EVALUATION OF CNS STIMULANT POTENTIAL OF DELONIX REGIA LEAF EXTRACTS

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ABSTRACT

The leaves of Delonix regia grow alternately on the stem, biparpipinnate with stout petiole. The extraction ability of different solvents for recovering extractable components from leaves followed the order: methanol>ethylacetate>water>n-hexane. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaf of the plant. All the extracts were subjected to in vivo determination of CNS stimulant potential using photoactometer recognizable locomotion test and tail suspension test to evaluate the behavior of the mice. The results reveal that all the extracts except n-hexane extracted component was able to increase the locomotion in mice significantly ($p<0.05$) as evidenced by actophotometer test. The ethanolic extract exhibited better CNS stimulation compared to the standard drug caffeine at a dose of 200 mg/kg per oral. From the results that immobilization time of the ethanolic extract was comparable at both the evaluated doses was comparable to that of caffeine while the ethylacetate and aqueous extracts were able to reduce the immobility time by a lower margin compared to the control group. The n-hexane extract was unable to reduce the immobilization time in mice. Diazepam was used as the negative control to monitor immobility and lack of locomotor response while caffeine was used as the reference drug to compare the CNS stimulant potential.

Keywords: - *Delonix Regia; CNS Stimulant Potential; Photoactometer recognizable locomotion test, Mice.*

1. INTRODUCTION

Central nervous system stimulants are drugs or medicines that are used for the treatment of attention deficit hyperactivity disorder and, also in some cases, for narcolepsy [1]. Some CNS stiulatns like modafinil are used to treat excessive somnolence that can occur due to shift work disorder, narcolepsy and obstructive sleep apnea [2]. The two classes of drugs that are prominently used to stimulate the CNS are psychomotor stimulants and hallucinogens. The psychomotor stimulants usually cause excitement and euphoria while decreasing the feelings of fatigue, and increasing the motor activity. The hallucinogens produce reflective changes in thought patterns and mood, with minimally producing effects related to the brainstem and spinal cord [3].CNS stimulants have also been safely and effectively used for treating patients with post-stroke depression, and patients with human immunodeficiency virus (HIV)-associated depression [4]. However the CNS stimulants have been misused for recreational effects related to them and have a potential of abuse. The non-monitored used of the herbal recreational drugs and CNS stimulants leads to damage of the neuropsychobiological integrity of humans. They disrupt the psychological equilibrium of the human body by acutely stimulating multiple mood states and then impairing them during the recovery time after the effect of the drug is over. This uncertainty

in mood states can be an index for more intense psychological changes [5]. Cocaine, caffeine, Tea (*Camellia sinensis*), Coffee (*Coffea robusta/arabica*), Cocoa (*Theobroma cacao*), Cola Nut (*Cola nitida/acuminata*), Guarana (*Paullinia cupana*), Yerba Mate (*Ilex paraguariensis*), Ephedra, Khat (*Catha edulis*), Ginkgo, *Centella asiatica* and *Panax ginseng* are some herbs widely used world- wide for their CNS stimulant potential [6].

Delonix regia is a species of the flowering plant in belonging to family Fabaceae, subfamily Caesalpinioideae and is native to Madagascar. It is famous for its fern-like leaves and glitzy portray of orange-red flowers throughout summer. In several tropical regions of the world it is grown as an ornamental tree and is widely called as flamboyant, flame of the forest, or flame tree. In Hindi the plant is named as Gulmohar. The flowers of *Delonix regia* are big, with four scarlet or orange-red spreading petals up to 8 cm in length, and a fifth upright petal called the standard, which is slightly larger and with yellow and white spots. They appear in corymbs along and at the ends of branches. The pods are green and flaccid when young and turn dark-brown and woody on maturing. They can be up to 60 cm long and 5 cm wide. The seeds are small with an average weight of around 0.4 g. The compound (doubly pinnate) leaves have a feathery appearance and is a characteristic light, bright green. Each leaf is 30–50 cm long with 20 to 40 pairs of primary leaflets or pinnae, each divided into 10–20 pairs of secondary leaflets or pinnules. Pollen grains are elongated, approximately 52 microns in size. The leaves, flowers, seed and bark of this plant contain a range of medicinally active compounds, though the leaves are generally the richest source of most of these compounds. The plant is reported to have antibacterial, antidiabetic, antidiarrhoeal, antifungal, antiinflammatory, antimalarial, antimicrobial, antioxidant, cardio-protective, gastro-protective, hepato-protective and wound healing activity [7]. It is used in folk medicine to treat a range of disorders, including constipation, inflammation, rheumatoid arthritis, diabetes, pneumonia, and malaria. The active compounds include flavonoids, alkaloids, saponins, sterols, beta- sitosterol, lupeol, tannins, carotenoids, and phenolic acids [8].

Figure 1 *Delonix regia* Tree

The leaves, flowers, seed and bark of this plant contain a range of medicinally active compounds, though the leaves are generally the richest source of most of these compounds. The plant is reported to have antibacterial, antidiabetic, antidiarrhoeal, antifungal, antiinflammatory, antimalarial, antimicrobial, antioxidant, cardio-protective, gastro-protective, hepato-protective and wound healing activity.

2. MATERIALS AND METHODS

2.1. Selection of the Plant

Several studies have suggested that preventing the oxidative stress by use of antioxidants can lead to change in behavioral pattern (can reduce depression). This information formed the basis to select the plant and scientifically investigate its CNS stimulant potential.

2.2. Collection and Identification of Plant Material [9-11]

The leaves of D. regia were collected from the herbal garden of Technocrats Institute of Technology-Pharmacy, Madhya Pradesh in the month of November and authenticated at TIT Pharmacy, Bhopal. The voucher specimen was deposited in Department of Pharmacognosy, Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh for future reference.

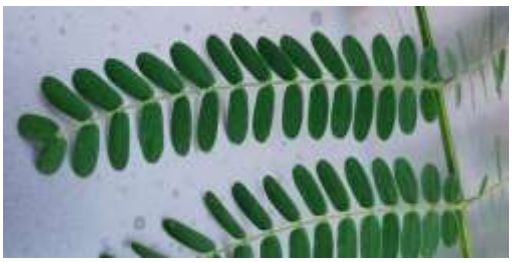

Figure 2.2 Leaf of Delonix regia

2.3. Extraction of leaves [12]

The powdered leaves were used for the extraction process. 500 g of powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity including n- hexane, ethyl acetate and ethanol (90%) by hot continuous extraction process for about 18 h. The aqueous extraction was carried out by cold maceration process after completion of the solvent extraction process. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extracts were concentrated by distillation to reduce the volume to 1/10. The concentrated extracts were transferred to 100 ml beaker and the remaining solvents were evaporated on water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture. The dried extracts were stored in desiccators for further processing.

2.4. Preliminary phytochemical screening [13]

All the extracts were evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in them.

2.4.1. Test for Alkaloids

2.4.1.1. Mayer's Test: To a few ml of plant sample extract, two drops of Mayer's reagent was added along the sides of test tube.

2.4.1.2. Wagner's test: A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube.

2.4.1.3. Hager's test: A few drops of Hager's reagent were added to few ml of plant extract along the sides of test tube.

2.4.1.4. Dragendroff's Test: A few drops of Dragendroff's reagent were added to 1 ml of each extract.

2.4.2. Test for Glycosides

2.4.2.1. Saponin glycosides

2.4.2.1.1. Froth test: 1 ml solution of the extract in water was placed in a test tube and shaken vigorously.

2.4.2.2. Cardiac glycosides

2.4.2.2.1. Kedde's test: The extract was extracted with chloroform and evaporated to dryness. One drop of 90% alcohol and 2 drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid Kedde's reagent) in 90% alcohol are added to the above residue. The solution is made alkaline with 20% sodium hydroxide solution.

2.4.3. Test for Tannins and phenolic compounds

2.4.3.1. Gelatin test: To the extract was added 1% gelatin solution containing 10% sodium chloride.

2.4.3.2. Ferric chloride test: To the extract was added a freshly prepared solution of ferric chloride.

2.4.3.3. Vanillin hydrochloride test: Test solution of the extract was treated with few drops of vanillin hydrochloride reagent.

2.4.3.4. Alkaline reagent test: Test solution of the extract was treated with sodium hydroxide solution.

2.4.4. Test for Flavonoids

2.4.4.1. Shinoda test: To the test solution of the extract, few fragments of magnesium ribbon were added and conc. hydrochloric acid was mixed drop wise to it.

2.4.4.2. Zinc hydrochloride reduction test: To the test solution a mixture of zinc dust and conc. hydrochloric acid was added.

2.4.5. Test for Proteins and amino acids

2.4.5.1. Millons test: Test solution of the extract was allowed to react with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).

2.4.5.2. Ninhydrin test: The solution of extract was boiled with 0.2% solution of ninhydrin.

2.4.6. Test for Sterols and triterpenoids

2.4.6.1. Libermann Burchard test: Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added from the sides of the test tube.

2.4.6.2. Salkowski test: The extract was dissolved in chloroform and a few drops of conc. sulphuric acid were added to it. The mixture was shaken well and allowed to stand for some time.

3. DETERMINATION OF CNS STIMULANT POTENTIAL [14]

Various animal models have been reported for evaluation of the CNS stimulant potential of drugs and molecules. These include the hole-board test, the actophotometer test, the rotarod test, the elevated plus maze test, the Y maze test and open field test.

The in vivo antidepressant action of the extracts was carried out in albino mice weighing between 25–30 g by Actophotometer and tail suspension test (TST). The protocol of the present work was approved by Institutional Animal Ethical Committee (IAEC), Technocrats Institute of Technology-Pharmacy Bhopal, India (TIT/IAEC/831/P'Col/2020/02).

The animal were grouped and housed in poly acrylic cages (38x23x10 cm) in the animal house of the institute. Not more than four animals per cage were housed and maintained under standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water ad libitum for one week for acclimatization before and during the experiments.

Animal were divided into 5 groups of 6 animals each for conducting the study. Group I was administered 1% CMC and served as the control, group II was administered with diazepam, 10 mg/kg (p.o) and served as negative control, group III & IV were administered with 100 & 200 mg/kg (p.o) of the extracts, whereas group V served as positive control and was administered with caffiene,10 mg/kg (p.o).

3.1. Actophotometer method [15]

The basal activity counts of each mouse were observed for 15 minutes 2 days before starting the evaluation of the extracts. A count was recorded when the beam of light falling on the photo electric cell of actophotometer was cut off by mice causing a count in the counter to observe the locomotor behavior.

The extracts and caffeine were dispersed in 1% CMC and administered orally in a standard volume of 0.5 mL per 20 g body weight, to each mouse 30 minutes prior to the test. The locomotor behavior of the mice was monitored and for a period of 15 minutes. The difference in the number of counts for each group was recorded.

3.2. Tail Suspension Test (TST) [16]

The extracts and caffeine were dispersed in 1% CMC and administered orally in a standard volume of 0.5 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the extract mice were individually suspended by tail using clamp (2 cm from the tip of the tail) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Minimal background noise was maintained and the testing was carried out in dark room. All animals were suspended for total 6 minutes, and the duration of immobility was observed and noted during the final 4 minutes of the test. Mice were considered immobile only when they hung passively and completely motionless. The animals were used only once for this test.

3.3. Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.05 ($P<0.05$) *vs* control.

4. RESULTS AND DISCUSSIONS

The present work focused on preparing successive solvent extracts of Delonix regia and establishing its CNS stimulant action in animal models The results obtained from the investigation are presented:

4.1. Extraction Yields: The extraction abilities of different solvents for recovering extractable components from leaves followed the order: methanol>ethylacetate>water>n- hexane. Shabir et al also reported that methanol provided maximum yield among various solvents used for extraction.

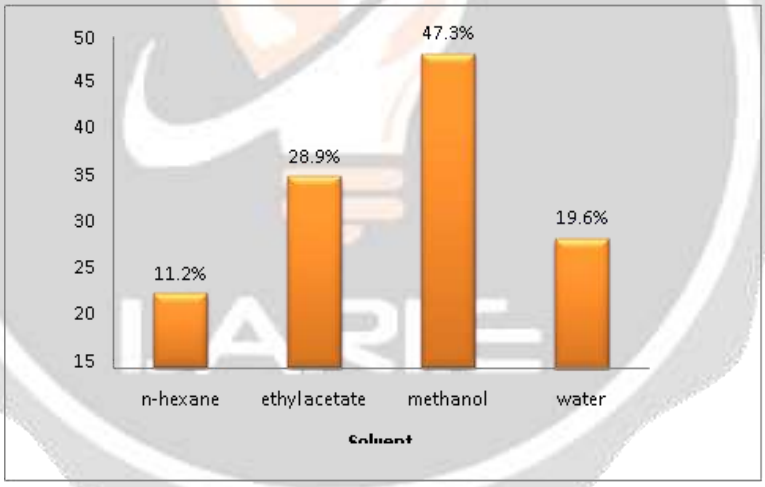

Chart 4.1 Extraction yields of different solvents

4.2. Evaluation of CNS stimulant Potential

All the dried extracts of the plant were subjected to in vivo evaluation of their capability to affect the neurological functioning in rodents by observing for their CNS stimulant potential as a function of their locomotor activity using TST and actophotometer method.

4.2.1. Actophotometer method

The photoactometer is a chamber used to observe locomotor activity of the mice. The instrument has continuous beams of lights, across the chamber and incident on corresponding photoelectric cell. When animal moves in the cage of the photoactometer, it interrupts the beam of light falling on the photoelectric cell producing a reading in the counter. The number of readings on the counter is a measure of the locomotor activity of the animal. Different groups of animal were allowed to move

in the photoactometer for a period of 15 minutes and the locomotion was recorded for each animal of each group.

Group	Average number of movements in 15 minutes	
	Count on actophotometer	% locomotion
Control	176.3±3.077	-
Diazepam	114.8±3.312	65.11
Caffeine	205.7±3.360	116.7
Extract 100 mg	141.2±5.913	80.09
Extract 200 mg	167.8±3.920	95.17

Table 4.2.1 Effect of n-hexane extract on locomotion (photoactometer test)

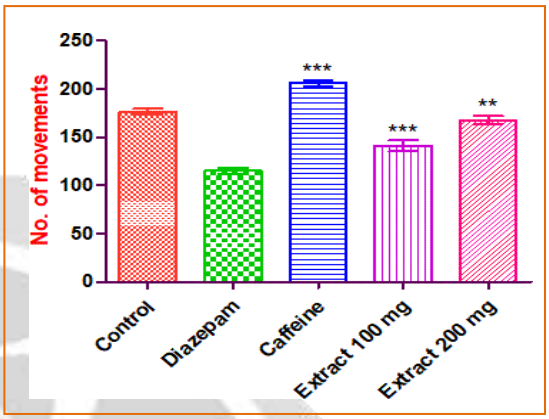

Figure 4.2.1 Effect of n-hexane extract on locomotion

All the extracts were able to make changes in the locomotor activity of mice though the n-hexane extract was not very significant in inducing locomotion. A comparative analysis of the results of locomotor activity for the different extracts is presented in Figure:

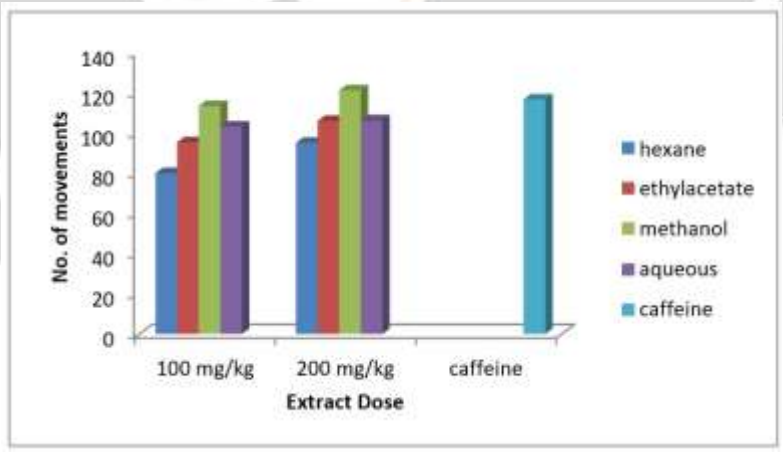

Figure: Comparative % locomotion exhibited by various extracts of Delonix regia

4.2.2. Tail Suspension Test

TST is used to measure the effect of drug on the ability of the animal to improvise or reduce mobilization. All the extracts were able to reduce the immobilization time of mice though the n-hexane extract was not significant in reducing immobilization time. A comparative analysis of the results of TST for the different extracts is presented in Figure:

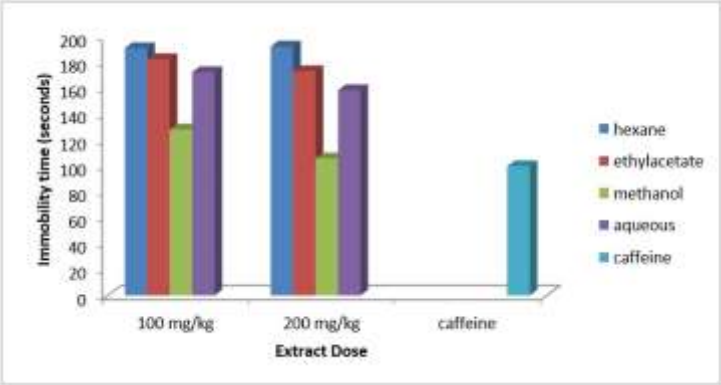

Figure: Comparative immobilization time exhibited by various extracts of Delonix regia

5. CONCLUSION

The objective of the present study was to assess the CNS stimulant potential of leaf extracts of Delonix regia using the in vivo models in mice. The results obtained led to the conclusion that Delonix regia leaves are a rich source of potential flavonoids. The CNS stimulant potential of the ethanolic extract was comparable to caffeine making it a potential herb for behavioral issues in human.

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