Studies on prevalence of gastrointestinal helminths parasites of *Capra hircus* (L) and evaluation of selected ethnoveterinary plants for anthelmintic activity

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SUMMARY

Goat farming has played an important role in the upliftment of the rural economy of India. Gastrointestinal (GI) helminths are most commonly prevalent parasites affecting almost all ages, gender of animals and one of the main causes of reduced productivity of the livestock. Several control methods have been established, but the use of synthetic anthelmintic drugs still remains the only option. Recurrent use of synthetic anthelmintic drugs results in the emergence of resistance. Hence, there is an urgent necessity to find out the other effective way of controlling gastrointestinal helminthiasis. Therefore, this problem can be overcome by exploiting traditional plants against gastrointestinal helminths. In this view, the present study was proposed to investigate the prevalence of gastrointestinal helminths and evaluation of ethnoveterinary plants against gastrointestinal helminths.

The first chapter of the thesis provides a brief description on the importance of livestock in the rural economy, parasitism, helminthiasis, life cycle, anthelmintic resistance of synthetic drugs, and ethnoveterinary plants along with the objectives related to the subject of the proposed topic.

The second chapter includes the Review of Literature, emphasizing the prevalence of helminths parasite, anthelmintic resistance of synthetic drugs and ethnoveterinary plants as an alternative source for helminthiasis.

The third chapter revealed identification and prevalence of gastrointestinal helminths during the time span from November 2014 to October 2016 to investigate the epidemiology of gastrointestinal helminth parasites of goats in Lucknow, Uttar Pradesh, India. A total of 355 gastrointestinal tracts were collected and examined for the infestation and it was found that 240 gastrointestinal tracts were found to be infected with different helminth species indicative of 67.6 % prevalence. Significantly ($P \leq 0.05$) higher prevalence of nematodes (65.1 %) was found in comparison to trematodes (18.6 %) and cestode (15.5 %).

On the basis of necropsy observation, six species of helminth parasites were identified by using a light bright field microscope (100X), and photographs were taken by Evos XL imaging microscope. Out of the six species, four were nematode- (*Haemonchus* spp. 62.3%, *Trichuris* sp. 56.3 %, *Oesophagostomum* spp. 36.1 %, *Bunostomum* sp. 27.9 %). One species of trematode i.e., *Paramphistomum* sp. (18.6%), and one species of cestode i.e., *Moniezia* sp.

(15.5%) were also found to be prevalent. All the gastrointestinal parasites differed significantly (P≤0.05) with their prevalence.

A month-wise prevalence study was carried out and it was found that the highest incidence rate of these parasites was recorded in the month of September (88.5 %), followed by August (87.9 %) whereas the lowest was reported in the month of December i.e., 37.0 %. Month-wise prevalence study of different parasitic species revealed that the nematode parasite namely *Haemonchus* spp., *Trichuris* sp., and *Oesophagostomum* spp. showed a significant (P≤0.05) increase in the prevalence rate from July (84.4 %, 68.8 %, and 43.8 % respectively) to September (88.5 %, 80.8 % and 65.4 %), except *Bunostomum* sp. which showed significantly(P≤0.05) higher prevalence rate in the month of September (61.5 %) followed by July (43.4%) and August (42.4 %), whereas all these parasites had shown a significant (P≤0.05) decrease in the prevalence rate in the month of December.

Furthermore, trematode namely *Paramphistomum* sp. showed a significant (P≤0.05) discrepancy in the prevalence rate and recorded highest in the month of July (40.6) followed by March (33.3) and not observed in the month of November and December. Although, in the case of cestode, *Moniezia* sp., a significantly (P≤0.05) higher prevalence rate was noticed only in the month of September (42.3) while no prevalence was recorded in the month of November, December, and January.

The prevalence of these parasites across the three seasons (winter, summer, monsoon) were also recorded and it was found that the significant (P≤0.05) higher incidence rate was seen in the monsoon season (87.4%), followed by summer (68.6%) and winter season (49.6%). Moreover, it was also recorded that all helminth parasites showed a significant (P≤0.05) disparity throughout all the three seasons; and in this respect the highest incident rate was noted down in the monsoon season (*Haemonchus* spp. 83.1 %, *Trichuris* sp. 69.5 %, *Oesophagostomum* spp. 51.7 %, *Bunostomum* sp. 42.4 %, *Paramphistomum* sp. 27.1 %, *Moniezia* sp. 29.7 %), followed by summer (*Haemonchus* spp. 61.0 %, *Trichuris* sp. 55.9 %, *Oesophagostomum* spp. 33.0 %, *Bunostomum* sp. 27.1 %, *Paramphistomum* sp. 18.6 %, *Moniezia* sp. 14.4 %) and winter (*Haemonchus* spp. 42.9 %, *Trichuris* sp. 43.7 %, *Oesophagostomum* spp. 23.5 %, *Bunostomum* sp. 14.3 %, *Paramphistomum* sp. 10.1 %, *Moniezia* sp. 14.4 %).

Furthermore, in the season-wise study, the mean intensity (MI) and relative abundance (RA) were recorded highest during the monsoon (MI= 63.1; RA= 53.5) followed by summer (MI= 31.5; RA= 20.6) and winter (MI=24.2; RA=12.0). The present study also reported higher mean intensity and relative abundance in the month of September (MI= 83.6; RA= 73.9) followed by August (MI= 62.8; RA= 55.5), July (MI= 55.4; RA= 46.8), October (MI= 50.9; RA= 39.6), June (MI= 32.4; RA= 20.1), May (MI= 28.2; RA= 22.1), April (MI= 32.5; RA= 21.7), March (MI= 33.8; RA= 22.6), February (MI= 35.2; RA= 21.6), January (MI=32.1; RA=15.5), November (MI= 12.1; RA= 6.1) and December (MI=9.6 ; RA=3.5).

The mean intensity of the parasite was recorded maximum for *Paramphistomum* sp. (30.5) followed by *Haemonchus* spp. (20.12), *Trichuris* sp. (8.2), *Oesophagostomum* spp. (7.9), *Bunostomum* sp. (8.7) and *Moniezia* sp. (1.6). At the same time, relative abundance was highest for *Haemonchus* spp. (12.5) followed by *Paramphistomum* sp. (5.7), *Trichuris* sp. (4.6), *Oesophagostomum* spp. (2.8), *Bunostomum* sp. (2.4) and *Moniezia* sp. (0.24).

The current study revealed that younger goats (87.5%) are significantly ($P \leq 0.05$) more susceptible to these parasites as compared to adults (51.3 %). Furthermore, this study also showed gender-wise significant disparity and revealed that female goats (74.2%) are significantly ($P \leq 0.05$) more prone to infection as compared to male goats (59.6%).

The fourth chapter evaluated the anthelmintic efficacy of selected ethnoveterinary plants along with the comparative study of treated and non-treated parasite with reference to standard drug. For this, all extract concentrations and drug solution were prepared freshly before starting the assay. Ten actively motile, same sized worms were chosen and placed in Petri dishes having 1 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ ml concentration of both aqueous extracts (AE) and methanolic extracts (ME) in PBS. PBS was taken as the control (negative control) and standard drug Albendazole was used as a reference (positive control). In the entire anthelmintic assay run, Albendazole (positive control) showed a significant difference ($P \leq 0.05$) with both the extract of all plants at all tested concentrations, except the 10 mg/ml concentration of methanolic extract of both *Trachyspermum ammi* (39.6±0.3 minutes) and *Hibiscus rosa-sinesis* (73.6 minutes) and aqueous extract of *Trachyspermum ammi* (44.0±2.0 minutes), wherein the time taken to get paralysis was less (32±2.3 minutes). Moreover, use of Albendazole also recorded lesser time taken by worms to die (57.3±5.5 minutes) and showed a significant disparity ($P \leq 0.05$) at all the concentrations using both the extracts of all plants except 10 mg/ml concentration of methanolic extract of *Trachyspermum* *ammi* (60.3 minutes). No mortality of the worm was observed in PBS (negative control). However, the highest and lowest anthelmintic efficacy was obtained at a concentration of 10 mg/ml and 1 mg/ml, respectively in all the plant extracts, within 12 hours and within one hour of exposure. This study also revealed that both the extracts showed significant ($P \leq 0.05$) anthelmintic potency as compared to negative control and methanolic extracts of all plants exhibited high anthelmintic efficacy than the aqueous extracts against *Haemonchus* spp., the abomasal nematode parasite of the goats.

Moreover, the present study on the basis of LC50 values revealed that aqueous extracts showed less potency as compared to methanolic extracts. The study categories the potency of the plant and the order was observed as follows- *Trachyspermum ammi* (LC50= 2.76 mg/ml, y = 7.917x + 28.134 and LC50 = 2.13 mg/ml, y = 7.5315x + 33.917 for both aqueous and methanolic extracts respectively), followed in the descending order of their anthelmintic efficacy by *Hibiscus rosa-sinesis* (LC50= 3.81 mg/ml, y = 8.9076x + 16.052 and LC50= 2.62 mg/ml, y = 7.8527x + 29.431both aqueous and methanolic respectively), *Trigonella foenum-graecum* (LC50= 4.31 mg/ml, y = 9.4351x + 9.3628 and LC50= 2.88 mg/ml, y = 8.0535x + 26.752 both aqueous and methanolic extracts respectively), *Curcuma longa* (LC50= 4.90 mg/ml, y = 10.434x — 3.506 and LC50= 3.62 mg/ml, y = 8.6988x + 18.518; R^2 = 0.931 both aqueous and methanolic extracts respectively), *Azadiracta indica* (LC50= 5.43 mg/ml, y = 10.411x — 6.502 and LC50= 4.20 mg/ml, y = 9.4672x + 10.214 both aqueous and methanolic extracts respectively), *Nigella sativa* (LC50= 5.71 mg/ml, y = 11.074x — 13.216 and LC50= 4.65 mg/ml, y = 9.8019x + 4.4163 both aqueous and methanolic extracts respectively), *Coriandrum sativum* (LC50= 5.95 mg/ml, y = 11.299x — 17.256 and LC50= 5.21 mg/ml, y = 10.195x — 3.1539 for both aqueous and methanolic extracts respectively), and *Ficus religeosa* (LC50= 6.10 mg/ml, y = 11.421x — 19.673 and LC50= 5.75 mg/ml, y = 10.513x — 10.473 both aqueous and methanolic extracts, respectively).

On the other hand, the present study also categorised the plants on the basis of dose-dependent efficacy (based on their R^2 value) which was found to be different as compared to anthelmintic potency. The best dose-dependent effects on adult motility were shown by *Azadiracta indica* (R^2 = 0.9914), followed by *Nigella sativa* (R^2 = 0.9912), *Curcuma longa* (R^2 = 0.986), *Ficus religeosa* (R^2 = 0.9718), *Coriandrum sativum* (R^2 = 0.977), *Trigonella foenum-graecum* (R^2 = 0.972), *Hibiscus rosa-sinesis* (R^2 = 0.9463), and *Trachyspermum ammi* (R^2 = 0.8671) in aqueous extract, whereas in methanolic extracts the order was

Coriandrum sativum (R^2 = 0.9961), Nigella sativa (R^2 = 0.987), *Ficus religeosa* (R^2 = 0.9595), *Azadiracta indica* (R^2 = 0.9528), *Curcuma longa* (R^2 = 0.9528), *Trigonella foenum-graecum* (R^2 = 0.8622), *Hibiscus rosa-sinesis* (R^2 = 0.8233), and *Trachyspermum ammi* (R^2 = 0.7666).

On the basis of LC50 value of all selected ethno-veterinary plants, the present study ranked the three most effective plants i.e., *Trachyspermum ammi* followed by *Hibiscus rosa-sinesis* and *Trigonella foenum-graecum*. The study also revealed that methanolic extracts exhibited high anthelmintic potential than the aqueous extracts. Based on these observations, the worms kept at higher concentration i.e., 10 mg/ml of methanolic extracts of these plants were taken to observe the comparative histological (anatomical) changes in the worms after *in vitro* treatment.

Histological comparison of the control worms and treated worms was done by observing the parasites under the light bright field microscope (100X and 400X), and photographs were taken by Evos XL imaging microscope. The results revealed that in a transverse section, the cuticular ridges appear like cuticular elevations (ridges) and covered by a cuticular sheath epicuticle enclosing a spine like core or strurt, which form a sort of exoskeleton to provide a mechanical asset to the worm. Below the cuticle, hypodermis is present comprising syncytial epidermis, giving rise to a complex layer of longitudinal muscles towards the outside and connective tissues towards the inner side. The middle portion of the body has a large body cavity called pseudocoel. A large intestine lies centrally in the cavity and runs through the length of the body. Near the intestine, two well-developed ovaries are present in females. The histological study of the three plants also revealed that similar to reference drug Albendazole, all these plants also caused the anatomical damage in the worm body but among these plants, *Trachyspermum ammi* showed more anthelmintic potency than other two plants. The worm treated with *Trachyspermum ammi* showed extensive damage in the epicuticle along with cuticular wall and cuticular ridges. A severe disintegration was found in the ovary wall and oviduct whereas, irregular disorganization occurred in the muscle layer and intestinal wall.

In the present study, the efficacy of most effective plant i.e., *Trachyspermum ammi* (methanolic extracts) was further evaluated through observation of surface morphological changes occurred in treated worm by using Scanning Electron Microscopy (SEM) (Jeol, Japan; JSM 6490 LV) at different resolutions as compared to control. The results revealed that control worm showed semi-circular rudimentary lips and dorsal lancet (DL) worm's blood sucking apparatus. A pair of cervical papillae was protuberant and spine-like. The cuticle was transversally striated with longitudinal cuticular ridges. Digital end of the body remained pointed and straight. The main changes observed in treated worm were, extreme damage of transverse striation and longitudinal cuticular ridges, the cuticle was completely ruptured. Buccal capsule showed severe distortion with blebbing of the lips at the anterior end. The posterior end, i.e. the tail was slightly more wrinkled and constricted.

The fifth chapter described the presence of phytochemical constituents by the screening of the effective plants. Preliminary qualitative analysis was performed for all plants and revealed that both aqueous extract and methanolic extract of *Trachyspermum ammi* seeds revealed the presence of carbohydrate, alkaloids, flavonoids, terpenoids, tannins, phenols, saponins, glycosides except the anthraquinone. Furthermore, methanolic extract showed the intense colour of precipitate for alkaloids, terpenoids, tannins, and phenols while saponins showed more foam formation than aqueous extract.

The preliminary qualitative analysis of *Hibiscus rosa-sinesis* leaves showed that both aqueous extract and methanolic extract of this plant exhibited the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, saponins, glycosides except carbohydrate, and anthraquinone. Also, the methanolic extract showed the intense colour of precipitate for alkaloids, tannins, and phenols than aqueous extract.

The qualitative analysis of *Trigonella foenum-graecum* seeds revealed that the aqueous extract showed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, saponin, glycosides whereas, methanolic extract showed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, and saponins except for glycosides. Moreover, the methanolic extract showed the intense colour of precipitate for carbohydrate, alkaloids, tannins, flavonoids, and phenols than aqueous extract.

The qualitative analysis of the *Curcuma longa* rhizome extracts indicated that the methanolic extract showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, anthraquinone, and carbohydrates while the aqueous extract showed the presence of alkaloids, carbohydrate, flavonoids, terpenoids, and saponins. Besides the methanolic extract showed the intense colour of precipitate for carbohydrate, alkaloids, tannins, flavonoids, and phenols while saponin showed more foam formation than aqueous extract.

The preliminary phytochemical screening of extract of *Azadiracta indica* leaves disclosed that the methanolic extract showed the presence of glycosides, alkaloids, flavonoids, tannins, saponins, terpenoids, phenol, and carbohydrates except for anthraquinone. In other hands, aqueous extract showed only the presence of alkaloids, tannins, flavonoids, and phenols.

The preliminary phytochemical analysis *Nigella sativa* seeds revealed that the aqueous extract showed only the presence of alkaloids and phenol whereas, the methanolic extract showed the presence of carbohydrate, alkaloids, phenol, tannin, flavonoids, and saponins.

The preliminary phytochemical analysis of the extract of *Coriandrum sativum* seeds has shown that methanolic extract has shown the presence of flavonoids, terpenoids, tannin, phenol, and glycosides while aqueous extract showed the presence of flavonoids, terpenoids, and phenol.

The qualitative phytochemical analysis of leaves extract of *Ficus religeosa* disclosed that the methanolic extract displayed the presence of carbohydrate, alkaloids, tannin, and phenol whereas aqueous extract showed the presence of carbohydrate, terpenoids, and phenols.

The quantitative analysis was performed for the methanolic extracts of the plants identified to be most effective in the present research for calculating the most important phytochemical compounds responsible for the anthelmintic activity.

The quantitative analysis data was tabulated in Table 5.4.2. The amount of total phenol was determined with the Folin-Ciocalteu reagent method using Gallic acid as a standard compound. The total phenols were expressed as mg/g Gallic acid equivalent using the standard curve equation: $y = 11.496x + 0.2134$; Where y is an absorbance at 765 nm and x is total phenolic content in the extract (Graph 5.4.2a). It is evident from the Table 5.4.2a that *Trachyspermum ammi* showed the highest amount of total Phenolic compound (52.85±2.9) followed by *Hibiscus rosa-sinensis* (48.53±0.12), and *Trigonella foenum-graecum* (44.65±1.3).

The amount of total tannin was also determined with the Folin-Ciocalteu reagent process using Tannic acid as a standard compound. The total tannin was expressed as mg/g Tannic acid equivalent using the standard curve equation: y = 7.0764x — 0.0914; Where y is absorbance at 725 nm and x is total phenolic content in the extract. *Trachyspermum ammi* showed the highest amount of total tannin compound (78.63±0.29) followed by *Hibiscusrosa-sinensis* (62.15±1.16), and *Trigonella foenum-graecum* (55.42±0.88).

The total flavonoid content was determined using Aluminum Tri Chloride (AlCl3) reagent method and Rutin was used as a standard. The total flavonoid content was expressed as mg/g Rutin equivalent using the standard curve equation: y = 13.129x— 0.1742; where y is the absorbance at 510 nm and x is total flavonoid content in the extract. The result revealed that the total flavonoid content was found maximum for *Trachyspermum ammi*, i.e. 37.65±0.6 whereas, *Hibiscus rosa-sinensis* showed 18.5±0.69, and lowest in *Trigonella foenum-graecum* 14.4±0.33.

The total alkaloids present in plant extract were determined by Bromocresol green-chloroform method. Atropine was used as a standard compound and the total alkaloid content was expressed as mg/g Atropine equivalent using the standard curve equation: y = 0.4394x - 0.0065; where y is the absorbance at 470 nm and x is total Alkaloid content in the extract. The data showed that the higher amount of alkaloids content was observed in *Trachyspermum ammi*, i.e. 104.9±2.4 while *Hibiscus rosa-sinensis* showed moderate 73.72±0.54, and low in *Trigonella foenum-graecum* 45.62±1.0.

The total Saponin present in plant extract was determined by Vanillin reagent method. Diosgenin was used as a standard compound and the total alkaloid content was expressed as mg/g Diosgenin equivalent using the standard curve equation: y = 0.3726x + 0.0217; where y is the absorbance at 544 nm and x is total Diosgenin content in the extract. The result disclosed that the higher amount of saponin was observed in *Trachyspermum ammi* (40.66±4.0) followed by *Hibiscus rosa-sinensis* (14.7±2.1), and lowest in *Trigonella foenum-graecum* (8.4±1.3).

The sixth chapter dealt with the significance and future scenarios of the presented research on the prevalence of gastrointestinal helminth parasites of *Capara hircus* (goat) and importance of ethnoveterinary plant as an alternative choice for the synthetic anthelmintic drugs.