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ANXIOLYTIC EFFECT OF *COUROUPITA GUIANENSIS*AUBL. FLOWER EXTRACTS IN MICE

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ABSTRACT

Despite the traditional use of *Couroupita guianensis* (CG) to treat various ailments, it had been less exploited from ethnopharmacological perspective. The aim of the present study was to evaluate aqueous and methanolic extract of CG for its anxiolytic activity in mice.Swiss albino male mice were orally administered both aqueous and methanolic extracts of CG at the dose of 100, 250 and 500 mg/kg/ml for 14 days. The elevated plus maze (EPM), light and dark (LD), and Open field test (OFT) models were used to evaluate the anxiolytic activity. Both the aqueous and methanolic extracts of CG at a dose of 500 mg/kg significantly showed an anxiolytic activity compared to vehicle control in EPM, LD, and OFT model in mice. This is the first reported study of CG flower extracts for its anxiolytic potential activity. Further study needs to be performed for isolating the active constituent to prove mechanism based activity.

KEYWORDS: **Anxiolytic, Elevated Plus Maze, Light and Dark, Open Field Test model.**

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INTRODUCTION

Anxiety has become an important area of research in psychopharmacology as 1/8th of the total world population being affected by this disorder[1, 2]. Due to rapid growth in scientific studies and discoveries of new drugs, there is increased interest to study the plant extract for its anxiolytic activity in animal models[3,4] . Benzodiazepines are the mainstay of drug treatment in anxiety disorders but are associated with sedation, muscle relaxant, ataxia, amnesia and pharmacological dependence[5]. Researchers have focussed more onto the development of new anxiolytic compounds that leads to quick onset of action with lesser side effects and wider therapeutic index. *Couroupita guianensis* [CG] Aubl. (Lecythidaceae) is a deciduous tropical tree with a peculiar type of flowers indigenous to the Amazon rainforest[6]. Various activities related to plant are reported like antidepressant[5], antimicrobial[8, 9], larvicidal[10] and immunomodulatory[11]. Apart from the reported, traditionally it is been used for stomachache pain[12], tumours[13], and inflammatory processes[14]. In flowers, the chemical constituents mainly reported were eugenol, linalool and (E,E)-farnesol [15].To add further, the plant is also a rich source of triterpenoids, a constituent responsible for anxiolytic activity as reported earlier[16]. A previous study on methanolic extract of roots of CG carried out in our lab suggests a potential anxiolytic activity[17]. However to demonstrate, for the first time we evaluated *C. guianensis* flower extract at a dose of 100, 250, and 500 mg/kg for its anxiolytic activity. The animal models used to evaluate anxiolytic activity were elevated plus maze (EPM), light-dark (LD) and Open field test (OFT) model.

MATERIALS AND METHODS

Preparation of Plant Extract

Flowers of CG collected from our Institute garden were identified by Prof. Ganesh Iyer, Department of Botany, Ruia College, Mumbai. They were shed dried and powdered using a mechanical grinder. The powdered flowers were extracted subsequently in Soxhlet apparatus with petroleum ether and methanol. Aqueous extract were also collected. Both *Couroupita guianensis* aqueous (CGA) and methanolic (CGM) extract were concentrated under reduced pressure using rota evaporator at 55º C. Extracts were kept in tightly closed containers in refrigerator till further analysis.

Animals

Swiss albino male mice (18-25g) were purchased from Haffkine Biopharmaceuticals Corporation Ltd, Mumbai, India. The animals were housed in a cage with controlled temperature at 22-25 ºC with 12h light: dark cycle (Humidity: 50-55 ºC). Food (M/s D S Trading, Mumbai, India) and water were available *ad libitum.* After 7 days of acclimatization period animals were used for experiments. All the manipulations were carried out between 9:00 to 15:00 h. The protocol for performing experiment was approved by the Institutional Animal Ethics committee as per guidelines of the Committee for Purpose of Control and Supervision of Experimental on Animals (CPCSEA No 87/1999). The acute toxicity study in mice was performed as per the OECD guidelines (No. 423**)** to evaluate the undesirable effects or toxicity of both CGA and CGM extracts.

Experimental Design

Forty-eight male mice were randomly divided into 8 groups of 6 mice in each. Group I received 0.1%Na CMC (10 ml/kg, *p.o.*) vehicle control and group II received diazepam (2 mg/kg, *i.p.*) served as positive control. Group III, IV and V received CGA extract and group VI, VII and VII received CGM extract in the doses of 100, 250 and 500 mg/kg, respectively *per orally*. The animals were dose for 14 days after a period of 7 days of acclimatization.

Elevated Plus Maze (EPM) Model

The EPM test was used to evaluate anxiety in animal model [18, 19, 20]. Briefly, EPM consisted of two open arms (30cm x 5 cm) and two closed arms (30cm x 5cm x 15 cm), with an open roof arranged, such that the two arms of each type was opposite to each other. The

maze had a central square (10 cm x10 cm) which gave the shape of a plus sign and was kept elevated 50 cm above the floor. The floor and the walls of each arm were wooden and painted black. Each mouse were placed individually on the centre facing towards open arm and the number of entries and time spent by the mice on the open and closed arms were recorded during a period of 5 min. Diazepam(2 mg/kg, *i.p*) was used as a positive control.

Light and dark (LD) model

The experimental procedure was described by Crawely and Goodwin (1980)[21]. The apparatus consisted of a Plexi glass box with compartments (25cm x 33cm x 24cm) which were connected by an opening (10cmx 10cm) through which mice were allowed to move from one box to the other. A 100 W incandescent bulb (Cema Electric Lighting, Kerala, India) emits visible light was placed in one of the compartment at height of 30cm while other remained dark. The transition between the light and dark box and time spent in it was recorded for 5 min immediately after the mouse stepped into the dark box.

Open Field Behaviour Test (OFT)

OFT isused to evaluate the exploratory activity and emotional response in animals[22]. The apparatus consisted of acrylic transparent walls and black floor (96 cm x 96 cm) divided into 16 squares and placed in dim light room. After 30 min of administration of test extract, standard drug and vehicle, treated mice were placed individually on one corner of the apparatus and observed for the next 15 min. The observed parameters were the number of squares crossed with the four paws and number of rearings.

Statistics

The data obtained were analyzed using the Graph Pad software program Version 5.0. Values are represented 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.

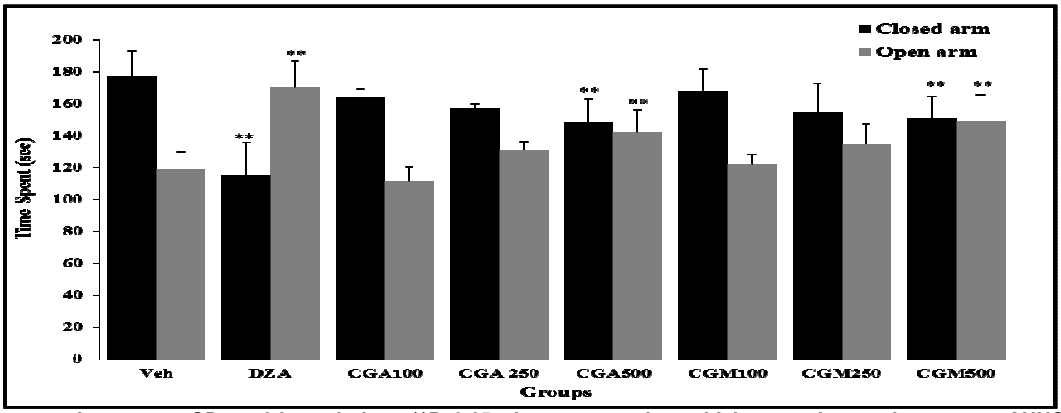
RESULTS

EPM model

In the EPM model, both the CGA and CGM extracts at 500mg/kg showed a significantly ($p<0.05$) increase in the time spent on the open arms compared to vehicle control (Figure1). Whereas concomitant decrease in the indices on the closed arm was observed in mice treated with diazepam (2 mg/kg, *i.p.*).

Figure 1

Effects of CGA and CGM in the elevated plus-maze model in mice.



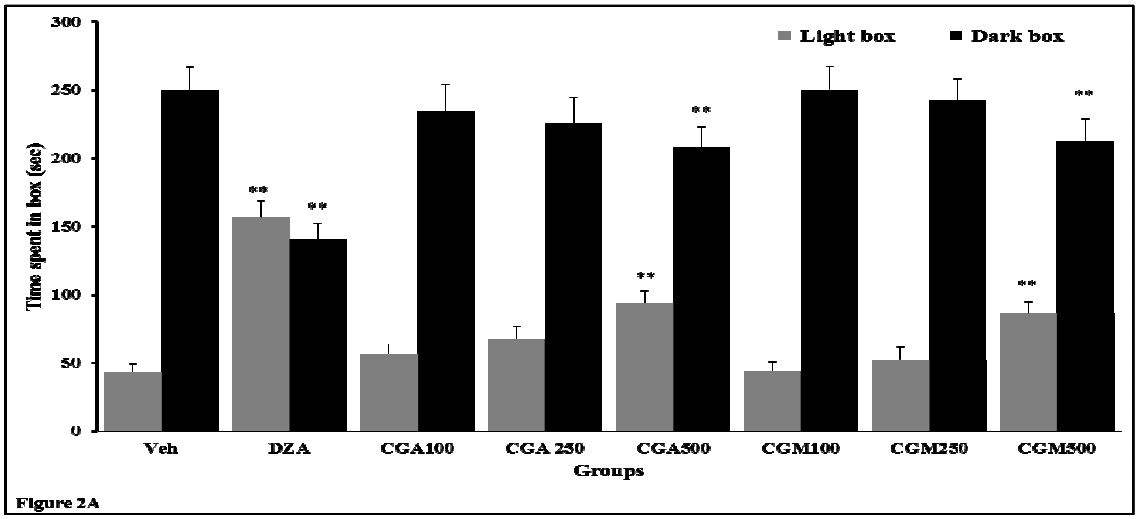
*Values are expressed as mean ± SD; n= 6 in each dose. **P<0.05 when compared to vehicle control group by one-way ANNOVA with post hoc Dunnett's test. Veh: Vehicle control (0.1% Na CMC, p.o.), DZA: Diazepam (2mg/kg, i.p.), CGA: Couroupita guianensis aqueous (100, 250 and 500 mg/kg dose, p.o.) extract and CGM: Couroupita guianensis methanolic (100, 250 and 500 mg/kg dose, p.o.) extract.*

Light and Dark Model

As represented in figure 2A and 2B after the administration of CGA and CGM extracts there was a significant ($p<0.05$) increased in the time spent on the illuminated side of LD apparatus compared to vehicle control. On the other hand the number of entries was also found to be significantly ($p<0.01$) increased only after the administration of the higher dose (500mg/kg). Similarly, significant ($p<0.05$) effects were observed with animals treated with diazepam (2 mg/kg, *i.p.*).

Figure 2A and 2B

Effects of CGA and CGM in the light dark model in mice.



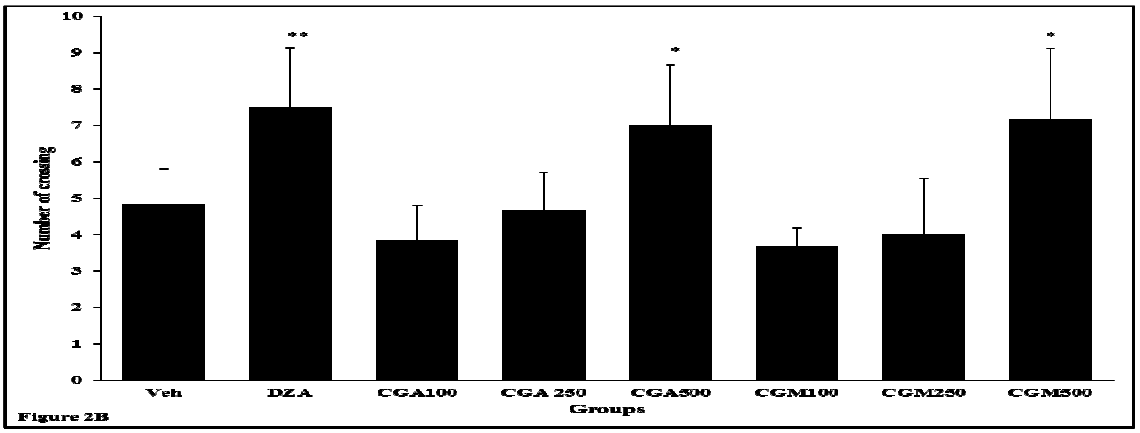
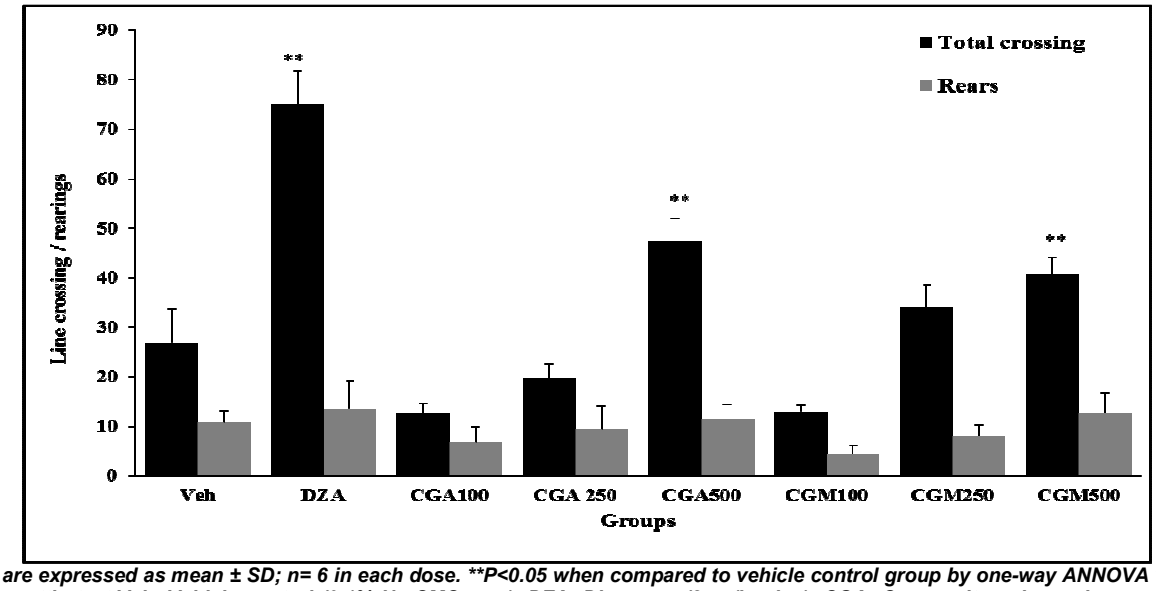


Figure 2A: Represents time spent in light (grey) and dark (black) compartment and figure 2B represents number of crossing. Values are expressed as mean ± SD; n= 6 in each dose. **P<0.01 and *P< 0.05 when compared to vehicle control group by one-way ANNOVA with post hoc Dunnett's test. Veh: Vehicle control (0.1% Na CMC, p.o.), DZA: Diazepam (2mg/kg, i.p.), CGA: Couroupita guianensis aqueous (100, 250 and 500 mg/kg dose, p.o.) extract and CGM: Couroupita guianensis methanolic (100, 250 and 500 mg/kg dose, p.o.) extract.

Open Field Behaviour Test (OFT)

The open field test was done in order to determine the effect of administration of the plant extract upon spontaneous motor activity. CGA and CGM extract at dose of 500 mg/kg produced significantly ($p<0.05$) an increase in the number of squares crossed (Figure 3). Diazepam (2 mg/kg, *i.p.*) produced a qualitatively similar response.

Figure 3
Effects of CGA and CGM in the open field test in mice.



*Values are expressed as mean ± SD; n= 6 in each dose. **P<0.05 when compared to vehicle control group by one-way ANNOVA with post hoc Dunnett's test.Veh: Vehicle control (0.1% Na CMC, p.o.), DZA: Diazepam (2mg/kg, i.p.), CGA: Couroupita guianensis aqueous (100, 250 and 500 mg/kg dose, p.o.) extract and CGM: Couroupita guianensis methanolic (100, 250 and 500 mg/kg dose, p.o.) extract.*

DISCUSSION

In the present study anxiolytic activity of both the aqueous and methanolic extracts obtained from *Couroupita guianensis* flower had been demonstrated. The EPM model was used to evaluate the anxiolytic effects of both the extracts. The time spent was defined as entry of mice into an arm placing all four legs over the line marking that area. The open arm signifies the exploratory activity, whereas the number of entries in the closed arms was considered as the locomotor activity. At 500 mg/kg, both CGA and CGM extracts showed increase in the time spent in open arms.The preference of mice towards the aversive space indicates anxiolytic effects of both the extracts. To validate further, LD model was used for screening anxiolytic or anxiogenic activity. It is based on the innate aversion of animals to illuminated areas and on spontaneous exploratory behaviour in response to mild stressors. It has been reported that the measurement of the time spent in the light area and not the number of transfer is the most consistent and useful parameter for assessing anxiolytic activity[23]. The transitions of animal between the light and dark box and time spent in the light box were recorded immediately for 5 min after the mouse stepped into the dark box. The present study showed that CGA and CGM at 500 mg/kg significantly increase the time spent and number of entries in light area. Similar effects were observed with diazepam (2 mg/kg) when used as standard drug for light and dark model. The effects produced by CG and diazepam (2 mg/kg) upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice, indicative of anxiolytic activity. Significant ($p<0.01$) increased in the number of squares crossed at the dose of 500 mg/kg for both CGA and CGM was observed.

CONCLUSION

The ongoing challenges for standardization of plant derived medicines, points out the need to identify, select and use only those plant parts that possess the maximum therapeutic efficacy [24]. In summary, the aqueous and methanolic extract of *C. guianensis* exhibits anxiolytic activity. In addition, our results suggest that this anxiolytic activity may be attributable in part to triterpene rich fractions within the plant extracts. It could be attributed

to involvement of specific central recognition sites coupled to GABAa receptors, facilitating GABAergic transmission involved in the physical expression of anxiety [25]. Further, the study needs to be carried out to isolate the active constituent that plays a significant role in anxiolytic activity.

REFERENCES

1. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van RM, Trends in alternative medicine use in the United States. The Journal of the American Medical Association. 280: 1569-1575, (1998).
2. World Health Organization (2004).The World Health Report 2004: Changing History, Annex Table 3: Burden of disease in DALYs by cause, sex, and mortality stratum in WHO regions, estimates for 2002.Retrieved from http://www.who.int /whr/2004/en/report04_en.pdf.
3. Basavaraj P, Shivakumar B, Shivakumar H, Anxiolytic activity of tabernaemontana divaricata (linn) R. Br. flowers extract in mice. International Journal of Pharma and Bio Sciences. 3: 65-72, (2011).
4. Avijit C, Amudha P, Geetha M, Surjit SN, Evaluation of anxiolytic activity of methanolic extract of Sapindus Mukorossi gaert n. in mice. International Journal of Pharma and Bio Sciences. 3: 1-8, (2010).
5. Lader M, Morton S, Benzodiazepine problems.British Journal of Addiction. 86: 823–828, (1991).
6. VaidyaratnamVPS, Indian Medicinal Plants, AryaVaidyaKottakkai Kerala, India, Orient Longman Ltd. (1994).
7. Gupta VH, Wankhede SS, Gunjal MA, Juvekar AR, Antidepressant like effect of Couroupita guianensis aubl.Flowers in animal model of depression.International Journal of Toxicological and Pharmacological Research.4:12-16, (2012).
8. Khan MR, Kihara M, Omoloso AD, Antibiotic activity of *Couroupita guianensis*. Journal of Herbs, Spices & Medicinal Plants.10: 95-108, (2003).
9. Al-Dhabi NA, Balachandran C,Raj MK, Duraipandiyan V, Muthukumar C,Ignacimuthu S, Khan IA, Rajput VS., Antimicrobial, antimycobacterial and antibiofilm properties of *Couroupita guianensis* Aubl. fruit extract. *BMC Complementary and Alternative Medicine.* 12:242, (2012). doi:10.1186/1472-6882-12-242,
10. Desal T, Golatakar SG, Rane JB, Ambaye RY, Kamath VR, Larvicidal property of *Couroupita guianensis* Aubl. Indian Drugs. 40: 484-486, (2003).
11. Pradhan D, Panda PK, Tripathi G, Evaluation of immunomodulatory activity of methanolic extract of *Couroupita guianensis* Aublet.flowers in rats Natural Product Radiance. 8: 37-42, (2009).
12. Sanz-Biset J, Campos-de-la-Cruz J, Epiquién-Rivera MA, Canigueral S, A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon). Journal of Ethnopharmacology.122: 333–362, (2009).
13. Kokate CK., Practical Pharmacognosy, 2nd ed Delhi Vallabh Prakash(1988).
14. Boyum A., Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1g. Scandinavian journal of clinical and laboratory investigation supplementum. 97: 77-89, (1968).
15. Eknath AA, Shivchandraji LK, β-Amyrinpalmitate isolation from Couroupita guianensis Aubl.leaves. Indian Drugs. 39: 213–216, (2002).
16. Zhang ZJ, Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Science. 75: 1659–1699, (2004).
17. Wankhede S, Gambhire M, Juvekar A, Anxiolytic effect of methanolic extract of root of Couroupita guianensis aubl. Pharmacologyonline. 3:193-206, (2009).
18. Pellow S, File SE, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacology

Biochemistry and Behavior. 24: 525–529, (1986).
19. Lister RG, The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 92: 180–185, (1987).
20. Nogueira E, Vassilieff VS, Methodological evaluation of the elevated plus-maze (EPM) test for anxiety in rats. RevistaCienciasBiomedicas. 17: 47–54, (1996).
21. Crawley J, Goodwin FK, Preliminary report of a simple animal behaviour model for the anxiolytic effects of benzodiazepines. Pharmacology Biochemistry Behavior. 13: 167–170, (1980).
22. Archer J, Tests for emotionality in rats and mice: A review. Anim. Behav. 21: 205–235, (1973).
23. Young R, Johnson DN, A fully automated light/dark apparatus useful for comparing anxiolytic agents. Pharmacology Biochemistry Behavior. 40: 739–743, (1991).
24. Bonati A, How and why should we standardize phytopharmaceutical drugs for clinical validation?. Journal of Ethnopharmacology. 32: 195-197, (1991).
25. Costa E, Guidotti A, Toffano G, Molecular mechanisms mediating the action of diazepam on GABA receptors. Brazilian Journal of Psychiatry. 133: 239–248, (1978).