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Anxiolytic effects of *Plumeria rubra* var. *acutifolia* (*Poiret*) L. flower extracts in the elevated plus-maze model of anxiety in mice☆

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ABSTRACT

Interest in alternative medicine and plant-derived medications that affect the "mind" is growing rapidly since last two decades. The aim of the present study was to investigate the effects of ethanolic extract of flower of *Plumeria rubra* (PR) along with its fractions in the elevated plus-maze (EPM) model of anxiety. The *P. rubra* extract or its fractions was administered orally to male Swiss mice, at graded doses, 1 h prior to behavioural assessment. The PR extract at the dose of 100 mg/kg p.o., significantly increased the time spent in the open arms of the EPM. Further, the anxiolytic properties of hexane, chloroform and butanolic soluble and insoluble fractions at one-fifth of the original dose were also observed in the EPM task. Out of which butanol insoluble fraction showed significant anxiolytic activity comparable to standard anxiolytic drug, diazepam. Further, pretreatment with crude ethanolic extract and butane insoluble fraction showed no significant effects in the horizontal activity, total distance travelled and stereotypy count in the animal activity monitor and had no motor in-coordination side effects in the rotarod test in mice. These observations suggest that the flower extract of *P. rubra* and its insoluble butanolic fraction might possess significant anxiolytic potential to be pursued further for drug development process.

1. Introduction

Anxiety is one of the most prevalent psychiatric disorders. In any given year, approximately 40 million adults are affected by anxiety disorder and can also precipitate or aggravate cardiovascular and behavioural disorders (Weissman et al., 1990). Approximately two-thirds of the anxious patients respond to the currently available treatments but the magnitude of improvement is still disappointing, besides, they also produce various systemic side effects and exhibit dependence and tolerance on chronic treatment which now have become a major concern about the use of currently used medicines. At present, the benzodiazepines (BZDs) are the most commonly employed medicinal treatments for anxiety. BZDs produce their pharmacological actions via specific high affinity binding sites on a supramolecular complex composed of GABA-A and a BZD receptor coupled with a chloride ion channel. Besides, the potential for drug dependence and side effects, BZDs also cause drug interactions by potentiating the effect of other CNS depressants such as alcohol, hypnotics, and neuroleptics if taken together. Other anti-anxiety medications include antidepressants, buspirone and β-blockers which though effective in many cases, also possess side effects like nausea, light headedness, dizziness, headache, dry mouth, constipation, diarrhea, etc. (Smith et al., 2012). Therefore, there is an urgent need of drug which possesses greater efficacy, lesser undesirable effects with minimum or no tolerance and dependence. In order to overcome these adverse effects, investigations has been extended for the search of novel and better biocompatible molecules from plant sources.

Herbs are widely accepted sources of medicine, which play an important role in health care programme worldwide (Verma et al., 2010). The search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade and their therapeutic potential has been assessed in a variety of animal models (Carlini, 2003; Zhang, 2004; Chatterjee et al., 2012). Our previous studies also involved evaluation of some Indian medicinal plants like *Ocimum sanctum* (Chatterjee et al., 2011b) and *Bacopa monera* (Chatterjee et al., 2010) for their anxiolytic effects. In this study, we extended our search for such herbal medications to evaluate the neuropharmacological effects of flower extract of another Indian medicinal plant, *Plumeria rubra*. *P. rubra* (PR) syn. *Plumeria acutifolia* is also known as Plumeria tree, temple tree, West Indian jasmine or frangipani. This shrub or small tree is native to Mexico, and is well-known for its strongly

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perfumed flowers that can be of several colors. Various species of Plumeria has been previously shown to possess antioxidant (Ruiz-Terán et al., 2008), hypolipidemic (Merina et al., 2010), hypoglycemic (Zaheer et al., 2010), antimicrobial (Rasool et al., 2008) and cytotoxic activities (Kardono et al., 1990). The medicinal properties of plant also include protection against ulcers, skin diseases, inflammation, arthritis and constipation (Hamburger et al., 1991; Gupta et al., 2006; Zaheer et al., 2010).

Despite the widely popular use of this plant, the available scientific information about the potential effects of PR in animal models of psychiatric disorders especially in anxiety is limited. Therefore, the present study was conducted to evaluate the anxiolytic effect of PR in different models in mice.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by our Institutional Ethical Committee following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Albino male Swiss mice weighing 20–25 g were employed in the study. Mice were housed in six per cage at constant room temperature (22 ± 2 °C) and 12-h light/12-h dark (8:00 a.m.–8:00 p.m.). Mice were fed standard laboratory food and water was given ad libitum. Each animal was used once in the behaviour tests.

2.2. Preparation of extract

2.2.1. Collection of the flowers

Fresh flowers of *P. rubra* were collected from Lucknow botanical gardens during the month of October and identified by Dr. R.K. Sharma, Head, Botany Division of the Central Drug Research Institute (CDRI), Lucknow. The specimen has been preserved in the Botany Division and has been allotted the CDRI extract no 117.

2.2.2. Extraction and fractionation procedure

Fresh flowers (500 g) were extracted with 90% ethanol (4 × 700 ml) at room temperature. The combined ethanolic extract was filtered and concentrated in a rotavapour below 50 °C to a viscous mass which was dried under high vacuum to remove the last traces of the solvent to get crude extract (12.6 g). The ethanolic extract (10.0 g) was successively fractionated with hexane (0.9 g), chloroform (1.2 g), n-butanol soluble (3.8 g) and n-butanol insoluble (4.1 g) fractions (Fig. 1). All these fractions were concentrated under reduced pressure below 50 °C separately in a rotavapour and all were submitted for anxiolytic activity. The n-butanol soluble fraction and n-butanol insoluble fractions were found showing some similar spots on TLC plates. Therefore these fractions were mixed and chromatographed over a column of silica gel, rechromatography of some column fractions yielded 4 pure compounds, which were identified by co-TLC with the authentic samples and also by comparison of physicochemical data provided in literature (Dubois et al., 2005; Kuigoua et al., 2010).

2.3. Drugs and treatment schedule

Diazepam (DZP) was obtained from M/s. Sigma (St. Louis, MO, USA). All compounds were suspended in 0.5% gum acacia. Mice were either treated with vehicle or the extract or fractions daily for 3 days prior to the experiment. Drugs were prepared fresh daily before administration. DZP was administered once at a dose of 1.5 mg/kg, 1 h prior to the experiment. Compounds were administered per orally at a volume of 0.1 ml/10 g body weight

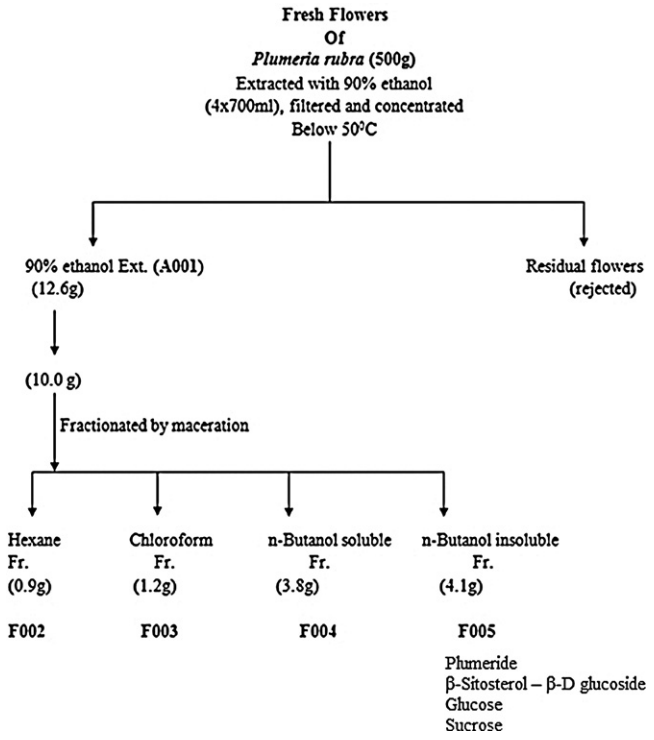

Fig. 1. Diagrammatic representation of extraction procedure of *P. rubra* flowers.

2.4. Behavioural observations

Mice were randomly divided into following groups of 8 mice each.

(a) Control group: mice were treated with vehicle (0.5% gum acacia).
(b) Treatment group: mice treated with graded doses of extract or fractions.
(c) Standard drug treated group: mice were treated with diazepam (1.5 mg/kg p.o.).

2.5. Procedures

2.5.1. Elevated plus maze

This test has been widely used to measure anxiety in rodents (Lister, 1987). Mice were treated daily for 3 days with extract or fractions or DZP (single dose) 1 h prior to the experiment. Each animal was placed at the centre of the maze, facing one of the open arms. The time spent in enclosed and open arms was recorded for 5 min test. The movement of animals across the arms is calculated by interruption of beams which was analysed by maze tracking software (M/s Columbus Instruments, USA). After each test, the maze was carefully cleaned up with a wet tissue paper (70% ethanol solution).

2.5.2. Spontaneous motor activity

Gross open field activity was studied using Digiscan Infrared Photocell system [Omnitech Electronics, Columbus, Ohio] in 42 × 42 × 30 cm Plexiglass arenas, fitted into infrared beam containing metallic grid. Activity of animals was observed by the interruption of infrared beams (Chatterjee et al., 2011a).

(a) Horizontal activity: the total number of beam interruptions that occurred in the horizontal sensor in the duration of 2 min.
(b) Total distance travelled: it is the distance travelled by the animal in a given sample period, indicated in centimetres. Total