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PHARMACOLOGICAL EVALUATION OF ANTI DEPRESSANT ACTIVITY OF CAESALPINIA PULCHERRIMA IN ANIMAL MODELS

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ABSTRACT

Caesalpinia pulcherrima belongs to the family fabaceae. Depressions are widespread psychiatric disorders affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants have been used to treat anxiety and depression for thousands of years. The present study was designed to evaluate the antidepressant activity of the alcoholic and aqueous extracts of Caesalpinia pulcherrima leaves in rodents. The antidepressant activity was tested by using forced swim test and Open Field Test. The results infer that reduced immobility time elicits antidepressant activity. It was concluded that alcoholic and aqueous extracts of Caesalpinia pulcherrima leaves having antidepressant activity. Alcoholic extract of Caesalpinia pulcherrima leaves showing more significant activity over the aqueous extract.

Keywords: Caesalpinia pulcherrima, Antidepressant activity, forced swim test, Open Field Test.

1. INTRODUCTION

World Health Organization reported that 80% of the world's population depends on medicinal plants for their primary health care. In the Plant Kingdom, Medicinal plants form the largest single grouping of plants. It is estimated that 30,000 species worldwide fall in this group, of which around 33% are trees[1] Plants are known to be the source of many chemical compounds. In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins[2].

The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized. Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity[3].

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants[4]. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry.

About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments[5], which also forms a rich source of knowledge. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments[6]. In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases[7]. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects.

2. MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation

including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India. Diazepam was collected from Nicholos Piramal Ltd.

2.1.Experimental animals:

Wistar rats (150-200 g) and Swiss albino mice (18-22g) of either sex selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and12:12 h light – dark cycle. Animal studies had approval of IAEC.

2.2.Plant Material Collection:

The fresh leaves of Caesalpinia pulcherrima was collected from local market. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

2.3.Preparation of Aqueous Extract:

Fresh leaves of Caesalpinia pulcherrima were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

2.4.Preparation of Alcoholic Extract:

Fresh leaves of Caesalpinia pulcherrima leaves were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

2.5.Selection of dose for animal study:

The dose considered for the experiment on rats was obtained from conversion of human dose of Caesalpinia pulcherrima (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice (Ghosh 1984). Hence the calculated dose for the rats (considering human dose3 and 5 g/kg) is 200 mg/kg and for mice is 20 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

2.6.Pharmacological evaluation:

2.6.1.Preparation of extracts:

The aqueous and alcoholic extracts of Caesalpinia pulcherrima suspended in water in presence of 3%v/v Tween-80 solution. All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

2.6.2.Acute oral toxicity:

The acute oral toxicity of aqueous and alcoholic extracts of Caesalpinia pulcherrima was determined by using rats and mice which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

2.6.3.Screening for antidepressant activity:

The aqueous and alcoholic extracts of Caesalpinia pulcherrima leaves were tested for antidepressant activity using despair swim test and tail suspension test.

2.7.Treatment:

Animals were divided into four (I-IV) groups.

Group I: Control group received distilled water (1ml, p.o).

Group II: Standard group received Diazepam (10mg/kg i.p).

Group III: Test group received aqueous extract of Caesalpinia pulcherrima (200mg/kg p.o).

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Group IV: Test group received alcoholic extract of Caesalpinia pulcherrima (200mg/kg p.o).

2.8.Despair Swim Test Apparatus:

For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10cm, at 25 ± 2°C. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

2.9.Tail suspension test:

Tail suspension test was performed based on the method prescribed. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remain motionless.

2.10.Open field test (OFT):

This test was carried out on mice's to evaluate the effects of investigational drug on mobility of animal. Open field equipment was made of plywood which is white in colour and measured 72 by 72 and wall is 36cm long In this test mice's were treated individually with DMSO, standard drug Diazepam (10mg/kg) and testing drugs ethanolic extracts of Caesalpinia pulcherrima L. (200/ml). Then placed them independently in the middle of the open field for 5minutes to count Total Locomotion (TL) i.e. the total number of square crossed both outer and inner ones, Peripheral Locomotion (PL), and Central Locomotion (CL) respectively. The other factors, which were also evaluated, are number of rearing, leaning, grooming and defecation

2.11.Statistical analysis:

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparision had made. i.e. Normal control Vs All treated groups. Differences between groups were considered significant at $P<0.001$ and $P<0.05$ levels.

3. RESULTS AND DISCUSSION

3.1.Antidepressant activity by Forced Swim Test:

Antidepressant activity of aqueous and alcohol solvent soluble fraction of the leaves of Caesalpinia pulcherrima studied at a dose of 200 mg/Kg, using Forced Swim Test experiment. The anti-depressant activity of Aqueous Extract of Caesalpinia pulcherrima (AQECP) and Alcoholic Extract of Caesalpinia pulcherrima (ALECP) was assessed using Forced Swimming Test in Swiss albino rats were illustrated in table No: 1 and Open Field Test in Swiss albino rats were illustrated in table No: 2 It was observed that AQECP and ALECP at a dose of 200mg/kg exhibited significant reduction in immobility time when compared to control in dose dependent manner. Similarly the animals treated with diazepam (10mg/kg) as expected showed significant decrease in immobility time.

Table 1: Effect of extracts of Caesalpinia pulcherrima on Anti-depressant activity

S.No	Group	Dose (i.p; mg/kg)	Immobility period		% change in activity
			Before	After	
1	Control	5ml/kg	130	--	---
2	Diazepam	10mg/kg	183	65	65.73%
3	AQECP	200mg/kg	175	69	61.93%
4	ALECP	200mg/kg	303	197	35.14%

*The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

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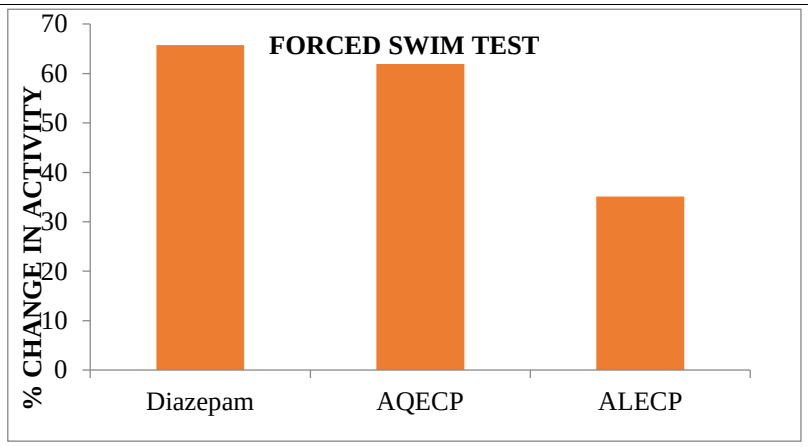

Fig-1: Effect of extracts of Caesalpinia pulcherrima on Anti-depressant activity

Table 2: Effects of Caesalpinia pulcherrima on duration of immobility time by Open Field Test

Treatments	Dose (mg/kg)	TL	CL	PL	L	G	D
Control	---	140.1±3.1	30.1±6	110.1±5.14	8.0±1	1.5±1.51	0.31±1.00
Diazepam	10	152.6±2.72	36±2.08	116.6±2.33	9.0±1.52	0.33±0.32	0.0±0.0
AQEMP	200	119.3±5.24	24.3±2.4	95.0±5.5	11.3±2.02	1.33±0.32	0.0±0.0
ALEMP	200	135.6±13.5	27.3±5.04	27.3±5.04	10.3±1.20	2.33±1.20	0.0±0.0

*Values are expressed as Mean ± S.E.M (n=10). *P <0.05, **P<0.01, ***P<0.001 when compared with control groups. TL: Total Locomotion, PL: Peripheral Locomotion, CL: Central Locomotion (CL), L: leaning, G: grooming, D: defecation.

For the open field test number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration and/or a lower level of anxiety. The number of central square entries and the duration of time spent in the central square are measures of exploratory behavior and anxiety. A high frequency/duration of these behaviors indicates high exploratory behavior and low anxiety levels.

3.2.Phytochemical analysis:

The Phyto constituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis reveals the presence of alkaloids, flavonoids, tannins, terpenoids and saponins have associated with various degree of anti-microbial, anti-bacterial, anti-fungal, anti-oxidant and anti-termites. Therefore, the anti-diabetic, hypoglycemic, anti-depressant, anti-anxiety, skeletal muscle relaxant property, locomotor activity, anti-inflammatory, analgesic and diuretic activities were observed in this study may be due to the presence of chemical constituents in both aqueous and alcoholic extracts of Caesalpinia pulcherrima.

3.3.Behavioral activities:

3.1.Anti-depressant activity:

3.1.1.Open Field Test (OFT):

Open field behavioral model was used to study exploratory and locomotor activity in this investigation. Reported studies have shown that stress factors account for the decreases in mobility and functional responses against novel environment. The purpose of including this test was to assess the general activity of the animals after performing FST. The results observed in the open field test showed that i.p administration of aqueous and alcoholic extracts of Caesalpinia pulcherrima (200 mg/kg) did not significantly increase the locomotor activity in unstressed groups of rats as compared with their control groups. However, aqueous and alcoholic Caesalpinia pulcherrima administered rats following the exposure to repeated restraint stress showed significant ($p<0.01$) increases in locomotor / exploratory activity on an open field arena. It is therefore, suggested that the extract has the ability to reverse or normalize the locomotor suppressant behavior in laboratory animals and hence may help to cope with immobility factor associated with depression in humans. In the present study that administration of aqueous and alcoholic Caesalpinia pulcherrima at the dose of 200 mg/kg significantly altered the behavioral deficits induced by injections of atypical neuroleptic, haloperidol and increased brain serotonin metabolism in mice. The results are in general agreement with our previous studies in continuation to this plant and indicating its antidepressant-like activity in behavioral models of depression.

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3.1.2.Forced Swim Test:

Mood disorders are one of the most common mental illnesses, with a lifetime risk of 10% in general population. Prevalence of depression alone in general population is estimated to be around 5% with suicide being one of the most common outcomes. Commonly used Antidepressants often cause adverse effects, and difficulty in tolerating these drugs is the most common reason for discontinuing an effective medication, for example the side -effects of Selective Serotonin Reuptake Inhibitor (SSRIs) include: nausea, diarrhea, agitation, headaches. Sexual side-effects are also common with SSRI's. The Food and Drug Administration requires Black Box warnings on all SSRIs, which state that they double suicidal rates (from 2 in 1,000 to 4 in 1,000) in children and adolescents. Side effects of Tricyclic Antidepressants (TCA's) include drowsiness, anxiety, emotional blunting (apathy/anhedonia), confusion, restlessness, dizziness, akathisia, hypersensitivity, changes in appetite and weight, sweating, sexual dysfunction, muscle twitches, weakness, nausea and vomiting, hypotension, tachycardia, and rarely, irregular heart rhythms.

In the present study we have evaluated the antidepressant activity of Caesalpinia pulcherrima of both aqueous and alcoholic extracts in FST. The development of immobility when rodents are placed in an inescapable cylinder of water during FST reflects the cessation of their persistent escape-directed behavior. Conventional drugs reliably decrease the duration of immobility in animals during this test. This decrease in duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents. Exact mechanisms underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of Phytochemical in the Caesalpinia pulcherrima. However, the antidepressant activity may be attributed to the presence of saponins, flavonoids and tannins in the extract. It is possible that the mechanism of anxiolytic action of AQMP and ALMP could be due to the binding of any of these phytochemical to the $GABA_A$ -BZD_S complex.

4. CONCLUSION

The results obtained in this study indicate that the n-hexane, ethyl acetate and methanol fractions of the leaves of Caesalpinia pulcherrima have significant CNS Depressant activities in animal model systems. The medicinal values of the plant leaves may be related to their constituent phytochemical. So, further detailed investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be done. It will help in the development of novel and safe drugs for the treatment of different types of CNS disorders.

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