

Comparative efficacy of neem leaves extract and levamisole against ascariasis in chicken

Biddut Kumar Saha^{1*}, Md. Abdullah-Al-Hasan², Mohammad Afazur Rahman¹, Md. Maruf Hassan³, Nurjahan Begum²

¹Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

²Department of Parasitology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

³Upazilla Veterinary Hospital, Kushtia Sadar, Kushtia, Bangladesh

ABSTRACT

Ascariasis is very common in indigenous chicken causing retarded growth, low productivity and mortality. Considering the problem of *Ascaridia galli* in chicken, anthelmintic resistance, high cost and human health hazard of chemical anthelmintic the use of medicinal plant is an alternative choice. An experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh during the period from May, 2010 to September, 2010, to evaluate the efficacy of Neem leaves extract and patent anthelmintic Etrax® (Levamisole) on clinical findings of ascariasis in chicken. Indigenous medicinal plants Neem (*Azadirachta indica*) was studied both in vitro and in vivo in comparison with levamisole against ascariasis in chicken. In vitro neem leaves extract were incubated at the dose rate of 1, 2, 4, 20 mg/ml to adult worms. Shortest time of parasite paralysis was observed at dose rate 20mg/ml. Levamisole was incubated at the dose rate of 0.1, 0.2, 0.4, 0.6mg/ml to adult worms. Shortest time of parasite paralysis was observed at dose rate 0.6mg/ml. Efficacy was recorded as compared to the control. Neem leaves extract showed gradual increase of efficacy up to day 14th and 21st of treatment. Post-mortem worm count revealed that neem leaves extract showed considerable efficacy against worm burden. The aqueous extract of plant did not show any adverse effect on haematological parameter and physiological condition of treated birds but increase values TEC, Hb and PCV were observed in chicken after treatment with levamisole.

Key words: Ascariasis, chicken, neem leaf extract, levamisole.

*Corresponding author.

E-mail address: biddutvet@yahoo.com (BK Saha)

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INTRODUCTION

The poultry industry in Bangladesh plays a crucial role in economic growth and simultaneously creates numerous employment opportunities. There are around 150,000 poultry farms in Bangladesh, of which approximately half are backyard farms, with an annual turnover of approximately US\$750 million, and about 4 million Bangladeshi people directly or indirectly associated with poultry business (ICDDR,B, 2008). The Poultry population stabilized at around 140 million until 2006 (FAO STAT

<http://faostat.fao.org/default.aspx>). The total contribution of livestock sub-sector to the GDP (Gross Domestic Product) is approximately 6.5% (DLS, 1998).

In Bangladesh, about 19.8% source of protein comes from chicken (Anonymous, 1985). But chicken farming in Bangladesh are facing various hindrances. Among these, parasitic infections play a vital role. The high percentage of chicken mortality due to various diseases including parasitic one, is a discouraging factor for the farmers who want to raise more chicken. Among

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parasitic diseases ascariasis is very common in indigenous chicken and causing great economic loss, young birds are heavily infected when they are 3-4 weeks old and they may die at an early stage (Lapage, 1956).

There are various types of anthelmintics, which are being frequently used for the deworming of animals and birds in Bangladesh. Among them piperazine, levamisole, albendazole etc. are widely used for the treatment of ascariasis in poultry (Hoque et al., 2006). On the other hand there are several indigenous medicinal plants such as neem, pineapple, tobacco, have anthelmintics action and used against both ecto and endo parasites in Bangladesh, India, Thailand and several countries (Sivdas, 1980, Mannan, 1997, Nath, 1983). World Health Organization (WHO) has recognized the necessity for investigation and mobilization of ancient medicinal practice to fulfill the primary health care of the animals and realizes that the traditional system of medicine may play an important role in the development of livestock of the third world countries (WHO, 1993). Moreover, the rapid development of drug resistance, associated with the high cost of availability of drugs, has limited the success of poultry diseases control, thus studying medicinal plants as an alternative source is the aim of this research. The present study was undertaken to evaluate the effect of neem leave extract against ascariasis in indigenous chicken.

MATERIALS AND METHODS

Collection and processing of plant material

Neem leaves were collected locally. 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 in a refrigerator at 4°C to maintain the active ingredients of juice.

Collection and maintenance of *A. galli*

Adult *Ascaridia galli*, were collected from the intestine of chickens. Small intestines were collected, 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 prominently visible *A. galli* were collected with a needle and placed in a petridish containing normal saline.

In vitro assay

In a petridish parasites were treated with neem leave extract in a 50 ml media containing NaCl, KCl, Trisodium citrate, dihydrate, glucose, anhydrase. The Petridishes were incubated at room temperature, observed and time was recorded to attain paralysis of worms. For each experiment fresh leaves juice was used for treatment. Levamisole was used as a reference anthelmintic. Physical activity of the nematodes was observed and time was recorded to attain paralysis of worms and complete immobility was noted.

Sixty indigenous chickens of 45 to 60 days old were randomly selected. The chickens were allowed to acclimatize for 7 days in the experimental shed. During acclimatization the chicken were supplied with recommended feed and water at morning, noon and afternoon. Aqueous extract of neem (10%) was freshly prepared and levamisole (Etrax®) was purchased from local market for this experiment.

In vivo assay

All the 60 infected chicken randomly divided into 3 groups (A, B and C). Group 'A' was kept as control and was not treated, group 'B' was treated with neem leaves extract, administered orally @ 1g/kg body weight by dropper by consecutive 7 days, and group 'C' was treated with levamisole (Etrax®) that was administered orally @ 30 mg/kg body weight by dropper for 7 consecutive days. All the chicken of treated and control groups were closely observed for 21 days after treatment and body weight, feed consumption and water consumption was recorded before and during administration of drugs. The number of parasite was count following postmortem examination of both treated and control chickens.

Hematological parameters

Blood samples were collected from neck vein of chicken of both control and treated groups at pre-

feeding and during feeding (21 days) period at 7 days interval. Total erythrocyte count (TEC), Hemoglobin estimation (Hb), Packed cell volume (PCV), determination of Total erythrocyte count (TEC) were performed following the method described by Lamberg and Rothstein (1977).

Statistical analysis

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

RESULTS AND DISCUSSION

In vitro assay

A. galli were collected from naturally infected chickens' intestine and effectively survived for 48.00 ± 0.67 h in the medium without treatment (Table 1). The nematodes were persistently active, but once their movement ceased, death ensued abruptly.

Dose dependent significant effects on survival of *A. galli* were observed for both neem extract and levamisole. The maximum survival time of 13.60 ± 0.22 h was observed at highest concentration (0.1mg/ml) of levamisole used in this experiment. Whereas maximum survival time of 11.75 ± 0.25 h was observed for highest concentration (1 mg/ml) of neem leave extract. The minimum survival time of 2.0 ± 0.10 h and 6.08 ± 0.05 h were observed for lowest concentration of levamisole (0.6mg/ml) and neem leave extract (20mg/ml), respectively. In comparison of patent anthelmintics neem leaves showed moderate paralysis of *A. galli*. Similar observations were reported by Islam (2008) and Begum et al. (2010).

In vivo assay

Effect on body weight

There was 0-5% increase of live body weight following administration of drugs (Figure 1). The increased values of body weight in chickens of the treated groups were not significant on day 7 and

day 14 but that were significant on day 21 compared to control.

Table 1

Concentration dependent efficacy of levamisole and neem extract on the survival of *A. galli*.

Incubation medium	Concentration (mg/ml)	Time (h) survival (mean \pm SD)
Control	0	48.00 ± 0.67
Levamisole	0.1	$13.60 \pm 0.22^*$
	0.2	$9.28 \pm 0.15^*$
	0.4	$2.25 \pm 0.12^*$
	0.6	$2.0 \pm 0.10^*$
Neem leaves extract	1	$11.75 \pm 0.25^*$
	2	$10.22 \pm 0.14^*$
	4	$8.25 \pm 0.15^*$
	20	$6.08 \pm 0.05^*$

*means are significantly different at $P < 0.0$.

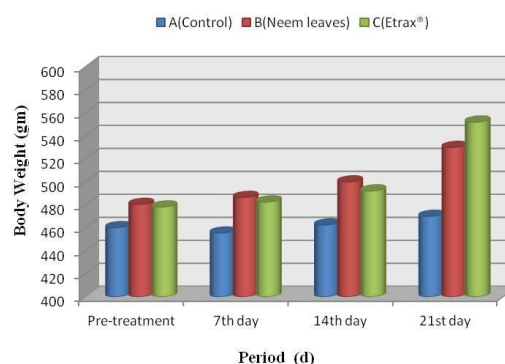


Figure 1

Effects of neem leaves extract and Etrax® on body weight (gm) in chickens.

The anthelmintics significantly increased the percentage of mean body weight of chicken of groups B and C as 11.89% and 13.62% respectively on 21st day after treatment whereas the percentage of body weight was increased as low as 2.20% on 21st day after treatment. The significant increase of body weight in group B and C might be due to decreasing the parasitic load as a result of neem and levamisole treatment which might increase proper absorption and metabolism of feed nutrients. This study is in contrast with the report of Hoque et al. (2006) and Begum et al. (2010). Lonkar et al. (2008) reported that the inclusion of garlic powder and, Neem seed cake and their combination could not affect the crude protein content of breast and thigh meat in broiler.

Effect on hematological parameters

Effect total erythrocyte count (TEC)

The oral administration of neem leaves extract and levamisole increased the number of erythrocytes of chickens in groups B and C respectively. The highest number of cells was recorded on 21th day after application of drugs.

The administration of neem leaves extract and Etrax® increased the number of erythrocytes of chicken. The highest number of cells was recorded on 21st day of treatment. TEC was increased, because of destruction of parasites by the anthelmintic action of neem and levamisole. But in control group TEC was decreased due to effect of parasitic infestation on hematopoietic system and

RBC. This report supports the findings of Hoque et al. (2006) and Begum et al. (2010), who showed that TEC values was increased after administration of levamisole in all treated group.

Effect on Hb estimation

The oral administration of Etrax® and neem leaves extract significantly increased the hemoglobin level in chickens. The increase of hemoglobin level was highest (11.15 gm%) on 21st day after treatment. But in control group Hb content was decreased slightly due to effect of parasitic infestation. The present findings support the report of Hoque et al. (2006) and Begum et al. (2010), who showed that in all treated groups, haemoglobin estimation was increased after administration of levamisole.

Table 2

Effects of neem leave extract and Etrax® on TEC (million/cu mm) in chickens.

Group of chicken	Drug, dose and route	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	3.76±0.03	3.86±0.27	3.95±0.05	4.33±1.05
C	Levamisole	3.50±0.01	3.62±0.23	3.27±0.26	3.86±0.05

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

Table 3

Effects of neem leave extract and Etrax® 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	Control (untreated)	10.60±0.24	9.70±0.06	10.0±1.08	9.0±0.05
B	Neem	10.76±0.02	10.87±0.03	11.01±0.06	11.15±0.09
C	Etrax®	10.25±0.02	10.72±0.14	10.85±0.05	11.36±0.53

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

Packed cell volume (PCV)

The oral administration of neem leaves extract and Etrax® in ascarid infected chicken showed a significant effect on PCV. The increase of PCV values was highest on 21th day after treatment.

On the other hand, PCV level were decreased in control group. The present findings supported the report of Hoque et al. (2006) and Begum et al. (2010) where PCV was increased after administration of levamisole in all treated groups (Table 4).

Table 4
Effects of neem leaves extract and Etrax® on PCV (%) in chickens.

Drug and dose	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)		
		7 th day	14 th day	21 st day
Control	21.35±0.31	21.30±0.72	20.40±0.26	19.39±0.27
Neem leaves extract	21.45±1.74	22.0±0.22	22.75±0.11	23.47±0.47
Etrax®	22.58±0.16	22.65±0.42	23.65±0.2	23.73±0.58

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

Table 5
Effects of neem leaves extract and Etrax® on number of parasites in chickens.

Drug and dose	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)		
		7 th day	14 th day	21 st day
Control	24±0.50	22±0.45	25±0.40	29±0.95
Neem leaves extract	10±0.65	5±0.22	2±0.21	0
Etrax®	18±0.52	0±0.50	0±0.28	0±0.00

Values given above represent the mean ± SD of 5 chickens

Effect on parasite load in chicken

Before and after 14 and 21 days of treatment 3 birds from each group was slaughtered to count number of parasites (*A. galli*) and observed if there were any pathological changes present. There was no significant pathological change in any internal organs of the birds of treated groups were found.

Neem leaves extract and levamisole as anthelmintics decrease the number of *A. galli* in chicken in group B and C on day 14th and 21st (Table 5). The highest decrease number of parasites was recorded on 21st day of post treatment. Similar finding has been reported by similar finding has been reported by Varma et al. (1998), Islam.(2008), Hoque et al. (2006) and Begum et al. (2010).

Ascariasis is a major problem in indigenous chicken in Bangladesh. Indigenous medicinal plant neem was tested for their efficacy against ascariasis. Neem was found effective after 21st days of oral administration. However this is a

preliminary work, 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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