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Effect of Neem extract on the Gastro-intestinal Nematodes of Sheep and Goat: *in vitro* and *in vivo* study

Dr. Jatinderpal Singh[1*], Manpreet Kaur Saini[2] and Satveer Kaur[3]

[1]Associate Professor & Head, Department of Zoology, Baring Union Christian College, Batala-143505, Punjab, India
[2]Ph. D. Scholar, Department of Zoology, School of Bioengineering and Biosciences Lovely Professional University, Phagwara, 144411, Punjab, India
[3]M.Phil. Scholar, Department of Zoology, Baring Union Christian College, Batala-143505, Punjab, India
*Corresponding author Email id: jpsbucc@gmail.com

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ABSTRACT

The study examined the *in vitro* and *in vivo* effects of neem leaf extract and neem seed oil on gastro-intestinal nematodes of sheep and goat. Neem leaf extract and neem seed oil were discovered to have the potential to control gastrointestinal nematodes. All three species of gastrointestinal nematodes selected for this study, *Haemonchus contortus*, *Oesophagostomum columbianum*, and *Trichuris ovis*, were affected by different concentrations of neem leaf extract and neem seed oil. Neem leaf extract and neem seed oil reduced motility in all three nematodes. Variations in neem leaf extract and neem oil concentrations killed the nematode species. The treated nematode worms had greatly changed histology. The body wall's cuticle and muscular layers showed the most histological changes. Neem-treated *Haemonchus contortus* worms lost the intestine's border membrane and damaged the vaginal, vulvar walls and eggshell. Fertilised ova, which are normally discharged at the two-celled stage, developed into morulas. Vaginal musculature abnormalities prevented their evacuation. Neem leaf extract and seed oil primarily affected *Oesophagostomum columbianum's* body wall and intestinal brush border. Neem extracts affected nematode body wall. Nematode worms' cuticle flake and etch. Muscles were damaged. Intestinal brush boundary epithelium erupted. Erosion of the intestinal brush barrier may have hampered nutrient absorption and killed worms. Male and female worm reproductive tracts remained unchanged. Neem leaf extract and seed oil altered histological structure of digestive system in case of *Trichuris ovis.* Microvillar brush border and intestinal epithelial damage was noticed. The reproductive organs merely lost bounding membranes of gonads.

Keywords: Nematodes, Neem, *Azadirachta indica*, *Haemonchus contortus*, *Oesophagostomum columbianum*, *Trichuris ovis*

INTRODUCTION

Parasitic diseases are among the major public health problems of tropical countries including India, resulting in great economic losses. Parasites not only infect man but also domestic animals and wildlife. Our country is harbouring a large number of helminthic species of all varieties of animals. However, nematodes are the most important parasitic organisms affecting all domestic animals. Nematodes are the most numerous multicellular animals present on the earth (Maggenti, 1981). They parasitize man, livestock, and crops and have a deleterious effect on all. Though a number of workers have contributed to understanding the histomorphology of some gastrointestinal nematodes; Rauther (1918 and 1930), Anya (1964), Inglis (1964), Jennings and Colam (1970), Beams and Sekhon (1972), Wright and Sommerville (1985), Mackinnon (1987), Weber (1987), Fujino and Ishii (1988), Johal (1988), Brunanska (1991, 1992 and 1997), Fok et al. (1992) Lee et al. (1993), Takahashi et al. (1994 and 1995), Post and Pinder(1995), Patel and Wright (1998) Weinstein (1999), Modha et al.(1999) and Singh (2000), but information regarding the histological changes induced by anthelmintic agents is scanty. Different histochemical parameters such as proteins, lipids, carbohydrates and nucleic acids have been described in the cuticle, digestive and reproductive organs in various nematodes by Johal (1988) and Singh (2000). But the attention on the histology and histochemistry of anthelmintic treated worms remains overlooked. Helminth infections are widespread in animal and human populations. In India, considerable work has been done to tackle this problem. There have been good monographs (Srivastava and Dutt, 1962; Dutt 1980; Gupta, 1993), review articles (Mukherjee and Chauhan, 1965; Chauhan et al. 1973; Baugh, 1978; Chaudhary and Tada, 1994; Sood, 2003; Singh, 2005; Tandon and Dhawan, 2005) on helminthic infections with special reference to India. Obviously, initial attention was paid on taxonomy, morphology and life cycle of the parasites. Whereas biochemical, epidemiological and immunological aspects are emerging areas of concern. The main aim of all the studies was to minimize helminthic diseases with an enhancement of animal production and the extension of cost-effective technologies to the field. The development of new and improved drugs for use in human and veterinary medicine is the primary objective of researchers involved in chemotherapeutic research. Many anthelmintic drugs are limited in their usefulness because of a narrow spectrum of action, safety, high cost or impractical delivery system. Some of these are discontinued because of unforeseen problems such as the occurrence of drug resistance which needs more attention than it presently receives. Drug development is costly and time-consuming and requires exacting interdisciplinary interactions between researchers using the most stringent quantitative tests to determine toxicity, safety, mode of action and pharmacogenetics before a drug is ever used in clinical trials. Control of internal parasites of sheep and goats in the country and abroad is mainly anthelmintic dependent. The haphazard use and total reliance on anthelmintics have led to emergence of the anthelmintic resistant strains of parasites. Sanyal (1996) stated that keeping in view the problem of anthelmintic resistance, there is a global need for sustainable integrated parasite management. The rising cost of broad-spectrum anthelmintics and concern about the development of resistance against these compounds in the parasites and their toxicity to the host are posing a serious threat to the continued use of synthetic drugs. Due to this, scientists are trying to test the anthelmintic properties of their traditional medicinal plants. Numerous traditional medicinal plants have been used for centuries for anthelmintic treatment (Satrija et al. 1994; Evans and Gujatt, 1995; Coles and Mceillie 1997). The *in vitro* anthelmintic activity of *Embelia ribes* was indicated by Guru and Mishra (1964), Gupta et al. (1976). Garg and Mehta (1982) observed *in vitro* anthelmintic activity of *Butea frondosa* and *Embelia ribes*. In the present study, three gastrointestinal nematode parasites of sheep and goats were chosen. The effect of neem extract was tested on them in both *in vitro* and *in vivo* conditions. *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichuris ovis* are the three important species of nematodes affecting the sheep and goat population of Punjab, Himachal Pradesh, Haryana, Rajasthan and Uttar Pradesh. Roundworm infestation is one of the major health problems, more so in tropical countries. *Haemonchus contortus* selected for the present study is a serious nematode parasite of sheep (*Ovis aries*) and goat (*Capra hircus)* of cosmopolitan distribution. This pathogenic nematode causes severe anaemia resulting in weight loss, poor milk yield and wool production. Medium infection causes sheep to lose condition and heavy infection may result in death. Thousands of worms may occur in a single ruminant stomach and it has been estimated

that 4000 worms suck about 63 cm^3 of blood per day (Smyth, 1996). It was estimated by Baker et al. (1959) that a single worm causes an average daily loss of 0.08ml of blood. The effect of an indigenous formulation from the different parts of the Neem tree (*Azadirachta indica,* Family: Meliaceae) was tested on the gastrointestinal nematodes of sheep and goats. In India, the importance of neem in veterinary medicine dates back to the Mahabharata as reported by Indologist Vartak and presently neem products have internationally received significant attention (Randhawa and Parmar, 1996). The neem tree is very popular in most tropical and subtropical regions of the world. It is a hardy tree that grows vigorously in desert areas and in harsh climates. In rural areas, the people of India have been using neem leaves for centuries for different medicinal purposes.

MATERIAL AND METHODS

The present study was conducted on three gastrointestinal nematode parasites of sheep (*Ovis aries*) and goat (*Capra hircus*). These three gastrointestinal nematodes are *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichuris ovis*. The effect of neem leaf extract and neem seed oil was tested on these gastrointestinal nematodes. The anthelmintic properties of neem have been assessed *in vitro* and *in vivo* conditions.

Collection of Parasites:
The infected stomachs and intestines of sheep and goats were collected from the local abattoirs of Batala. For collecting *Haemonchus contortus,* the abomasum portion of the stomach was cut open by a longitudinal incision and the contents were thoroughly searched. For collecting *Oesophagostomum columbianum* and *Trichuris ovis* the intestine was cut open. All three species of nematode parasites were placed in separate containers and washed several times with 0.85 per cent sodium chloride solution.

Preparation of Neem Leaf Extract:
The neem leaf extract was prepared with the method given by Vyas and Mistry (1996). Mature green leaves of neem (*Azadirachta indica*) were collected, washed thoroughly and dried in the shade. These dried leaves were crushed to powder with the help of an electric grinder. The powdered leaves were soaked in water in a ratio of 1:5 overnight. The aqueous extract thus prepared was strained through a fine muslin cloth. This was taken as a stock solution.

Preparation of Neem Seed Oil:
The kernels were collected from the ground under the neem tree, de-pulped, and washed with water. These were dried and crushed using a crucible and blender. The oil was obtained by pressing the crushed kernels.

To Study the Effect of Neem Extract:
For determining the anthelmintic properties of neem leaf extract, different concentrations viz. 10 per cent, 20 per cent, 30per cent, 40 per cent and 50 per cent were prepared. The suitable controls were run simultaneously. The fresh and active nematode worms were divided into 5 groups and placed in various concentrations of neem leaf extract plus Tyrode's solution. The neem leaf extract and Tyrode's solution were taken in a ratio of1:1 at each dose. Similarly, five different concentrations of neem seed oil i.e.10 per cent, 20 per cent, 30 per cent, 40 per cent and 50 per cent were used. The nematode worms were placed in these concentrations of neem seed oil. At each concentration, the Tyrode's solution was used as an artificial medium. The Tyrode solution and neem seed oil were taken in a ratio of 1:1 at each experimental dose.

To Study the External Morphological Changes:
For studying the external characteristics of the parasites, these were stained in 1 per cent methylene blue at 60°C for 18 hours. Then these were differentiated in 80 per cent alcohol, cleared in lactophenol and mounted in the same. The slides were observed under the research microscope to record the morphological changes induced by neem extract.

To Observe the Histological Changes:
Various fixation techniques using Bouin's, Carl's and Carnoy's fixatives were tried. Alcoholic Bouin's fixative was found to be most suitable for histological studies. The nematode parasites were fixed in Bouin's fixative for 12-24 hours at room temperature. Before fixation, each worm was straightened and cut into 3 pieces. After fixation, these were washed in 70 per cent alcohol. Then these worms were dehydrated through a graded series of alcohol, cleared in xylene and embedded in paraffin wax (melting point of 58°C). Transverse sections were cut at 7 µm and longitudinal section at 5 µm by using a rotary microtome. Ribbons were mounted on albuminized slides, stretched and

dried. After dewaxing the slides were hydrated and brought to water. These slides were stained with Heidenhain's haematoxylin and Harris's haematoxylin. After differentiation in acid water, the slides were dehydrated upto 90 per cent alcohol and then counter-stained in eosin. The slides were further dehydrated, cleared in Xylene and mounted in DPX. The slides were observed under a research microscope to note the histological changes induced by the neem extract. The final conclusions were drawn after comparing the histological structure of treated and untreated worms. To study the *in vivo* effect of neem leaf extract and neem seed oil, 21 animals including sheep and goats were selected with 5 controls.These animals showed clinical evidence of gastrointestinal parasitic infection. These animals were stall-fed and were also allowed for grazing in the field. However, all the animals showed varying degrees of infections of *Haemonchus contortus, Oesophagostomum columbianum* and *Trichuris ovis*. The effect of neem treatment was assessed by comparing pre-treatment and post-treatment faecal egg counts from 1st to the 21st day after treatment. The actual effectiveness of treatment was assessed by the percentage reduction of egg counts.

Collection of Faecal Matter:

Faecal samples of treated and control animals were collected in small plastic bags from freshly dropped faeces and then taken to the laboratory for further examination. Identification slips on which information regarding the age, sex, area, general condition of the animal, feed used and weight of the animal was noted, were tagged along the sample. Various procedures were adopted for the faecal examination. The direct smear method was used to have a rough idea of infection. If this test was found positive, the ova were concentrated by the Willis floatation technique for proper identification and the Stoll dilution method was used for counting eggs per gram of faeces (EPG). For rapid examination, faecal smears were prepared by taking a small amount of faecal matter mixed with saline water. It was spread on a slide to form a smear. The slide was observed under the microscope for the presence of eggs. Simple Floatation Technique (Willis Technique) was used. In this technique about one gram of faeces was taken in a glass container and a few drops of the saturated salt solution were added. It was then stirred with a glass rod so as to make an even emulsion. A more saturated salt solution was added to form a meniscus. A clean glass slide was placed on the meniscus and allowed to remain for 40-45 minutes. The glass slides were quickly lifted up and a cover slip was placed on them for the examination under the microscope. If positive for infection then the proper examination was done under high power.

The Stoll Dilution Egg Count Technique:

For counting nematode eggs, 3 grams of faeces were taken into a large test tube. N/10 sodium hydroxide was added to the 45 ml mark of the test tube. Ten small glass beads were added. The tube was then closed with a rubber stopper and shaken vigorously to give a homogenous suspension of the faecal matter. After shaking, a 0.15ml sample of well-mixed suspension was immediately drawn off with a graduated pipette and placed on a slide. The total number of eggs in the 0.15ml sample was then counted and the number multiplied by 100 gives the total number of eggs in 1gm of faeces.

RESULTS AND DISCUSSION

a) Effect on the Motility and Mortality of Nematode Worms

Haemonchus contortus:

Observation on the motility of nematode worms revealed that when freshly put in Tyrode's solution and various concentrations of neem leaf extract, the worms were equally motile in all the groups. A sluggishness started appearing in the nematode worms placed in various concentrations between the third and fourth hours. After six hours a further decrease in motility was seen in the worms put indifferent concentrations of the neem leaf extract. In lower concentrations of neem leaf extract (i.e. 10 percent, 20 percent and 30 percent) the worms were still active after a pinprick, whereas in higher concentrations (i.e. 40 percent and 50percent) the onset of paralysis was evident. The motility of worms was decreased considerably. The nematode worms showed a slight motility after the pin prick. After 8 hours the nematodes kept at 50percent concentrations were completely immobile even after repeated pinpricks. After placing the nematode worms in warm saline and carefully observing them under the binocular microscope, the worms were found to be dead. Further, at 10-hour interval examination, the nematodes placed in 40 percent concentration were also found to be dead. At that time the nematode worms placed at 30 percent concentration showed very feeble movement.14-16 hours after treatment

with neem leaf extract, all the worms were dead whereas controls showed adequate movements. When the fresh medium i.e. Tyrode's solution was added the worms of the control group become more active. Such revival of motility was not observed in the neem leaf extract-treated worms. The worms placed in higher concentrations i.e. 40 percent and 50 percent of Neem seed oil died only after 6 and 8 hours respectively. The motility of those worms is decreased after 4 hours. They showed little motility after pin prick. After 4 hours of50 per cent neem seed oil treatment, their movement ceased and ultimately resulted in their death. In lower concentrations of the neem seed oil i.e. 10 percent and 20 percent the worms died after 10 hours of treatment. However, after 4 hours their motility was considerably decreased. The present results were found to be in conformity with the previous studies conducted by Singh (2000 and 2015).

Oesophagostomum columbianum:
The nematodes of *Oesophagostomum s*pecies were more susceptible to the neem leaf extract and neem seed oil treatment as compared to *Haemonchus contortus*. In 10 percent and 20 percent neem leaf extract the worms died after 12 hours. The motility ofthe nematode worms decreased after 6 hours. After 10 hours of treatment with a lower concentration of neem leaf extract the worms showed little motility. In higher concentrations of neem leaf extract i.e. 40 percent and 50 percent the motility of nematode worms becomes feeble after4 hours and 6 hours respectively. After an interval of 8 hours and 6hours, the worms placed in 40 percent and 50 percent concentrations showed no motility. After carefully observing the worms under the microscope and placing them in saline solution they were found to be dead. In 50 percent concentration of neem leaf extract, 100 percent mortality of *Oesophagostomum columbianum* occurs after 6 hours. The various groups of nematode worms placed in neem seed oil died earlier as compared to those placed in the same concentrations of neem leaf extract. In 40 percent and 50 percent concentrations of neem seed oil, the nematode worms showed feeble motility after 4hours of treatment. They become sluggish and even after repeated pinpricks, they showed little motility. The complete mortality of nematodes placed in 40 percent and 50 percent concentrations was after 8 hours and 6 hours respectively. The lower concentrations of neem seed oil were also very effective. Both the 10 percent and 20percent concentrations of neem seed oil caused the death of nematode worms after 10 hours. The worms placed in these concentrations showed moderate motility after 8 hours and 6 hours respectively.

Trichuris ovis:
Trichuris ovis placed in different concentrations of neem leaf extract show motility for varying time intervals. The worms placed in lowermost concentrations of neem leaf extract i.e. 10 per cent show moderate motility after 8 hours. Furthermore, their motility becomes feeble after 12 hours. After 14 hours of treatment with 10 per cent of neem leaf extract, the movements of nematodes stopped altogether. After repeated pinpricks, they showed no motility and presumed to be dead. Even when they were placed in Tyrode's solution alone their motility was not reversed. In higher concentrations of neem leaf extract i.e. 40 percent and 50 percent, *Trichuris ovis* died after 10 hours and 8 hours respectively. In a 50 per cent concentration of neem leaf extract, the motility of nematode worms becomes moderate after 4 hours of treatment. They showed feasible motility after 6 hours. The death of worms took after 8 hours of neem leaf extract treatment. The effect of neem seed oil on *Trichuris ovis* was quicker as compared to neem leaf extract. The nematode worms placed in higher concentrations i.e. 40 percent and 50 percent showed decreased motility after 6 hours of treatment. In 50 percent concentration of neem seed oil, the death of nematode worms took place after 8 hours. The lower concentrations of the neem seed oil showed decreased motility after 6 hours. Moderate motility was observed in worms placed in 10percent, 20 percent and 30 percent concentrations of the neem seed oil after 6 hours. Their motility became feeble after 8 hours of treatment. The death of worms placed in 10 percent, 20 percent and 30 percent concentrations was observed after 12 hours, 12 hours and 10 hours respectively. The nematode worms placed in Tyrode's solution alone, i.e. control group, show no decrease in motility even after 24 hours. They survived for longer time intervals if the *in vitro* culture medium i.e. Tyrode's solution was replaced regularly.

b) Effect on Histology

Haemonchus contortus:
The histological structure of freshly dead worms was studied in comparison to the control (living) worms at the same time interval. No histological alteration was observed in the nematode worms of the control group.

The histological structure of the control group was the same as that of the untreated worms. In *Haemonchus contortus* the histological changes were observed in the body wall, bounding membranes of internal organs and in some parts of the female reproductive system. In the anterior-most region of the body of nematode worms, large infoldings of the cuticle were observed. There was no peeling or disruption of the cuticular layers. In the nematode worms which were placed in lower concentrations of neem leaf extract and neem seed oil (i.e. 10percent, 20 per cent and 30 percent), superficial etching in the cuticle was observed. Deep indentations were also observed in some nematode worms. The worms which were placed in higher concentrations (i.e.40 percent and 50 percent) of the extract show wrinkling and peeling of the cuticle. The cuticular alterations were mainly seen in the epicuticle and cortical layers only. The flattening of the muscle layer was seen in *Haemonchus contortus* placed in higher concentrations of neem leaf extract and neem seed oil. The muscular defects were more pronounced in the posterior portion of the nematode worms where the struts were absent in the cuticle. Due to the absence of supporting struts, the cuticle show marked alterations in the posterior half of the nematodes. The bounding membrane of the digestive system disappeared in some places. The vaginal and vulvar muscles were seen to be affected. The fertilized ova, which is normally found in a two-celled stage in normal worms, was developed up to the early morula stage in the genital tract of neem-treated worms. The eggshell wall and uterine epithelium were found to be altered in treated worms.

Oesophagastomum columbianum:
The histological studies on treated worms of *Oesophagastomum columbianum* showed that the body wall and intestinal brush border was affected. The alterations in the body wall were peeling and etching of the cuticle layer. The muscle layer was also severely damaged. The erosion of the intestinal microvillar brush border indicated that it may have affected the absorption of nutrients and ultimately led to the death of worms. However, no marked change was observed in the gonads and genital tracts of male and female worms.

Trichuris ovis:
The effect of neem leaf extract and neem seed oil on the histological structure of *Trichuris ovis* was also on the histological structure of the gut. The intestinal epithelium of neem treated worms showed structural deformities including broken microvillar brush border and detachment of cells from bounding membrane. The disappearance of bounding membranes of gonads was seen to be the prominent effect in all the histological slides prepared from treated worms.

c) *in vivo* Study: The *in vivo* studies on the effect of neem on gastrointestinal nematodes revealed a decrease in egg count. The animals treated with neem showed a decline in faecal egg count after a few days. The efficacy of different anthelmintic drugs was tested on various nematode species by a number of workers. The micromorphological changes induced by anthelmintic piperazine were tested by Abdulazizov (1975). He assessed the *in vivo* effect of piperazine on *Ascaris lumbricoides.* Boulaqu et al. (1979) found that the anthelmintic levamisole causes serious alterations in the tissues of *Ascaris lumbricoides*. The histological alteration reported by them includes the thinning of muscle cells and their detachment from each other. The atrophy of excretory ducts and nerve cords was also reported. Xiao et al. (1989) reported the effect of tribendimidin on *Necator americanus*. The drug-induced histological changes were the cuticular swelling, fusion of transverse striations, peeling and erosion of female worms. The effect was mainly on the somatic muscle cells of adult *Brugia* and *Litomosoides carini*. The electron density of the cytoplasm surrounding the myofilaments in the fibrillar portion of muscle cells is increased. The appearance of a light zone in between the fibrils was also reported. In *Litomosoides carini*, the muscle cells do not increase in size but their mitochondria swell up and disintegrate. The disintegration of the myofilaments and vacuolation of the cytoplasm was also reported. In a study conducted on the adults and larvae of *T. spiralis*, they found that the cuticle disintegration took place between hypodermal pores which appear somewhat thickened and irregular. Wrinkling of the outer layer of cuticle and bullae formation was also reported by them. Both these anthelmintics have been reported to block the contractility of nematode axial muscle by causing sustained depolarization of the muscle membrane and also the neuromuscular transmission. Ambuet al. (1995) found that ivermectin has a destructive effect on the cuticle, epidermis and muscular layers of the body wall of *Angiostrongylus malaysiensis*. In the present study on *Haemonchus contortus*, the effect of neem leaf extract and neem seed oil was mainly on the cuticle and muscle layers of

the body wall. The histological alteration includes etching, peeling and wrinkling of the cuticle. These alterations confined to the epicuticle and cortical layers of the body wall. From this observation, it can be concluded that the neem extract and seed oil has been able to penetrate the acid muco-polysaccharide barrier present on the surface of the body wall of *H. contortus*. The second affected area in the case of *Haemonchus contortus* was muscle cells. The muscle cells of the body wall have undergone flattening and detachment from the cuticle. These defects can be due to the loss of fundamental activity of the muscle layer due to interruption of the glycolytic pathway of metabolism. Previously, Gandhi et al. (1999) have also reported that the neem leaf extract blocked the activity of enzymes of carbohydrate metabolism of *Trichuris globoulosa,* especially glucose- 6-phosphate. Neem leaf extract and neem seed oil treated *Haemonchus contortus* showed deep infoldings of the body wall in the anterior region of the body. However other types of degenerative changes were not observed in this portion. It may be due to the presence of a well-developed exoskeleton, in the form of struts, in that region. These struts provide rigidity and strength to the cuticle. Some weak points do appear which cause the deep infoldings and rigidity of the cuticle to be lost which may be hampering the locomotion of the parasite. No marked effect was seen on the different layers of the body wall of *Trichuris ovis*. In the case of *Oesophagastomum columbianum* the main effect of neem leaf extract and neem seed oil was on the body wall and intestinal brush border. The body wall of the neem-treated worms showed marked alterations. The cuticle layer of these nematode worms shows peeling and etching. The musculature was also damaged. Bogoyavlenskii et al. (1975) described that the treatment with piperazine or napthamon deteriorated the structure of the genital tract due to condensation of the cytoplasm. They also reported a reduction in the glycogen level in the wall tissues. No marked change was observed in the reproductive tracts of male and female worms of *Oesophagostomum columbianum*. Similarly, no effect was seen on the reproductive organs of *Trichuris ovis* except the disappearance of bounding membranes of gonads. The extensive damage to male reproductive system and the formation of abnormal spermatozoa was also reported by them. Bogoavlenskii et al. (1975) found that no alteration takes place in the reproductive system of nematodes following treatment with drug tetramisole. In the present study on *Haemonchus contorts* treated with neem leaf extract and neem seed oil, the female reproductive system have undergone marked changes like erosion of uterine epithelium and eggshell walls. Moreover, the fertilized ova were found to develop to the morula stage. They were not expelled at the normal two-celled stage. This may be due to the damage to the vaginal and vulvar musculature. Boulaqu et al. (1979) reported the effect of levamisole mainly on the intestine of *Ascaris lumbricoides*. The columnar cells of the intestine have acquired an irregular shape and became degenerated. Their nuclei have displaced from normal positions and the cytoplasm undergo vacuolations. The destruction of the nuclei and cytoplasmic organelles and the destruction of microvilli have been reported in *Bunostomum trigonocephalum* following treatment with fenbendazole (Semenkov and Akil' Zhanov, 1988).

Following treatment with thiophenate and fenbendazole, the deterioration of the absorptive surface of *Haemonchus contortus* was reported by Kaur and Sood (1992).In the present study, it was observed that the effect of neem leaf extract and neem seed oil was on the bounding membrane of the intestine of *Haemonchus contortus*. The intestinal epithelium and the microvillar border remains unaltered. These findings agree with the research work of Singh (2000 and 2015). The brush border of the intestinal epithelium of *Oesophagostomum columbianum* erupted. The erosion of the intestinal brush border indicated that it may have affected the absorption of nutrients and ultimately led to the death of worms. The effect of neem leaf extract and neem seed oil on the histological structure of *Trichuris ovis* was also on the digestive system.

CONCLUSION

The present study was conducted on *in vitro* and *in vivo* effects of neem leaf extract and neem seed oil on gastro-intestinal nematodes of sheep and goat. It was found that neem leaf extract and neem seed oil have the potential to control gastro-intestinal nematodes. All the three species of gastrointestinal nematodes chosen for present research work i.e. *Haemonchus contortus, Oesophagostomum columbianum* and *Trichuris ovis* were affected by various concentrations of neem leaf extract and neem seed oil. The nematodes of all three species showed decreased motility when placed in neem leaf extract and neem seed oil. The

death of the nematode species took place under the effect of varying the concentration of neem leaf extract and oil. The histological structure of the treated nematode worms was severely altered. The histological alterations were mainly found in the cuticle and muscle layer of the body wall. In *Haemonchus contortus*, the other histological changes in the neem-treated worms were the disappearance of the bounding membrane of the intestine. The damage to vaginal and vulvar musculature and egg shell walls was also noticed. The fertilized ova which were normally expelled at two-celled stage were found to be developed up to the morula stage. Probably their expulsion was not possible due to the development of defects in vaginal musculature. In the case of *Oesophagastomum columbianum* the main effect of neem leaf extract and neem seed oil was on the body wall and intestinal brush border. The body wall of the neem-treated worms showed marked alterations. The cuticle layer of these nematode worms showed peeling and etching. The musculature was also damaged. The brush border of intestinal epithelium erupted. The erosion of the intestinal brush border indicated that it may have affected the absorption of nutrients and ultimately led to the death of worms. However, no marked change was observed in the reproductive tracts of male and female worms. The effect of neem leaf extract and neem seed oil on the histological structure of *Trichuris ovis* was also on the digestive system. The microvillar brush border and intestinal epithelium were badly damaged. No effect was seen on the reproductive organs except the disappearance of bounding membranes of gonads. The *in vivo* studies on the effect of neem on the gastro-intestinal nematodes chosen for the present study revealed a decrease in egg count. The animals treated with neem showed a decline in faecal egg count after a few days. The present research work revealed that the neem tree (*Azadirachta indica*) has strong anthelmintic properties. Future research work can be aimed to check the effect of different components of neem on gastro-intestinal nematodes.

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Correspondence and requests for materials should be addressed to JP Singh.

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