

ANXIOLYTIC ACTIVITY OF ROOT EXTRACTS OF *DECALEPIS HAMILTONII* W.A. IN MICE

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Summary

The petroleum ether, alcoholic and water extracts of *Decalepis hamiltonii* root were evaluated for anxiolytic activity using elevated plus maze model (EPM) and open field test (OFT) in mice. The alcohol extract exhibited potent anxiolytic effects at 10, 30 and 100 mg/kg., i.p. in both methods whereas petroleum ether and water extract were less effective. Presence of saponins, phenols and tannins may contribute to the anxiolytic activity of *Decalepis hamiltonii* root.

Keywords: *Decalepis hamiltonii*, anxiolytic, elevated plus maze, open field test

Introduction

Decalepis hamiltonii W.A (Asclepidaceae) commonly known as Shwet Sariva. Roots of *D.hamiltonii* has traditionally been used as demulcent, diaphoretic, diuretic and tonic. It is useful in the loss of appetite, fever, skin diseases, diarrhoea, nutritious disorders, as blood purifier [1, 2], antimicrobial [3, 4] and in treatment of epilepsy and central nervous system disorders [4]. In our previous pre clinical study we have reported anticonvulsant activity of petroleum ether extract of *D. hamiltonii* [5].

The phytochemicals presents in *D. hamiltonii* roots are ellagic acid [6], volatile oil (0.68 %) which contain 2-hydroxy-4-methoxy benzaldehyde (96 %), salicylaldehyde (0.018 %), benzaldehyde (0.017 %), methyl salicylate (0.044 %), benzyl alcohol (0.016 %), 2-phenylethyl alcohol (0.081 %), ethyl salicylate (0.038 %), p-anisaldehyde (0.010 %) and vaniline (0.45 %) [7], ketone, resinol, sterols, saponins, tannins [8], inositol [9], fatty acids [10], α -amyrin, β - amyrin acetate, and lupeol [11]

In context with use of *D. hamiltonii* for the treatment of CNS disorders and our previous study of the same plant for anticonvulsant activity, it is hypothesized that this plant may possess anxiolytic activity.

The objective of present study was to investigate anxiolytic activity of petroleum ether extract (PE), alcoholic extract (AE) and water extract (WE) of roots of *Decalepis hamiltonii* in mice.

Material and methods

Plant material:

Fresh roots of *Decalepis hamiltonii* W.A (Asclepidaceae) were collected in the month of June from Nashik district of Maharashtra region in India and dried in shade at room temperature. The plant material was authenticated by botanist Dr. A. M. Mujumdar, Agharkar Research Institute, Pune, India and a voucher specimen of the herbarium has been deposited at that institute.

Preparation of extract:

The roots (1kg) were powdered and extracted successively with petroleum ether (60-80 °C), alcohol (90%) and water by cold maceration process. The extracts was concentrated on water bath (50-55 °C) and stored in refrigerator at 4 °C. The yield of petroleum ether extract (PE), alcoholic extract (AE) and water extract (WE) was 2.7%, 7.4% and 13.8 % (as % of dry weight) respectively.

The dosage form of PE was prepared by using Tween 80 (2.5%) and that of AE with Tween 80 (2.5%) plus carboxy methyl cellulose (CMC 1%). Diazepam and water extract dissolved in water for injection. Volume administered was 5 ml/kg of body weight of animal.

Chemicals:

Petroleum ether, alcohol, Tween 80, and carboxy methyl cellulose were procured from Merk Ltd. Mumbai, whereas Diazepam injection (Calmpose ® Ranbaxy Ltd, Delhi and water for injection were purchased locally.

Animals:

Male Swiss albino mice weighing 25 ± 2 g obtained from animal house of Serum Institute of India Ltd. Pune, were housed in animal house facility of Poona College of Pharmacy, Pune, India. Animals were housed in group of 6-8 animals per cage at a temperature of $24 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ and relative humidity of 45-55 %. A 12:12 h dark: light cycle was followed during the experiment and the experiment was carried during 10:00- 16:00 hr. Animals had free access to standard laboratory feed (Standard chow pellet, Chakan oil mills, Sangli) and water *ad libitum*. The study was conducted in accordance with the internationally accepted principles.

The Institutional Animal Ethical Committee of Poona College of Pharmacy, formed under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved the pharmacological and acute toxicity protocol.

Methods:

Acute toxicity:

Acute intraperitoneal toxicity was performed in mice at limit dose 2000 mg/kg following the dosing schedule and procedure described in OECD Guideline (AOT 425).

Elevated plus maze (EPM)

The elevated plus maze apparatus consisted of two open arms (30 x 5 cm) and two closed arms (30 x 5 x 20 cm) emanating from a common central platform (5 x 5 cm). Two pairs of identical arms were opposite to each other. Entire apparatus was elevated to a height of 50 cm above the floor level.

Animals were divided into 13 groups of 6 mice per group. Group I, VI, and X received vehicle, group II, III, IV received PE 10, 30 and 100 mg/kg, group VII, VIII, XI received AE 10, 30 100 mg/kg group XI, XII, XIII received WE 10, 30 and 100 mg/kg and group V received diazepam 0.5 mg/kg. All the extracts, vehicle or diazepam were administered by intra-peritoneal route 45 min before start of session. To start session mouse was placed at the center of maze, its head facing closed arm and allowed to explore maze for 5 min. During this 5 minutes time spent in open arm and number of entries in open were recorded. An entry was defined as all four paws on the arm. The plus maze was carefully wiped with hydrogen peroxide and dried with sponge after each trial. Test was conducted in quite room to avoid disturbances to animals [12].

Open field test [OFT]:

Open-field test apparatus consisted of a wooden box (68 x 68 x 45 cm³) with dark gray floor subdivided into 16 equal fields. Box was placed in a quiet dark room except open field apparatus, which was illuminated with a 40W bulb focusing onto the field from a height of about 100 cm.

Mice were divided into 13 groups and received vehicle, extracts or standard drug (diazepam 0.5 mg/kg) as per treatment schedule mentioned in above method. After 45 min of treatment mice were placed individually in a corner square of apparatus and changes in the following behavioral parameters were observed during next 5 min: ambulation (the number of squares crossed at periphery) and activity in center (number of central squares crossed). Open field was carefully wiped with hydrogen peroxide and dried with sponge after each trial [13].

Phytochemical Analysis.

Extracts were subjected to phytochemical analysis using method described by Khandelwal 2003 [14].

Statistical Analysis:

The data was presented as Mean \pm SEM and statistical difference between mean time (in sec) spent in open arm were analyzed by one way Analysis of Variance (ANOVA) followed by Dunnett's test, whereas data of number of entries in open arm and number of squares traveled in open field was analysed by Mann-Whitney *U*-test. The difference was considered significant at 5% level.

Results

Acute toxicity study:

Decalepis hamiltonii root extract PE, AE and WE was found to be safe and no mortality occurred upto 2000 mg/kg i.p.

Elevated plus maze:

PE (10 mg/kg) significantly ($P<0.01$) increased time spent and number of entries in open arm. Similar results were obtained in PE (30 mg/kg) except open arm entries, which were changed with less significance ($P<0.05$). Significant ($P<0.01$) increase in time spent in open arm and number of entries in open arm were found in a group which received AE (10 and 30 mg/kg). Results obtained in AE 100 mg/kg were more significant ($P<0.01$) than the results obtained at lower doses (10 and 30 mg/kg) ($P<0.05$). WE showed significant ($P<0.05$) increase in time spent and number of entries in open arm however results are less significant than PE and AE.

Table 1. Effect of petroleum ether extracts of *D. Hamiltonii* roots on behavior of mice in EPM

Treatment (mg/kg, i.p)	Time (in Sec) Spent in Open Arm	No. of Entries Open Arm
Vehicle (Tween 80, 2.5%)	25.25 ± 1.57	3.83 ± 0.40
PE (10)	61.50 ± 5.60**	8.17 ± 0.65**
PE (30)	59.50 ± 4.13**	6.00 ± 0.63*
PE (100)	34.33 ± 3.22	4.67 ± 0.92
Diazepam (1)	78.00 ± 6.52**	17.18 ± 1.59**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. vehicle group.

Table 2. Effect of alcohol extracts of *D. Hamiltonii* roots on behavior of mice in EPM.

Treatment (mg/kg, i.p)	Time (in Sec) Spent in Open Arm [#]	No. of Entries Open Arm ^{\$}
Vehicle (tween 80, 2.5% + CMC 1%)	22.33 ± 1.48	3.50 ± 0.43
AE (10)	68.67 ± 7.77**	8.33 ± 1.26**
AE (30)	65.33 ± 6.68**	9.00 ± 1.13**
AE (100)	71.34 ± 6.11**	12.17 ± 2.03**
Diazepam (1)	78.00 ± 6.52**	17.18 ± 1.59**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. vehicle group.

Table 3. Effect of water extracts of *D. Hamiltonii* roots on behavior of mice in EPM.

Treatment (mg/kg, i.p)	Time (in Sec) Spent in Open Arm [#]	No. of Entries Open Arm ^{\$}
Vehicle (Water)	24.50 ± 1.61	4.00 ± 0.45
WE (10)	45.11 ± 1.14*	5.67 ± 0.76
WE (30)	44.67 ± 0.96*	6.50 ± 0.50**
WE (100)	38.00 ± 2.41	4.67 ± 0.49
Diazepam (1)	78.00 ± 6.52**	17.18 ± 1.59**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. Vehicle.

Open field test:

In the open field test PE (30 mg/kg) and AE (10 and 100 mg/kg) showed significant ($P<0.01$) increase in ambulation and activity at center. PE (10 mg/kg), AE (30 mg/kg) showed significant ($P<0.01$) increase in activity at center without significant increase in ambulation. However, WE (10, 30 and 100 mg/kg) did not show significant increase in ambulation and activity at center.

Table 4. Effect of petroleum ether extracts of roots of *D. Hamiltonii* on behavior of mice in OFT

Treatment (mg/kg, i.p)	No. of squares traveled	
	Wall side (Ambulation)	Central (Activity at center)
Vehicle(Tween 80, 2.5%)	43.50 ± 3.58	00 ± 00
PE (10)	64.67 ± 8.36	4.00 ± 0.63**
PE (30)	73.33 ± 8.48*	10.17 ± 1.58**
PE (100)	46.67 ± 6.78	4.83 ± 0.40
Diazepam (1)	105 ± 7.36**	10.33 ± 1.34**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. vehicle group.

Table 5. Effect of alcohol extracts of roots of *D. Hamiltonii* on behavior of mice in OFT

Treatment (mg/kg, i.p)	No. of squares traveled	
	Wall side (Ambulation)	Central (Activity at center)
Vehicle (tween 80, 2.5% + CMC 1%)	43.50 ± 3.34	00 ± 00
AE (10)	80.33 ± 4.40**	10.67 ± 1.21**
AE (30)	55.17 ± 5.24	4.83 ± 0.48**
AE (100)	78.17 ± 7.59**	10.67 ± 1.86**
Diazepam (1)	105 ± 7.36**	10.33 ± 1.34**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. vehicle group.

Table 6. Effect of water extracts of roots of *D. Hamiltonii* on behavior of mice in OFT

Treatment (mg/kg, i.p)	No. of squares traveled	
	Wall side (Ambulation)	Central (Activity at center)
Vehicle (Water)	47.50 ± 1.98	2.33 ± 0.42
WE (10)	66.33 ± 6.72	7.67 ± 0.85
WE (30)	62.16 ± 6.75	5.16 ± 0.60
WE (100)	54.66 ± 6.38	00 ± 00
Diazepam (1)	105 ± 7.36**	10.33 ± 1.34**

Values are mean ± SEM., n=6; **P- 0.01, *p<0.05 vs. vehicle group.

Phytochemical analysis:

Phytochemical analysis of petroleum ether extract showed the positive test for steroids and oils, alcoholic extract contains carbohydrates proteins, amino acids, tannins and phenols whereas water extract contains carbohydrates, proteins, amino acids, tannins, phenols and saponins.

Discussion

Fear and anxiety are defined as the response of a subject to real or particular threats that may impair its homeostasis, this response may include physiological or/and behavioral. Measuring anxiety like behavior in mice has been mostly undertaken using a few classical animal models of anxiety such as the elevated plus maze and open field test. All these procedures are based upon the exposure of subject to unfamiliar aversive place [15].

Increase in time spent and number of entries of animal in the open arm indicates anxiolytic activity of drugs [16]. In present study significant increase in time spent on open arm, as well as significant increase in number of entries proves anxiolytic activity of *D. hamiltonii*. Maximum activity was observed at 100 mg/kg of AE in elevated plus maze.

Many anxiolytic agents act differently in the elevated plus maze with regard to arm entries, time spent on open arm suggesting non-specific nature of anxiety or multiplicity of drug action. Diazepam increased time spent on open arm and entries on open arm. On the contrary, drugs with potential anxiolytic properties but also showing sedative effect may mask the anxiolytic behavior [17]

In open field test, when animals were placed in a different environment from home cage, they express their anxiety and fear by decrease in ambulation and exploratory behavior. These behavioral changes are attenuated by classical anxiolytic agent and augmented by anxiogenic agent [18, 19]. In present study increase in ambulation, tendency to travel in central squares by animals treated with AE at 10 and 100mg/kg and PE at 30mg/kg suggests anxiolytic activity of *D. hamiltonii*. However WE was devoid of significant anxiolytic activity.

It is well known that plants [20, 21, 22], isolated constituents from plants [23, 24] and synthetic drugs like benzodiazepines and Phenobarbital [25] possess anxiolytic and sedative activity. At the higher doses diazepam (20 mg/kg p.o.) in rats

[26] chlordesmethyldiazepam (benzodiazepine receptor full agonist) and desmethyldiazepam (benzodiazepine receptor agonist, metabolite of diazepam) at 5 mg/kg i.p., in mice showed decreased activity in open field test which was concluded as sedative effect [27]. In the present investigation decrease in ambulation at higher dose (100 mg/kg) of PE and WE was observed which might be due to the sedative property of the extracts. This decrease in activity at higher doses of PE and WE in EPM and OFT can be correlated well with each other.

Animals treated with AE, PE and WE at 2000mg/kg i.p. showed no toxic sign or mortality in acute toxicity study.

The petroleum ether extract and water extract was found to be less potent anxiolytic than alcoholic extract of roots of *D. hamiltonii* in EPM and OFT in present investigation. Phytochemical analysis of AE showed presence of phenols and tannins and which might be contributing in part to the observed pharmacological effects. These phytochemicals have been reported to show potent activity against various CNS disorders e.g. saponins have been shown to possess anxiolytic activity [28, 29, 30] and nootropic activity [31]. Tannins have been also been shown to possess activity against many CNS disorders [32] including Alzheimer's disease [33] and epilepsy [34].

Conclusion

It is concluded from the present study that alcoholic extract of *D. hamiltonii* possess potent anxiolytic at doses 10, 30 and 100 mg/kg., i.p which can be attributed to presence of and tannins and phenols.

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