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Reproductive activities of *Heliotropium indicum* isolate against *Helopeltis theivora* and toxicity evaluation in mice

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Abstract

A new compound E was isolated from the methanolic extract of the leaves of *Heliotropium indicum* by chromatographic fractionation. In the present study, the effect of the compound E on reproduction of *Helopeltis theivora* has been evaluated. The acute toxicity study (LD_{50}) and sub-acute toxicity studies (haematological, biochemical and histopathological parameters) in albino Swiss mice were carried out to evaluate the safety aspect of the compound E. The compound showed significant inhibitory effect on the reproductive life of *H. theivora*. The oviposition period, fecundity and hatching percentage of *H. theivora* were found to be 15.67days, 39.33 and 28.00 % respectively after treatment with 2% compound E, whereas the control value were found to be 20.33 days, 77.67 and 77.33% respectively. The LD_{50} of the compound was found to be 780 mg kg^{-1} in Swiss albino female mice. The compound did not show any toxicity in mice at sub-lethal dose treatment (78 mg kg^{-1} b. wt., once daily) for 21 days as evident from different haematological, biochemical and histopathological parameters in compound E treated group when compared with control.

Key words

Helopeltis theivora, Insecticidal properties, *Heliotropium indicum,* Reproductive inhibition.

Introduction

Currently used synthetic insecticides cause environmental problems and they also have harmful effects on non-target organisms including human beings. To overcome these problems, scientists have developed interest in plant-derived compounds as alternatives to the synthetic insecticides (Cornelius *et al.,* 1995) as they are biodegradable and relatively safe to natural enemies and higher organisms (Williams and Manish, 1993).Tea mosquito bug, *Helopeltis theivora,* is a major pest of tea plant in North East India and on an average 25% crop (sometimes up to 50%) is lost annually (Prasad, 1992; Gurusubramaniam and Bora, 2007). Many plant chemicals are known to have antifeedant and insecticidal properties (Tripathi and Tripathi *et al.,* 2000; Sharma *et al.,* 1999; Roychoudhury, 1994; Gudddewar *et al.,* 1991; Reddy *et al.,* 1990; Schumutterer 1990). Deka *at el.* (1998) reported the effects of some indigenous plant extracts on fecundity and viability of egg of *H. theivora*. In our laboratory, several plants were screened for their insecticidal activity against *H. theivora* and other insects (Gogoi, *et al.,* 2001; Gogoi *et al.,* 2003; Gogoi *et al.,* 2005; Rahman *et al.,* 2005; Dolui and Debnath, 2010). By chromatographic fractionation of the methanolic extract of *Heliotropium indicum,* a new insecticidal compound E (2-ethyl-1,2,3,4-tetrahydro-6-hydroxymethyl isoquinoline) was isolated in our laboratory (Dolui *et al.,* 2011). Anti-inflammatory (Srinivas *et al.,* 2000) wound healing (Reddy *et al.,* 2002) and antifeedant property (Dolui *et al.,* 2010) of *H. indicum* has already been reported earlier. The taxonomic position of the plant is Kingdom-Plantae, Subkingdom-Tracheobionta, Division-Magnoliophyta, Class-Magnoliopsida, Subclass-Asteridae, Order-

Lamiales, Family-Boraginaceae, Genus-*Heliotropium* L. and Species-*Heliotropium indicum* L.

In the present study, the effect of the compound E, an isolate of *Heliotropium indicum* on reproduction of *H. theivora* was evaluated. The acute toxicity study (LD_{50}) and sub-acute toxicity studies (haematological, biochemical and histopathological parameters) in albino Swiss mice were carried out to evaluate the safety aspect.

Materials and Methods

Isolation of compound E from *Heliotropium indicum*: Methanolic extract of leaves of *H. indicum* was chromatographed on a silica gel column with the eluting solvent ethyl acetate to obtain different fractions. Fraction E was dissolved in acetone: water (1:1) and crystallized. The compound was again re-crystallized from the same solvent to get a gray crystalline powder.

Reproductive inhibitory tests in *Helopeltis theivora:* Fresh tea shoots were collected from the experimental plot. They were sprayed with different concentrations viz. 0.5, 1.0 and 2.0 % of compound E with pre-calibrated hand atomizer to the point of no dripping using 2 ml of solution. The sprayed shoots were allowed to dry for 5 min. Three of the treated shoots were then kept in water in 50 ml conical flask. One pair of newly immerged adult (male and female) *H. theivora* were released on the treated shoots and covered with glass chimney. The treated shoots were replaced after 5 days and the subsequent shoots given were untreated ones. Three replications for each treatment and control were maintained.

The total number of days after which the insects laid eggs was recorded as the oviposition period for all the treatment and control. The adult female was allowed to lay eggs and number of eggs laid by the adult of different treatment was recorded. The hatching percentage was observed by recording the number of eggs hatched from the total number of eggs laid by treated female on each treatment.

Toxicity studies in mice: To study the mammalian toxicity of the compound, Swiss albino mice (20-25 g) were reared in plastic cages and fed a standard pellet diet and given tap water *ad libitum.* The animals were used in the experiments after approval from the Institutional Animal Ethical Committee of Tripura Medical College, Agartala, Tripura.

LD_{50} determination: Lethality tests were performed according to OECD-423 (2001) guidelines. Swiss mice (n=3) of female sex selected by random sampling technique were employed in the study. The animals were fasted for 4 hrs with free access to water only, after which the compound E was administered orally by intragastric tube and observed for 3 days. If mortality was observed in 2-3 animals, then the same dose was repeated to confirm the toxic dose. If mortality was observed in one animal, then also the same dose was repeated to confirm the toxicity. If mortality was not observed, the procedure was repeated further with higher doses to determine the lethality of the compound E.

Sub-acute toxicity studies: Adult albino Swiss mice of both sexes were used in this study. The animals were randomly divided into two groups of six mice each. A sub- lethal dose ($1/10^{th}$ of the LD_{50} i.e. 78 mg kg^{-1} b. wt.) of the compound E was administered orally once every 24 hrs for 21 consecutive days. One day after the final administration the mice were sacrificed by decapitation and blood of each mouse was collected.

Haematological examination: The heparinised blood samples were taken from both the groups to determine and compare the haematological parameters viz RBC count, total WBC count, haemoglobin content and some blood indices viz. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), packed cell volume (PCV) by standardized laboratory methods (Baker *et al.,* 1998).

Biochemical analysis: Diagnostic kits obtained from Roche Diagnostic were used for all biochemical determination using spectrophotometer. The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined using Reitman and Frankel's method (1957). Alkaline phosphatase was carried out by phenopthaline monophosphate method (Babson *et al.,* 1966), urea was analyzed by modified method of Bethlot Searcy's (Searcy, 1967). Creatinine was determined by method described by Larsen (1971). Total cholesterol was estimated by method described by Allain *et al.* (1974) and blood glucose was estimated by using the enzymatic GOD-PAP method (Trinder, 1969). The values obtained after compound E treatments were compared with those of control group.

Histopathological studies: After collection of blood, liver and kidney samples were excised from the control and treated groups of animals and washed with normal saline separately. The tissues were dehydrated with graded concentration of alcohol, cleaned with xylol and embedded in paraffin bath. The paraffin blocks were cut at 5 µm in a microtome and the sections were stained with haematoxylin and eosin and mounted with D.P.X. The sections were examined carefully under the microscope at low and high power magnification. Any histopathological changes deviant from the normal were carefully recorded.

Statistical analysis: Results were expressed as mean ± standard error (SE). Statistical significance was determined by paired t- test. The data obtained from experiments were analysed by using student t-test. $P<0.01$ was considered significant.

Results and Discussion

Results indicate that the treated insects had short oviposition period and the effect was dose dependent. The highest inhibitory activity was recorded at 2.0 % where oviposition period reduced to 15.67 days against the normal oviposition period of 20.33 days.

(Table. 1) Reduction in oviposition period indicated the effectiveness of compound E. Pandey and Khan (1998) and Gogoi *et al.* (2001) reported that the extracts of *Clerodendron siphonenthus* inhibited oviposition with no or reduced egg laying. The reduction in oviposition period might be due to reduced food intake or due to abnormalities in the ovaries generated by the treatment. Leaf extract of *Melia azedarach* L. strongly deterred oviposition by *Aedes aegypti* with a significantly lower proportion of eggs being laid on ovitraps containing extract in comparison with water or ethanol solution control (Coria *et al.,* 2008). Nathan *et al.* (2005) also reported the strong ovipositional deterrency of *Melia azedarach* extracts on *Anopheles stephensi*.

The result indicates that there is a considerable reduction in the number of eggs laid per female after the treatment. The lowest fecundity was 39.33 when treated with 2.0 % compound (Table 1). Treatments with other concentrations also had inhibitory effects on fecundity as compared with the control, and this indicates the efficacy of the compound. The range of number of eggs laid per female were 58.00 to 39.33 after 0.5 to 2.0 % treatment respectively, whereas in control the value was 77.67. These findings are in agreement with those of Srivastava and Mann (1999) who reported reduced fecundity in *Callosobruchus chinensis* treated with extracts of *Peganum harmala*. Deka *et al.* (1998) reported the effects of some indigenous plant extracts on fecundity and viability of egg of *H. theivora*.

The hatching percentage was 48.00, 39.00 and 28.00 after 0.5, 1.0 and 2.0 % compound E treatment respectively, whereas in control it was 77.33 (Table 1). Here also significant dose dependent inhibitory effect was seen. Gajmer *et al.* (2002) reported that methanolic extracts of neem seed exhibited severe adverse effects on hatching of *Earias vittella* eggs.

The LD_{50} value of the compound E was calculated to be 780 mg kg^{-1} b.wt. in female albino mice. (Table 2). According to WHO (1991), extract or agent with LD_{50} above 3000 mg kg^{-1} b.wt. is essentially safe. Based on the classification of Loomis and Hayer (1996), substances with LD_{50} between 500 and 5000 and between 5000 and 15000 mg kg^{-1} b.wt. are regarded as slightly toxic and practically non-toxic. According to Klassen *et al.* (1995) the compound can be classified as slightly toxic.

No significant change in the values of RBCs, WBCs, haemoglobin, PCV, MCV, MCH, MCHC of treated animals was observed when compared to those of control group (Table 3). Such findings suggest that the compound E produced no toxicity in the animal at sub-lethal dose ($1/10^{th}$ of LD_{50}) for a period of 21 days.

No significant changes were observed in the biochemical parameters of the treated animals (78 mg kg^{-1} day^{-1}) as compared to the control group. Moreover, no lethality was recorded when sub-lethal dose of the extract was administered orally for 21 days of treatment (Table 4).

Table 1 : Effects of compound E on oviposition periods, fecundity and hatching percentage of *Helopeltis theivora*

Concentrations of compound E (%)	Oviposition periods (days)	Fecundity (No. of eggs laid)	Hatching percentage
Control	20.33 ± 1.20	77.67 ± 2.60	77.33 ± 0.33
0.5	20.00 0.58	58.00 ± 1.53**	48.00 ± 1.00**
0.1	17.67 ± 0.67	48.67 ± 0.88**	39.00 ± 1.53**
2.0	15.67 ± 0.67**	39.33 ± 1.45**	28.00 ± 0.58**

Values are mean of three replicates ±SD. Significance P <0.01 (**) as compared to control

Table- 2: Determination of LD_{50} value for the compound E from methanolic extract of leaves of *H.indicum*

Groups	Dose(mg kg^{-1} b.wt.)	No. of animals	No. of dead mice	% of dead animals	LD_{50} value (mg kg^{-1} b.w)
1	300	3	0	0	
2	500	3	1	33	
3	550	3	1	33	
4	690	3	1	33	
5	725	3	1	33	
6	750	3	1	33	
7	760	4	1	25	
8	770	4	2	50	
9	780	4	2	50	780.0
10	800	3	2	67	
11	900	3	2	67	
12	990	3	2	67	
13	1000	3	2	67	
14	2000	3	3	100	

The compound E did not induce any damage to the liver and kidney as examined by clinical blood chemistry. SGOT, SGPT, ALP were produced in the liver and are good markers of damage to liver cells (Achliya *et al.,* 2004). There were no significant changes in the level of SGOT, SGPT, ALPs in serum of the treated group when compared with control group indicates that the compound E did not affect liver function. Bilirubin level is another important indicator of liver damage, which is increased during haemolytic anaemia (Tripathi, 2003). No significant changes in the level of bilirubin of treated group as compared to control group indicated that the compound E has no deleterious effect on liver function. Urea and creatinine determinations are the markers of kidney functions (Mitchell and Kline, 2006). No significant change in the treated group when compared with the control implies that the compound E is not associated with kidney damage and subsequent renal failure.No changes in total cholesterol and glucose level also indicate that the compound E has no harmful effect on animals.

Histological examination of kidney and liver from both treated and control animals showed normal architecture, suggesting no detrimental effects of compound E treatment (78 mg kg^{-1} day^{-1}) on both the kidney and liver. This compliments the biochemical finding and confirms the safety of the compound E.

Table 3 : Haematological parameters in mice after 21 days of oral treatment of compound E (78 mg kg^{-1} b.wt. day^{-1})

Parameters	Control	Treatment
Red blood corpuscle (x10^6 mm^{-3})	7.16 ± 0.148	7.07±0.053
White blood corpuscle (x10^3 mm^{-3})	10.42±0.621	10.45±0.541
Haemoglobin (g dl^{-1})	12.43±0.270	12.23±0.359
Packed cell volume (%)	41.43±0.903	43.13±0.950
Mean corpuscular volume (fl)	57.87±0.818	61.03±1.487
Mean corpuscular haemoglobin (Pg)	17.39±0.538	17.30±0.503
Mean corpuscular haemoglobin concentration (g dl^{-1})	30.05±0.811	28.50±1.380

Values shown are mean of six replicates ± SE. Experimental groups were compared with control

Table 4 : Biochemical parameters in mice after 21 days of oral treatment of compound E (78 mg kg^{-1} b.wt. day^{-1})

Parameters	Control	Treatment
Bilirubin(g dl^{-1})	0.48±0.0568	0.49±0.058
SGOT (U l^{-1})	56.58±1.00	57.7±0.867
SGPT (U l^{-1})	27.20±0.540	27.2±0.516
Creatinine (g dl^{-1})	0.25±0.009	0.23±0.011
Alkaline phosphatase (U l^{-1})	127.96±0.942	127.75±0.745
Urea (g dl^{-1})	2.21±0.0294	2.22±0.047
Total Cholesterol (g dl^{-1})	2.02±0.044	1.98±0.05
Glucose (g dl^{-1})	68.08±0.68	67.95±0.57

Values shown are mean of six replicates ±SE. Experimental groups were compared with control

From the present study it may be concluded that the compound E isolated from *H. indicum* has a very good potential for use as an insecticide with little chance of toxicity to mammals. The compound has successful inhibitory effects on the reproductive life of *Helopeltis theivora*.

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