IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Evaluation of Anti- Anxiety activity of leaves extract of Adansonia Digitata Linn

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Abstract: Anxiety is a normal emotional behavior, however, becomes pathological precipitating cardiovascular and psychiatric disorders when it is severe. Many allopathic drugs are available to treat anxiety disorders. The Present work summarizes as follows:-in preliminary phytochemical screening Ethanolic extract showed positive results for Flavonoids, Carbohydrates, Saponins compound and Tannins & proteins. Aqueous extract showed presence of carbohydrate, alkaloids, glycosides, steroid, flavonoids and proteins. Ethanolic and Aqueous Adansonia *Digitata* Linn. Extract was studied for acute oral toxicity as per revised OECD guideline no.- 425 *Adansonia Digitata* Linn. Was devoid of any toxicity up to 2000 mg/kg in mice by oral route. Hence for further studied doses of 150- 200 mg/ kg of *Adansonia Digitata* Linn. was used. In Elevated plus Maze Significant increase in time spent in open arm was also observed in each animals treated with standard (Diazepam) drug. The numbers of entries were significant increase in animals treated with ethanol and Aqueous extract and standard drug Diazepam when compared to control animals. In Light Dark Model significant increase in time spent in light area was also observed in each animal treated with standard (Diazepam) drug. The numbers of Transitions were signification increase in animals treated with Ethanol and Aqueous extract and standard drug Diazepam when compared to control animals. the Ethanolic extract of *Adansonia Digitata* Linn. (200 mg/kg) markedly increased the percentage of average time spent by the animals in the open arms. The anti- anxiety effect of both the doses (150 mg/kg ,200 mg/ kg) showed significant activity at being that 2000 mg/ kg show higher activity. The anti-anxiety activity effects of Ethanolic and Aqueous extract of *Adansonia Digitata* Linn. may be attribute to any of our combination of chemicals present in the extract.

***Index Terms - Adansonia Digitata* Linn, anti- anxiety effect.**

I. INTRODUCTION

1.1 Medicinal Plant

The origin of ayurveda or the indian science of life is life is linked with the origin of universe and developed from out of the various vedic hymns describing fundamentals, philosophies about the world and life, diseases and medicines. around 1000 bc, the knowledge of ayurveda was compr-ehensively documented in charak samhita and sushruta samhita. in the present study 197 plant species are identified which are used in ayurvedic terminology, curative disease and diversity status etc. those plants which are falls in the category of vulnerable, rare and endangered are recommended for germplasm collection and to take up cultivation and propagation activities through modern agronomical techniques. the knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as ayurveda, siddha and unani. in india it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular source of medicinal preparation in the pharmaceutical industries. during the last few decades there has been an increasing interest in the study of medcinal plants and their traditonal use in diffrerent parts of the world. in india about 90% of plant materials are collected from wild source, many of the plants have become rare, threatened, endangered or vulnerable due to the destructive harvesting. the ethno- botanical survey indicates that about 8,000 species of medicinal plants in different parts of india. the combination of these five element are represented in the form of tridosha eg. vata, pitta and kapha.[(1,2,3)]

1.2 Characteristics of medicinal plants

Medicinal plants have many characteristic when used as a treatment, as follow-

i) Synergic medicine:- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

ii) Support of official medicine:- In the treatment of complex cases like cancer diseases the component of the plants proved to be very effective.

iii) Preventive medicine:- It has been proven that the component of the also characterize bt their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e. reduce the side effect of synthetic treatment.[4]

1.3 Anxiety

The twenty-first century with rapid changes in environmental structure has been called a stressful, anxious and pressured century. Therefore, psychological disorders have been increasing among people. Anxiety is the most prevalent psychiatric disorder; it is pervasive, and unpleasant, causing physical symptoms such as sweating, palpitation, chest muscle spasm, gastrointestinal diseases and agitation, which are created as a response to internal and external stimulation and it tends toward cognitive, emotional, physical and behavioral symptoms. Although Anxiety is not so serious, people experience it everywhere and constantly within all cultures. But the educational system is worried about student's Anxiety which can be intolerable for some. Adolescents, today are living in an increasingly Anxiety ridden atmosphere. They live in a world where nothing seems to be guaranteed with certainty and at the same time they are expected to perform at every front, the main being the academics. Adolescents often lack in academic motivation and performance, as their attention is divided among a lot many things especially at creating an identity for themselves. Anxiety is one of the most studied phenomena in psychology. It is a normal human response to stress.[5, 6, 7]

1.4 Types of Anxiety

Anxiety is like other affective factors such as: tiredness, boredom, anger and emotional disorders. It is entirely related to the psychology of the individual. It does not occur as a single issue. It can rather acquire forms of manifestation and can be categorized as: state and trait anxiety, situation specific anxiety situation specific anxiety, and facilitative versus debilitative anxiety.[8,9, 10]

- Existential Anxiety
- Test, Academic and Performance Anxiety
- Stranger and Social Anxiety
- Generalize Anxiety
- Trait Anxiety
- Choice or Decision Anxiety

1.5 Adansonia digitata Linn

Adansonia digitata Linn. is an important arboreal species which is being threatened of going into extinction. The plant is a largest succulent tree in the world and is a deciduous, massive, majestic tree upto 25 m height. It is a very long-lived tree with multipurpose uses; it is thought that trees are over 1000 yrs old. [11-14]

II. MATERIAL AND METHODS

The Leaves of *Adansonia Digitata* Linn. Were collected from in and around the hilly regions of Mandu (Dhar), Madhya Pradesh for the study.The collected plant was Authenticated by Dr. Bhursingh Solanki Assistant Professor, Department of Botany Govt. P. G. Collage, Khargone, Madhya Pradesh. Preparation of extract by using the following solvents as:-Water (Continuous maceration method) Ethanol (Soxhlet Extraction method) [15-19]

2.1 Phytochemical Studies:

Tests for carbohydrates and Glycosides by

Molisch test**,** Fehling's test**,** Benedict's test

Test For Alkaloids by
Mayer reagent, Dragondroff's reagent, Wagner's reagent

Test for proteins and Flavonoids
Shinoda test, Test for Saponins, Foaming test

Test for Glycosides
Liebermann's test , Modified Borntrager's test, Killer- killani test
Test for Protein
Millon's test, Biuret test, Test for Steroid, Salkowaski Reaction[19-25]

III. EXPERIMENT

Albino mice (20-30 gm) of either sex were procured from institute of Animal Health and Veterinary Biological Rasalpura, Mhow (M. P.) India. Animal Experiments for current study were performed in Charak Institute of Pharmacy, Mandleshwar under CPCSEA, Registrations No.: 1465/ a/ 10 / CPCSEA.Evaluation of Anti- Anxiety Activity by using following models: Elevated Plus Maze and Light Dark Model.

The data were be expressed as mean standard error mean (SEM). The significance of different among the groups assessed using one way analysis of variance (ANOVA) by prism software. The test was followed by Dunnett's $p< 0.05$ considered as significance.

IV. RESULTS

4.1 Preparation of Extract:-

Extraction of *Adansonia Digitata Linn*. Leaves with Ethanol**,** The materials were refluxed with each solvent for 72 hours at 40-70 ^{0}C. Extracts were collected and them evaporated at 40 ^{0}C using hot air oven. Dried extract was kept in desiccators for two days and stored at 5 ^{0}C in airtight containers**.** Maceration Extraction of *Adansonia Digitata* Linn. with Distilled water. The Leaves of *Adansonia Digitata* Linn. Were washed thoroughly with tap water followed by double distilled water to remove dust and dirt.

Extractive values of extracts are given

Plant Name	Parts Used	Method	Ethanol (95%)	Aqueous
Adansonia Digitata Linn.	Leaves	Soxhlet and Maceration	23%	18%

Table No. 1:- Extractive values of different extracts of *Adansonia Digitata* Linn.

4.2 Preliminary Phytochemical Screening

Preliminary Phytochemical screening of ethanolic and aqueous extracts of Leaves of *Adansonia Digitata* Linn. Performed to identify presence of Carbohydrates, Alkaloids, Flavonoids, Saponins, Glycosides, proteins and Steroids.

S.No.	Phytochemical Constituent	Ethanol 95 %	Aqueous Extract
1	**Test for Carbohydrates**		
	Molisch Test	+	+
	Fehling′ Test	+	–
	Benedict′s Test	+	+
2	**Test for Alkaloids**		

	Mayer′s Test	–	–
	Dragendorff′s Test	–	+
	Wagnor′s Test	+	+
3.	**Test for Flavonoids**		
	Shinoda Test	+	+
4	**Test for Saponins**		
	Foaming Test	–	+
5	**Test for Glycosides**		
	Libermann Test	+	+
	Modified Borntrager′s Test	+	–
	Keller- Kilani Test	–	–
6	**Test for Phenol and Tannins**		
	Dil. Ferric Chloride	+	+
7	**Test for Proteins and Free Amino acid**		
	Millon′s Reagent	–	+
	Biuret′s Test	+	+
8	**Test for Steroid**		
	Salkowaski Test	+	–

Absent (–)	**Present (+)**

Table No.2:-Results of preliminary phytochemical screening of Ethanolic and Aqueous extracts of Leaves of Adansonia *Digitata Linn.*

4.3 Determination of LD_{50} of the Ethanolic and Maceration extracts of Leaves of *Adansonia Digitata* Linn.

4.3.1 in mice by Acute Toxicity Studies

Acute oral toxicity study was done according to OECD guideline (AOT 425) on albino mice.All the animals survived any symptom or toxicity during the observations up to 24 hrs. Based on the above observation, LD_{50} of the compound was confirmed to be greater than 2000 mg/kg for the test compound.Any dose below 2000 mg/kg could be used as a dose for animals. The biological evaluation of Anti-anxiety activity was carried out at doses of 150 and 200 mg/kg body weight. The result of this study were analysed on the mean ± SEM from 6 animals. Statistical analysis well be carried by using one-way analysis of variance (ANOVA). Test and by the Dunnett's test using Prism Software. $P<0.05$, $P<0.001$ was considered significant.

4.3.2 Antianxiety activity by Elevated Plus Maze model

Antianxiety activity of Ethanol and Maceration extract of Adansonia Digitata Linn. was determination by using mice of either sex weight between (20- 30 gm). The Adansonia Digitata extract, both Ethanolic and Aqueous extract (150-200 mg/kg) were mixed in saline water (5mg/0.5ml and 10mg/0.5ml). The Prepare dose were administered for 5 day once daily (p.o.) and the last dose was given on 5th day before 60 min. of commencement of experiments. The standard drug (Diazepam) we given at a dose of (2 mg/kg i.p.), 60 min. before starting experiment. After proper treatment each mice was placed at the maze with their heads facing the open arm. During the 5 min. experiments, the behaviour of the mice was recorded as the number of the entries into the open and closed arm and time spent by the mice in each of the arms. The results of this activity are shown in table no.3 and graph no. 1.

Information about group used is as follows:

Treatment

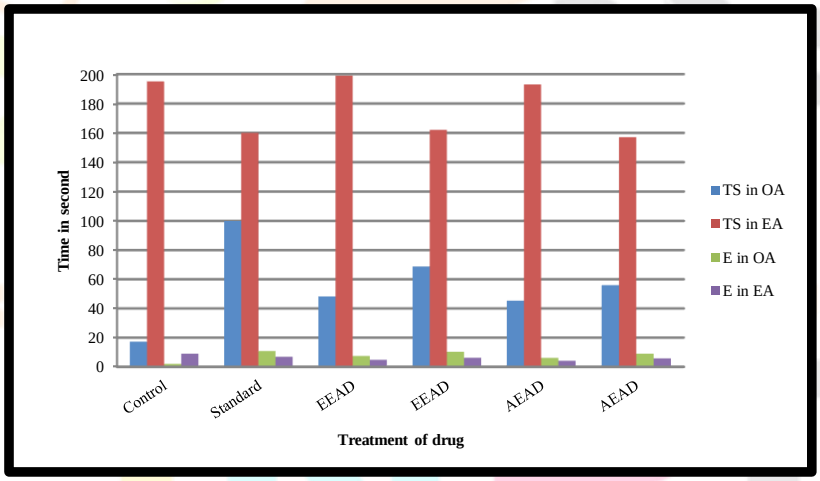
Animals were divided into 6 (I-VI) groups

Group I: Control group received Saline

Group II: Standard group received Diazepam (2 mg/kg i.p.)
Group III: Test group received Ethanolic extract of Adansonia Digitata Linn.(150 mg/kg p.o.)
Group IV: Test group received Ethanolic extract of Adansonia Digitata Linn.(200 mg/kg p.o.)
Group V: Test group received aqueous extract of Adansonia Digitata Linn. (150 mg/kg p.o.)
Group VI: Test group received aqueous extract of Adansonia Digitata Linn. (200 mg/kg p.o.)

Group	Treatment	Time spent in open arm(s)	Time spent in enclosed arm(s)	No. of entries in open arm(s)	No. of entries in enclosed arm(s)
I	Control	17.2± 1.13	195± 2.53	2±0.33	9.01±1.23
II	Standard(Diazepam 2 mg/kg)	100±1.95***	160±2.75***	10.8±1.21***	6.81±0.47***
III	Ethanolic Extract (150 mg/ kg)	48.2± 5.24	199±5.03	7.4±0.76	4.8±0.49
IV	Ethanolic Extract (200 mg / kg)	68.7±3.73**	162±3.44**	10.3±0.48**	6.21±0.51**
V	Aqueous Extract (150 mg/ kg)	45.3±2.23	193±1.74	6.11±0.65	4.12±0.49
VI	Aqueous Extract (200 mg/ kg)	56±3.21	157±2.20	9.03±0.79	5.80±0.21

Table No. 3 :- Effect of *Adansonia Digitata* Linn. Leaves on Mice in EPM model



Graph No. 1:- Effect of various fraction of *Adansonia Digitata* Linn. on EPM

4.3.3.Anti-anxiety activity by Light Drak Model

Anti-anxiety activity of Ethanol and Maceration extract of *Adansonia Digitata* Linn. was determination by using mice of either sex weight between (20-30 gm). The *Adansonia Digitata* Linn. extract, both Ethanolic and Aqueous extract (150-200 mg/kg) were mixed in saline water (5mg/0.5ml and 10mg/0.5ml). The Prepare dose were administered for 5 day once daily (p.o.) and the last dose was given on 5th day before 60 min. of commencement of experiments. The standard drug (Diazepam) we given at a dose of (2mg/kg i.p.) 60 min. before starting experiment. The apparatus consist of two plastic boxes one was dark and the other was transparent. Each animal was allowed to move from one box to the other though an open door between the two boxes. A 100W bulb which was placed 30 cm above the floor of the transparent box was the only light source in the room. Each animals was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min. immediately after the animal stepped into the dark box. The apparatus was cleaned thoroughly between trials. All behaviour recordings were carried out with the observer of the treatment the mine had received. The results of this activity are shown in table no.9 and graph no. 2. Information about group used is as follows:

Treatment

Animals were divided into 6 (I-VI) groups

Group I: Control group received Saline

Group II: Standard group received Diazepam (2 mg/kg i.p.)

Group III: Test group received Ethanolic extract of *Adansonia Digitata* Linn. (150 mg/kg p.o.)

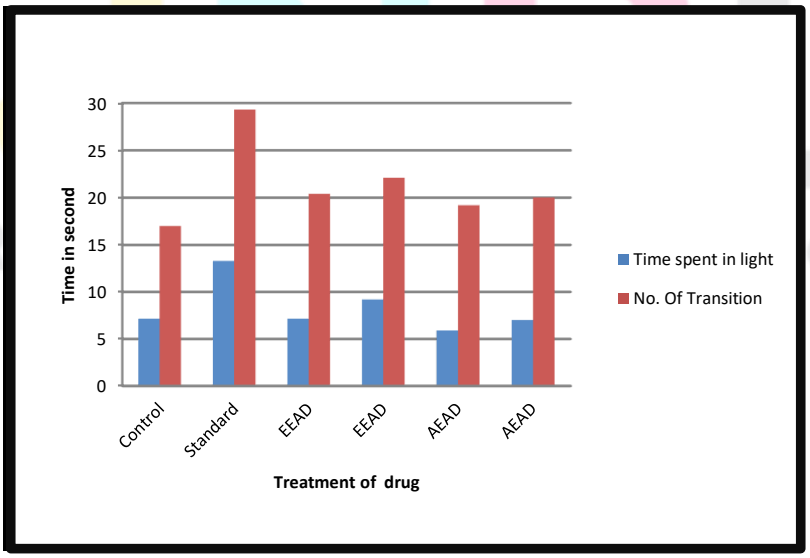
Group IV: Test group received Ethanolic extract of *Adansonia Digitata* Linn.(200 mg/kg p.o.)

Group V: Test group received Aqueous extract of *Adansonia Digitata* Linn. (150 mg/kg p.o.)

Group VI: Test group received Aqueous extract of *Adansonia Digitata* Linn. (200 mg/kg p.o.)

Group	Treatment	Time spent in light	No. of Transition
I	Control	7.16±0.46	17±0.3
II	Standard (Diazepam 2 mg/kg	13.3±0.87***	29.3±2.46***
III	Ethanolic extract (150 mg/kg)	7.15±0.55	20.4±0.52
IV	Ethanolic extract (200 mg/ kg)	9.2±0.68**	22.1±2.30**
V	Aqueous extract (150 mg/kg)	5.9±0.64	19.2±1.22
VI	Aqueous extract (200 mg/kg)	7.01±1.06	20.2±2.81

Table No.:-4 Effect of *Adansonia Digitata* Linn. Leaves on Mice in LDM



Graph No. 2:- Effect of various fraction of *Adansonia Digitata* Linn. on LDM

V. DISCUSSION

The analysis of Ethanolic extract of *Adansonia Digitata* Linn. indicated the presence of Flavonoids, Saponins, Carbohydrate, Tannins & Proteins. Aqueous extract showed presence of Flavonoids, Steroid, Alkaloids and Glycosides, Proteins& Steroids. Different solvents were prepared by ethanol and aqueous extract using standardized procedure and also subjected to anti-anxiety activity *Adansonia Digitata* Linn. exhibited significant anti-anxiety activity with respect to control.

Acute Toxicity study

The present study showed that the Ethanolic and Aqueous extract of *Adansonia Digitata* Linn. Possess anti-anxiety activity as evidenced by its significant effect by Elevated Plus Maze and Light Dark Model in mice.

Elevated Plus Maze

The result obtain from the EPM model, indicates that ethanol and Aqueous extract showed significant ($P< 0.05$) anti- anxiety activity as compared to Diazepam. The average time spent in open arm increase 17.2±1.13(sec) in control to 48.2±5.24 (sec) and 68.7±3.73 in Ethanol extract at a dose of 150 and 200 mg/kg and 45.3±2.23(sec) and 56±3.21 (sec) in Aqueous extract at a dose of 150 and 200 mg/kg.

Light Dark Model

In Light Dark Model the time spent in light were significantly ($P< 0.001$) increased in each animals treated with Ethanol and Aqueous extract at 150 mg/ kg and 200 mg/ kg when compared to control animals. The average time spent in light increased from 7.16±0.46 (sec) in control to 7.15±0.55 (sec) and 9.2±0.68 (sec) in Ethanolic extract at dose of 150 and 200mg/kg. And 5.9±0.64 (sec) and 7.01± 1.06 (sec) in Aqueous extract at a dose of 150 and 200mg/kg.

VI. SUMMARY

The present study of *Adansonia Digitata* Linn. Leaves extract were studied against experimently anti-anxiety activity studies. The results are summarizes as follows:-in preliminary phytochemical screening Ethanolic extract showed positive results for Flavonoids, Carbohydrates, Saponins compound and Tannins & proteins. Extract was studied for acute oral toxicity as per revised OECD guidline no.- 425 *Adansonia Digitata* Linn. Was devoid of any toxicity up to 2000 mg/kg in mice by oral route. In Elevated Plus Maze, numbers of entries were significant increase in animals treated with ethanol and Aqueous extract and standard drug Diazepam when compared to control animals and In Light Dark Model significant increase in time spent in light area was also observed in each animal treated with standard (Diazepam) drug. The numbers of Transitions were signification increase in animals treated with Ethanol and Aqueous extract and standard drug Diazepam when compared to control animals.

VII. CONCLUSION

The present work demonstrates that has anti- anxiety activity in mice by Elevated Plus Maze and Light Dark Model. Anti- anxiety at both the dose level which is comparable with the standard. the extract (200 mg/kg) markedly increased the percentage of average time spent by the animals in the open arms and anti- anxiety effect of both the doses (150 mg/kg ,200 mg/ kg) showed significant activity at being that 2000 mg/ kg show higher activity.

The anti-anxiety activity effects of extract may be attribute to any of our combination of chemicals present in the extract. Further studies are required to identify the acute phytoconstituent responsible for the observed anti- anxiety activity effect of Ethanolic and Aqueous extract. It is suggested and assumed that a further exploration of the present research work is needed to come up with an active Anti-anxiety activity agent.

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