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(Research Article)

Neuropharmacological Evaluation of the Methanolic Extract of *Couroupita guianensis* Aubl. Flower in Mice

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ABSTRACT

In the present study, effects of the methanolic extract of Couroupita guianensis (Family: Lecythidaceae) were studied on spontaneous motor activity, rota-rod performance and Phenobarbital sleeping time in mice. Preliminary phytochemicals analysis of extract revealed the presence of alkaloids, glycosides, tannins and flavonoids. The extract (100, 250 and 500 mg/kg) in dose dependent manner showed a significant reduction in spontaneous motor activity but had no effect on motor coordination as determined by the performance on rota-rod. These extracts also produced reduction of the onset and duration of pentobarbitone induced hypnosis. These results suggest that the extract contained an agent with its activity on both central and peripheral nervous system and the plant should be studied for neuropharmacological effects.

Key Words: *Couroupita guianensis*,Acute Toxicity, Spontaneous Motor Activity, Lecythidaceae,Sleeping time.

INTRODUCTION

Medicinal herbs represent a great deal of untapped reservoir of drugs and the active component molecules in future are targeted for lead compounds[1]. *Couroupita guianensis* Aubl. (*C .guianensis*) belonging to family Lecythidaceae is a deciduous tropical tree 90 feet tall and indigenous to Amazon rainforest. In, India it has been growing for past two to three thousand years at least. The flower are stunning fragrant, waxy aromatic smelling growing directly on the bark. Native Amazonian people used the infusion or teas obtained from leaves, flower and bark of *C. guianensis* to treat hypertension, tumours, pain and inflammatory processes[2]. In Orissa decoction of flowers has been used to boost the immune system to fight number of disease[3, 4]. Apart from this, extracts of flower had also been screened for antimicrobial activity[5], larvicidal activity against vector[6], cold, intestinal gas formation and stomachache[7] and immunomodulatory activity[8].

A number of natural product scientist believed that the initial selection of plants with diverse application in tradition medicine might be encouraged by their easily noticeable psychotropic effects[9]. Due to lack of scientific data on *C .guianensis*, the plant was less exploited from ethno-pharmacological point of view particularly for its central nervous system related activity. On the basis of this we evaluated the methanolic extract of *C. guianensis* flower for spontaneous motor activity; pentobarbitone induced sleeping time and motor coordination test using rota rod. To evaluate presence of secondary metabolites and the safety of this plant, phytochemicals investigation and acute toxicity study were also undertaken.

MATERIAL AND METHODS

Plant Material

Flower of *Couroupita guianensis* (CG) Aubl. were collected from the garden of Institute of Chemical technology, Matunga, Mumbai-400019 in the month of July-September. The sample was identified and authenticated by Prof. Ganesh Iyer, Department of Botany, University of Mumbai, Mumbai.

Preparation of Flower Extract

The flower was cleaned, shed-dried and powdered using mechanical grinder. The dried powdered specimen was first extracted with petroleum ether (60- 80 ºC) to remove the fatty contents and the extract was discarded. The residue was exhaustively extracted in a Soxhlet apparatus for at least 12 h with methanol and extract was used for experiment. The solvent from extract was removed under reduced pressure and controlled temperature (40-50 ºC). The yield of methanolic extract was 15.3% w/w. The dried semisolid *Couroupita guianensis* methanolic (CGM) extract was kept in tightly closed container in refrigerator till further analysis. Preliminary Phytochemical analysis of the extract was performed as per the procedure given in standard reference book.

Animals

Swiss albino mice of either sex (18-25 g) were obtained from Haffkine biopharmaceuticals Ltd, Mumbai. Animal were housed in group of 6-8 per cage under controlled conditions (room temperature 25±2 ºC, 12 h light: dark cycle, relative humidity 55±3 ºC). The animals were fed with pellets (M/s. D S trading, Mumbai) and water *ad libitum*. After 7 days of acclimatization animals were used for experiments. The experiments were carried out in accordance with the ethical guidelines laid down by Committee for the Purpose of Control and Supervision of Experimental on Animals and approved by Institutes Animal Ethic committee (CPCSEA No 87/1999).

Acute Toxicity Study

The acute toxicity study in mice was performed as per the OECD guidelines (No. 423) to evaluate the undesirable effects or toxicity. CGM extract were administered once orally at a dose of 2000 mg /kg body wt. to Swiss albino mice divided into 3 animals per group. The mice were then critically observed for clinical signs, gross behavioural changes and mortality after 30min, 1hr, 2hr, 3hr and then after 24hr. These observations were continued for a period of 7 days. Based on the results of acute toxicity study, 100 mg/kg, 250 mg/kg and 500 mg/kg body wt. were the dose narrowed down for further experiment.

Drugs and Chemicals

Diazepam (Zepose 5mg, Cipla Limited, Mumbai), Pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd, Queenborough, Kent) and Sodium Carboxy methyl cellulose (Na-CMC). Other chemical used were of analytical grade.

Experimental Design

The animals were divided into five groups. The group I animals were administered with 0.1% Na-CMC (Vehicle control, 10 mg/ml *p.o*), group II, III and IV animals received 100 mg/kg, 250 mg/kg and 500 mg/kg of CGM extract respectively and group V were administered standard drugs (Diazepam and Pentobarbitone) intraperitoneally.

Spontaneous Motor Activity

This test was determined using actophotometer (Bhooshan Scientific, Mumbai, India). Test involves placing the mice in cage which had photoelectric cells connected to circuit with a counter. When the beam of light is cuttoff due to movement of mice, locomotion count was recorded by the instrument[10]. The mice were singly placed in cage and spontaneous motility was recorded by actophotometer for 10 min sessions. The group I animals were administered with 0.1% Na-CMC (10 mg/ml *p.o*), group II, III and IV animals received 100mg/kg, 250 mg/kg and 500 mg/kg of CGM extract respectively and group V were given Diazepam (2 mg/kg *i.p.*) as a standard drug.

Pentobarbitone Induced Sleeping Time

The mice were randomly divided into five groups consisting of six mice each. Group I received 0.1% Na CMC (10ml/kg, *p.o.*) served as vehicle control, Group II, III and IV received orally the test extract at the doses of 100mg/kg, 250 mg/kg and 500 mg/kg respectively while group V received diazepam (2 mg/kg, *i.p.*). Thirty minute later, all the animals were administered with pentobarbitone (50 mg/kg, *i.p.*) to induce sleep. The animals were observed for the latent period (administration of pentobarbitone until loss of righting reflex) and duration of sleep (time of loss to regaining of the righting reflex)[11].

Test for Motor coordination

This test was performed using a horizontal rotating rod (Medicraft Rota rod apparatus, Inco, Ambala, India) 32 mm diameter set at a rate of 16 revolutions per minute. Mice that were able to remain on the rod longer than 3 min were selected and divided into five groups of 6 mice each[12]. The animals in each group received control vehicle or *C. guianensis* methanolic extract (at different dose of 100 mg/kg, 250 mg/kg and 500 mg/kg, *p.o.*), diazepam (2mg/kg, *i.p.*) and after 30 min each mouse was placed on the rod for 3 min at intervals of 30 min, up to 3 hours [12, 13].

Statistical Analysis

Values are given as mean ± S.D and significances calculated using one way analysis of variances

followed by Dunnett's test. The values of P <0.05 were considered statistically significant.

RESULTS

Preliminary Phytochemical Analysis

Phytochemical analysis of the extract showed the presence of glycosides, alkaloids, tannins and flavonoids.

Acute Toxicity Study

Acute toxicity study was carried out in accordance with OECD guideline. Methanolic extract of *C. guianensis* flower in dose of 2000 mg/kg (*p.o.)* did not cause any mortality in mice when observed for period of 7 days. The mice did not show any behavioural changes also clinical symptoms were normal.

Spontaneous Motor Activity

The *C. guianensis* methanolic extract at a dose of 100, 250 and 500 mg/kg reduced the spontaneous motor activity in mice (Table-1). A significant reduction in spontaneous motor activity was observed in dose dependant manner when compared to vehicle control group.

Pentobarbitone Induced Sleeping Time

Table-2 suggests that prior administration of *C. guianensis* methanolic extract at doses of 100, 250 and 500 mg/kg significantly ($p<0.01$) induced the sleep and also prolonged the duration of sleeping time in test animals as compared to vehicle control group. However, potentiation of sleep in test animals was not in dose dependant manner.

Test for Motor Coordination

The *C. guianensis* methanolic extract at a dose of 100, 250 and 500 mg/kg *p.o.* did not exhibit significant effect on the rota rod performance of the mice as all the animals stayed on the rod for 3 min without falling (data not shown). There was no failure of motor coordination at all the dose mentioned above.

DISCUSSION

Behavioural pharmacology field make use of the concepts that are derived from pharmacology and psychology to study behaviour in animal models[14]. The discovery of new compounds which act on CNS processes (either CNS depressant or CNS stimulant) will provide clinical useful information for validation of animals. This will also provide a new insight to researcher to understand the physio-pathological and neurochemical processes involved in investigation of new compounds.

As shown in present investigation the methanolic extract of *C. guianensis* flower produced central inhibitory effects in mice. The extract significantly reduced spontaneous motor activity in dose dependant manner which gives an indication of the level of excitability of the central nervous system[15] and this decrease may be closely related to sedation resulting from depression of the central nervous system[16]. Also, the extract did not abolish the flexor and extensor when administered at a dose of 2000 mg/kg for acute toxicity study.

Further evidence of the central depressant activity of the extract is provided by the extract ability to potentiate pentobarbitone induced sleeping time, an effect that may be attributed to an action on the central mechanism involved in the regulation of sleep[17, 18]. The prolongation of pentobarbital induced sleeping time may be attributed to an inhibition of pentobarbital metabolism[19]. The CGM extract prolongation of pentobarbital sleep suggest that barbital induced hypnosis is a good index of central nervous system depressant activity [20].

To validate neuropharmacological activity of *C. guianensis* motor coordination using rota rod test was selected with the goal of determining the muscle relaxant, sedative and depressive effect on CNS. The test allows determination of whether there is neurological damage with the implication of muscular tone caused by action of muscle relaxant, anticonvulsants or depressors on CNS. The methanolic extract of *C. guianensis* did not show any sedation and motor in coordination.

CONCLUSION

In conclusion it appears that the methanolic extract of *C. guianensis* can cause a central nervous system depression indicates active sedative properties which may be the basis of its folkloric use in traditional medicine to reduce pain[2]. However our results need to be further corroborated with different models to speculate on the mechanism of action.

Table 1: Effect of *C. guianensis* flower methanolic (100, 250 and 500 mg/kg, *p.o.*) extract and diazepam (2 mg/kg, *i.p.*) on spontaneous motor activity

Groups	Treatment	Dose (kg $^{-1}$)	Mean Activity Counts
I	Vehicle Control	10 ml	345.83 ± 38.80
II	CGM	100 mg	231.66 ±11.87**
III	CGM	250mg	272.66 ±15.61**
IV	CGM	500 mg	246.50 ±16.04**
V	Diazepam (*i.p.)*	2 mg	227.83 ±22.71 **

Note: Values are expressed as mean ± SD, n=6, **P < 0.01, when compared to vehicle control group by one-way ANNOVA followed by Dunnett's test.

Table-2: Effect of *C. guianensis* flower methanolic (100, 250 and 500 mg/kg) extract and diazepam (2 mg/kg) on pentobarbitone induced sleeping time.

Groups	Treatment	Dose (kg $^{-1}$)	Duration of Sleep (min)
I	Vehicle *p.o* + Thiopentone *i.p*	10 ml + 50 mg	9.44 ±3.97
II	CGM *p.o.* + Thiopentone *i.p*	100 mg + 50 mg	69.6 ±6.17 **
III	CGM *p.o.* + Thiopentone *i.p*	250mg + 50 mg	67.8 ±4.16 **
IV	CGM *p.o.* + Thiopentone *i.p*	500 mg + 50 mg	70.5 ±8.66 **
V	Diazepam *p.o.* + Thiopentone *i.p*	2 mg + 50 mg	134.4 ±11.09)**

Note: Values are expressed as mean ± SD, n=6, **P < 0.01, when compared to vehicle control group by one-way ANNOVA followed by Dunnett's test.

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