Research Article

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ANTIANXIETY EFFECT OF ETHANOLIC EXTRACT OF LEAVES OF *TYLOPHORA INDICA* IN WISTAR ALBINO RATS

Shyamjith Manikkoth, Chandrashekar R, Rao S N*
Department of Pharmacology, Yenepoya University, Mangalore, India

Received on: 08/10/12 Revised on: 30/11/12 Accepted on: 16/12/12

***Corresponding author**
E-mail: silentkillersm@gmail.com
DOI: 10.7897/2277-4343.04142
Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT
Pathological anxiety is one of the most common mental disorders in humans. Anxiolytic drugs, mostly belonging to the benzodiazepine (BDZ) group and serotonergic groups are widely used to treat anxiety. However the clinical uses of these established drugs are associated with lot of adverse effects. Therefore the development of new agent possessing anxiolytic effect with minimal or no adverse effects would be of great importance in the treatment of anxiety related disorders. Very high importance is given to the phytoconstituents by the scientific community for a new anxiolytic agent which is devoid of side effects/ minimal adverse effects. In this research work the ethanolic extract of *Tylophora indica* (300 mg/kg body weight) was administered oral for ten days to Wistar albino rats for screening its anxiolytic effect by using Elevated Plus Maze (EPM) and Light Dark Arena (LDA) test. The results of our study demonstrated that the ethanolic extract of *Tylophora indica* has significant anxiolytic activity in EPM and LDA models of anxiety.
Keywords: Anxiety, Wistar albino rats, *Tylophora indica,* Ethanolic extract, Anxiolytic activity

INTRODUCTION

Anxiety is a kind of emotional reaction [1]. It is an unpleasant emotion that most people feel when they face challenges [2]. 'Anxiety' is like some sort of 'worry'[3]. Mild anxiety can help people perform at their best[4]. But when anxiety becomes more intense, it causes distress, lasts for a longer time and interferes with daily activities, which is a medical concern[5]. Anxiety disorder is one of the most common mental disorders in humans[6]. Drugs, belonging to the benzodiazepine group have a prominent position among anxiolytic drugs [5, 6, 7]. Currently the drugs affecting the serotonergic system are recommended as first choice, in anxiety disorders[8]. Despite the use of benzodiazepines, antidepressants and 5-HT1$_A$ agonists in the pharmacotherapy of anxiety disorders, only a small percentage of the patients attain a symptomatic free state during the course of the therapy [9]. Remission rates with serotonergic drugs are lower than the conventional benzodiazepines [10]. Therefore, for the treatment of anxiety disorders, a novel agent with a good therapeutic effect and with a better compliance is needed [10]. Consequently, there is still, a need for improved animal tests for anxiety which are capable of predicting the anxiolytic efficacy of a novel drug [8]. Very high importance is given to the phytoconstituents by the scientific community for a new anxiolytic agent which is devoid of side effects/ minimal adverse effects.

Tylophora indica is a branching climber found in the southern and eastern regions of India. This plant belonging to Asclepiadaceae family, was traditionally used for the treatment of bronchial asthma, jaundice & inflammation. The leaves and roots have emetic, expectorant, diaphoretic, laxative and purgative properties. It has also been used for the treatment of allergies, cold, dysentery, hay fever and arthritis. Various studies have shown that this plant posses antitumor, anti-inflammatory, antiasthmatic, analgesic, anticonvulsant, antirheumatic, antioxidant and hepatoprotective activities. Tylophorine a major alkaloid of *Tylophora indica* has shown to have anti-inflammatory activities. There are no reports on the anxiolytic activity of this indigenous plant. So in this pre- clinical study we have evaluated the anxiolytic role of *Tylophora indica* by using rat models.

MATERIALS AND METHODS

Animals

Young adult Wistar Albino rats of either sex weighing 175–200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (YU/IAEC/1/2009), Yenepoya University. The rats were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) in Dept of Pharmacology, Yenepoya University, Mangalore. The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water ad libitum. Animals were acclimatized under standard laboratory condition and were kept in 12hr day and night cycle for seven days before conducting experiments.

Drugs / Dose / Route of administration

Diazepam (Cipla Ltd) was obtained from Yenepoya Hospital Pharmacy in Mangalore. It was administered at a dose of (1 mg/ kg i.p).

Instruments

Soxhlet apparatus was used to prepare the plant extract. Elevated Plus Maze (EPM) apparatus and Light Dark Arena (LDA) apparatus was used for screening anxiolytic activity.

Plant materials

Tylophora indica plant was cultivated during the month of June. The fresh leaves were collected in the month of September. Authentification of the plant was done by Dr.Noeline. J.Pinto, Head of the Department, Botany, St.Agnes College, Mangalore, Karnataka, India. A voucher specimen of the plant Ref No: YU/TI/22/8/2011 has been kept in the Pharmacology departmental museum, Yenepoya University, Mangalore. The leaves were shade dried, grinded into coarse powder and used for extraction.

Preparation of the extracts

***Tylophora indica* ethanolic extract (TIEE)**

A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60ºc. The percentage yield of the extract was 10%. The ethanolic extract was suspended in distilled water. TIEE was administered at a dose of 300 mg/kg/bodyweight/day orally.

Experimental design

Thirty six animals were used in this study. The animals were divided into three groups. Each group consisting of 6 males and 6 females (n=12).

Group I : Normal Saline (0.1ml) i.p for 10 days
Group II : TIEE (300 mg/kg/day orally) for 10 days
Group III : Diazepam (1 mg/ kg i.p) for 10 days.

On 10th day, after half an hour of the administration of test compounds, the animals were taken for the following tests for screening of anxiolytic activity.

Elevated plus maze (EPM)

This test has been widely validated to measure anxiety in rodents. The plus-maze combines three potential anxiogenic factors – novelty, height and open space. Briefly, the cross-shaped maze consists of four arms that are interconnected by a central platform. Two opposing arms are surrounded by side- and end-walls (closed arms), whereas the remaining two arms are unprotected (open arms).The set-up consists of a maze of two open arms (25 cm ×5 cm), crossed with walls (35 cm high) and central platform (5 cm ×5 cm). The maze is suspended 50 cm above the room floor. The animal was placed on the central platform, facing one of the enclosed arms and observed for 5 minutes. During the 5-min test period, the time spent in open and enclosed arms were recorded [16-22].

Light Dark Arena (LDA)

Light-dark exploration test is one of the few tests specifically designed for use in rats. The original maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment) whereas the remaining 2/3 was open and illuminated (light compartment). The door between the two compartments permits rat to move from one side to another. Each rat was released in the light compartment and observed for 5 minutes. Time spent in light and dark compartment, were recorded [16-22].

Statistical analysis

Results were expressed as mean±SD. One-way analysis of variance (ANOVA) was carried out and the statistical comparisons among the groups were performed with Tukey Krammer test using a statistical package program. $p<0.05$ was considered as significant.

Table 1: Anxiolytic effect of TIEE by Elevated Plus Maze test

Groups	Drugs	Time spent in each arm in seconds	
		Open	Closed
I	Normal saline	8.06±1.40	262.33±6.21
II	TIEE	55.13±8.90***	152.46±11.53***
III	Diazepam	52.58±1.68***	160.27±7.21***

One Way ANOVA, followed by Tukey Kramer multiple comparison test, Results are expressed as mean±SD; n = 12. *** $p< 0.001$ →extremely significant, TIEE-Ethanolic Extract of *Tylophora indica*

Table 2: Anxiolytic effect of TIEE by Light Dark Arena test

Groups	Drugs	Time spent in each arena in seconds	
		Light	Dark
I	Normal saline	34.01±7.332	252±6.44
II	TIEE	138.27±3.32***	160.85±9.51***
III	Diazepam	122.53±3.44***	169.73±4.35***

One Way ANOVA, followed by Tukey Kramer multiple comparison test, Results are expressed as mean±SD; n = 12. *** $p< 0.001$ →extremely significant, TIEE-Ethanolic Extract of *Tylophora indica*

RESULTS

Elevated plus maze

TIEE treated animals (Group II) showed a significant ($p<0.001$) increase in the time spent in open arms (Table 1) by EPM test on comparing with the normal (Group I). But there was no significant difference between the TIEE treated animals (Group II) and Diazepam treated ones (Group III).

Light Dark Arena

TIEE treated animals (Group II) showed a significant ($p<0.001$) increase in the time spent in bright arena (Table 1) by LDA test on comparing with the normal (Group I). But there was no significant difference between the TIEE treated animals (Group II) and Diazepam treated ones (Group III).

The above observations suggest that *Tylophora indica* has anxiolytic activity.

DISCUSSION

The neurobiology of anxiety disorders is not fully known[6]. Low level of GABA in CNS is most frequently associated with anxiety disorders [23]. In addition to GABA, 5-HT plays an important role in the development and the persistence of anxiety disorders[24]. Many studies have shown that patients with anxiety disorders have genetic polymorphisms in the 5-HT transporter[8]. Anxiety

disorders can also be due to free radical induced damage to GABAergic and serotoninergic systems [25].

Relatively little information exists on the CNS activity of *Tylophora indica*. In our study, ethanolic extract of *Tylophora indica* showed significant anxiolytic activity. The anxiolytic activity of *Tylophora indica* can be due to its GABA agonistic activity or by its antioxidant property. Its role on other neurotransmitters like Serotonin, Acetylcholine and Nor-epinephrine cannot be ruled out.

Further studies are ongoing to elucidate the exact mechanism by which this plant exerts the anxiolytic activity.

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Cite this article as:
Shyamjith Manikkoth, Chandrashekar R, Rao S N. Antianxiety effect of ethanolic extract of leaves of Tylophora indica in wistar albino rats. Int. J. Res. Ayur. Pharm. 2013; 4(1):127-129

Source of support: Nil, Conflict of interest: None Declared