Research Article
CODEN (USA): IJPLCP

ISSN: 0976-7126
Jaiswal *et al.,* 12(1):10-16, 2021

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Antianxiety effect of ethanolic extract of leaves of *Ipomea mauritiana* in swiss albino mice

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Article info

Received: 11/12/2020

Revised: 25/12/2020

Accepted: 24/01/2021

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Abstract

Ipomea mauritiana commonly known as giant potato belonging to the family convolvulaceae. The objective of this study was to evaluate antianxiety activity of ethanol extract of the leaf of *Ipomea mauritiana* in swiss albino mice. swiss albino mice were divided into four groups (n=6)group I and group II received normal saline and diazepam respectively. while group III and group IV received *Ipomea mauritiana* orally at dose 200mg/kg and 400mg/kg body weight. Anti-anxiety was assessed by using elevated plus maze (EPM) and light and dark model methods. The ethanolic extract exhibit anxiolytic effect in experimental mice. So it is supports the use of *Ipomea mauritiana* as anxiolytic agents. Further investigation should be made to elucidate the active constituent of responsible for the activity.

Keywords: Anxiety, Ethanolic extract, Elevated plus maze, *Ipomea mauritiana.*

Introduction

Anxiety is psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral component, associated with significant disability, uncomfortable emotional state, negative feelings about the future, or distress that triggers a sense of defense that serves as a warning so that the individual can prepare to face a possibly dangerous situation. Anxiety disorders are psychiatric disorders affecting nearly 25% of the adult population at some purpose in their life. The prevalence of anxiety disorders is 30.5% and 19.2% in women and men respectively. The prevalence of anxiety disorders is remarkably high in young people.

A survey has also stated that less than 14% of people with such psychiatric disorders receive treatment. Anxiety can aggravate many physical and mental ailments and also impede recovery from any other problems. Anxiety has become a significant are of psychopharmacological research during this decade, as it affects around one-eighth of the total population of the world.

Plants with medicinal properties have been known for 1000 of years and have been used as traditional medicine by the people to treat diseases. Due to many side effects of drugs of medical science and their high cost, the traditional medicines are being used all over the world. Botanically derived medicines have played an important role in human society throughout history and prehistory.

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Ipomea mauritiana (Giant potato) is a member of the family, convolvulaceae and it is all over there all over the tropics it is naturalized in many parts of world . this vine has stems that grow to 10 m. leaf blade in circular in outline,7-18x 7-22 cm. unusally palmately 5-7 divided to or beyond middle, rarely entire or shallot lobed. Flowers are pink or reddish purple with darker center funnel forms 5-6 across. The tubers of this plant are used as tonic, alterative, aphrodisiac, galactogogue, demulcent lactagogue, purgative, cholagogue and have antioxidant and immunomodulatory activities33. It is mainly used to increase secretion of milk, enlarged liver and spleen, increases weight, moderate menstrual discharge, poor digestion also for menorrhagia, debility and fat accumulation.

Active Ingredients of plants are tiglic rhizome of the herb contains betasitosterol and taraxerol acetate. Ergonovine, isobutyric(S)-2methylbutyric n-decanoic, n-dodecanoic, cinnamic acids, and tto glycosidic acids, quinollinic acid A and operculinic acid. The major phytochemical are present in leaves of *Ipomea mauritiana* are alkaloids, flavonoids, tannins, gums, carbohydrates, phenols, glycosides.

Material and Methods

Plant material

Leaves of *Ipomea mauritiana* were collected from Star square, Indore (M.P.) India. And when authentication by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, janata PG College, APS, University, Rewa (M.P.),India.

Treatment of plant part

The leaves of the plant were cleaned, dried under shade and powdered by a mechanical grinder.

Preparation of ethanolic extract

Hundred grams of coarsely powder of leaves was defatted with petroleum ether using soxhlet apparatus. The defatted marc was further extract with ethanol using soxhlet and the extract obtained was concentrated using rotary evaporator. Then, the percentage yield of extracts was 15g and stored in a desiccator.

Phytochemical screening

Phytochemical investigation of ethanolic extract of *Ipomea mauritiana* leaves.

Table 1: Phytochemical constituents of ethanolic extract of *I.mauritiana*

Name of the chemical constituent	Ethanolic extract of *I.mauritiana*
Alkaloid	Present
Sterols	Present
Gums	Absent
Flavonoid	Present
Saponins	Absent
Glycoside	Present
Tannins	Present
Carbohydrates	Present
Phenols	Present

Drug protocol

Diazepam (0.5mg/kg, *i.p.*) was used as standard drug obtained in the form of ampule. It was diluted with normal saline to required strength before use.

Experimental animal

Swiss albino mice (males; 20-25 g) were produced from disease free small animal house, Swami Vivekanand College of pharmacy, Indore M.P. (India). Since, estrogen are the female sex hormones, found to have neuroprotective effect, therefore, we have excluded female mice and used only male mice for the present of study. The mice were kept at constant temperature (22±2ºC) and 12-h light and 12-h dark. Mice were fed standard laboratory food and water was given *ad libitum.* The animal were acclimatized to the laboratory condition before experimental. Experiments were carried out between 09:00 AM- 04:00 PM. The experiment protocol was approved by Institutional Animals Ethics Committee (IAEC), Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India, New Delhi. (Registration No. 1839/OP/ERe/S/15/CPCSEA).

Acute toxicity study

Acute toxicity study procedure was followed as per OECD 423 guidelines. The *Ipomea mauritiana* ethanolic extract was administered orally at the dose of 500mg/kg, 1500mg/kg, 2000mg/kg and 2500mg/kg. Control group of administered saline (10ml/kg of animal). The literature search of conventional LD_{50}test shown not any sing of toxicity or mortality was observed at the higher dose of 2000mg/kg during the

observation for mortality up to 48 hrs. After administration of the doses. During the acute toxicity study, animal were observed for 14 days to study their behavior neurological toxicity.

Experimental Design

Table 2: The animals were divided into four groups with six animals in each group for both models

Group (*n*=6)	Treatment
Group I	Control: Normal saline (10ml/kg)
Group II	Standard: Diazepam (0.5mg/kg)
Group III	Test: *I.mauritiana* ethanolic extract (200mg/kg)
Group IV	Test: *I.mauritiana* ethanolic extract (400mg/kg)

Elevated Plus maze

The plus maze apparatus consisted of two open arms, measuring (16x5 cm), and two closed arms, measuring (16x5x12 cm), and an open roof with the entire maze elevated (25 cm) from the floor. Swiss albino mice 20-25g was treated with normal saline, diazepam, extract 30 min before being placed individually in the center of elevated plus maze, facing a closed arm. The spent in bath open and closed arms was recorded for 5 min. the time spent was measured in seconds. The numbers of entries into the open and closed arms were counted during the test.

An entry was defined as having all four paws with the arm.

Light and Dark Model

The Light and dark apparatus consisted of open top wooden box. Two distinct chambers, a black chamber (25 cm long×35 cm wide×35cm deep), painted black and made dark by covering its top with black plywood, and a bright chamber (25 cm long×35 cm wide×35cm deep), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. Two chamber were connected through a small open doorway (7.5 cm long x7.5 cm wide) situated on the floor level at the center of the partition. The mice were placed individually in the center of the light box after 30min of oral treatment and observed for 5 min.

Statistical analysis

The result were expressed as Mean ± SEM. The statistical significance was determine by One–Way Analysis of Variance (ANOVA) following by Tukey's *post-hoc* test, $p<0.05$ was considered statistically significant.

Results and Discussion

Acute oral toxicity study

During acute oral toxicity studied, the extract was administered orally at dose of (500mg/kg, 1500mg/kg, 2000mg/kg and 2500mg/kg). Animal were observed for gross behavior and morphological change at 14 days. The extract did not produce any significant change in the normal behavior of animal, and no toxic symptoms were seen at the dose levels studied. Dose level $1/10^{th}$ and $2/10^{th}$ of 2000mg/kg dose were selected for the anxiolytic activity.

Elevated plus maze model

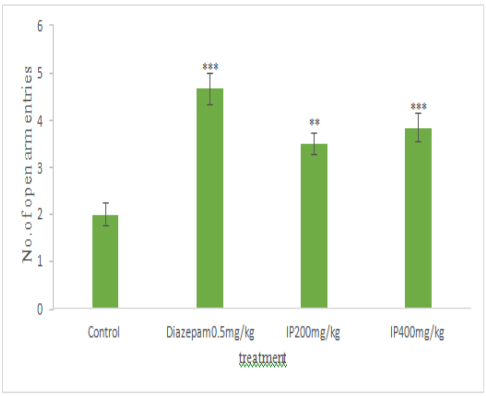
Administration of diazepam significant increase in the number of open arm entries, timespent in the open arms and number of rears in the open arm. They showed a reduction in time spent in close arms. Plant extract treated mice exhibited significant increase in the number of open arm entries,time spent in the open arm,number of total arm entries and number of rear sinthe open arms, but decrease in time spent in the closed arms. (Table3)

Table 3: effect of administration of *Ipomea Mauritiana* ethanolic extract on mice behavior in elevated plus maze

Treatment	Number of open arm entries	Number of total arm entries	Percentage of open arm entries	Time spent in open arm (s)	Time spent in closed arm(s)	Percentage of total time spent in open arm	Number of rears in open arm
Control	2±0.25	6.5±0.61	44.44±4.36	24.66±2.77	275.33±2.77	8.22±0.92	1.16±0.16
Diazepam (0.5 mg/kg)	4.66±0.33***	11.16±0.54***	71.42±3.01***	103.33±4.99***	196.16±4.99***	34.60±1.66***	3.83±0.30***

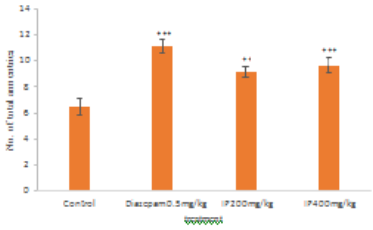
IP (200 mg/kg)	3.5±0.22**	9.16±0.40*	61.66±2.68**	84.16±15.62**	215.83±15.62**	28.05±5.20**	2.5±0.34*
IP (400mg/kg)	3.83±0.30***	9.66±0.61**	65.23±1.81***	101±8.64***	199±8.64***	33.66±2.88***	3.5±0.42***

All values are mean ± SEM, No of animal (n) =6, Statistical analysis by one-way ANOVA followed by tukey's *post-hoc* test, *p<0.05, **p<0.01, ***p<0.001



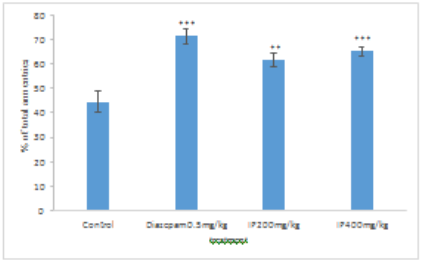
Graph 1: Number of open arm entries in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, **p<0.01, ***p<0.001 compare with control group.



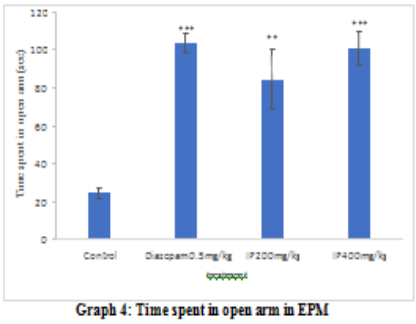
Graph 2: Number of total arm entries in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, *p<0.05, ***p<0.001, compare with control group



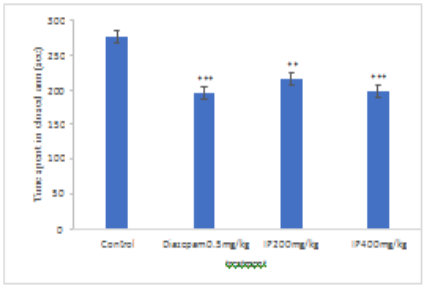
Graph 3: Percentage of open arm entries in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, **p<0.01, ***p<0.001, compare with control group.



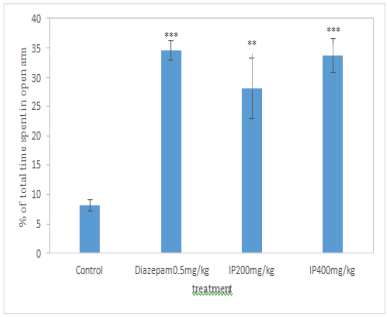
Graph 4: Time spent in open arm in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, **p<0.01, ***p<0.001, compare with control group.



Graph 5: Time spent in closed arm in EPM

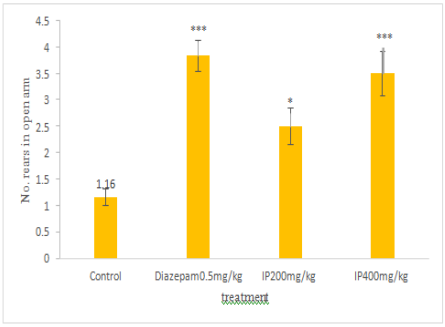
Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, **p<0.01, ***p<0.001, compare with control group.



Graph 6: Percentage of total time spent in open arm in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, **p<0.01, ***p<0.001, compare with control group.

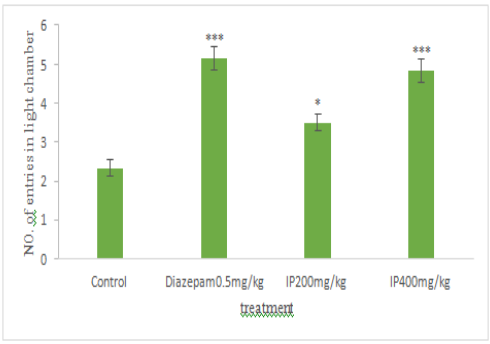
ISSN: 0976-7126
Jaiswal *et al.*, 12(1):10-16, 2021



Graph 7: Number of rears in open arm in EPM

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test, *p<0.05, ***p<0.001, compare with control group.

also showed a reduction in duration of immobility at all two doses. (Table 4)



Graph 8: Number of entries in light chamber

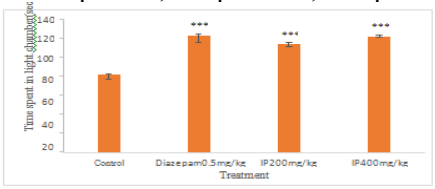
Light and dark model

The standard drug diazepam treated mice have spent increased time in light area and also rear significant. Plant extract treated mice showed an increase in the time spent in light area and they

Table 4: Effect of administration of *Ipomea Mauritiana* ethanolic extract on mice behavior in light and dark model

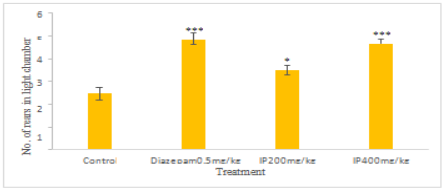
Treatment	Number of light chamber entries (s)	Time spent in light chamber (s)	Number of rears in light chamber (s)	Duration of immobility(s)
Control	2.33±0.21	81.5±4.18	2.5±0.22	75.5±3.17
Diazepam (0.5mg/kg)	5.16±0.30***	123±7.24***	4.83±0.30***	42.83±1.13***
IP (200mg/kg)	3.53±0.22*	113±2.84***	3.5±0.22*	57±2.46***
IP (400mg/kg)	4.83±0.30***	122±1.39***	4.66±0.21***	44.16±1.07***

Values are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test. *p<0.05, ***p<0.001, compare with control group.

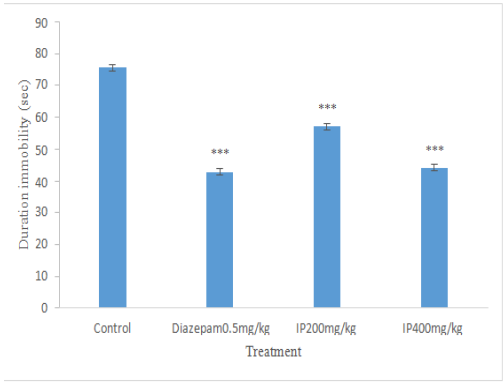


Graph 9: Time spent in light chamber

ues are expressed as Mean ± SEM. Data was analyzed by one-way ANOVA followed by tukey's *post-hoc* test. ***p<0.001 compare with control group.



Graph 10: Number of rears in light chamber



Graph 11: Duration of immobility in light darkmodel

Anxiety disorder are due to involvement of GABAergic, serotonergic, involvement. This two, adrenergic and dopaminergic system played important role in anxiety. Since 40 years BZA used in of anxaiety, but due to their unwanted side effect, alternative treatment strategies with treatment favorble side effect profile. Medicinal plants are good source to find new remedies for these disorders. Despite the wide spread traditional use of *Ipomea Mauritiana* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that the *Ipomea Mauritiana* extract had anxiolytic activity in mice by elevated plus maze, light dark model.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety. In the elevated plus maze, the open arms are more fear provoking to compare than closed arms. The reduction in entry and time spent in open arms are indications of high level of fear and anxiety. Then Number of entries and time spent in the open arms have been found to be increased by anxiolytic and reduced by anxiogenicagents. A significant increase in the time spent in open arms was observed after treatment with all two doses of *Ipomea Mauritiana* A significant increase in both times pent in open arms is observed after treatment with 400mg/kg of *Ipomea Mauritiana* extract suggesting anxiolytic activity.

The light/dark exploration test is based on the natural aversion of mice to brightly lit places. Reduction in the number of entries, time spent and rearing behavior in the light chamber are regarded as markers of anxiety. Anxiolytics reduce the natural aversion to light and increase the time spent in the light compartment. In this model, *Ipomea Mauritiana* extract in the dose of 200mg/kg and 400mg/kg produces significant increase in the time spent in the lighted box and reduction in immobility at all two doses ,thus demonstrating its anxiolytic like activity.

All these behavioral changes in both paradigms are suggestive of decreased anxiety, decrease aversion to light and increased exploratory behavior of the animal which are comparable changes produced by the standard drug diazepam.

Conclusion

Anti-anxiety activity was evaluate by Elevated plus maze (EPM) model and Light-dark model. Diazepam was taken as standard reference drug. All the extract have been shown a significant activity when compared to control it concluded that active constituents responsible for anti-anxiety activity might be present in the leaf extracts. However, further investigation are necessary to find the exact mechanism of anxiolytic effect and to isolate the active compound (s) responsible for this pharmacological activity.

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Cite this article as:
Jaiswal P., Pyathi A.R., Sujane S. and Khan T. (2021). Antianxiety effect of ethanolic extract of leaves of *Ipomea mauritiana* in swiss albino mice , *Int. J. of Pharm. & Life Sci.*, 12(1): 10-16.

Source of Support: Nil
Conflict of Interest: Not declared
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