higher with inhibition percentage of 82.8% and 75.58% by methanolic and hexane extracts, respectively. The present study concludes that methanolic extracts were more potent than hexane extracts in all assays. The study also showed that inhibitory effect of *A. theophrasti* was concentration dependent. The current research evidenced the anthelmintic potential of the fruitbody of *A. theophrasti* as natural medicine against this parasite for the savior of livestock.

Keywords: Abutilon theophrasti, Haemonchus contortus, anthelmintic potential, egg hatch test, larval motility test

INTRODUCTION

Helminth infestations are one of the foremost infectious disease restraining production of livestock in different places of the world (Abdo et al., 2017). Significant financial crises by gastrointestinal helminths are caused in the form of increased vulnerability of small ruminant towards different diseases, morbidities, and mortalities, particularly in severly affected animals. Moreover, these infections also lead to restrained animal segregation because of limited symptoms and ultimately huge loss Akhtar et al. (2000), increasing expense of veterinary treatment, declined weight gain, reduced milk production and animal propagation, decreased foodconsumption, reduced growth and developmental rates in animals (Athanasiadou et al., 2001). Amongst these gastrointestinal helminths, nematodes are the key reason for poor health and decrease in the overall growth of goat and sheep (Badaso & Mekonnen, 2015; Bakunzi et al., 2013; Blackie, 2014). Various examinations showed Trichostrongylid nematodes, which include *Haemonchus contortus* (barber's pole worm) as the crucial parasite diagnosed in small ruminants (Bakunzi et al. 2013; Carvalho et al., 2012). Previous studies investigated that *H. contortus* is one of the significant gastrointestinal parasite in sheep (Coles et al., 1992). In the warm tropical and subtropical areas of the world, *H. contortus* is the most financially expensive and pathogenic helminthic parasite in small ruminants (Ćujić et al., 2016; Dinu et al., 2010). Severely parasitized goats and sheep display weakness, pale mucous layers, edema (bottle jaw) and dark colored feces and unexpected death. A chronic ailment may lead to decreased apetite, weight reduction, and reduced blood levels in these parasitized animals (Elshahawy et al., 2014). Small ruminants normally possess increased parasitic load in comparision with grown-up sheep and goats. In addition to this, possibility and severity of *H. contortus* infection rely on the precipitation as well as the temperatures of any particular region (Blackie, 2014). Heavy rainfall along with favorable temperatures help speedy egg hatching in grazing pastures thereby causing fast infection. The frequently caused infections of *H. contortus* in a herd give a straight indication about the severity of parasitic damage to the level of ruminant production. Most favorable temperature, environment humidity, pattern of grazing by the host, and the nature of grazing field are important factors responsible for the dispersal of this gastrointestinal helminths. The abrupt increase in parasitic infestation is generally observed in warm and moist atmospheres (Falcão & Araújo, 2018). Some other potential factors like host species, age, sex, breed, body condition and power of worm contaminations influence the development of gastrointestinal parasites (Fleming et al., 2006).

The general prevalence of *H. contortus* contamination in ruminants has been found fluctuating by various studies which was reported to be in the range of 52.7% to 83% (Gadahi et al., 2009; Hammond et al., 1997). An Ethiopian investigation revealed a prevalence of infection 56.6% and 67.2% in goats and sheep, respectively [9], pointing out greater vulnerability of sheep. In South Africa, the occurrence of *H. contortus* infestation in sheep has been reported as 68% through FAMACHA score and 100% with the Hawkesleymicrohematocrit method (Jaja et al., 2017). A similar report revealed that *H. contortus* was the most widespread parasite, which has been already confirmed by some earlier findings (Jaja et al., 2017). It has also been observed from different studies that the predominance of *H. contortus* infectivity is persistently higher in sheep than in goats in geographically similar regions. An examination performed in Rwanda, found *Haemonchus* spp. to be the most prevalent parasitic species in young ruminants with extremely high infestations rates (Juvénal et al., 2011).

Throughout the world, the disease control is mostly accomplished by the utilization of anthelmintics in combination with grazing management[19]. The synthetic anthelmintics have numerous disadvantages, which include resistance. It has been reported that *H. contortus* was resistant to a wide range of anthelmintics (Manzi et al., 2013; Martin, 1995). Reports have shown that resistance was observed in various regions of Ethiopia against albendazole, tetramisole, levamisole and ivermectin (McDonald et al., 2004).

One pragmatic approach to ensure less economical and prevailing anthelmintics is to investigate some effective indigenous herbal medication (Nsereko et al., 2015). Nowadays, evidence for anthelmintic activities from some important medicinal plants is getting more and more attention. There are some earlier reports, especially from Africa, which highlight the role of some plant products in effectively controlling the helminth infections in animals (O'Grady & Kotze, 2004; Patterson, 1995). The conventional utilization of some important medicinal plants in developing nations, *in vitro* and *in vivo* analysis have been accomplished to explore the capability of some plant species as possible anthelmintics to treat gastrointestinal infections in ruminants. The *Abutilon theophrasti* (Figure 1) has broadly been utilized in Traditional Chinese Medication (TCM), its correlative research on its principal constituents has hardly been explored. *A. theophrasti* is known to be originated from China. In China, it is being grown for jute like fiber since around 2000 BC. It is also known as "*button-weed, China-jute, velvetleaf*". *A. theophrasti* is a hebaceous annual plant growing upto 1 m in height. It is a short-day plant which grows in diverse habitats like agricultural land and high altitudes in Kashmir valley. The short day photoperiods enhances its flowering. However, increased plant height, its internode length and fruit weight with increasing photoperiods was observed under glass house conditions (Powers et al., 1982). A medicinal plant is always loaded with a number of secondary metabolites. The principal components present in this plant include phenols, phenolic acids, flavonoids, coumarins, lignans, lignins, and tannins, which provides this plant its important therapeutic role.

Traditionally, *Abutilon* is used for the treatment of a range of diseases. The roots of *Abutilon* are considered useful as demulcent, diuretic, in chest infections and urethritis. The decoction of leaves is used in toothache and for inflammation of the urinary bladder. The bark is used as anthelmintic, laxative, and diuretic (Prichard, 1990). The ayurvedic pharmacopeia of India prescribes the use of roots in gout, polyuria, piles, gonorrhea and hemorrhagic diseases (Rampedi et al., 2016). Besides this *A. theophrasti* is also claimed to have various cytotoxic and antioxidant activities.

In view of the conventional use of *A. theophrasti* as a therapeutic agent and its previous research regarding its phytochemical analysis which indicates a high content of tannins, the main objective of the current study was to assess the *in-vitro* anthelmintic effects of *A. theophrasti* from its methanolic and hexane fruitbody extracts against eggs, infective larvae of the parasitic nematode *H. contortus*.

MATERIALS AND METHODS

Collection of plant matrerial and its authentication

Abutilon theophrasti was collected from "*Lower Munda*" District Qazigund of Jammu and Kashmir (latitude 33.56 and longitude 75.20). The identified plant was registered (Reg. No.2113-KASH) at Herbarium center for Biodiversity and Taxonomy, "*University of Kashmir*", India.

Preparation of plant extracts

The collected plants were washed with distilled water and were shade dried at room temperature for about 15 days. The plant samples (dried) were powdered by mechanical

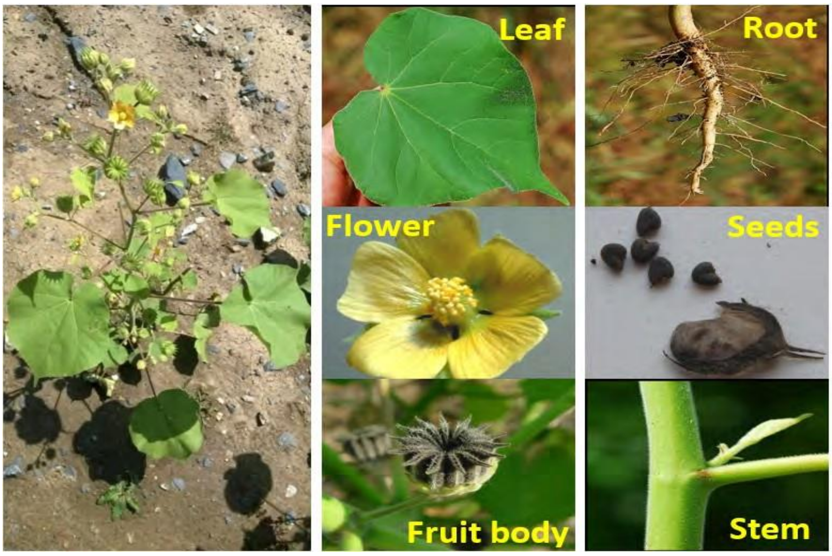

Figure 1. Abutilon theophrasti (velvet leaf).

crushing to fine mesh, stored in polythene bags at room temperature before extraction. The solvents used for extraction were methanol and hexane. The maceration technique was utilized for extraction. The crushed plant powder was placed in a container filled with the respective slovents. These containers were kept as such for several days with intermittent shaking until the complete extraction of the plant material (Rizwan et al., 2017). The macerated extracts were concentrated to dryness using rotary evaporator.

Parasites

The eggs for egg hatch test (EHT) and for prepration of larvae culture were obtained from fecal matter from *H.contortus* infected sheep. The confirmation of nematode infection was done by egg counting test (eggs per gram feces) (Rodriguez et al., 2007).

Anthelmintic activity

The anthelmintic assay was carried outon Indian earthworm (*Pheretima posthuma*) by following the methods adopted by Vagdevi et al (Roeber et al., 2013). Healthy adult Indian earthworm *P. posthuma* (Annelida, Megascolecidae) were used for assessmentof anthelmintic activity because of its physiological and anatomical resemblance with that ofhuman intestinal round worm parasites.A range of doses were used to ascertain thecorelation between dose strength and pharmacological activity and to develop the minimum and maximum dose that can be therapeutically effective against this worm in comparison to the standard drug. The crude extracts of methanol and hexane each with 10mg/ml, 20mg/ml, 50mg/ml and 100mg/ml concentrations were made.For the reference drug, Albendazole 10 mg/ml was used.Time for paralysis was noted when no movement was observed except when the worms were shaken vigorously. Time for the death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in lukewarm water.

Egg Hatch Test (EHT)

From the infected sheep (>2000 EPG), about 20 g of feces were collected rectally.The fecal matter was mixed in water and was passedthrough a series of compiled sieves (500, 150, 90, and 20 µm). Eggs which were retained at last sieve were obtained with a saturated saline solution by flotation. The prepared solution of 100µl containing approximately 100 eggs were distributed in microdilution plates. Different crude stem extracts (500, 250, 125 and 62.5 mg/ml) were added to the plates. Levamisole (2 mg/ml) was taken as a positive control and distilled water was taken as the negative control. Total time taken to complete the experiment was 24 hours and the temperature was maintained at 27°C (Schweizer et al., 1982). Eggs and first stage larvae (L_1) were counted as suggested by the World Association for the Advancement of Veterinary Parasitology (WAAVP). The obtained results were expressed as percentage inhibition of egg hatches.

Larval motility test (LMT)

About 20 g of feces were taken and homogenized at a concentration of about 2000 EPG with the proportion of 1:2 v/v and incubated for 7 days at room temperature. The incubated material was moistened daily with distilled water. The L_3 larvae were collected by spontaneous migration with the help of warm water (37°C). About 50µl suspension with 50 $L_3$was placed in microdilution plates. Crude extracts (500, 250, 125, 62.5 and 31.25 mg/ml) were added to these L_3 containing microdilution plates and were incubated for next 24 h at 27°C. After close observation, motile and non-motile L_3 larvae were counted based on the presence or absence of smooth sinusoidal movements. Levamisole (2mg/ml) and distilled water were taken as a positive and negative control, respectively (Rodriguez et al., 2007). Results were expressed as percentage inhibition of larval motility as a representation of three independent experiments performed in triplicate.

RESULTS

Anthelmintic activity

Both *in vitro* assays showed that crude extracts of *A. theophrasti* have promising adulticidal inhibitory effects. The extracts (methanolic and hexane) produced anthelmintic effect against *Pheritima posthuma* in a concentration based pattern (Figures 2, 3, 4, 5).The methanolic extracts were observed to be much more effective for paralysis and death of wormscompared to hexane extract. At highest concentration (100mg/ml) of methanolic extract time taken for paralysis (TTP) as well as time taken for death (TTD) was calculated as 9.56 minutes and 10.53 minutes, respectively, while in case of the hexane extract at same concentration, the time taken was 12.56 minutes and 15.53 minutes for paralysis and death, respectively. Albendazole (10mg/ml) was noticed to take 12.96 and 15.5 minutes for paralysis and death, respectively.

Egg Hatch Test

The result of egg hatch test (EHT) of crude methanolic and hexane extracts of *A. theophrasti* are shown in Figures 6, 7, 8. The results displayed relatively comparable egg hatching inhibitory effect by both the extracts with reference to drug Levamisole. The methanolicfruitbody extract required a maximum concentration of 500mg/ml to induce 90.68% egg hatch inhibition while as hexane extracts induced 80.65% inhibition at the same concentration. At 250mg/ml concentration, egg hatching inhibitory effect of methanolic and hexane was found 83.86% and 74.14%, respectively. Least inhibition was observed at 62.5mg/ml concentration with inhibition percentage of 42.15% and 40.58% for selected extracts, respectively, followed by 125mg/ml concentration which displayed 65.61% and 56.19% for methanolic and hexane extracts, respectively. Levamisole (2mg/ml) showed 95.6% inhibition, which clearly reveals the efficacy of the reference drug.

Larval motility test (LMT)

The results for larval motility test (Figure 9) of fruitbody extracts (methanolic and hexane) of *A. theophrasti* demonstrated the highest inhibition at 500mg/ml concentration with inhibition percentages of 82.70 % and 75.58 %, respectively. Methanolic extracts showed more potent inhibition than hexane extracts, i.e., 78.37% and 71.23% inhibitions at 250mg/ml. Likwise, at 125mg/ml extract concentration, inhibition percentages noticed were 70.50% and 64.88% for methanolic and hexane extracts,

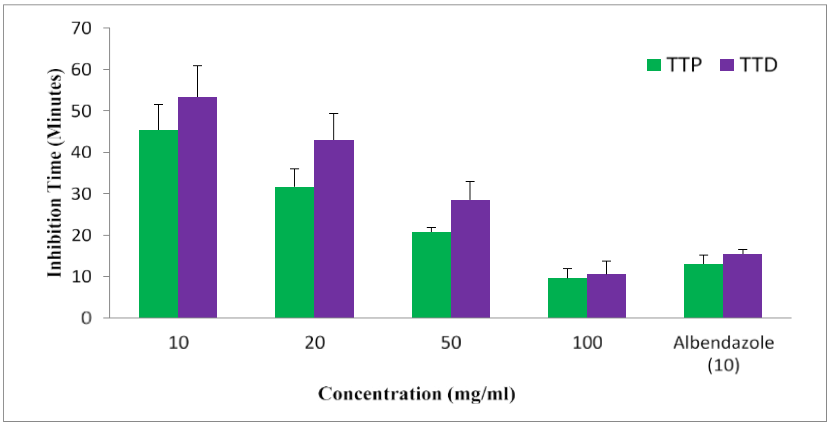

Figure 2. Antihelmintic activity of methanolic extracts of Abutilon theophrasti fruitbody against Pheritima posthuma. [TTP: Total time for paralysis; TTD: Total time for death].

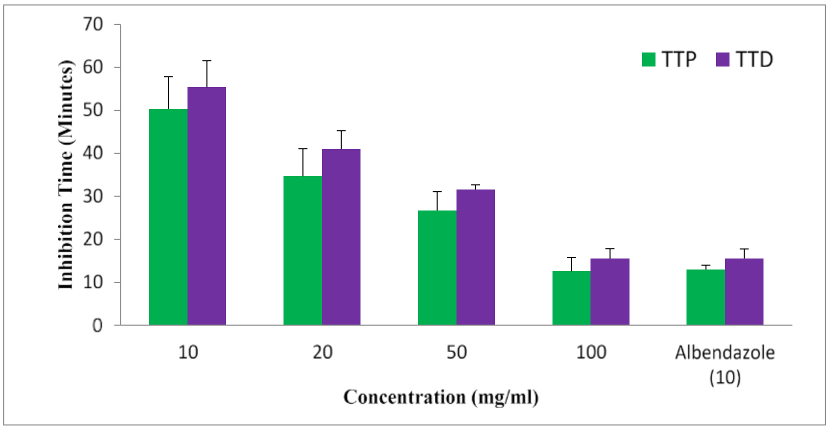

Figure 3. Antihelmintic activity of hexane extracts of Abutilon theophrasti fruitbody for against Pheritima posthuma. [TTP: Total time for paralysis; TTD: Total time for death].

Figure 4. Images depicting the anthelmintic activity of hexane fruitbody extracts against Pheritima posthuma.

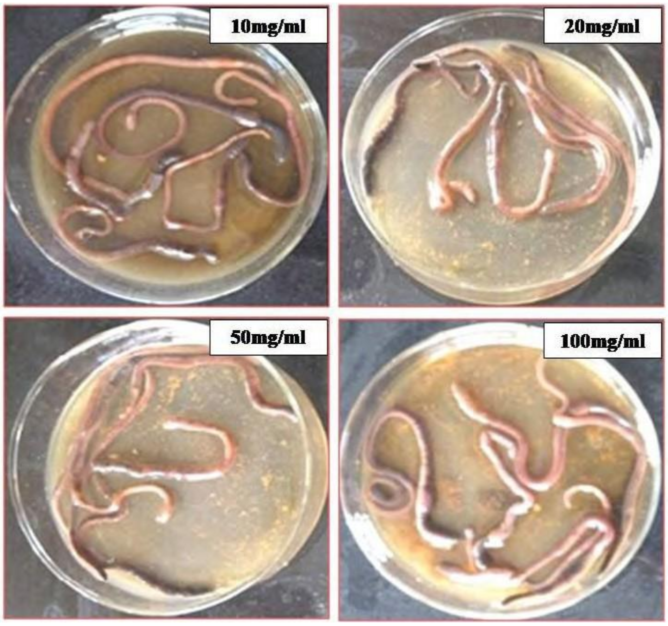

Figure 5. Images depicting the anthelmintic activity of methanolic fruitbody extracts against Pheritima posthuma

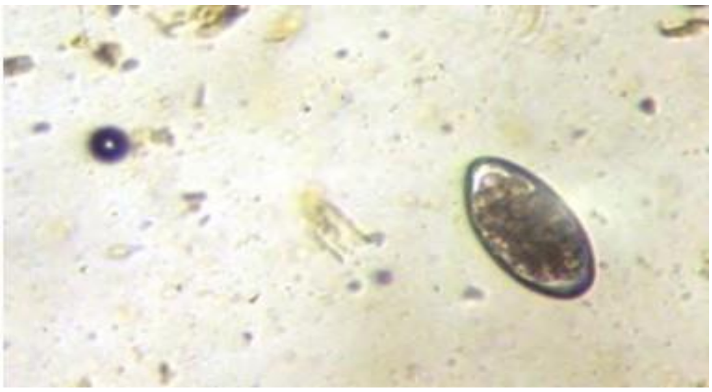

Figure 6. Haemonchus contorts egg.

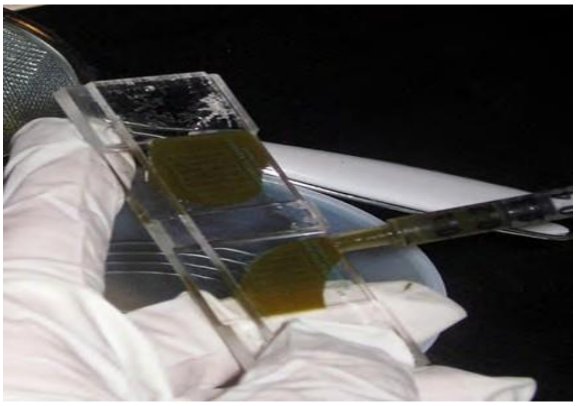

Figure 7. Mcmaster slide being filled for egg check of Haemonchus contortus.

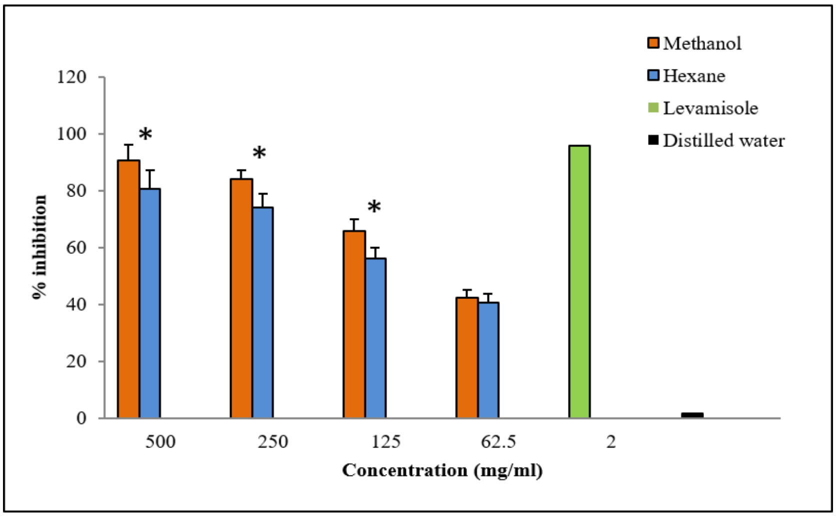

Figure 8. Egg hatch test (EHT) of methanolic and hexane extracts of Abutilon theophrasti fruitbody showing significant difference against H. controtus.

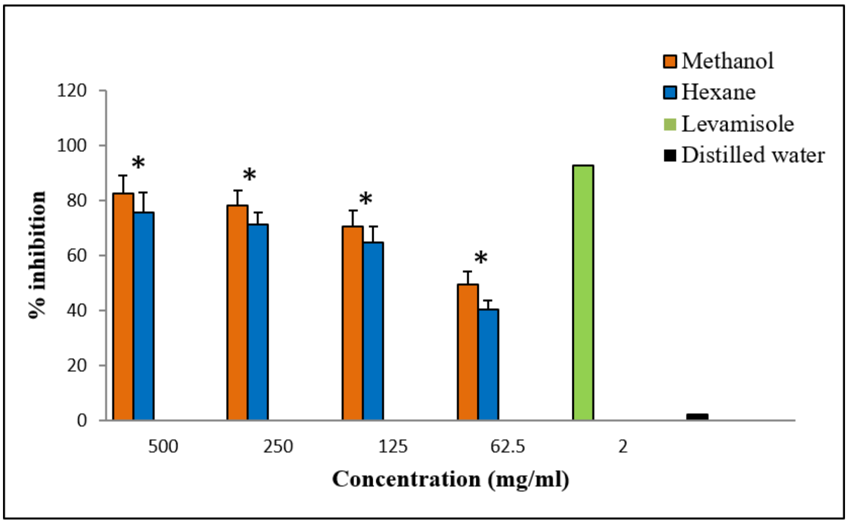

Figure 9. Larval motility test (LMT) of methanolic and hexane extracts of Abutilon theophrasti fruitbody showing significant difference against H. contortus.

respectively. Lowest inhibition percentages (49.62% and 40.46%) were observed at 62.5mg/ml for methanolic and hexane extracts, respectively. The reference drug levamisole (2mg/ml) displayed inhibition percentage of 92.88%.

Inhibition percentages of our selected extracts as seen in Figures 2,3 are clearly near to the inhibition percentage of Levamisole (control), but the crude extracts were used at higher concentrations as compared to control.

DISCUSSION

For the elucidation of some novel biologically active compounds from *A. theophrasti,* various parts have been screened from time to time for making this plant to be placed in the medicinal arena. The *A.theophrasti* is a well-known weed but at the same time,itsmedicinal value cannot be underestimated as the previous research supports its ethnomedicinal uses. The role of this plant as an expectorant, laxative and diuretic activities in traditional Chinese medicine also glorifies its peculiar nature in the medicinal world. In addition to this, various phytochemistrystudies have shown the presence of multifarious chemical constituents like tannins,rutin, quercetin, syriacusin, gallic acid Sharma & Subha (2016) and triterpenoid sapogeninin this plant (Singh & Swarnkar, 2008).

This study is mainly focused on the antihelminticactivityof A. theophrasti which confirmed that the plant has this attribute. The anthelmintic effect of this plant was examined against the model organism (P. posthuma). The different hexane and methanolic fruitbody extract concentrations were used to check for the time taken for paralysis (TTP) and time taken for death (TTD).

Various studies reported the anthelmintic properties of tannins in several plants [35,36]. Tannic acid (polyphenolic compound) affects helminth parasite by interfering with its energy productionand causes fatality by disturbing oxidative phosphorylation (Tariq et al., 2010) or interference with glycoproteins on the parasite epidermis (Thompson & Geary, 1995). The collagen-rich extracellular matrix (ECM) found in nematode membrane which is providing a protective cuticle, thereby forming the exoskeleton and is a key factor for itsvitality. In the industries which are meant for leather manufacturing, the tanning agents extracted from vegetables are commonly used in tanning purposes for leather processing (Tian et al., 1998). It is the reaction between tanning agents and dermal matrix of leather which confers collagen stability, thereby allocating collagen molecules to assemble into fibers causing flexibility check in the collagen matrix. Probably, a similar type of reaction is possible between the stratum corneum of P. posthuma and the tannins of A. theophrasti. The toughness of skin is achieved through this reaction. With the result worms become immobilized and dysfunctional, leading to their paralytic death. Therefore, proper isolation and further investigation of the active ingredients may assist the finding of some inovative compounds which couldsuccessfully inhibit a range of parasitic infections.

Some *in vitro* tests are being conducted for the isolation of natural anthelmintics to assess preliminary studywith different plants. In these tests, the parasitic eggs or larvae are being tried to grow directly on plant extracts to evaluate the effect on egg hatching and larval development (Ueno & Pedro, 1988). In thepresent study, both methanolic and hexane extracts contained active substances responsible for the anthelmintic action against *H. contortus*. Nevertheless, the anthelmintic activity present inthe methanolic extracts was more effective than hexane extracts, since methanolic extracts inhibit egg hatching and larval development higher than hexane extracts. Many triterpenoid sapogenins are also reported to be present in the fruitbody of *A.theophrasti*. Majority of the pant phytochemicals are attributed with anthelmintic activity (Vagdevi et al., 2001).

Diversity in the anthelmintic activity of the *A. theophrasti* extracts may be attributed to the discrepancy in the targets and qualitative and quantitative differences in the active phytochemicals of the fruitbody.The targets on which the anthelmintic effects were observed may also differ in various parasitic stages.The absolute use of the anthelmintic compounds will be defined at a particular parasitic stages (Befekadu & Teka, 2015). According to the guidelines of WAAVP, inhibition of worm egg hatching and larval motility by more than 90% should come under effective anthelmintic agents and the inhibition between 80–90% should be kept in moderately effective group (Waller, 1997). The *in vitro* results obtained with *A. theophrasti* extract against *H. contortus* eggs and larvae, particularly in their higher concentrations lead to the conclusion that the tested extractsshould be declearedas moderately effective. There are reports that accumulation of synthetic anthelmintics in the ruminants pose potential public health hazards through consuming meat of such animals (Whittier et al., 2009). Henceforth, the need of hour is to recognise some novel and promising anthelmintic extracts from various plants like *A. theophrasti.* Such extracts may contributeto the development of new phytotherapic products that could be more cost-effective, easily accessible, safer and provide a lower risk of resistance than the conventional therapeutic.

CONCLUSION

The present study confirmed that *Abutilon theophrasti* contains phytoconstituentswhich are having antihelmintic activity, hence needs further deep insight into the plant to elucidate some novel chemical molecules with pathogen killing properties.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

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