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The role of serotonergic system in the anxiolytic-like effect of Emblicaofficinalis in mice using hole board test.

Aravinda Kumar B, Bethi Yugandhar, Nishanthini D, Isabella Topno, Balakrishnan Sadasivam

Abstract:
BACKGROUND:*Emblicaofficinalis (EO), popularly known as amla is a common household remedy used in Indian indigenous system of medicine for several common ailments.*
Objectives:*To evaluate the role of serotonergic system in the anxiolytic-like effect of EO aqueous extract in mice after acute and chronic administration using hole board test.*
Materials And Methods:*Hole board test (Brown et al) was used to evaluate the anxiolytic-like effect of EO extract in mice after acute and chronic administration (10 days). Probable mechanism of action of the extract was investigated by pretreatment with pindolol (a β-blocker with 5HT1A antagonist activity). The number of dips &locomotor activity of mice were the parameters recorded and analyzed using One-way ANOVA statistical test.*
Results And Conclusions:*EO extract produced a significant increase in the number of dips and the locomotor activity after acute and chronic administration in the hole board test indicating a significant anxiolytic-like activity. Our study also showed that the anxiolytic-like effect was significantly reversed by pretreatment with pindolol. Hence we conclude that the anxiolytic-like activity of EO aqueous extract was due to its modulatory effect on the 5-HT1A receptors in the central nervous system. Our results contribute towards validation of the traditional use of EO as an adjuvant treatment of anxiety disorders.*
Key Words:*anxiolytic, Emblicaofficinalis, hole board test, buspirone, pindolol*

I. Introduction

The Indian gooseberry (Phyllanthusemblica. syn. Emblicaofficinalis) is a deciduous tree of Euphorbiaceae family. Fruits of Phyllantusemblica (a rich source of vitamin C) and related species have been extensively used in Unani and Ayurvedic systems in medicine in India for the treatment of wide variety of diseases [1]. Its fruits are very commonly available everywhere and have been reported to possess various activities like anti-depressant, anxiolytic, anti-epileptic, anti-cataleptic, hypoglycemic, hypolipidemic, antiulcer, hepatoprotective, antipyretic and analgesic, anti-inflammatory, antibacterial, immune-modulatory, antioxidant and gastro protective activities[2].

The World Health Organization estimated around80% population in developing countries rely on plant derived traditional medicines. It also estimated that more than 75% of people with mental, neurological and substance use disorders living in developing countries like India do not receive any treatment or care. [2, 3]

Anxiety response is an important mechanism by which we adapt and respond to real dangers. Dysregulation of this healthy response resulting in 'marked, persistent, and excessive or unreasonable fear', culminating in a significant interference in normal life can be described as an anxiety disorder [4]. There is abundant evidence for abnormalities of the serotonin (5HT) neurotransmitter system in anxiety disorders. Reproducible increase in serotonergic function is found to accompany treatment with anxiolytics [5].

Though selective serotonin reuptake inhibitors (SSRIs), selective serotonin norepinephrine reuptake inhibitors (SNRIs) and benzodiazepines (BZDs) are available as first line medications to treat these disorders, they produce various systemic side effects or exhibit tolerance upon chronic use [6]. Hence there is a need to identify newer drugs from plants.

*Emblicaofficinalis*fruits were selected to evaluate its anxiolytic-like effect in mice. It can be used to effectively treat anxiety disorders, if found to be effective in animal models. Earlier studies have proven the anxiolytic-like effect of Emblicaofficinalis aqueous extract, but the mechanism responsible for this effect was not studied.

II. Materials and Methods

2.1. Plant material

The test drug EO was purchased from M/s. Natural Remedies Pvt. Ltd., Bangalore, India. The extract was prepared by dissolving the powder in distilled water. Phytochemical analysis of the dried powder of EO gave positive results for tannins, alkaloids, carbohydrates, polyphenols and amino acids. HPLC analysis of the

powder was found to contain tannic acid > 30% and gallic acid > 10% as the main constituents. Estimation and purity of active principle was done by the Quality Control Laboratory, M/s. Natural Remedies, Bangalore, India (lab reference no.FP1012062, dated 28-12-2010).

2.2. Drugs

Buspirone (Buspin), a 5-HT_{1A} receptor partial agonist, was obtained from Intas Pharmaceuticals Ltd. (Ahmedabad, India) and used as the standard drug. Pindolol, a beta-blocker with 5-HT_{1A} antagonistic activity was obtained from Sigma Chemical Company (St. Louis, MO, USA) to study the probable mechanism of action.

2.3. Animals

Female albino mice weighing 20-25g body weight were obtained from the Central Animal House facility of Pondicherry Institute of Medical Sciences (Pondicherry, India). The animals were housed in clean polypropylene cages in groups maintained under standard laboratory conditions with natural 12 hours light and dark cycles and ambient room temperature with free access to food and water. The animals were acclimatized to laboratory conditions before testing. Experiments were conducted between 9.00 and 14.00 hours. All procedures in the study were reviewed and approved by the Institutional Committee for Ethical use of animals. The care of animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.4 Drug administration

The mice were divided into five groups of six in each. The first three groups received vehicle (distilled water), standard drug (buspirone – 1.25mg/kg body weight intraperitoneally) and test drug (EO aqueous extract – 4mg/kg body weight per oral) respectively and were used to evaluate both acute and chronic anxiolytic-like effect of EO extract. Probable mechanism of action of the extract was investigated in the remaining two groups by pretreatment with pindolol (a β-blocker with $5HT_{1A}$ antagonist activity at a dose of 30mg/kg body weight per oral).

2.5 Hole-board test

The method described by Brown et al, was used to assess the exploratory behaviour of mice. The hole-board apparatus consisted of a wooden board measuring 24 × 24 inches and it was raised 50 cm above the ground on a wooden stand. Sixteen holes (each 1 inch in diameter) were cut on the floor of the board and lines were drawn with a chalk to form squares by joining the holes. The apparatus was placed in a small testing room with dimmed white lighting. The stand of the apparatus was open on all sides, allowing the floor or objects to be dimly lit.

The mice were injected with drugs or vehicle and, thirty minutes later, each animal was placed in the centre of the hole-board, and allowed to freely explore the apparatus for 5 min. The total number of head dips into the holes until both the eyes are invisible (anxiolytic activity) and the number of squares crossed with both the forelimbs and hind limbs completely placed in the adjacent square (locomotor activity) were the parameters recorded.

2.6 Statistical analysis:

All results were expressed as mean ± standard deviation (SD) and analyzed by using one-way ANOVA with Post-hoc Bonferroni correction. GraphpadInstat software version 3.06 was used to analyze all the results. P value of less than 0.05 was considered to be statistically significant.

III. Results

3.1 Anxiolytic activity

After acute and chronic administration of the EO aqueous extract, the number of dips into the holes significantly increased when compared to the standard (buspirone) and control groups ($p<0.001$).

It was also found that the number of dips into the holes significantly decreased when the test group was pretreated with pindolol compared to the group which received EO extract alone ($p<0.001$)(Fig.1-A).

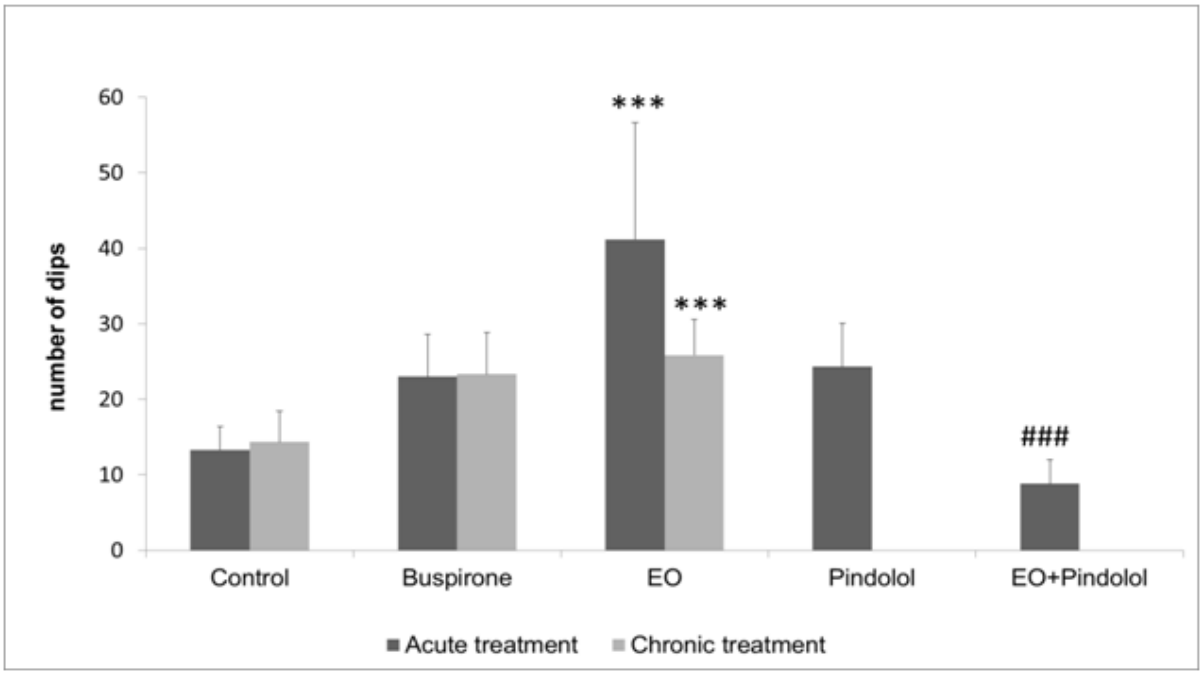
3.2 Locomotor activity

After acute and chronic administration of the EO aqueous extract, the number of squares crossed significantly increased when compared to the standard (buspirone) and control groups ($p<0.001$).

It was also found that the number of squares crossed significantly decreased when the test group was pretreated with pindolol compared to the group which received EO extract alone ($p<0.001$). (Fig.1-B)

Figure: 1

A]



B]

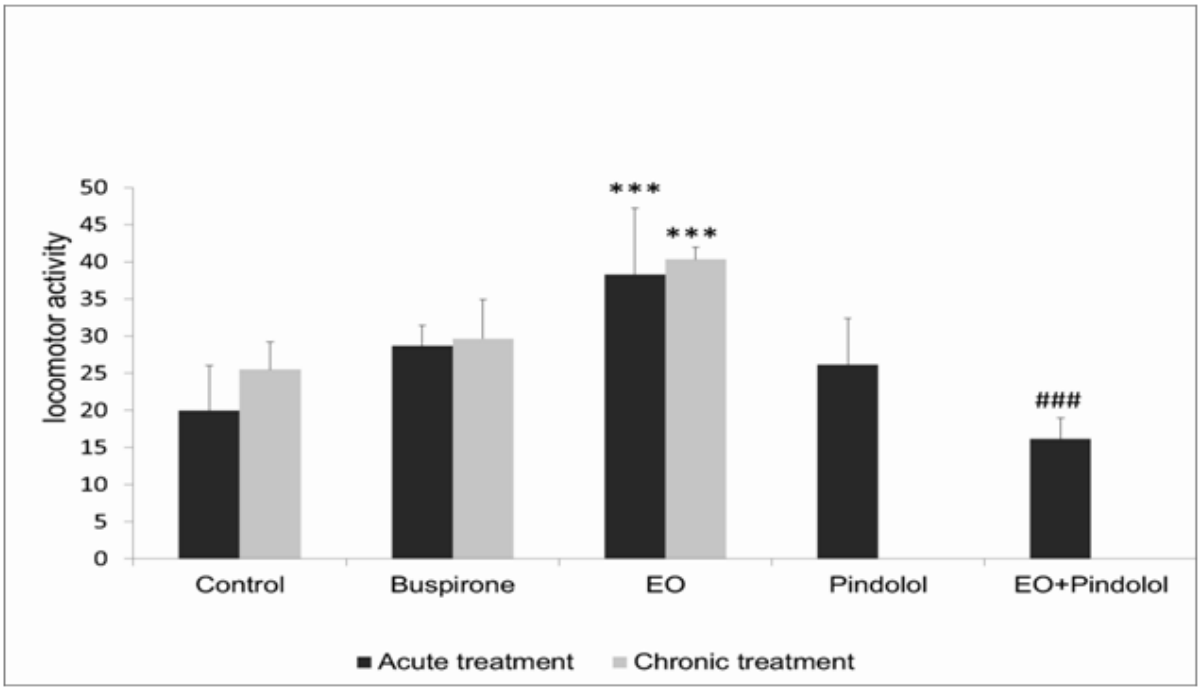


Figure: 1 Effect of acute and chronic treatment of mice with Emblicaofficinalisextract (4 mg/kg p.o.), buspirone(1.25 mg/kg i.p) and pindolol (30 mg/kg p.o.) in the hole-board test with respect to (A) number of dips and (B) locomotor activity. Each column represents the mean ± S.D. of 6 animals. *** P<0.001 when compared with the vehicle treated control. ###P < 0.001 when compared with EO extract alone.

IV. Discussion

This study was done to establish the probable role of serotonergic system in the anxiolytic-like effect of aqueous extract of Emblicaofficinalis, a plant commonly known for as a common house hold remedy for many ailments. The results presented here clearly indicate that the aqueous extract of Emblicaofficinalis has anxiolytic-like and locomotoracivities (Figs.1A and 1B).

Behavioral models of anxiety rely on the introduction of a stimulus to create a novel state within the organism. The nature of that state and the interference of anxiety, are assessed by the response of the subject to that stimulus in the presence and absence of drugs known to be efficacious anxiolytics in man. Stimuli used to induce anxiety state in animals can be broadly classified into two types: exteroceptive (those originating outside the body) and interoceptive (those originating within the body).

The present study used an exteroceptive animal anxiety model named hole-board test which is said to assess the exploratory behavior in mice. This model is also said to discriminate between the exploratory activity and other non-specific motor activity in rodents. The key measure used to assess exploration in this anxiety test is the number of head-dips and the number of squares crossed. Less anxious mice explore the holes over the board in this test more which reflects in the increase in the frequency of head dips and the number of squares crossed. Anxious mice on the other hand will avoid the holes as much as they can hence a decrease in the frequency of head-dips and squares crossed [7].

The dose selection of the aqueous extract was done based on the previous studies done by Pemminati S etal. where three doses was tested and the most effective dose (4mg/kg body weight of mice) was chosen in this study. Toxicity studies were also done previously by Pemminati S et al. and was found to be safe at the given dose.

Hence EO extract and buspirone significantly increased the frequency of head dips and number of squares crossed which confirms their anxiolytic effects.The results presented here show that Emblicaofficinalispossess both anxiolytic-like and locomotor activity in mice probably by its action on 5-HT_{1A} receptor.

V. Conclusion

In conclusion, the present study has shown that the aqueous fruit extract of Emblicaofficinalishas anxiolytic-like properties which is probably mediated through 5-HT_{1A} receptor. Further human trials are required to establish its clinical efficacy either alone or along with routinely prescribed drugs in various anxiety disorders.

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