

STUDIES ON MEDICINAL PLANTS AGAINST GASTROINTESTINAL NEMATODES OF GOATS

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ABSTRACT

A detailed investigation was performed with the aim to find out the indigenous medicinal plants having anthelmintic action. Ten (10) indigenous medicinal plants were primarily selected and the ethanol extracts were prepared for anthelmintic trial and determination of anthelmintic properties *in vitro* and *in vivo* against the gastro-intestinal nematodes in goat during the period from July 2006 to December 2006. Screening of ethanol extracts of selected plants showed the anthelmintic activity against gastrointestinal nematodes at lower concentration (50 mg/ml). *In vivo* screening (by oral administration) of four plant extracts (ethanol) showed variable degree of efficacy in experimentally infected goats, as measured by faecal egg count reduction test. A relatively higher efficacy was recorded in ethanol extract of neem treated animals in comparison to other plants extracts. Ethanol extracts of korolla also showed significant efficacy. The results obtained in this study showed that ethanol extract of Labanga, Neem, Karolla and Pineapple at the dose of 100mg/kg showed a significant and potent antinematodal effect. These findings indicate that the adult gastrointestinal nematodes are more vulnerable to selected indigenous plants. Within these ten (10) plants 4 showed more than 70% efficacy at a concentration of 100mg/mkg.

Key words: Medicinal plants, anthelmintics, nematodes, fecal egg count, goat

INTRODUCTION

A larger number of plants are naturally available in the indo-Pak-Bangladesh subcontinent, which possess narrow or broad spectrum anthelmintic activities. For both developed and less developed countries, recognition and development of herbal medicine offer treatment methods that are more environmentally benign, since they tend to be less toxic, produce fewer unanticipated side effects and apparently do not trigger anthelmintic chemoresistance. The phytochemical analyses of naturally available plants and control anthelmintic trials along with contemporary knowledge of parasite control strategies may offer new opportunities for effective and economical control of parasitic diseases. But, there are problems connected with the use of herbal medicine, the largest being the lack of scientific evaluation. The most effective approach to obtain such evaluation is the ethnobotanical approach, which assumes that indigenous uses of plant indicate the presence of biologically active compounds in the plants. Ethnoveterinary research, development and extension also play an important role in this context. The present study is a part of these research, development and extension that may help to evaluate effective, available and low cost anthelmintics of plant origin.

Livestock is an important prospective sector which may contribute to solve problems of marginal farmers. Livestock is also capable of helping poverty alleviation. It plays an important role to promote human health by supplying animal protein of high caloric value in the form of meat and milk. It is also important in earning substantial amount of foreign exchange by exporting leather, leather products and other products made from bones, horns and teeth per year (Alam, 1993). Livestock sub-sector contributes to solve the problems of small and marginal farmers and play important role in poverty alleviation. Livestock provides milk, meat, skin, fuel, organic fertilizer and draft power. The total contribution of livestock to the Gross Domestic Product (GDP) is approximately 6.5%. It generates 13% of total foreign currency and provides full time employment to about 30% and partial employment to about 50% of the rural population (Alam, 1993). The skin and hides of domestic animals are important commercial material if they are raised properly and protected against parasitic infestation. Leather is a surplus and export commodity of Bangladesh. About 10% of the available leather is required to meet domestic demands and the rest 90% is exported. Skin and hides earn 9% of the total foreign currency (Jabbar, 1985). As such to improve our present situation simultaneously with national economy, it becomes essential to increase animal population and their products. Goats have been considered most important part of livestock throughout the country.

There are about 30 million goats in Bangladesh and by exporting the skin of goats we can earn about 149 crore taka per year as foreign currency (Razzak, 2002). The production performances of these goats are very low in Bangladesh because of wide spread occurrence of harmful parasites (Rahman and Razzak, 1973; Qadir, 1981). Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Waller, 1987). Parasitic diseases are considered important in causing enormous economic losses through morbidity and mortality in livestock. Among the parasitic diseases, gastro-intestinal (g/i) nematodes such as *Haemonchus contortus*, *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum columbianum*, *Trichuris* spp. and *Strongyloides papillosus* are most common in Bangladesh (Qadir, 1981; Rahman and Mondal, 1983). This group of gastrointestinal nematodes is also associated with anaemia and gastroenteritis resulting loss of body weight, stunted growth, diarrhoea etc. that greatly hamper the normal growth and production of goats (Soulsby, 1982).

Control of GI nematodes is mainly based on regular anthelmintic treatment (Waller, 1987). Imported manufactured anthelmintics have long been considered the only effective way of controlling parasitic infection. However, as these are very expensive and unavailable to farmers in rural areas, livestock producers are not interested to these anthelmintics. Furthermore, some serious disadvantages of using these anthelmintics, notably the development of resistance to helminths (Waller and Prichard, 1985) to various anthelmintic compounds and classes, as well as chemical residue and toxicity problems (Kaemmerer and Butenkotter, 1973). For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific interest despite extensive use of synthetic chemicals in modern clinical practices all over the world (Akhtar *et al.*, 2000). The plant kingdom known to provide a rich source of botanical anthelmintics, antibiotics and insecticides (Satyavati *et al.*, 1976). In this context, investigations on indigenous medicinal plants might contribute to develop effective but low-cost herbal anthelmintics. These are mostly used in crude forms and their pharmacological preparations, dosages and mode of actions are not based on strong scientific evidence.

From the foregoing discussions of is assumed that parasitic infection is one of the major impediments for growth and development of goat in Bangladesh. Very little works have been performed in our country to investigate the anthelmintic properties of indigenous medicinal plants in livestock. For this reason, the present study has taken great impetus. This research work provides useful information on anthelmintic properties of some common medicinal plants to select the potential plants to be used against gastrointestinal nematodes of goats.

MATERIALS AND METHODS

The present study was conducted during the period from July, 2006 to November, 2006 in the laboratory of the Department of Parasitology and Department of Pharmacology and Central Laboratory, Bangladesh Agricultural University (BAU), Mymensingh with the aim for screening of indigenous medicinal plants for their anthelmintic activities against gastro-intestinal (g/i) nematodes in goat. Ten (10) indigenous medicinal plants Anaros (Pineapple: *Ananas comosus*), Ata (Custard apple: *Annona reticulata* Linn), Durba grass (Couch grass: *Cynodon dactylon*), Karolla (Bitter gourd: *Momordica charantia*), Katakura (Katakura: *Amaranthus spinosus*), Labanga (Cloves: *Eugenia caryophyllus*), Neem (Neem: *Azadirachta indica*), Pan (Betel leaf: *Piper betle*), Pat (Jute: *Corchorus oleriosus*) and Tamak Tobacco(*Nicotina tabacum*), were collected from BAU campus and its surrounding rural areas. These plants were screened for their anthelmintic activity *in vitro* using g/i worms. But *in vivo* screening of four (Anaros (Pineapple: *Ananas comosus*), Karolla (Bitter gourd: *Momordica charantia*), Labanga (Cloves: *Eugenia caryophyllus*) and Neem (Neem: *Azadirachta indica*), selected plants was performed using experimentally infected goats to determine their efficacy. After collection and bringing them to the laboratory, all fresh leaves, seeds and bark were washed in running tap water and cut into small pieces. Firstly, the plant materials were dried in shade and then they were dried in the oven at 55-60°C. Dust was prepared by pulverizing the dried leaves, seeds and barks with the help of a manual grinder. A 25-mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container, till their use in extract preparation.

Previously prepared plant materials were used for preparation of plant extract. 10 gram each category of dust were taken in a 500 ml beaker and separately mixed with 100 ml ethanol. Then the mixture was stirred for 30 minutes by a magnetic stirrer (1000 rpm) and left stand for next 24 hrs. The mixture was then filtered through

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Whatman filter paper. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50°C for ethanol up to final volume of 10 ml. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottle and stored in a refrigerator until their screening for anthelmintic property.

Adult nematodes were obtained from the g/I tracts of goats slaughtered in the local markets. Briefly the abomasai, small and large intestines were collected and brought to the laboratory. They were opened in a plastic bucket separately and the contents were washed in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult g/i worms were collected with the help of a needle and placed in a petridish containing PBS (Phosphate Buffer Saline). Petri-dish containing the worms was kept in incubator at 38°C until required for experiment on the same day. In vitro screening with pharmacological preparations (Ethanol extracts) of different plants was performed using adult g/i nematodes. The following techniques were followed for in vitro screening.

In vitro screening with adult worms

The ethanol plant extracts were used at various concentrations i.e., 10 mg/ml, 20 mg/ml, 50 mg/ml and 100 mg/ml using adult worms. A 200µl PBS containing 10 adult worms was pipetted on to a petri-dish and a 800µl of ethanol extracts of each concentration was then added at room temperature. The non-motile (dead) worms were counted and the percentage was calculated.

In vivo screening of plant extract

The ethanol extracts of above mentioned selected plants showed potential wormicidal effects following in vitro screening were further tested in vivo using a group of experimentally infected goats. Only 4 plants among 10 were included for in vivo study. Fifteen (15) goats aged between 15 to 24 months were used. The animals were divided into five (5) groups each containing three goats. The animals were treated with ethanol extract of four plants separately. EPG count was done on day 5, 7 and 9 post-treatment by McMaster egg counting technique described previously. The efficacy of different treatment was determined by faecal egg count reduction test following the formula mentioned below:

$$\text{Efficacy} = \frac{\text{EPG prior to treatment} - \text{EPG post - treatment}}{\text{EPG prior to treatment}} \times 100$$

Two grams of fecal samples were taken in a beaker and 30 ml of tap water was poured and stirred to dilute the sample. Then 30 ml of saturated salt solution was added and shaken. A small amount of the diluted sample was withdrawn by a pipette and run into the counting chamber to fill all the space. The slide was then put to stand for some times allowing the eggs to float under the surface of the upper slide of the McMaster chamber. The slide was then examined under microscope using low power objective (X10) and eyepiece (X6) and the total eggs within each ruled area were counted. The number of eggs per gram of feces was calculated by using the following formula. EPG of feces was counted on day 0 (pre-treatment), before treatment and on day 5, 7 and 9 of post-treatment. Fecal samples were counted from each animal of both treatment and control groups.

$$\text{Number in one gram} = \frac{\text{Number in two chamber}}{0.3} \times \text{dilution factor}$$

$$\text{*dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of faeces}}$$

RESULTS AND DISCUSSION

The efficacy of alcoholic extract of 10 plants such as, Anaros, Ata, Durba grass, Karola, Katakura, Labanga, Neem, Pan, Pat and Tobacco against the g/i nematodes of goat were studied in *in vitro* at different concentration @ 1% (10mg/ml), 2% (20mg/ml), 5% (50mg/ml) and 10% (100mg/ml) (Table1). The ethanol extract of 10 plants showed that all of them have more or less wormicidal effect. Four out of selected ten indigenous medicinal plants showed potential *in vitro* activity against gastrointestinal nematodes. Within these ten (10) plants 4 showed 60-80% and other showed 50% at a concentration of 100mg / ml. The most common plants had highly

significant activity against adult g/i nematodes *in vitro* were Anaros (Pineapple leaves), Karolla (fruit), Labanga (flower bud) and Neem (leaves) (Table 2). All the animals were highly infected with gastrointestinal nematodes which were determined by the faecal account by McMaster technique.

From the observation of this study, it is obvious that plants have potential effects on gastrointestinal nematodes of goats. Sharma *et al.* (1971) reported that with rotational grazing and pasture with 20-25% legumes, the worm problems can be practically controlled in cattle. Goat treated with ethanol extract of Pineapple leaves, Karolla, Labanga and Neem at the dose of 100 mg/kg body weight showed 73%, 78%, 85% and 81% efficacy on 9th day, respectively (Table 2). It was also observed that the efficacy of ethanol extract of Pineapple leaves gradually increased from days 5 to 9 post treatment.

Table1. Comparative *in vitro* efficacy of ethanol extract of plants against g/i nematodes of goat

S/N	Experimental plants			Percent of dead parasite			
	Local name	English name	Botanical name	1% (10mg/ml)	2% (20mg/ml)	5% (50mg/ml)	10% (100mg/ml)
1	Aam	Mango	<i>Mangifera indica</i>	30	40	40	50
2	Durba grass		<i>Cynodon dactylon</i>	30	40	40	50
3	Pat	Jute	<i>Corchorus</i> spp.	40	50	50	60
4	Karolla	Bitter gourd	<i>Momordica charantia</i>	70	70	90	100
5	Katakhura	Katakhura	<i>Amaranthus spinosus</i>	30	40	50	60
6	Labanga	Clove	<i>Eugeni</i> spp.	70	70	80	100
7	Neem		<i>Azadirachta indicaicu</i>	60	70	80	100
8	Pan	Betel leaf	<i>Piper betle</i>	40	50	50	70
9	Anaros	Pineapple	<i>Ananas comosus</i>	60	70	90	100
10	Tamak	Tobacco	<i>Nicotina tobacum</i>	60	60	70	80

Table 2. Comparative *in vivo* efficacy of ethanol extract of plants against g/i nematodes of goat

S/N	Local name	English name	Botanical name	EPG pre-treatment (0 day)	EPG post-treatment (percentage reduced)		
					5 th day	7 th day	9 th day
1	Anaros	Pineapple	<i>Ananas comosus</i>	986	664 (32%)	482 (51%)	260 (73%)
2	Karolla	Bitter gourd	<i>Momordica charantia</i>	862	520 (39%)	312 (63%)	185 (78%)
3	Labanga	Clove	<i>Eugeni caryophyllus</i>	1280	806 (39%)	442 (65%)	195 (85%)
4	Neem	Neem	<i>Azadirachta indicaicu</i>	844	495 (41%)	286 (66%)	162 (81%)
5	Control group			956	1220	1488	1550

A considerable number of plants showed strong wormicidal activities against gastrointestinal nematodes in goats. These findings indicate that the adult gastrointestinal nematodes are more vulnerable to selected indigenous plants. So, it may be suggested that those 4 plants which have strong wormicidal activity can be used as anthelmintic agent in the treatment of gastrointestinal nematodes in goat. In the present study it was observed that, the mortality of worm depends on the concentration of plants extract and the types of plants. This observation gave the indication that mortality percentage varied depending on the plants, solvents and doses.

The results obtained in this study showed that ethanol extract of Labanga, Neem, Karolla and pineapple at the dose of 100mg/kg showed a significant and potent antinematodal effect. However, the present study encourages further need for research into these cheap indigenous althelmintic medicinal plants against the gastrointestinal nematodal infection in goat.

Plant anthelmintics have been in the forefront of this growing awareness (Hammond *et al.*, 1997). A reason for this could be that they fall into the category of readily applicable elements of ethnoveterinary medicine in livestock development (McCorkle and Mathias-Mundy, 1992). Due to poor economic condition and high cost of patent drugs, farmers are not able to buy these drugs. So, they depend on ethnoveterinary medicine mainly on herbal products for the treatment of their livestock and other animals. Indigenous system of medicine reports a numbers of plants for their anthelmintic efficacy. A number of researchers (Neogi *et al.*, 1964; Sharma *et al.* 1971; Kalesaraj, 1974 and Lal *et al.*, 1976) were also worked on the anthelmintic activity of medicinal plants.

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