

## Evaluation of Anti-anxiety Activity of *Justicia gendarussa* Burm

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### ABSTRACT

**Background:** Anxiety is associated with diverse range of psychiatric conditions. *Justicia gendarussa* Burm. (Acanthaceae) has been traditionally used for the treatment of various ailments such as rheumatism, inflammation, nerve diseases, lumbago and chorea. Despite a long tradition of use, no systematic phytochemical and pharmacological work has been carried out on this potential plant. **Objective:** Thus, *Justicia gendarussa* Burm was subjected to preliminary anti-anxiety screening studies, with a view to ascertain the verity of its traditional use as an anxiolytic. **Materials and method:** In the present investigation the aerial parts of plant parts were extracted with ethanol. The plant extract (EJG) at the dose of 250 and 500 mg kg<sup>-1</sup> were evaluated for anti-anxiety screening studies, with a view to ascertain the verity of its traditional use as an anxiolytic. EJG administered orally in two different doses of 250 and 500 mg kg<sup>-1</sup>, was able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light-dark test, as well as caused significant reduction in freezing time in comparison with control animals. This effect was comparable to that of the benzodiazepine diazepam at the dose of 2.0 mg kg<sup>-1</sup>. **Conclusion:** These results indicate that ethanolic extract of *Justicia gendarussa* Burm has an effective anti-anxiety effect.

**Key words:** *Justicia gendarussa* Burm, anti-anxiety, elevated plus maze, diazepam

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### INTRODUCTION

According to the World Health report<sup>1</sup>, approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020<sup>2</sup>. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models<sup>3</sup>. Anxiety and depression are extremely dramatic and debilitating multifaceted disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of depression and anxiety. Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as antianxiety agents are limited by their unwanted side

effects. Therefore, the development of new pharmacological agents from plant sources are well justified.

*Justicia gendarussa* Burm. an evergreen scandent shrub belonging to the family Acanthaceae is commonly known as Vadaikkuthi in Tamil and widely distributed throughout river beds of Southern India. In traditional medicinal system, different parts of this plant have been mentioned to be useful in a variety of diseases. The leaves and tender shoots are diaphoretic and used in chronic rheumatism. Fresh leaves are used to treat edema and earache. The plant has been used by the native medical practitioners and tribes to treat various ailments including liver disorders, tumours, inflammation and skin diseases<sup>4</sup>. Literature review revealed that this plant is having reverse transcriptase inhibitory activity<sup>5</sup>, anti-angiogenic<sup>6</sup> and hepatoprotective activity<sup>7</sup>. Our previous study on this plant showed potent anti-inflammatory and analgesic activity<sup>8</sup>. Based on these considerations, the present study is to evaluate the anti-anxiety activity of alcoholic extract of aerial parts of *Justicia gendarussa* Burm.

### MATERIALS AND METHODS

**Collection of plant and preparation of the extract:** The aerial parts of plant *justicia gendarussa* were collected

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from the Pattanamthitta, Kerala in the month of December 2008. The plant was then authenticated by the Joint director, The Botanical Survey of India, Coimbatore, Tamilnadu, India (No. BSI/Sc/5/23/08-09/tech.-45). The aerial parts were shade dried and pulverized. The course powder was treated with petroleum ether for dewaxing and removal of chlorophyll. Later, it was packed (250 g) in a soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 mL of ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a dessicator. The percentage yield was found to be 4.5% w/w.

**Animals:** Swiss albino mice (20-25 g) of either sex obtained from Swamy Vivekanadha College of Pharmacy animal house were used for study. They were maintained in standard laboratory conditions  $21 \pm 2^\circ\text{C}$  and relative humidity of 55-60%. They were fed with standard pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Ethical Committee.

### Evaluation of antianxiety activity

**Experimental design:** The animals were divided into four groups and each comprising six animals. Group I served as a control received 0.3% w/v CMC by oral route. Group II animals were administered with standard drug Diazepam ( $2.0 \text{ mg kg}^{-1} \text{ b.wt./p.o.}$ ) suspended in 0.3% CMC solution. Group III and IV animals were treated with ethanolic extract of *Justicia gendarussa* Burm at a dose of 250 and 500  $\text{mg kg}^{-1} \text{ b.wt.}$  by oral route for 21 days, respectively. After 21 days dosing period the animal's anxiety level was observed by screening methods such as elevated plus maze and light dark model.

**Elevated plus-maze test:** The elevated plus-maze comprised two open ( $30 \times 5 \times 0.25 \text{ cm}$ ) and two enclosed ( $30 \times 5 \times 15 \text{ cm}$ ) arms that radiated from a central platform ( $5 \times 5 \text{ cm}$ ) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 50 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00-14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into and the time spent in each of the two types of arm were counted during a 5 min test period. The percentage open arm entries and percentage open arm time were used as indices of anxiety. A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received<sup>9</sup>.

**Light dark test:** The apparatus consisted of two  $20 \times 10 \times 14 \text{ cm}$  plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100 W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received<sup>10</sup>.

**Statistical analysis:** The results have been expressed as Mean  $\pm$  SEM. One way ANOVA followed by Dunnett's multiple comparison test was done to compare the different groups using inst graph pad software.  $p < 0.05$  was considered significant.

## RESULTS

**Effect of EJG on elevated plus maze:** Anxiolytic effect of ethanolic extract of *J. gendarussa* was presented in Table 1. Mice received the standard diazepam showed a significant increase in the time spent and the rears in open arms when compared to normal. This suggests decreased fear, an increased exploratory behavior and the behavioral disinhibitory effect of the standard anxiolytic. The animals treated with EJG at both the dose levels significantly increase the time when compared to control. But there is no significant difference was observed when compared to standard group which indicates that the extract is equipotent to the standard diazepam. Moreover the extract produce dose dependent activity. All these results suggested that the extract is having anxiolytic activity.

**Effect of EJG on light dark model:** A significant increase in time was observed in the animals treated with extract of *Justicia gendarussa* Burm 250 and 500  $\text{mg kg}^{-1}$  when compared to control animals. A significant increase in time spent in light area was also observed in animals treated with standard (Diazepam  $2 \text{ mg kg}^{-1}$ ) drug. The number of transitions was significantly increased in animals treated with *Justicia gendarussa* 250 and 500  $\text{mg kg}^{-1}$  and standard drug Diazepam when compared to control

Table 1: Antianxiety activity of EJG using elevated plus maze

Treatment	Open arm	
	No. of entries	Time in sec
0.3% CMC ( $1 \text{ mL kg}^{-1} \text{ p.o.}$ )	$8.16 \pm 0.47$	$48 \pm 8.16$
Diazepam ( $2 \text{ mg kg}^{-1} \text{ p.o.}$ )	$10.6 \pm 1.05$	$154.83 \pm 22.1^{ab}$
EJG ( $250 \text{ mg kg}^{-1} \text{ p.o.}$ )	$10.3 \pm 0.88$	$136 \pm 15.8^a$
EJG ( $500 \text{ mg kg}^{-1} \text{ p.o.}$ )	$10.5 \pm 0.76$	$151.3 \pm 18.05^a$

n = 6, All values are expressed in Mean  $\pm$  SEM, <sup>a</sup> $p < 0.01$  vs control, <sup>b</sup> $p < 0.01$  vs. Diazepam, Data were analyzed by using Dunnett's test

Table 2: Antianxiety activity of EJG using light dark model

Treatment	Time spend in light (sec)	No. of transitions
CMC-0.1 g 10 mL <sup>-1</sup>	6.10±0.20	17.51±0.63
Diazepam 2 mg kg <sup>-1</sup>	15.20±0.52 <sup>ab</sup>	34.40±0.53 <sup>ab</sup>
EJG 250 mg kg <sup>-1</sup>	10.39±0.36 <sup>ab</sup>	22.29±0.45 <sup>ab</sup>
EJG 500 mg kg <sup>-1</sup>	13.96±0.53 <sup>a</sup>	34.32±0.89 <sup>a</sup>

n = 6, All values are expressed in Mean±SEM, <sup>a</sup>p < 0.01 vs control, <sup>b</sup>p < 0.01 vs. diazepam, data were analyzed by using Dunnet's test

animals. The plant extract produce the activity in dose dependent manner. The results were displayed in Table 2.

## DISCUSSION

The etiology of most anxiety disorders are not completely understood, but various studies has exposed the involvement of GABA, serotonergic neurotransmission in etiology, expression and handling of anxiety<sup>11</sup>. The adrenergic and dopaminergic systems have also been given away to play a role in anxiety. Despite the widespread conventional use of *Justicia gendarussa* Burm for treating various disorders there are no information of scientific assessment of its anxiolytic activity. The present work established that the ethanolic extract of *Justicia gendarussa* Burm had anxiolytic activity in mice in several animal models of anxiety like Elevated Plus Maze (EPM) and Light Dark test models. The conformist plus maze is highly receptive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA benzodiazepine complex<sup>12</sup>. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines. In EPM, male swiss albino mice will usually favor to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics<sup>13</sup>.

The EPM test has been in use as a rodent model of anxiety for a decade, and is spokesperson of those tests that are based upon the study of unprompted behavior patterns and which have high ecological validity. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a comparatively narrow raised platform, moreover it is known that anxiolytic agent increases the incidence of entries and time spent in open arm of the EPM<sup>14</sup>. In agreement with previously published reports, diazepam augmented the percentage time spent on open arms and the figure of entries on open arms. Total digit of open arm entries and number of closed arm entries are usually employed as measures of general activity. In the present study it is noted that administration of EJG lengthened the time spent in the open arms and the number of entries into open arms.

The light/dark box is also widely used for rodents as a model for screening anxiolytic or anxiogenic drugs, based on the inborn aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of mice in response to mild stressors, that is, a novel surroundings and light. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolytic action. The present study showed that EJG (250 and 500 mg kg<sup>-1</sup>) could boost the time in the light area, suggesting again that EJG possesses anxiolytic properties. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavanoids, saponins and tannins possess activity against many CNS disorders.

Phytochemical tests of EJG revealed the presence of saponins, flavanoid, alkaloids and steroids. It may possible that the mechanism of anxiolytic action of EJG could be due to the binding of any of these phytochemicals to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor<sup>15</sup>. The aerial parts of *Justicia gendarussa* Burm also contains flavones which may liable for its anti-anxiety activity. So, the anti-anxiety activity of EJG might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect.

From the above observations, we can conclude that ethanolic extract of *Justicia gendarussa* Burm possesses anti-anxiety activity at both the dose level which is comparable with the standards. However, further studies are under process to isolate the active constituent responsible for the activity and to elucidate the mechanism of action.

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