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Research Article

Evaluation of Anti-anxiety Property of Alcoholic Extract of *Abutilon indicum* Leaves in Albino Mice

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Abstract

Abutilon indicum native to tropic and subtropical regions and sometimes cultivated as an ornamental plant and is considered invasive on certain tropical islands. The whole plant is often used in traditional system of medicine to treat different medical conditions. Various studies were conducted for anti stress and antioxidant property of the plant *Abutilon Indicum* but not on anti anxiety action. This study is done to elucidate the anti anxiety property of the plant *Abutilon Indicum*. Alcoholic Extract of *Abutilon Indicum* leaves (AEAIL) were prepared using soxhlets apparatus. Five groups of mice, each include five animals. Group I was taken as control and Normal saline was given orally. Group II was taken as standard and diazepam 2mgs/kg was given orally and groups III, IV, V were given Alcoholic extract of *Abutilon Indicum* leaves (AEAIL) in the dose of 100, 200 and 400mgs/kg respectively. All test drugs were given orally, 60 minutes prior to experiment. Using Elevated plus Maze (EPM), the number of entries, time spent and rearing behavior of albino mice in open arm was noted. There was dose dependent increase in both parameters and there was dose dependent increase in percentage of time spent in open arm with AEAIL which indicates its Anti-anxiety Property. Statistical Analysis was done by one way ANOVA followed by Dunnet's test and P-values < 0.05 were considered statistically significant.

1.0 Introduction

Abutilon indicum Linn. (Family : Malvaceae) commonly known as 'Thuthi /Duvenna Kaya' is distributed throughout the hotter parts of India and also in sub-Himalayan tract and hills up to 1,200 m and used in our Traditional System of Medicine¹. The leaves are effective in ulcer, for the treatment of diabetes, diuretic infection and gingivitis. The bark is used as febrifuge, anthelmintic, alexeteric, astringent and diuretics. The seeds are used in piles, laxative, aphrodisiac, and expectorant, in chronic cystitis, gleets and gonorrhea^{2, 3, 4, 5}. In Siddha system of medicine, it used as a remedy for jaundice, piles, ulcer and leprosy⁶. Alkaloids, flavonoids, steroids, terpenoids and saponins have been isolated and characterized from genus *Abutilon*^{7, 8}. Previous phytochemical investigations of *Abutilon indicum* showed the presence of seven flavonoids, two sesquiterpenes lactones⁹, Gallic acid, β -sitosterols, geraniol and aryophylline⁹. In traditional medicine, Leaves of *A. indicum* is used as a demulcent, aphrodisiac, laxative, diuretic, and pulmonary and sedative. The bark is astringent and diuretic; laxative, expectorant, anti-inflammatory and anthelmintic (plant); analgesic (fixed oil); diuretic and for leprosy (roots)¹⁰. The leaves can also be used to treat ulcers, headaches, and gonorrhea and bladder infection and the decoction of the leaves is used in toothache, tender gums and internally for inflammation of bladder⁹. The seeds are used in piles, as laxative, expectorants, in chronic cystitis, gleet and gonorrhea^{11, 12, 13}. Medicinal plants have been found to possess several phytochemical active compounds which possess wide range of biological activities that are responsible for the observed curative effects of herbal medicines. Various studies are there for anti-stress property of the plant

*Abutilon indicum*¹⁴. As stress and anxiety are inter related, we attempted the evaluation of anti-anxiety property of *Abutilon indicum* plant.

2.0 Materials and Method

The plant was collected from forest at Khammam and authenticated by official agency. The plant was shade-dried and powdered and ethanolic extraction was done by Soxhlets apparatus in the department of pharmacology.

2.1 Experimental Animals

Adult Wister albino mice (30-40g) of either sex were used for the study. The mice were fed with standard pellet and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by IAEC.

2.2 Acute Oral Toxicity Study

Acute toxicity study was performed in healthy albino mice (30-40g) as per guidelines (AOT 425) suggested by the Organization for Economic Co-operation and Development (OECD). From this data and pilot study reports; three different doses 100, 200 and 400 mg/kg were selected for this study. It is found that AEAIL was safe at limit dose of 2000 mg/kg with no mortality in studied animals. 1/10th of these doses i.e. 200 mg/kg and doubling of that dose that is 400 mg/kg and half of 1/10 dose that is 100mg/kg were used in the subsequent study respectively.

2.3 Elevated Plus Maze

The EPM consisted of two open arms measuring 16 × 5 cm and two closed arms, measuring 16 × 5 × 12cm, connected to a central platform 5 × 5 cm. The Maze was elevated to a height of 25cm above the floor. Each mouse was put at the centre of the elevated

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plus Maze with its head facing towards an open arm¹⁵ and the stop watch was started and following parameters were noted for 5 min. a) First preference of mice to open and closed arm. b) Number of entries in open and closed arms (An arm entry defined as the entry of four paws into the arm) c) Average time each animal spends in each arm (Average time = Total duration in the arm / Number of entries). In EPM test, the percent time on the open arms was determined as follows:

$$\% = 100 \times \text{Number of seconds spent on open arms} / 300 \text{ Total seconds (5 minutes observation time)}$$

2.4 Experimental Protocol

All the mice in experimental groups were exposed to EPM for normal duration of five minutes to assess the anxiety levels in rodents¹⁶. Group 1 was taken as control and normal saline was given. Group 2 was taken as standard and diazepam 2mgs/kg was given and groups 3, 4, 5 were given Alcoholic extract of *Abutilon Indicum* leaves (AEAIL) in the dose of 100, 200 and 400mgs/kg respectively. All test drugs were given orally, 60 minutes prior to experiment. Using Elevated Plus Maze (EPM), the number of entries, time spent and rearing behavior of albino mice in open arm was noted. The apparatus was thoroughly cleaned with 5% ethanol before placing each mouse in the cage.

2.5 Statistical Analysis

All the values were expressed as Mean±SEM. The differences were compared using one way Analysis of variance (ANOVA) followed by Dunnet's test. The p values <0.05 were considered significant and p-values <0.000 were considered highly significant.

3.0 Results and Discussion

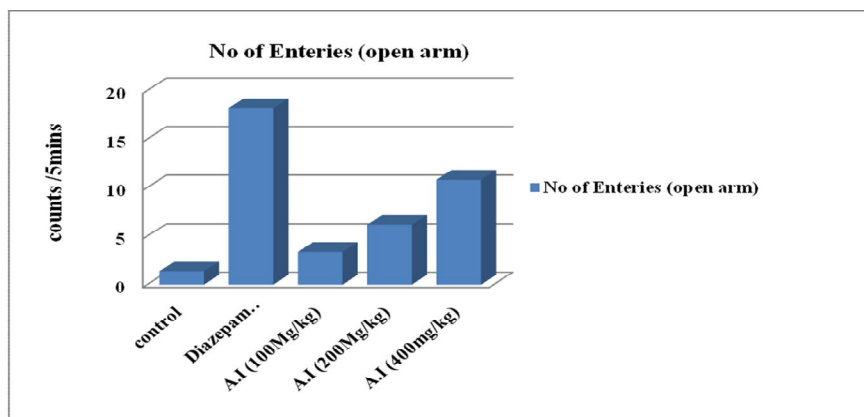
Administration of Diazepam 2mgs/kg increased the amount of time spent in open arms and the percentage of open arm entries

significantly (p<0.0001) to normal saline treated group (Table1). Alcoholic extract of leaves of *Abutilon indicum* (AEAIL) in the dose of 100, 200 and 400mgs/kg significantly (p<0.05) increased the amount of time spent in open arms. Entries in the open arm increased significantly at 100mg (p<0.05), 200mg (p<0.001) and 400mg (p<0.000). That is there was dose dependent increase in both parameters as shown in Table-1 and Bar Diagrams-1 and 2. There was dose dependent increase in percentage of time spent in open arm as shown in Table-1.

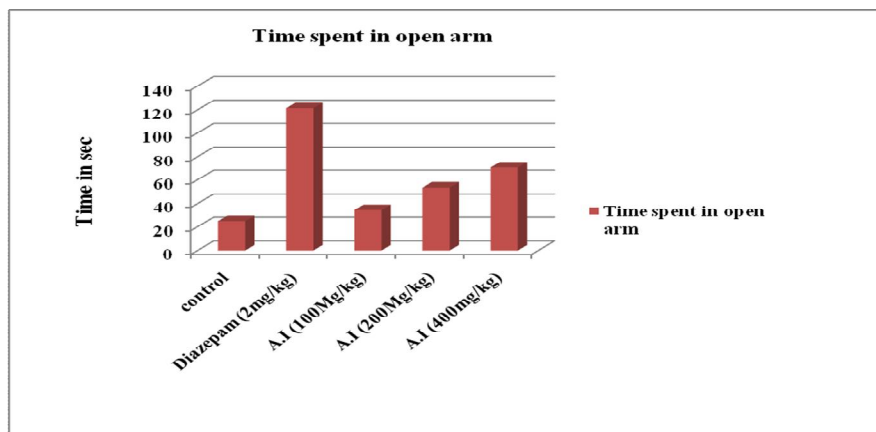
Table 1: Anti-anxiety activity of alcoholic extract of *Abutilon indicum* leaves on Elevated plus Maze Model

Groups (n = 5)	No. of entries (counts/5 min)		Time spent in arm (sec/5min)		Time in Open arm (%)
	Open arm	Closed arm	Open arm	Closed arm	
Group 1 Control (Normal saline)	1.4 ± 0.5099	15 ± 1.183	24.8 ± 4.923	207 ± 10.24	8.26
Group 2 Diazepam 2mg/kg	20.2 ± 3.426**	12.8 ± 1.2	121.4±19.59**	142±10.92	40.4
Group 3 AEAIL 100mg/kg	3.4 ± 1.327	14.7 ± 1.288	34.4 ± 7.846	201.6±7.897	11.4
Group 4 AEAIL 200mg/kg	6.2± 1.393*	13.8 ± 1.02	53.4 ± 10.17*	182.2±12.28*	17.8
Group 5 AEAIL 400mg/kg	10.8±1.281*	12.6± 0.6782	70.6±9.704*	166.8 ± 7.21*	23.5

*P<0.05, **P<0.0001, n = No of animals, All values are expressed as Mean ± SEM. SEM-standard error of mean. AEAIL - Alcoholic Extract of *Abutilon indicum* leaves



Bar diagram: 1



Bar diagram: 2

Mood and anxiety disorders have been found to be associated with different medical conditions. Stress is interconnected with anxiety. The most widely used medications for anxiety disorders are the benzodiazepines, which are being used for different types of anxiety for the last five decades because of their minimal or almost nil side effects. Behavioral effects of diazepam generally thought to be a reflection of its anxiolytic properties¹⁷. Still research is going on for newer molecules especially on traditionally used medicinal plant products. Psychiatric disorders such as depression and anxiety are associated with stress. Several phytochemical active compounds are present in medicinal plants which possess wide range of biological activities that cure different diseases. The AEAIL were found to contain carbohydrates, proteins, amino acids, saponins glycosides, flavonoids, alkaloids, tannins and phenolic compounds. TLC analysis showed the presence of flavonoids¹⁸. Anti-stress activity was reported with *Abutilon indicum* which contains biologically active chemicals such as flavonoids, saponins, alkaloids, proteins, and fixed oils. The anti stress activity may be due to the presence of these constituents. *Abutilon indicum* also claimed to possess antioxidant property¹⁹. The validation of antioxidant claim, usefulness of *Abutilon indicum* to combat stress and role of oxidative stress as major determinant in variety of pathological states¹⁷ have been investigated. As stress and anxiety are inter related, it is worthwhile to investigate anti-anxiety effect of *Abutilon indicum*.

The Elevated plus Maze model induces stress in the experimental animals which in turn causes anxiety in those animals. *Abutilon indicum* with three different doses showed dose dependent increase in the time spent in open arm. The elevated plus maze (EPM) is a mice model of anxiety that is used as a screening test for putative anxiolytic compounds such as diazepam and as a general research tool in neurobiological anxiety research^{20,21}.

The conventional indices of anxiety in this test, number of open arm entries and percent time spent in the open arm, are exquisitely sensitive to agents thought to act via the GABAA receptor complex (i.e., benzodiazepines, barbiturates, ethanol, and neurosteroids). For this reason, we have taken this EPM model to investigate the anxiolytic potential of *Abutilon indicum*. The Model is based on animal's aversion of open spaces. This aversion leads to the behavior which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space which is termed thigmotaxis^{22, 23}. In EPM this translates into a restriction of movement to the enclosed arms. Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open arms), and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open arms)²⁴. Studies have confirmed that full and partial benzodiazepine receptor agonists produce behavioral changes in the maze consistent with anxiety reduction²⁵. In the present study, increase in the proportion of number of entries and time spent in open arm are the best index of reduced anxiety levels in mice following the oral administration of alcoholic leaf extract of *Abutilon indicum* in three different doses (Table-1).

4.0 Conclusion

Several pharmacological properties have been observed with *Abutilon indicum*. It has been used in traditional system of medicine as a demulcent, aphrodisiac, laxative, diuretic, and pulmonary and sedative, hepatoprotective, wound healing, immunomodulatory, analgesic, anti-malarial, antimicrobial, hypoglycemic activity, as anti-stress and as antioxidant because of their chemical constituents such as carbohydrates, steroids, glycosides, flavonoids, tannins and Phenolic compounds. In our study it was observed that *Abutilon indicum* also has anti-anxiety action. To confirm this claim, further extensive research has to be carried out to obtain a new anti anxiety molecule.

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