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Evaluation of anti-anxiety activity of *Lawsonia inermis* Linn.

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Abstract

Anxiety is one of the most common mental disorders, characterized by changes in mood, behavior, somatic function, and cognition. Benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety. Synthetic drugs available for treatment of anxiety have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease. *Lawsonia inermis* commonly known as henna is a perennial herbaceous plant belonging to family Lythraceae. Traditionally it has been used to treat skin diseases, dysentery, bronchitis, anemia and inflammation. The aim of present study was to investigate the anxiolytic activity of methanolic extracts of *Lawsonia inermis* leaves in mice. Methanolic extracts were administered orally at a dose of 200 and 400mg/kg bw. The results were analyzed by One Way Analysis of Variance (ANOVA).The results showed the extracts of leaves showed significant anxiety relatively methanolic extract. The findings concluded that *Lawsonia inermis* leaves exhibit anxiety and further studies are suggested to isolate the active principles responsible for the activity.

Keywords: *Lawsonia inermis*, Anxiety, Methanolic extract

Introduction

Lawsonia inermis(henna) belonging to the lythraceae family is a very widespread medicinal plant and natural dye in the world[1].have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interesinthe use of herbal remedies. Further more many western drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. Given their potential to produce significant therapeutic effect, they can be useful as drug or supplement in the treatment / management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compound(s) have demonstrated spectrum of biological activities have[2].

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Henna is an important source of phytochemicals such as naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones, flavonoids, gallic acid, hennotannic acid and mannitol which are effective as immunomodulators and other allied agents[3]. In the present study anti-anxiety activity of methanolic extracts of *Lawsonia inermis* is carried out.

Material and Methods

Selection of plant

The plant was selected based on the traditional claims

Collection, identification and authentication of Plant material

Plant material (fruits) were collected from local market in Indore and authenticated by Dr. S. N. Dwivedi, Professor of Botany, Janata PG College, APS University, Rewa, (M.P.).

Preparation of extracts

Coarsely powdered leaf was extracted using soxhlet apparatus with petroleum ether. After drying, the residue was extracted with methanol and was filtrated. The methanolic solution (filtrate) was evaporated to dryness to get methanolic extract. The methanolic extract obtained was screened for phytochemical analysis.

Animal

Albino mice, weighing 20-30gm, were obtained from the animal house of the Department of Pharmacology of the Swami Vivekanand College of Pharmacy, Indore, India. Animals was housed at four per cage, allow them, free access to water and food, and was maintained under constant temperature (23±1^{0}C) and humidity (60±10%) under 12-h light/dark cycle. Animal treatment and maintenance was conducted accordance to the Principles of Laboratory Animal Care[4].

Anti-anxitey activity

Experimental design

Elevated plus maze (EPM) test

This model is based on natural behavior of mice for open spaces and fear of height. Mice always tend to avoid the open areas and stay in darker areas, more enclosed spaces. When animal is placed on EPM anxious animals spend more time in enclosed arms and non-anxious animals explore and spend more time open arm.

The plus-maze consists of two open arms, 43 × 15 cm (L ×W), and two enclosed arms, 43 × 15×23cm (L ×W×H), opened to the top, arranged in such way that the two open arms are faced opposite to each other. The maze is elevated to a height of 70 cm. The mice weighing 25-30gms body weight are randomly selected irrespective of sex and grouped into 4 groups so that each group consisting of 5mice. (LD50 of AM is >2000mg. After one hour of oral administration of the test drug or the standard, the rat is placed at the centre of the maze, facing towards one of the enclosed arms. After 5min of observation the following parameters are noted: The number of entries into open arm and closed arms and time spent in the open and enclosed arms.[5]

Light and dark transition test

The albino mice of either sex divided into 4 groups of 5 animals each. Group I received 0.1ml normal saline (NaCl) orally for seven days as a control group. Group II received 5mg/kg of diazepam orally for seven days as a standard drug. Group III received 200 mg/kg methanol extract orally for seven days. Group IV received 400mg/kg methanol extract orally. Anxiety was induced in all groups by put on dark chamber in mice. On the second day caused anxiety

The apparatus was cleaned thoroughly between trials.

A total number of 20 mice were divided into four groups of five mice each:

Group I: control (Normal Saline, 0.1ml/kg)

Group II: standard (Diazepam5mg/kg)

Group III: Test (methanolic extract of *Lowsonia inermis* Linn 200mg/kg)

Group IV: Test (methanolic extract of *Lowsonia inermis* Linn400mg/kg)

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of mice in response to mild stressors, that is, novel environment and light. The apparatus for light/dark transition test consist of two compartments: one light area (15 L.15 W x 30 H cm), illuminated by 100 W desk lamp was painted white, and the other dark area (15 L x 15 W x 30 H cm) was painted black. The two compartments were separated by a partition with a tunnel to allow passage from one compartment to the other A mice was put into the light box facing the hole. The alteration among the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received5.

Statistical analysis

All the data represent mean±S.E.M. values. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. All analyses were performed using the SPSS statistical software. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

Results and Discussion

Methanolic extract was subjected preliminary phytochemical screening; the results were presented in table 1.

Table 1: Indicating presence of various phytochemical constituents

S.NO.	Test	Positive/negative
1	Carbohydrate	+
2.	Terpenoids	+
3.	Flavone glycoside	+
4.	Phenolic compound	+
5.	Flavonoids	+
6.	Saponins	-

Note:+=present: -=absent

The methanolic extract of leaves of *Lawsonia inermis* Linn. were subjected to evaluation of anti-anxiety activity.

Table 2: Result of elevated pluse maze

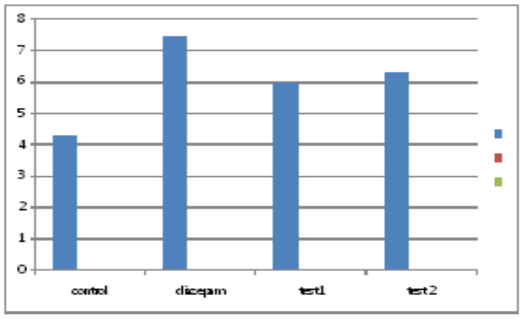
Drugs	Dose	No.of entries in open arm	Time spent in open arms
Control	0.1mg/lkg	4.25±0.35	215±8.5
Diazepam	5mg/kg	7.45+0.57*	356±7.5**
Test 200mg	200mg/kg	5.97±0.42**	246±6.3**
Test 400mg	400mg/kg	6.27±0.31**	275±6.2*

All reading are expressed as mean±S.E.M, Values obtained was compared with Turkeys test and found to be statistically significant* p<0.05; **p<0.001

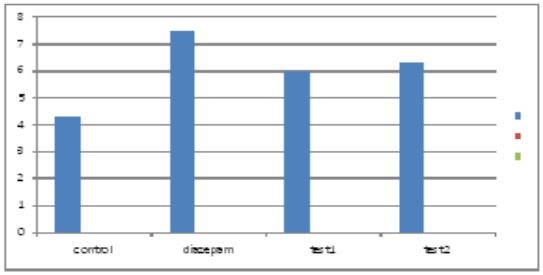
Table 3: Results of light and dark model test

Drug	Dose (mg/kg)	Light area (sec) mean+S.E.M.	Dark area (sec) Mean+S.E.M.
Control	0.1mg/kg	20.4±1.5	6.3±2.20
Diazepam	5mg/kg	70.2±2.5**	16.3±3.28**
Test 200mg	200mg/kg	29.5±4.2*	8.3±3.19*
Test 400mg	400mg/kg	49.2±3.1**	11.21±1.20**

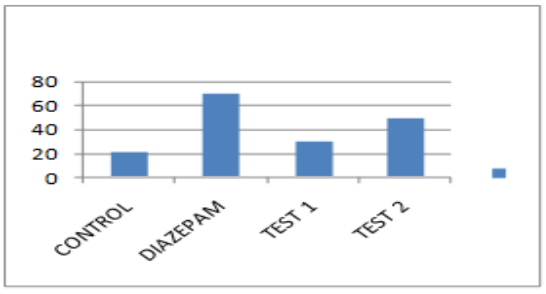
All reading are expressed as mean±S.E.M, Values obtained was compared with Turkeys test and found to be statistically significant* p<0.05; **p<0.001



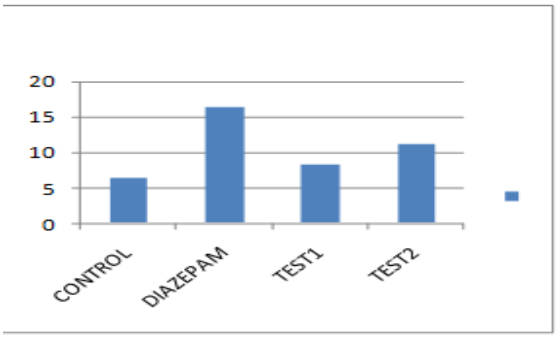
Graph 1: Plusemaze in no. of entries in open arm



Graph 2: Plusemaze in time spent in open arms



Graph 3: Light and dark model in light area



Graph 4: Dark area

Conclusion

The results obtained from these experimental models clearly confirmed that the anti-anxiety activity of methanolic extracts of *lawsonia innermis* leaves. The acute treatment with (200mg/kg) clearly demonstrates a dose dependant anti-anxiety effect comparable to diazepam (5mg/kg; i.p) in all experimental models of anxiety. The phytoconstituent like chrysin flavonoids were reported for their anti-anxiety activity and these constituents were present in *lawsonia innermis* leaves so this active principle might be responsible for anti-anxiety activity. The mechanism of anti-anxiety activity of *lawsonia innermis* leaves extracts is unclear hence further studies are needed to identify the mechanism and the phyto-constituents responsible for the effects of the methanolic extract of *lawsonia innermis* leaves.

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