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Study of antianxiety activity of *Acalypha indica* Linn. leaves using elevated plus maze model

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Abstract

Acalypha indica L. is an annual erect herb found throughout various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa. The plant has wide uses in the traditional medicines of various countries and reportedly possesses diuretic, purgative and anthelmintic properties, besides being also used for bronchitis, asthma, anxiety, pneumonia, scabies and other cutaneous diseases. So, the present study have been designed to study the anxiolytic effects of leave extracts of Petroleum ether, Chloroform, Methanol and water using elevated plus maze model in swiss albino mice by different doses 100,200,400mg/kg. Diazepam 2mg/kg use as standard. Results displayed that methanol extract of leaves 400mg/kg dose *Acalypha indica* increased the average time spent in the open arms of the EPM which was compared with the effect of diazepam. Hence this study shows that the plant has potentially antianxiety agent.

Keywords: *Acalypha indica*, Petroleum ether, EPM, antianxiety agent

Introduction

Anxiety is a characteristic human response that includes both personality and body [1]. Anxiety is characterized by a diffuse, undesirable, unclear feeling of misgiving. Usually attending via autonomic side effects, for example, sweat, palpitations, cerebral pain, and snugness in the chest [2]. In spite of the fact that, benzodiazepines have been known as a viable treatment of tension issue, they have a few unwanted symptoms. Consequently, further research is important to discover new anxiolytic medications with less unfavorable impacts [3-4].

Writing audit uncovered that the utilization of plants in the administration of ailments has been since time relic, and ceaselessly developed after some time as correlative medication since they were promptly and economically accessible medicinal services choices. Medications segregated from conventional plants may have conceivable restorative consequences for uneasiness. Research led to think study anti-anxiety compounds natural for alternative therapy [5].

Acalypha indica L. is an annual erect herb found throughout various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa. The plant has wide uses in the traditional medicines of various countries and reportedly possesses diuretic, purgative and anthelmintic properties, besides being also used for bronchitis, asthma, pneumonia, scabies and other cutaneous diseases [6]. A drug used for prevention and reversal of atherosclerotic disease process in the Sidha system of Indian medicine, Anna Pavala Sindhooram, contains the leaves of this plant as one of the ingredients [7]. Chemical constituents reported from this plant include acalyphamide (as acetate), aurantiamide and its acetate, succinimide calypho-lactate, and its β -D-glucoside (leaves); a cyanogenetic glucoside, acalyphine, two alkaloids, viz, acalyphine and triacetanamine, an essential oil n-octacosanol, kaempferol, quebrachitol, β sitosterol acetate and tannin (whole plant); stigmasterol (root) [8]. Recently, four kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin have also been isolated from the flowers and leaves of this plant [9]. Petroleum ether and ethanol extracts of the whole plant demonstrated post-coital antifertility activity in female albino rats [10]. Ethanolic extract of the plant showed promising wound healing activity in rats [11]. Administration of ethanol leaf extract of the plant has been shown to significantly inhibit in a dose dependent manner, the Viper russelli venom-induced lethality, haemorrhage, necrotizing and mast cell degranulation in rats and cardiotoxic and neurotoxic effects in isolated frog tissue [12]. Based on reports of the use of *acalypha indica* in Bangladesh traditional medicine, the present study was conducted to study the analgesic effect of *acalypha indica* leaves The literature review revealed that the plant leaves have been used for anxiety treatment but no biological data are available to support such facts. So, the present study have been designed to study the anxiolytic effects leaves extracts of Petroleum ether, Chloroform, Methanol and water using elevated plus maze model in swiss albino mice.

Material and methods

Authentication

The leaves were collected from Jamadarpali a place in the Sambalpur district of Orissa. Two herbarium were prepared one was sent to Botanical Survey of India at Central National Herbarium P.O.: Botanical Garden, Howrah-711103 for proper authentication and the other one was kept in M.Pharm Pharmacognosy Lab. of Khalsa College of Pharmacy. The sample was identified to be *Acalypha indica* (L.) family: Euphorbiaceae

Animals

The experimental animals Swiss albino mice (20-25 gm) of either sex were procured from the central animal house, Animal House, Pinnacle Biomedical Research Institute (PBRI), Bhopal The animals were given standard laboratory feed and water ad libitum., both being withdrawn 12 hrs prior to experimentation. The experiments were performed between 8.00 to 12.00 hrs. The experiments were conducted in a semi sound proof laboratory. The biological studies were carried out as per the guidelines of institutional ethical committee.

IAEC Approval

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (CPCSEA Reg. No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-17029.

Preparation of leaf extracts

The petroleum ether, chloroform, methanol and aqueous extract of dried leaves (100gm) were prepared by successive Soxhlet extraction (Fig. 1). Solvent was distilled off, extracts were weighed and percentage was calculated in terms of dried weight of plant material.

Phytochemical screening

The extracts were tested for the presence or absence of alkaloids, saponins, flavonoids, carbohydrates, tannins and proteins [13-15].

Evaluation of anxiolytic activity

Treatments

- Control: vehicle {simple syrup I P + tween 80 (5%)} 0.25 ml.
- Standard drug: diazepam {2mg/kg orally}
- Test extracts:leaves extracts {petroleum ether, chloroform, methanol & aqueous extract at different doses i.e. 100, 200, 400 mg/kg suspended in vehicle.

Elevated Plus Maze Model

The elevated plus maze is a well established animal model for testing anxiolytic drugs [16-17]. It has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds by decreasing anxiety, increase the open arm exploration time, anxiogenic have the opposite effect. Plus maze apparatus consist of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) with an open roof and is elevated to a height of 25cm [18].

Procedure

Weighing and numbering of the animals was done. Then they were divided into different groups, each consisting of 5 mice. One group was used as control (vehicle) and second group for

standard drug (diazepam) treatment and other test groups for different extracts.

The animals were placed individually in the center of the maze, head facing towards open arm and the stop watch was started and following parameters were noted for 5 min.

- First preference of mice to open or enclosed arm.
- Number of entries in open and enclosed arms (an arm entry defined as the entry of four paws into the arm).
- Average time each animal spends in each arm (average time = total duration in the arm/ number of entries).

Vehicle was administered to the control group. Diazepam was administered to the standard drug treatment group and extracts were administered to the various test groups. After 45 minutes animals were placed individually in the centre of the maze and all parameters as described under step 2 were noted.

Statistical Analysis

The anxiolytic activities of the extracts, Diazepam and control was analysed by ANOVA, the test groups were compared with standard/control by Tukey's Multiple Range Test. Difference were considered significant at $p < 0.05$.

Results

Petroleum ether extract

Treatment with petroleum ether extract at dose 100, 200, 400 mg/kg did not show any significant increase in the time spent by mice in open arms of EPM (Table 1) and (Fig. 1)

Table 1: Effect of Petroleum ether extract of *Acalypha indica* leaves on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Pet. ether	100	5	10.576 ± 0.828 *
4	Pet. ether	200	5	10.664 ± 0.788 *
5	Pet. ether	400	5	11.516 ± 1.389 *

n=no. of animals

The values are expressed as mean ±S.E.M.

* $p < 0.05$ as compared to Diazepam

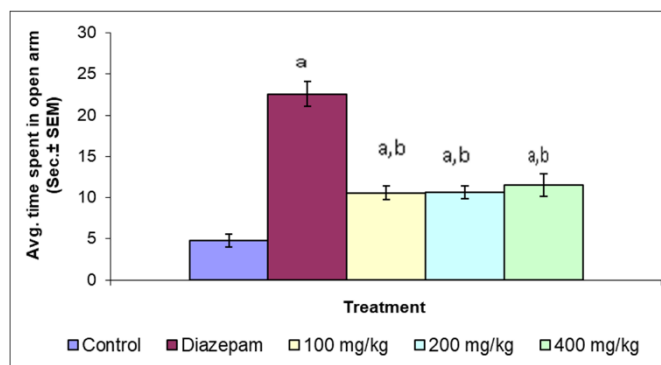


Fig 1: Effect of Petroleum ether extract of *Acalypha indica* leaves on time spent in open arm

The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.

a= $p < 0.05$ vs. Control (Vehicle); b= $p < 0.05$ vs. Diazepam (Standard drug)

Chloroform extract

Treatment with Chloroform extract at dose 100, 200, 400 mg/kg did not show any significant increase in the time spent by mice in open arms of EPM (Table 2) and (Fig. 2).

Table 2: Effect of Chloroform extract of *Acalypha indica* leaves on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Chloroform extract	100	5	13.864 ± 1.629*
4	Chloroform extract	200	5	14.984 ± 1.556*
5	Chloroform extract	400	5	6.946 ± 0.651*

n=no. of animals

The values are expressed as mean ±S.E.M.

* $p < 0.05$ as compared to Diazepam

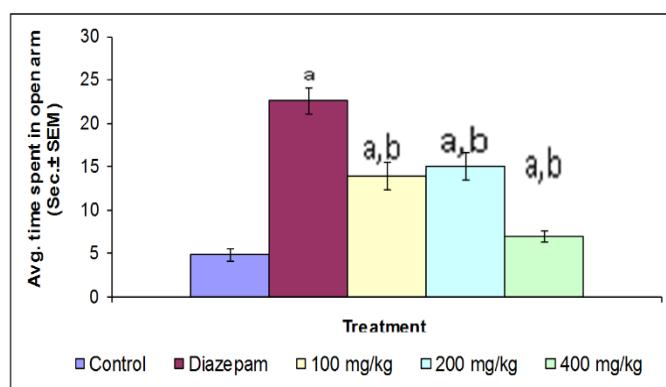


Fig 2: Effect of Chloroform extract of *Acalypha indica* leaves on time spent in open arm

The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.

a= $p < 0.05$ vs. Control (Vehicle); b= $p < 0.05$ vs. Diazepam (Standard drug)

Methanol Extract

Treatment with Methanol extract at the dose of 100mg/kg showed a significant increase in the time spent by mice in the open arms of EPM (Table 3) and (Figure 3).

Table 3: Effect of Methanol extract of *Acalypha indica* leaves on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg. time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Methanol extract	100	5	21.858 ± 1.487
4	Methanol extract	200	5	20.386 ± 0.968
5	Methanol extract	400	5	11.162 ± 0.831*

n=no. of animals

The values are expressed as mean ±S.E.M.

* $p < 0.05$ as compared to Diazepam

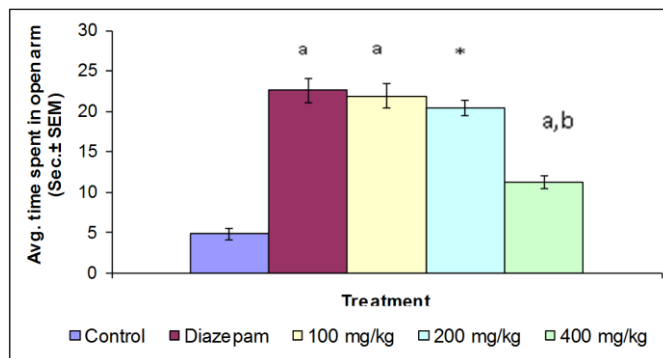


Fig 3: Effect of Methanol extract of *Acalypha indica* leaves on time spent in open arm

The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.

a= $p < 0.05$ vs. Control (Vehicle); b= $p < 0.05$ vs. Diazepam (Standard drug)

Aqueous Extract

As the dose was increased, increase in the anti anxiety activity was found but treatment with aqueous ext. at dose 100, 200, 400 mg/kg did not show any significant increase in the time spent by mice in the open arms of EPM (Table 4) and (Figure 4).

Table 4: Effect of Aqueous extract of *Acalypha indica* leaves on time spent in open arms as measured on EPM in mice

S. No	Treatment	Dose (mg/kg)	n	Avg time spent in open arm (Sec.± SEM)
1	Control		5	4.772 ± 0.773
2	Diazepam	2	5	22.564 ± 1.519
3	Aqueous extract	100	5	9.910 ± 0.844 *
4	Aqueous extract	200	5	10.754 ± 0.656 *
5	Aqueous extract	400	5	13.406 ± 1.587 *

n=no. of animals

The values are expressed as mean ±S.E.M.

* $p < 0.05$ as compared to Diazepam

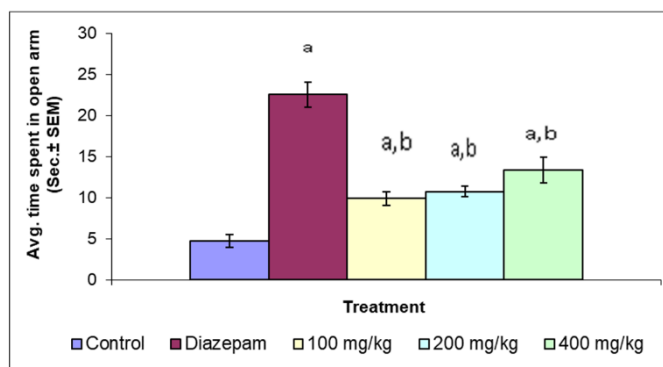


Fig 4: Effect of Aqueous extract of *Acalypha indica* leaves on time spent in open arm

The data was analyzed by one way ANOVA and post hoc Tukey's multiple range test.

a= $p < 0.05$ vs. Control (Vehicle); b= $p < 0.05$ vs. Diazepam (Standard drug)

Phytochemical screening

The results of phytochemical screening of leaves extracts as shown in (Table 5).

Table 5: The results of phytochemical screening of leaves extracts

Test	Petroleum Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
Alkaloids	—	—	—	—
Carbohydrate	+	—	+	+
Saponins	—	—	+	+
Proteins and Amino acids	—	—	—	—
Triterpenoids	—	—	+	—
Phenolic compounds and Tannins	—	—	+	—
Phytosterols	—	—	+	—
Flavonoids	—	—	+	—

Discussion and Conclusion

The essential oils obtained from the genus *Acalypha indica* leaves are recommended for the treatment of anxiety in aromatherapy. The present investigation was aimed at evaluating the anti-anxiety activity of various leaves of *Acalypha indica*. Well authenticated leaves of *Acalypha indica* were subjected to successive and exhaustive extraction with standard solvents in the increasing order of polarity with a view to segregate their constituents on the basis of polarity. Aqueous extract was observed to give higher yield of extractives.

Anti-anxiety activity of various extracts and volatile oil was evaluated using a standard model of anxiety - "Elevated Plus Maze". This model can be used to evaluate both anxiolytic as well as anxiogenic effect. The model was chosen as it is very effective, cheap, simple, less time consuming, requires no preliminary training to mice and does not cause much discomfort to the animals while handling. The model is principally based upon the observations that exposure of animals to the elevated and open maze alley evokes an approach avoidance conflict which is manifested as an exploratory cum fear drive.

In elevated plus maze model various results shows that methanol extract at a dose of 100mg/kg shows significant antianxiety activity comparable to diazepam. The methanol extracts at a dose of 100mg/kg shows values 21.858 ± 1.487 as compared to diazepam 22.564 ± 1.519 in average time spent in the open arm as show in Table 3 and Figure 3 where as Petroleum ether extract, chloroform extracts, and aqueous extracts devoid of antianxiety activity because there values are very less as compared to diazepam as shown in Table 1 and Figure 1, Table 2 and Figure 2 and Table 4 and Figure 4. The phytochemical screening shows the presence of carbohydrates, saponins, flavonoids, phenolic compounds and tannins, phytosterols in the methanol extracts which is responsible for antianxiety activity. Further investigations are necessary for providing pharmacological products of *Acalypha indica* and better understanding of anxiolytic properties and neurobiological mechanisms of *Acalypha indica*

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