

# Anti-anxiety Activity of *Cymbopogon citratus* (dc.) stapf Leaves Extracts on the Elevated Plus-Maze Model of Anxiety in Mice

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## ABSTRACT

The Methanolic extract of *Cymbopogon citratus* leaves at the dose of 200 mg/kg increased the percentage of time-spent and the percentage of arm entries in the open arms of the elevated plus-maze (EPM) and decreased the percentage of time-spent in the closed arms of EPM. Moreover, it prolonged the ketamine-induced latency to sleep but had no significant effects on total sleeping time induced by ketamine. Also, the locomotor activity was affected but not to the same extent as observed for diazepam. The anxiolytic effects of methanol extract *Cymbopogon citratus* leaves may be related to their content of flavonoids. This study validates the traditional use of the plant in management of anxiety.

**Key words:** *Cymbopogon citrates*, Diazepam, Elevated plus maze.

## INTRODUCTION

Anxiety disorders are the most common type of psychiatric disorders, with an incidence of 18.1% in total world population and a lifetime prevalence of 28.8%.<sup>[1-2]</sup> Anxiety is an important component of many other psychiatric or medical conditions.<sup>[3]</sup> Anxiety disorders are the most common mental illness in the world and became a very important area of research interest in psychopharmacology. Interest in alternative medicine and plant-derived medications that affect the 'mind' is growing. Self administration of herbal medicines was the most popular alternative therapies to the official medicine. The use of herbal medications by physicians in Europe and Asia is becoming very common and researchers are exploring the traditional remedies to find a suitable cure for these 'mind affecting diseases'.<sup>[4]</sup>

Lemon grass (*Cymbopogon citratus*, Stapf) family Graminae is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in

flavour, fragrancng and aromatherapy, medicinal tea, culinary herb,<sup>[5]</sup> and treatment for skin diseases.<sup>[6]</sup> It is known as a source of ethnomedicines.<sup>[7]</sup> *C. citratus* is used in different parts of the world in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains.<sup>[8]</sup> Essential oil of Lemon grass was evaluated for sedative/hypnotic activity through pentobarbital sleeping time, anxiolytic activity by elevated plus maze and light/dark box procedures and anticonvulsant activity through seizures induced by pentylenetetrazole and maximal electroshock. Essential oil was effective in increasing the sleeping time, the percentage of entries and time spent in the open arms of the elevated plus maze as well as the time spent in the light compartment of light/dark box. In addition, essential oil delayed clonic seizures induced by pentylenetetrazole and blocked tonic extensions induced by maximal electroshock, indicating the elevation of the seizure threshold and/or blockage of seizures spread.<sup>[9]</sup>

A review of literature revealed that *Cymbopogon citratus* is highly reputed plant, and has been widely employed in herbal medicine and aromatherapy but no significant work has been carried out on the anxiolytic effects of the plant extracts. So, the present study was designed to evaluate effect of the methanolic extract of *Cymbopogon citratus* on CNS in mice.

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## MATERIAL AND METHODS

### Plant material

*Cymbopogon citratus* (DC.) stapf. leaves were collected in the month of February 2007, from Panjab University, Chandigarh, India. The taxonomic identity of the plant was confirmed by Dr. H.B. Singh, Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (CSIR), New Delhi 110067.

### Extraction

The dried leaves of *Cymbopogon citratus* was minced (200 g) and were macerated in 600 ml of methanol and water. The extract was concentrated in a rotating evaporator under reduced pressure to give a residue (10%, w/w). The residue was dissolved in normal saline for final suitable concentrations.

### Animals

Swiss albino mice (20-30 gm) of either sex were housed in standard environmental conditions. Food and water were available ad libitum. All experiments were carried out between 09.00 and 13.00 h.

### Elevated plus-maze (EPM)

The EPM is apparatus comprised of two open arms (35 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) that extended from a common central platform (5 cm × 5 cm). That was elevated to a height of 50 cm above floor level.<sup>[10]</sup> Mice were given a single i.p. dose of the plant extract 30 min before their placement on the EPM. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal. Diazepam at dose of 0.5 mg/kg i.p. was used as standard.

### Locomotor activity

The actions of *Cymbopogon citratus* leaves methanolic extract on spontaneous locomotor activity were measured automatically by breaking of infrared beams as described by Rabbani et al.<sup>[11]</sup> The units of the activity counts were arbitrary and based on the beam breaks by movement of mice. Each mouse was i.p. injected with the plant extract (100 and 200 mg/kg) and after 30 min placed in a novel cage in the infrared apparatus. The locomotor activity was measured at 5-min interval for 15 min. Six mice were used for each treatment group. Diazepam at dose of 0.5 mg/kg i.p. was used as reference drug.

### Ketamine-induced sleeping time

The effect of the studied extract on ketamine-induced sleeping time was measured as described by Mimura et al.<sup>[12]</sup> After 30 min pretreatment with the plant extracts (200 mg/kg i.p.)

or vehicle, animals (six for each group) were injected with ketamine (100 mg/kg, i.p.). The interval between the administrations of ketamine until the loss of the righting reflex was recorded as onset of sleep. The time from the loss to regaining of the righting reflex was considered as duration of sleep.<sup>[13]</sup> Diazepam (0.5 mg/kg) was used as standard drug.

### Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Tukey test.  $P < 0.05$  was considered significant. All data are expressed as mean ± S.E.M

## RESULTS

### Effect of *E. sphaericus* fruits extract on the elevated plus-maze

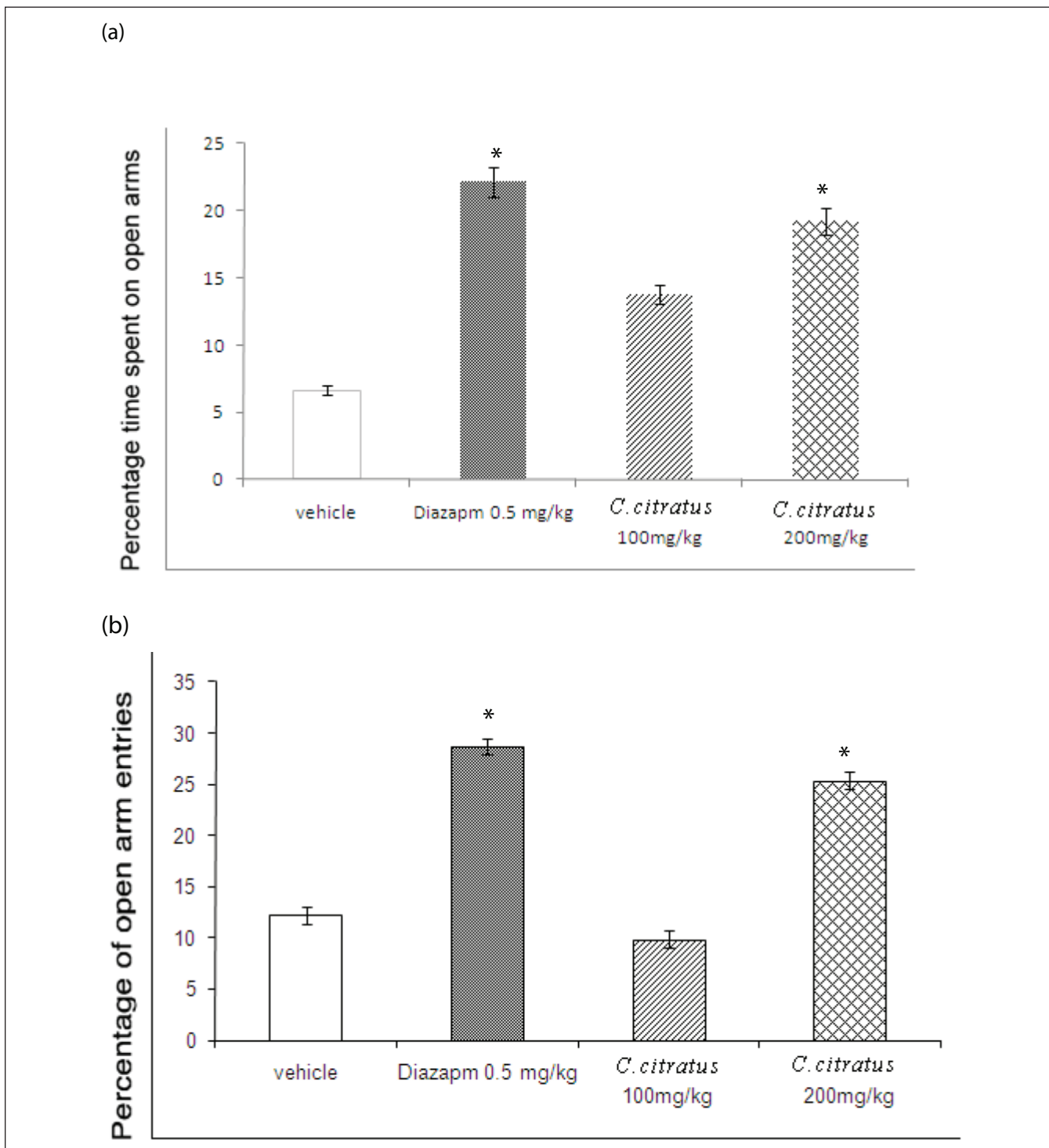
In the elevated plus-maze, the behavior observed confirmed the anxiolytic activity of diazepam as reported previously.<sup>[14]</sup> The methanolic extract of *Cymbopogon citratus* leaves at a dose of 200 mg/kg increased the percentage of time spent and percentage of arm entries in the open arms ( $P < 0.05$ , Fig. 1a,b) and decreased the percentage of time spent and percentage of arm entries in the closed arms ( $P < 0.05$ , Fig. 2a,b). The extract at 100 mg/kg had no significant effects on any of the measured parameters (Figs. 1a,b and 2a,b). In a similar fashion to the studied extract, diazepam increased the percentage of time spent and percentage of arm entries in the open arms ( $P < 0.05$ , Fig. 1a, b). The magnitude of the anxiolytic effects of 200mg/kg mg/kg of *Cymbopogon citratus* leaves of methanolic extract were very close to that observed with 0.5 mg/kg of diazepam.

### Effects of of *Cymbopogon citratus* leaves extract on spontaneous locomotor activity

Fig. 3 shows the cumulative locomotor activity during 15 min of test. In animals pretreated with of *Cymbopogon citratus* leaves methanolic extract (200 mg/kg), the locomotor activity was decreased by 22% compared with vehicle treated controls. Administration of diazepam at 0.5 mg/kg suppressed the locomotor activity to a greater extent (83%).

### Effects of of *Cymbopogon citratus* leaves extract on ketamine-induced sleeping time

Results are reported in Fig. 4. In saline treated animals the righting reflex was lost after 118"2s of ketamine injection. Injection of *Cymbopogon citratus* leaves extract (200 mg/kg) and diazepam significantly suppressed the latency to sleep by 34% and 57%, respectively ( $P < 0.05$ , Fig. 4). On the contrary, the total duration of the sleep was not affected by *Cymbopogon citratus* leaves (+23%,  $P > 0.05$ ) In animals treated with diazepam, however, the duration of sleep was significantly increased by 116%.

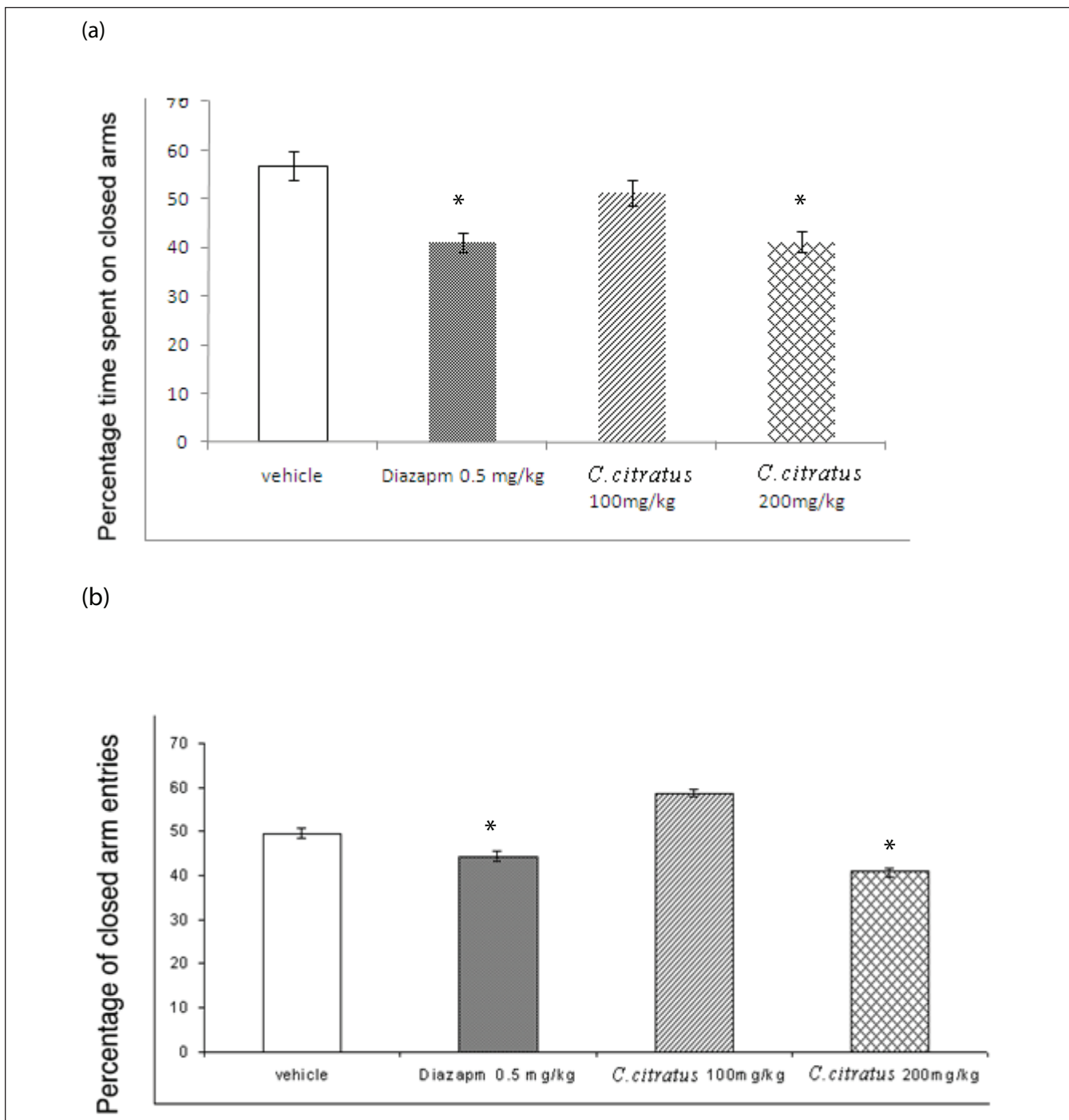


**Figure 1:** Effects of *Cymbopogon citratus* leaves methanolic extract on: (a) the percentage of time spent in the open arms; and (b) the percentage of open arm entries of the elevated plus-maze (EPM). Data are presented as means  $\pm$  S.E.M from a group of six mice each. \* $P < 0.05$  compared with vehicle-treated control.

## DISCUSSION AND CONCLUSION

The aim of the present study was to evaluate the anxiolytic effect of Methanolic extract of *Cymbopogon citratus* leaves. Various doses of the plant extract were tested on the EPM. Only at 200 mg/kg, the plant extract

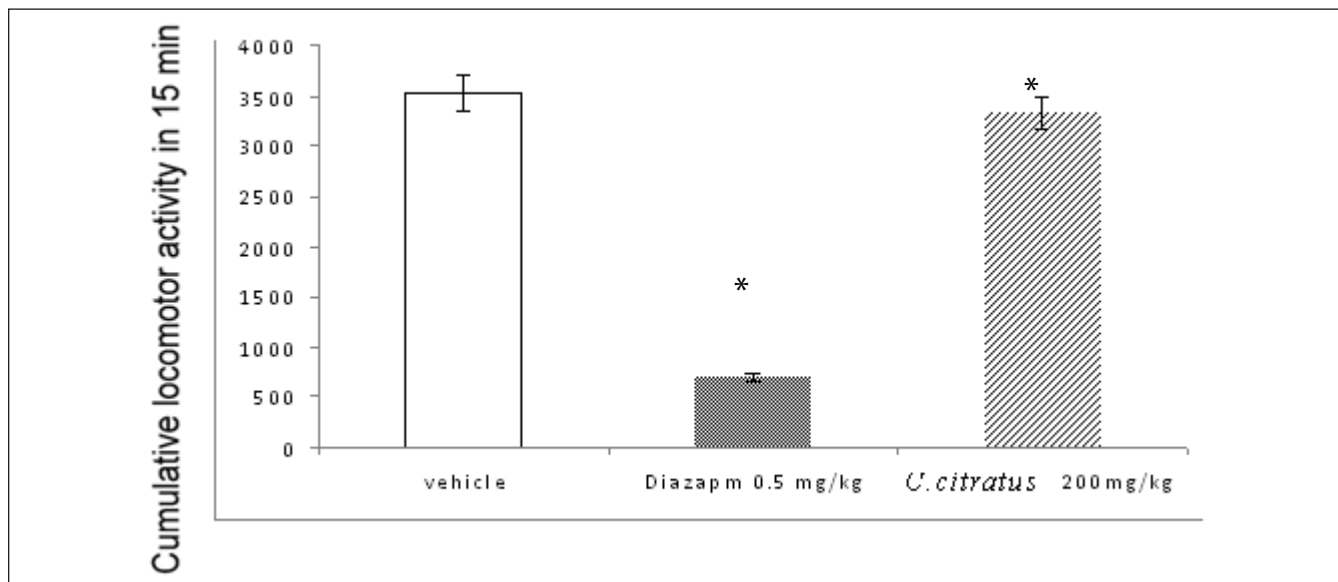
produced anxiolytic effect with a mild sedative action, at doses lower than 200 mg/kg, there was no significant changes in the behavior parameter that was measured on the EPM. Doses higher than 200 mg/kg produce severe sedative effects (data not shown) and were not considered suitable for further evaluation. As expected, diazepam



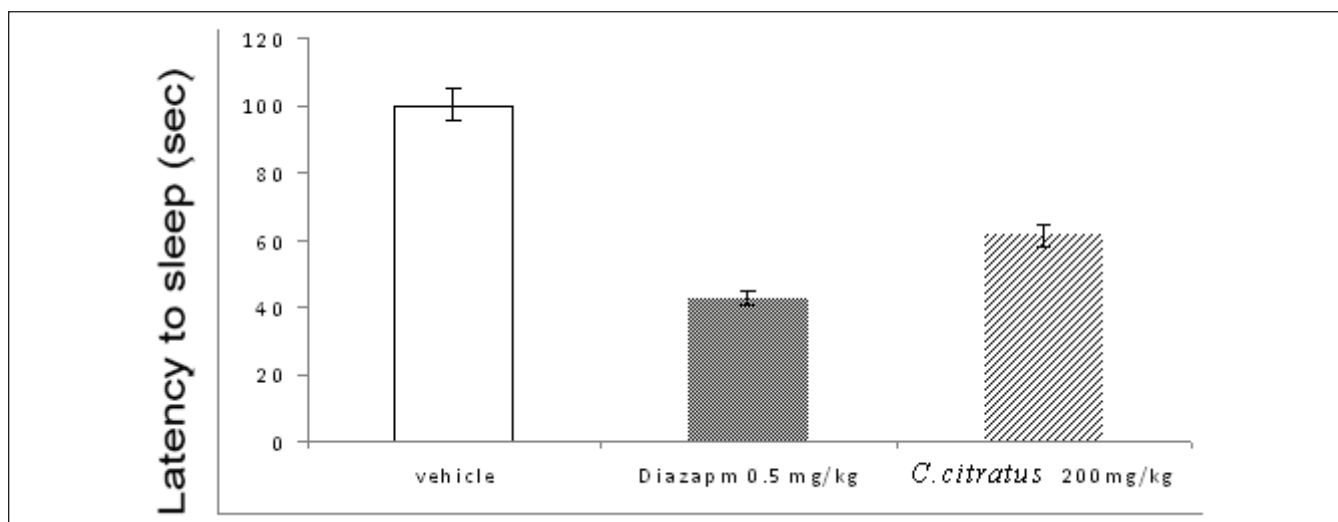
**Figure 2:** Effects of *Cymbopogon citratus* leaves methanolic extract (a) the percentage of time spent in the closed arms; and (b) the percentage of closed arm entries of the elevated plus-maze (EPM). Data are presented as means  $\pm$  S.E.M from a group of six mice each. \* $P < 0.05$  compared with vehicle-treated control.

produced significant increases in open arm time and in number of entries into the open arms. These data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce significant anxiolytic effects in a variety of anxiolytic screening procedures, including elevated plus-maze test procedures.<sup>[15-19]</sup> The decrease aversion to the open arms is a result of an anxiolytic

effect expressed by an increased number of open arm entries and time spent in the EPM. This primary index of anxiety is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds.<sup>[20]</sup> The decreased time spent on the central platform is another indication of a reduced 'decision-making' behavior. Both parameters are accepted as reliable indicators of anxiety and fearfulness.<sup>[21]</sup>



**Figure 3:** Effects of *Cymbopogon citratus* leaves methanolic extract on locomotor activity in mice. Data are presented as means  $\pm$  S.E.M from a group of six mice each. \* $P < 0.05$  compared with vehicle-treated control.



**Figure 4:** Effects of *Cymbopogon citratus* leaves methanolic extract on the latency to loss of righting reflex. After 30 min pretreatment with the plant extracts or vehicle, animals were injected with ketamine (100 mg/kg, i.p.). The interval between the administrations of ketamine until the loss of the righting reflex was recorded as onset of sleep. Results represent means  $\pm$  S.E.M. from six mice. \* $P < 0.05$ , compared with vehicle-treated control.

The other behavioral parameter that was altered in the plus-maze was the number of entries into the enclosed arms. The fact that the number of entries into enclosed arms was lower in animals treated with the plant extract or diazepam indicates that these compounds could have sedative properties. Number of entries into the enclosed arms has been taken as a measure of locomotor activity by a number of researchers.<sup>[20-22]</sup> In the locomotor study, both the plant extract (at anxiolytic dose) produced a significant reduction in locomotor activity count suggesting a sedative property, although the diazepam sedative effect was more severe.

In addition to the locomotor study, the data from the interaction of the plant extract with ketamine also showed a different profile of activity from diazepam. The extract of *Cymbopogon citratus*, shortened the latency to sleep induced by ketamine but did not significantly change the duration of sleep. Diazepam reduced the latency to sleep and increase significantly the sleeping time. Further phytochemical screening showed the presence of flavonoids, saponins and triterpenoids in methanolic extract.

In summary, the present results demonstrated an anxiolytic-like effect from *Cymbopogon citratus* leaves methanolic extract

with a mild sedative action. Further pharmacological investigations are underway to identify the active constituents of the plant extract responsible for the showed activities.

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