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Efficacy of Neem (*Azardirachta indica*) oil on common poultry worms *Ascaridia galli* and *Heterakis gallinae*.

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Abstract: Neem oil was triturated with tween 20 and suspended is distilled water to make 16% stock solution was tested for its in vitro anthelmintic efficacy against common poultry worms *Ascaridia galli* and *Heterakis gallinae* under laboratory condition (Tem 410C). This suspension proved to be 100% effective at 2, 4 and 6% as it caused mortality (indicated by loss of motility) after an incubation of 12 and 10 hours at 4% concentration mortality was cuased after 14 and 12 hrs respectively. The effect of Neem oil was also examined on glucose uptake glycogen content, phosphomonoesterase activity and lactic acid level of the parasite.

Key words : *Azardirachta indica*, Anthelmintic, *Ascardia galli*, *Heterakis gallinae.*

I. INTRODUCTION

Helminths with chemotherapy has been practised since long but recent studies indicate that parasite are developing resistance against most of the anthelmintics. Besides, all these drugs are highly toxic and exhibit large number ofside effects in host animals. A potent anthelmintic drug without in desirable effets on host animal is yet to be discovered. The present investigations aim at evaluating the efficacy of neem oil against two avian nematode parasite *A. galli* and *H. gallinae*, which are known to cause heavy loss to poultry. Efforts have also been made to find out the possible mode of action of the extract.

II RESEARCH METHODOLOGY

The parasites *A. galli* and *H. gallinae* were obtained from the intestine and caecum respectively, of the common fowl (*Gallus gallus*) slaughtered in local poultry farms. After several washings in normal saline they were transferred in saline (pH 7.2) to which 1 g of glucose/100ml was added. The requisite quantity of the extract was added to the incubation medium to obtain the required concentration and its effect was compared with untreated controls. Worms were incubated at 380C. Death was assumed to have occurred when all signs of movement had ceased.

Glucose uptake was determined by the method of Ahmad and Nizami (1987). Glycogen was estimated in the homogenates (20% w/v) of these worms according to the method of Good et al. (1933) as modified by Montgomery (1957). Rate of oxygen consumption was measured manometrically by the method of Warburg as described by Umbreit et al. (1964). Lactic acid production was measured by the method of Baker and Summerson (1941). Acid and alkaline phosphomonoesterase activity was also determined in homogenates, according to Bergmeyer (1971), whereas cholinesterase activity was measured by the method of Huerga et al. (1952), using acetylcholine as substrate. The chemicals used were of analytical grade It the present studies the *Azardirachta indica* oil was used after triturating with tween 20% at 2,4 and 6% concentration.

III. RESULTS AND DISCUSSION

A. Effect of *A. indica* oil on the parasite incubated *in vitro* :

The effect of 2-6% oil was examined on the mortality of adult *A. galli* and *H. gallinae* incubated in vitro. Neem oil (6%) caused mortality after an exposure of 12 and 10 hrs. in *A. galli* and *H. gallinae*, while at 4% mortality was caused after an exposure of 14 and 12 hrs. respectively.

B. Effect of A. indica oil on the biochemical activities of the parasites :

i. **Glucose uptake** : Neem oil (6%) caused reduction in glucose uptake by 61% and 47% in *A. galli* and *H. gallinae*, respectively (Table 1).

ii. **Glycogen contents** : As shown in Table 1, 6% neem oil reduced the glycogen contents by 44 in *A. galli* and 41% in *H. gallinae*.

iii. **Rate of oxygen consumption** : Changes in the rate of oxygen consumption are given in Table 2. Neem oil (6%) suppressed the rate of oxygen consumption by 45 and 44% in *A. galli* and *H. gallinae*, respectively.

iv. Lactic acid production : An increase of 71 and 78% was observed in lactic acid production with 6% neem oil in *A. galli* and *H. gallinae*, respectively (Table 2)

v. **Acid phosphomonoesterase activity** : As shown in Table 3 on in vitro treatment with 6% neem oil, the activity of acid phosphomonoesterase was diminuted by 54 and 53% in *A. galli* and *H. gallinae*, respectively.

vi. **Alkaline phosphomonoesterase activity** : A significant ($P<0.05$) inhibition of alkaline phosphomonoesterase activity was recorded in both *A. galli* and *H. gallinae*, incubated in vitro with 6% neem oil (Table 3).

vii. **Cholinesterase activity** : As shown in Table 3, the cholinesterase activity was not affected significantly ($P>0.05$) with 6% neem oil, in either of the parasite.

Results of Descriptive Statics of Study Variables

Table-1

Changes in glucose uptake (mg/g wet weight) and glycogen contents (% wet wt.) in *A. galli* and *H. gallinae* after *in vitro* incubation with different concentrations of *A. indica* oil.

Parasites	Concentration			
	Control	2%	4%	6%
Glucose uptake				
A. galli	6.4±0.14[a]	3.4±0.1 (46.87)	3.0±0.1 (53.12)	2.5±0.17 (60.93)
H. gallinae	4.9±0.14	3.8±0.14 (22.44)	3.3±0.12 (32.65)	2.6±0.17 (46.93)
Glycogen contents				
A. galli	7.2±0.37	5.6±0.2 (22.22)	4.2±0.14 (41.66)	4.0±0.55 (44.44)
H. gallinae	6.8±0.28	6.0±0.14 (11.76)	5.3±0.24 (22.05)	4.0±0.55 (41.17)

Mean ± S.D.

Value in parentheses are percent change of control values.

Table-2

Changes in the rate of oxygen consumption (µl/mg weight/hour) and lactic acid production (µ mol/gm wet weight) in *A. galli* and *H. gallinae* exposed to different concentrations of *A. indica* oil.

Parasites	Concentration			
	Control	2%	4%	6%
Rate of oxygen Consumption				
A. galli	8.3±0.14[a]	6.4±0.14 (22.89)	5.7±0.11 (31.32)	4.6±0.17 (44.57)
H. gallinae	7.5±0.22	5.6±0.1 (25.33)	4.8±0.12 (36.0)	4.2±0.12 (44.0)
Lactic acid production				
A. galli	3.5±0.14	5.4±0.14 (35.18)	5.7±0.13 (62.85)	6.0±0.14 (71.42)
H. gallinae	2.7±0.12	3.6±0.14 (33.33)	4.2±0.17 (55.55)	4.8±0.14 (77.77)

Mean ± S.D.
Value in parentheses are percent change of control values.

Table-3

Changes in acid and alkaline phosphomonoesterase (phosphatase units) and cholinesterase activity (µ moles acetylcholine/hour) in *A. galli* and *H. gallinae* following *in vitro* incubation with different concentrations of *A. indica* oil.

Parasites	Concentration					
	Control	2%	4%	6%	I[a]	r[b]
Acid Phosphomonoesterase						
A. galli	7.8±0.17[c]	5.8±0.14 (25.64)	4.7±0.3 (39.74)	3.6±0.14 (53.84)	5.572	0.9891
H. gallinae	5.5±0.26	4.3±0.1 (21.81)	3.7±0.1 (32.72)	2.6±0.22 (52.72)	5.692	0.9925
Alkaline Phosphomonoesterase						
A. galli	5.9±0.03	4.6±0.14 (22.03)	3.8±0.17 (35.59)	2.9±0.12 (50.64)	5.924	0.9940
H. gallinae	4.7±0.14	3.7±0.28 (21.27)	3.1±0.2 (34.04)	2.3±0.2 (51.06)	5.875	0.9961
Cholinesterase						
A. galli	3.8±0.14	3.5±0.14 (7.89)	3.2±0.14 (15.78)	2.9±0.12 (23.68)	12.668	1.0
H. gallinae	4.3±0.13	3.9±0.22 (9.30)	3.3±0.1 (16.27)	3.3±0.22 (23.25)	12.903	1.0

a. Concentration required for 50% inhibition.
b. r = correlation coefficient of the activity of control and treated samples.
c. Mean ± S.D.
Value in parentheses are percent change of control values.

Discussion :

Neem oil was screened for its anthelmintic activity in view of its reported antimalarial (Nadkarni, 1954), antitrypanosomal (Talakal et el., 1993), Insecticidal (Kumar and Dutta, 1987; Olaifa et al., 1987, Guddewar and Chandra, 1993; Bhathal and Singh, 1993; Stark and Walter, 1995 and Murugan et al., 1996); cyclopicidal (Bapna et al., 1988) and nematicidal (Guzman and Saxena, 1988 and Akhtar et al., 1985), activities.

During the present investigations the adult *A. galli* and *H. gallinae*, when exposed in vitro to 2, 4 and 6% neem oil exhibited 100% mortality after an incubation of 15, 14 and 12 hrs. and 14, 12 and 10 hrs. respectively. Shailaskar and Parashar (1989) also reported anthelmintic activity of neem extract against *A. galli*.

The results of the present studies indicated a significant effect of neem oil on the carbohydrate metabolism of both *A. galli* and H. gallinae. This was evidenced by depletion of glycogen contents and the reduction in glucose uptake (Table 1) and oxygen consumption (Table 2). This reduction was associated with concomitant rise in lactic acid production (Table 2) of both the nematodes.

The significant ($P<0.05$) inhibition of acid and alkaline phosphomonoesterase activity by neem oil (6%) as observed (Table 3) during present investigation is is also indicative of its interference in carbohydrate metabolism of the parasites, since phosphomonoesterases are reported (Pappas and Read, 1975) to play a signficant role in transport of glucose. Many modern anthelmintics are also reported (Sharma et al., 1987) to owe their anthelmintic action by interferring in carbohydrate metabolism of the parasites. Cholinesterase activity was however, not affected significant in either parasites.

During the present investigation neem oil did not show any adverse effect on the metabolic activities of the host tissues, some minor changes observed were not signficant. Therefore, neem oil appears to be montoxic and may be useful anthelmintic for the control of *A. galli* and *H. gallinae*.

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