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Efficacy of neem leaf extract against ascariasis in indigenous chicken

Jasim Uddin Khokon[1]*, Sharifuzzaman[2], Emran Hasan Sarker[1], Mohammad Afazur Rahman[1], James Jony Kisku[3], Mahbub Mostofa[1]

[1]Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Parasitology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Department of Medicine, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

The efficacy of a medicinal plant neem (*Azadirachta indica*) leaves and a reference anthelmintic (piperazine) was evaluated against adult *Ascaridia galli* in chicken in vitro and in vivo. The aqueous extracts of neem leaves and a rage of piperazine concentrations were screened for their anthelmintic effects against *Ascaridia galli* in indigenous chicken. The data revealed that the aqueous extract of neem leaves showed significant anthelmintic efficacy at a dose rate of ranging from 1 mg/ml, 2 mg/ml, 4 mg/ml and 20 mg/ml against the chicken ascariasis. Shortest time of parasite paralysis was observed at dose rate 20mg/ml. Piperazine was incubated at the dose rate of 0.1, 0.2, 0.4, 0.8 and 4mg /ml to adult worms. Shortest time of parasite paralysis was observed at dose rate 4mg/ml. Using the extract of neem leaves at a dose of 1gm/kg body weight (bd. wt.) and piperazine at a dose of 200mg/kg body weight single dose for seven consecutive days showed gradual increase of efficacy up to day 14th and 21st of treatment, respectively. In post-mortem worm count neem leaves showed considerable efficacy against worm burden. The live body weight was increased significantly in chicken after administration of neem leaves extract (1 gm/kg bd.wt) and Pipervet® (200mg/kg bd.wt) in groups 'B' and 'C' respectively. But in control group 'A' the change of body weight was not significant. Increase values of TEC, Hb and PVC were observed in chicken after treatment with Pipervet® (200mg/kg b.wt.) and neem leaves extract (1 gm/kg bd.wt). However, the aqueous extract of neem did not show any adverse effect on haematological parameter and physiological condition of treated birds.

Key words: *Ascaridia galli*, neem leaves extract, chicken

*Corresponding author. Tel.: + 8801816467202
E-mail address: jkhokon@ymail.com (JU Khokon)

INTRODUCTION

Bangladesh is an agricultural country, which is located at the sub-tropical region of the world. Livestock plays an important role as the back-bone of agriculture (Anonymous, 1985). Among livestock the importance of poultry in this country need not to be over emphasized. Poultry play important role in the national economy. A considerable amount of foreign exchange is being earned by exporting eggs and meats of poultry. Poultry husbandry also acts as a profession in unemployed young persons, landless farmers, poor, divorced women and children which can supplement their family income. The eggs and meats of poultry are the source of protein. The meats of poultry are nutritious, tasty and contain less fat.

The total population of domestic fowls in Bangladesh was estimated to be 7,64,46,000 (BBS, 1991). It provides protein need in our daily food menu in terms of meat and eggs. But it is not sufficient against need of our large population. Our food is very much deficient in protein of animal origin. In our country, 120 gm meat is needed/head/day but get only 12.61 gm/head/day and one egg is needed/head/day but get only 0.2/head/day. The average quantity of protein is

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9.5 gm/head/day (FAO, 1966), which is far below in comparison with developed countries.

The increasing demand of animal protein and the economic benefits obtained through chicken raising in both backyard and conventional farming systems have created a great deal of interest among the farmers in these days. But chicken farming in Bangladesh are facing various hindrances. Among these, parasitic infections perhaps play a vital role. Gastro-intestinal nematodiasis is a common problem of fowls in the tropical and subtropical countries of the world. Among gastro-intestinal nematodes ascarid infection rank top position in Bangladesh. Ascariasis is causing an appreciable loss to chicken population in Bangladesh. It is very difficult to find chicken flock that rear in free ranging system of this country free from ascarids infections. However, the economic losses due to ascariasis in chicken may be produced in different ways, such as, loss of meat, egg and loss due to death.

Bangladesh is plentiful with many plants, among them medicinal plants as a traditional system of therapy, have been used from ancient times to cure diseases of man and animals (Akhtar et al., 2000). Bangladesh, Pakistan and India bound with plants that were known to have medicinal properties.

Control of *Ascaridia galli* is mainly based on regular anthelmintic treatment. Because of high economic cost and unavailability of anthelmintics, our farmers cannot afford to purchase. Furthermore, frequent use of these anthelmintics increased the resistant population of nematodes (Waller, 1987). In this context, investigations on indigenous medicinal plants might contribute to develop effective but low-cost herbal anthelmintics. The “ayurvedic” and “unani” systems of medicine have used several hundreds of plants to cure many diseases in Bangladesh from time immemorial. However, these are mostly used in crude forms and their pharmacological preparations, dosages and mode of action are not based on strong scientific evidence. Till today a few works (Mostofa, 1983) have been performed in our country to investigate in vitro and in vivo anthelmintic properties of medicinal plants against *Ascaridia galli*. The present study was undertaken to evaluate the potential medicinal plant *Azadirachta indica* (neem leaves) against *Ascaridia galli* infection in chickens.

MATERIALS AND METHOD

Collection and processing of plant material

Neem leaves were selected for *in vitro and in vivo* evaluation for its effectiveness against *Ascaridia galli* in chicken. Mature and disease free neem leaves were collected from Bangladesh Agricultural University (BAU) campus. After washing, the fresh leaves were cut into small pieces and water was added at 1:10 ratio in a blender. Then juice were made by blending the leaves for 2-3 minutes and stored at 4°C until use.

In vitro assay

Adult *Ascaridia galli* were collected from the intestine of chickens. Small intestines were opened in a plastic bucket, and the contents were washed in tap water. The process was repeated for several times until parasites were seen easily. Then the visible *Ascaridia galli* were collected with a needle and placed in a petri dish containing normal saline.

Fresh leaves juice was used for preliminary treatment and the petri dishes were incubated at room temperature, observed and time was recorded to attain paralysis of worms. The parasite was then exposed to different concentration of the neem plant extracts and piperazine in separated petri dishes maintained at 37±1°C. Physical activity of the nematodes was observed until complete immobility of the treated parasite.

In vivo assay

Indigenous chickens of 45 to 60 days old were randomly selected. Sixty chickens were allowed to acclimatize for 7 days in the experimental shed. During acclimatization the chicken were supplied with feed and water. Ten percent aqueous extract of neem leaves was freshly prepared and administered orally by dropper. piperazine (Piper-vet®) was used as reference anthelmintic which

was purchased from local market and administered according to manufacturer instruction.

All the 60 infected chicken randomly divided into 3 groups (A, B, and C). Group 'A' was kept as non treated control. Group 'B' was treated with piperazine and the drug was administered orally @200 mg/kg body weight through drinking water or by dropper for consecutive 7 days. Group 'C' was treated with neem leaves extract, administered orally@1g/kg body weight by dropper by consecutive 7 days. All the chicken of treated and control groups were closely observed for 21 days after treatment.

Effect of treatment on clinical and hematological parameters

The effect of the neem leaves extract and piperazine on body weight, feed consumption and water consumption was recorded before and during administration of drugs. The weight of each chicken was taken in the morning, noon and afternoon. The average of these three weights was calculated and recorded. Mean live weight gain of each group of chicken on 7^{th}, 14^{th}, and 21^{th} days was recorded with the procedures described above.

Blood samples were collected from neck vein of chicken of both control and treated groups at pre-treatment and post treatment (21 days) period at 7 days interval to observed total erythrocyte count (TEC), hemoglobin estimation (Hb) and packed cell volume (PCV).

Parasites from the treated and non treated chicken were recovered and counted during postmortem examination. Three chickens from each group were slaughtered to count number of *Ascaridia galli* and observed if there were any pathological changes.

Statistical analysis

Statistical analyses were performed using Biostate 2007. Comparison of the mean values of the treatment against those of the control group was made using unpaired Student's *t*-test and the level of probability considered significant when $P<0.05$.

RESULTS AND DISCUSSION

In vitro assay

Ascaridia galli were collected from naturally infected chickens intestine and survived for 48.00±0.67h in the medium (NaCl, KCl, Trisodium citrate, dihydrate, glucose, anhydrase) at 37°C incubation.

Table1
Concentration dependent efficacy of piperazine on the survival of *Ascaridia galli*

Drug	Concentration (mg/ ml)	Time (hr) survival (mean ± SD)
Neem leaves extract	0 (control)	48.00±0.67
	1	11.75±0.25*
	2	10.22±0.14*
	4	8.25±0.15*
	20	6.08±0.05*
Piperazine citrate	0 (control)	48.00±0.67
	0.1	10.65±0.40*
	0.2	8.25±0.36*
	0.4	6.18±0.27*
	0.8	4.25±0.45*
	4	1±1.0*

*indicate the statistically significant values compared to control in respective treatment ($P<0.05$)

The extract of neem leaves showed concentration dependent efficacy against *Ascaridia galli*. Lowest survival time (6.08±0.05h) was observed at highest concentration of 20 mg/ml neem extract used in thi experiment. Piperazine was found to be highly effective anthelmintic exhibiting dose dependent activity at all concentrations tested (table 1). Significant mortality was observed at 10.65±0.40h in the lowest concentration (0.1 mg/ml), and at 1±1.0 h in the highest concentration (4 mg/ml). In comparison to piperazine neem leaves showed moderate paralysis of *Ascaridia galli*. The promising in vitro anthelmintic effects of neem leaves extract against *Ascaridia galli* in this study support the observations reported by Islam et al. (2008) and Begum et al. (2010).

Table 2
Effects of neem leave extract and Piper-vet® on body weight (gm) in chickens

Group of chickens	Drug and dose	Pre-treatment (gm) (mean ± SD)	Post-treatment (gm) (mean ± SD)			Mean weight gain (%)
			7th day	14th day	21st day	
A	Parasitic control (untreated)	460.2±4.5	455.5±3.8	462.5±1.22	470.1±0.80	2.20
B	Neem leaves 1g/kg	480.4±0.2	486.5±0.5	500±0.1	530±0.5	11.89
C	Piper-vet® @ 200mg/kg	450.5±2.4	460.8±2.5	468.5±1.65	532.0±2.8	17.30

Values given above represent the (mean ± SD) of 5 chickens

Table 3
Effects of neem leaves and Piper-vet® (piperazine) on TEC (million/cu mm) in chickens

Group of chicken	Drug and dose	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)		
			7th day	14th day	21st day
A	Control (untreated)	3.25±0.01	3.20±0.1	3.08±0.02	2.65±0.16
B	Neem leaves extract 1g/kg	3.76±0.03	3.86±0.27	3.95±0.05	4.33±1.05
C	Piper-vet® @ 200 mg/kg	3.54±0.80	3.56±0.82	3.70±0.85	4.05±0.90

Values given above represent the (mean ± SD) of 5chickens

Table 4
Effects of neem leaves and Piper-vet® on Hb estimation (gm %) in chickens

Group of chicken	Drug and dose	Pre- treatment (mean ± SD)	Post-treatment (mean ± SD)		
			7th day	14th day	21st day
A	Parasitic control (untreated)	10.60±0.24	9.70±0.06	10.0±1.08	9.0±0.05
B	Neem leaves extract@1g/kg	10.76±0.02	10.87±0.03	11.01±0.06	11.15±0.09
C	Piper-vet® @ 200mg/kg	9.45±0.02	9.56±0.03	10.05±0.02	10.58±0.01

Values given above represent the (mean ± SD) of 5 chickens

In vivo assay

Effect on body weight

The mean body weight gain was higher in piperazine treated group than the neem treated group. The body weight of chicken increased significantly in treated group compared to control which is in accordance with the report of Hoque *et al.* (2006) and Begum *et al.* (2010).

Effect on total erythrocyte count (TEC)

The oral administration of neem leaves extract and Pipervet® increased the number of erythrocytes of chickens (table 3). The highest number of cells was recorded on 21st day of treatment. This report

supports the findings of Hoque *et al.* (2006) and Begum *et al. (*2010), which showed that TEC values were increased after administration of Piperazine citrate in all treated group.

Effect on Hb estimation

The oral administration of Pipervet® and neem leaves extract significantly increased the hemoglobin level in chickens (table 4). Increased Hb content was highest on 21st day after treatment. But in control group-A Hb content was decreased slightly probably due to effect of parasitic infestation. The present findings support the report of Hoque *et al*. (2006) and Begum *et al. (*2010), where haemoglobin estimation was increased after administration of piperazine in all treated groups.

Table 5
Effects of neem leaves and Piper-vet® on PCV (%) in chickens

Group of chicken	Drug and dose	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)		
			7th day	14th day	21st day
A	Parasitic control (untreated)	21.35±0.31	21.30±0.72	20.40±0.26	19.39±0.27
B	Neem leaves extract@1g/kg	21.45±1.74	22.0±0.22	22.75±0.11	23.47±0.47
C	Pipet-vet® @ 200mg/kg	21.1±0.02	22±0.27	22.90±0.35	23.95±0.45

Values given above represent the (mean ± SD) of 5 chickens

Table 6
Effects of neem leaves and Piper-vet® on number of parasites in chickens

Group of chicken	Drug and dose	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)		
			7th day	14th day	21st day
A	Control	24±0.50	22±0.45	25±0.40	29±0.95
B	Neem leaves fresh extract @ 1g/kg	10±0.65	5±0.22	2±0.21	0
C	Piper-vet® @ 200mg/kg	8±0.37	0±0.22	0±0.40	0.00

Values given above represent the (mean ± SD) of 5 chickens

Effect on packed cell volume (PCV)

The oral administration of neem leaves extract and Pipervetin ascarid infected chicken showed a significant effect on PCV. The increase of PCV values was highest on 21th day after treatment (table 5). PCV level was decreased in control group- A. The present findings supported the report of Hoque *et al*. (2006) and Begum *et al*. (2010) that PCV was increased after administration of piperazine and levamisole in all treated groups.

Postmortem examination

At pretreatment and posttreatment three chickens from each group were slaughtered to count number of *Ascaridia galli* (table 6). Simultaneously any pathological changes were observed. There was no pathological change in any internal organs of the birds of treated groups were found.

Reduction of parasite count was found on 14th and 21th day in group B and C. Neem leaves extract and piperazine decrease the number of parasites in

chicken on day 14th and 21st. The highest decrease number of parasites was recorded on 21st day post treatment. Various internal organs of chicken group B and C were examined carefully. But there was not found any pathological changes. Whereas marked pathological changes in intestinal mucosa of chicken was observed in control group A. Similar finding has been reported by Verma et al. (1991) and Islam et al. (2008).

In this study, we used neem leaves extracts for comparative study with patent compound piperazine. The data demonstrated the promising effect of neem leave extract agains *Ascardia galli* in vitro and in vivo. However, further extensive research works should be carried out to explore the possible therapeutic use of neem leaves against ascariasis in chicken.

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