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Studies on the anthelmintic activity of *Tagetus patula* on common poultry worms *Ascaridia galli* and *Heterakis gallinae*

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Abstract:

Fresh petals of T. patula locally known as "Genda" was dired in shade and grind to obtain fine powder. This powder is subjected to extraction in soxhlet apparatus using alcohol as solvent for 20-24 hrs. The greasy mass obtained after the coplete removal of solvent is diluted to prepare different concentration i.e. 2%, 4% and 6% and tested for in vitro anthelmintic activity. 2% extract caused mortality in A. galli after an exposure of 12 hrs. and in H. gallinae after 10 hrs. At 6% concentration mortality was observed after 8 and 7 hrs. respectively in A. galli and H. gallinae. The effect of the extract was also examined on glucose uptake, glycogen contents, lactic acid production, acid and alkaline phosphomonoesterases and cholinesterase activity to find out the possible mode of action of the extract.

Keywords: *Tagetus patula, Anthelmintic, Ascaridia galli, Heterakis gallinae*.

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Introduction:

The present investigation aims at evaluating the efficacy of *T. patula* (Genda) alcoholic extract against two avian nematode *Ascaridia galli* and *Heterakis gallinae.*

Material and Methods:

It is a member of the family Composite and is locally known as 'Genda'. It is a hard annual herb growing about 60 cm. in height, erect and branched. It is distributed in Mexico and commonly grown in Indian gardens. Leaves are scented, pinnately divided segments lanceolate and serrate. Flowers are of one colour, the typical colour being lemon yellow, but it ranges from light to deep yellow. The flowers have a pungent bitter acrid taste, useful in fevers and epileptic fits (Ayurveda). The leaves are good for piles, kidney, toubles, muscular pain, their juice is used for earache and ophthalmia. The flower is also used as an astringent, carminative, stomachic, it is good teeth and gums, lessen inflammation, useful in scabies belching, scorpion and snake poisoning, liver complaints and bleeding piles. The leaves are used to cure boils and carbuncles. The yellow colouring matter of the flowers has been isolated and identified as the known quercetagetin.

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 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 38^0C. 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 alcoholic extract of Marigold petals has been tested for its anthelmintic activity against poultry worms.

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 *T. patula* oil.

Parasites	Concentration			
	Control	**2%**	**4%**	**6%**
Glucose uptake *A. galli*	5.5±0.17[a]	4.3±0.33 (21.81)	3.3±0.12 (40)	2.5±0.17 (54.54)
H. gallinae	6.2±0.17	4.6±0.31 (25.80)	3.4±0.1 (45.16)	2.5±0.17 (59.67)
Glycogen contents *A. galli*	7.3±0.14	6.7±0.14(8.21)	5.8±0.14 (20.54)	5.0±0.52 (31.50)
H. gallinae	6.7±0.14	6.1±0.14 (8.95)	5.3±0.244 (20.89)	4.6±0.31 (31.34)

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

Table-2: Changes in the rate of oxygen consumption (µl/mg wet. weight/hour) and lactic acid production (µ mol/gm wet weight) in *A. galli* and *H. gallinae* exposed to different concentration of *T. patula* petal extract.

Parasites	Concentration			
	Control	**2%**	**4%**	**6%**
Rate of oxygen consumption *A. galli*	5.5±0.17[a]	4.6±0.14 (16.36)	3.8±0.14(30.90)	3.3±0.22(40.0)
H. gallinae	4.9±0.2	3.8±0.14(22.44)	3.2±0.14(34.69)	2.5±0.17(48.97)
Lactic Acid Production *A. galli*	4.3±0.1	5.8±0.14(36.15)	6.6±0.12(54.92)	7.2±0.1(69.01)
H. gallinae	6.0±0.14	7.3±0.17 (21.66)	8.2±0.17 (36.66)	8.8±0.14 (46.66)

a. Mean ± S.D. Values in parentheses are percent 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 *T. patula* petal extract.

Parasites	Concentration					
	Control	**2%**	**4%**	**6%**	**I[a]**	**r[b]**
Acid Phosphomonoesterase *A. galli*	4.7±0.3[a]	3.6±0.28(23.40)	2.7±0.82(42.55)	2.2±0.14(53.19)	5.640	0.9836
H. gallinae	5.8±0.14	3.8±0.14(34.48)	2.4±0.14(58.62)	1.9±0.22(67.24)	4.461	3.5160
Alkaline Phosphomonoesterase *A. galli*	5.3±0.22	3.9±0.24(26.41)	3.1±0.2(41.50)	2.5±0.17(52.83)	5.681	0.9911
H. gallinae	4.7±0.14	4.0±0.14(14.89)	3.4±0.17(27.65)	2.7±0.82(42.55)	7.050	0.9859

Cholinesterase *A. galli*	7.0±0.3	5.7±0.14 (18.57)	4.8±0.14 (31.42)	4.1±0.1 (41.42)	7.243	0.9909
H. gallinae	6.2±0.3	5.5±0.26 (11.29)	4.4±0.31 (29.03)	3.6±0.14 (41.93)	7.154	0.9640

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.

RESULTS

Effect of *T. patula* petal extract on the parasites incubated *in vitro*.

The effect of alcoholic extract of the petals of *T. patula* was examined on the mortality in adult parasites incubated *in vitro.* 2% extract caused mortality in *A. galli* after an exposure of 12 hrs. and in *H. galiinae* after 10 hrs. (Table 2). At 6% concentration mortality was observed after 8 and 7 respectively in *A. galli* and *H. galiinae.*

B. Effect of T. *patula* petal extract on the biochemical activities of the parasites.

(i) Glucose uptake : On *in vitro* treatment, *T. patula* petal extract reduced glucose uptake by 55 and 60% in *A. galli* and *H. gallinae,* respectively at a "concentration of 6% (Table 1).

(ii) Glycogen contents : The glycogen contents of *A. galli* and *H. gallinae* after *in vitro* incubation with 6% extract were reduced by 31% in both *A. galli* and H. *gallinae* (Table 1).

Rate of oxygen consumption : Changes in the rate of oxygen consumption are presented in Table 2. T. *patula* extract reduced the rate oxygen consumption by 40 and 49% in *A. galli* and *H. gallinae,* respectively.

(iv) Lactic acid production : Lactic acid production was enhanced (Table-2) by 69 and 47% in *A. galli* and *H. gallinae,* respectively.

(v) Acid phosphomonoesterase activity : *T. patula* petal extract inhibited acid phosphomonoesterase activity by 53 and 67% in *A. gallinae* and respectively (Table-3).

(vi) Alkaline phosphomonoesterase activity : Alkaline phosphomonoesterase activity was diminuted by 53 and 43% in *A. galli* and *H. gallinae,* respectively (Table-3)

(vii) Cholinesterase activity : As shown in Table 3, the cholinesterase activity was inhibited by 41 and 42% in *A. galli* and *H. gallinae,* respectively at 6% concentration.

Effect of T. *patula* petal extract on host tissues:

No significant change was observed in the bio-chemical activities of the host tissue incubated with 2-6% extract of the petals of T. *patula* (Table 18).

DISCUSSION

The present investigations aimed at finding out the anthelmintic efficacy of the alcoholic extract of the petals of Marigold (T. *patula*) against avian worms *A. galli* and *H. gallinae* indicated that it possessed anthelmintic activity. The extract caused mortality in both the worms, when added to the incubation medium at all the concentrations used. The lower concentrations, however, required more time to cause 100% mortality. *H. gallinae* was found to be more sensitive as it required less time for complete mortality. No report is, however, available to substantiate the present findings. However, *T. petula* is reported (Singh and Kataria, 1985; Stein and Klingaut 1990 Gerard and Ruf, 1991) to possess a potent insecticidal activity. Uhlenbrock and Bijloo (1958) isolated the nematicidal principle occurring in the roots of Tagetes plant.

The studies on the biochemical activities of *A. galli* and *H. gallinae*
incubated *in vitro* with the alcoholic extract of Marigold petals indicated that it suppressed significantly the glucose uptake (Table 1) and oxygen consumption (Table 2) of both the parasites. The glycogen contents were also reduced (Table 1) and the lactic acid production was enhanced (Table 2) significantly. It probably indicated the interference of the petal extract in carbohydrate metabolism of the parasites. Acid and alkaline phosphomonoesterases, reported (Pappas and Read, 1975) to play an important role in the transport of glucose were also inhibited significantly in both *A. galli* and *H. gallinae.*

The inhibition of cholinesterase activity observed during present studies was also significant statistically ($P<0.05$). Anticholinesterase activity of some anthelmintic agents and medicinal plants has also been reported by Tinwin *et al.* 1994. Cholinesterase is related with neuromuscular transmissions in the parasites.

Though no specific mode of anthelmintic action of the petal extract of *T. patula* can be suggested from the above findings but it is evident (Table 18) that the extract did not cause any harmful effect to the host tissues. Further investigations involving *in vivo* experiments are however, necessary for final evaluation.

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