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Research Paper

Evaluation of the central nervous system effects of the oil and aqueous extract of *Cymbopogon citratus* (Poaceae) Stapf in Mice.

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ABSTRACT
This study was carried out to investigate the effects of the oil and aqueous extract of Cymbopogon citratus (Poaceae) Stapf on learning and memory, it further evaluated the anti-depressant and analgesic properties of the oil and aqueous extract in mice.
The essential oil of the plant was obtained by hydrodistillation using Clavenger apparatus while the aqueous extract was obtained using a reflux apparatus. Acute toxicity (LD_{50}) of both oil and extract were determined by Lorke's method for oral route only. The oil was evaluated for novelty-induced behaviours (locomotion, rearing and grooming) in the open field box, anxiolytic (hole board and elevated plus-maze), analgesic (hot plate model), anti-depressant (forced swimming test) and learning and memory (Y-maze) effects in mice.
The oil induced no significant effect on novelty induced rearing (NIR) but the extract (1000-2000 mg/kg) caused significant increase in locomotor activity when compared to control. The EO and AQ at all the doses caused significant increase in the number of head dips, and increase in the time spent in the open arms of the elevated plus-maze, respectively, indicating anxiolytic effects. The EO and the AQ at all the dose levels caused a significant reduction in the immobility time in the FST, indicating anti-depressant effect. Finally at these dose levels, the EO and AQ significantly caused increase in the reaction time on the hot plate model, indicating analgesic effects.
The essential oil and aqueous extract of C. citratusmay possessed significant anxiolytic, analgesic and anti-depressant properties.

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I. Introduction

Ethnomedicine is the study of traditional medical practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare seeking process and healing practices (Krippner, 2003).

The WHO estimates that up to 80% of the populations in sub- Saharan Africa make use of ethnomedicine (WHO, 2003). Indeed, in spite of widespread introduction of western-type medical services in sub-Saharan Africa; traditional medicine remains the most subscribed and accessible therapeutic system in the region. For instance, it is estimated that in Nigeria, ethnomedicine is actually the only healthcare resource accessible to a third of the population and scholars have attributed the popularity of ethnomedicine in Nigeria to its affordability, accessibility, cultural acceptability, and proven efficacy (Jegede, 1998; Udoh, 2000; Erinosho, 1989).

This research work focuses on a plant commonly used in Nigeria for various ailments – *Cymbopogon citratus* (lemongrass).

Lemongrass is an aromatic grass belonging to the family Poaceae and the genus *Cymbopogon*. Poaceae, is the grass family, which is a very large cosmopolitan family consisting of about 50 to 60 tribes, 660 genera and 9000 species throughout the world (Olorode, 1984; Hutchinson *et al*., 1972). Cymbopogon is a genus of about 55 species, which are indigenous to tropical and semi-tropical areas of Asia and are cultivated in South and

Central America, Africa and other tropical countries. The name Cymbopogon is derived from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement (Plants database, 2003).

Lemongrass is well known for its oil and it is one of the world's best known essential oils.

There are two main types of lemongrass namely East Indian and West Indian. The East Indian lemongrass oil is obtained from *Cymbopogon flexuosus*Stapf and is the genuine oil of commercial importance. The West Indian oil is extracted from *Cymbopogon citratus*(DC) Stapf that is mainly cultivated in Central and South America and also known in parts of Africa, South East Asia and the Indian Ocean Islands. The name lemongrass has been given because of its typical strong lemon - like odour, which is due to the high citral content.

The general uses of lemongrass include the following; cough, cold and sore throats, anxiety, high cholesterol, type 2 diabetes, colitis, indigestion, rough, dry scaly skin, acne, constipation, kidney detoxification, insomnia, relaxation and deep sleep

1.2.4 Ethnomedicinal uses

Decoction of leaf is taken orally with "mate" tea for sore throat, empacho, and as an emetic, in Argentina (Filipoy,1994). The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative in Brazil (Leite*et al*., 1986; Souza *et al*., 1986). Hot water extract of dried leaves is taken orally as an hypotensive, for catarrh and rheumatismin in Cuba (Carbajal *et al*., 1989). Hot water extract of dried leaves and stem is taken orally as a renal antispasmodic and diuretic in Egypt (Locksley et al.,1982). Fresh entire plant is said to repel snakes (Rao *et al.,* 1982). Two to three drops of essential oil, in hot water is taken orally for gastric troubles. For cholera, a few drops of oil with lemon juice are taken orally (John, 1984). Hot water extract of dried leaves is used for bathing in cases of severe headache and fever (Rao *et al*., 1982). A tea prepared from lemon grass is used as a sedative for the central nervous system, in India (Nair, 1977). Hot water extract of the entire plant is taken orally as an emmenagogue in Indonesia (Quisumbing, 1951). Hot water extract of the entire plant is taken orally as an emmenagogue in Malaysia (Burkhill,1977). Fresh entire plant is inhaled as a fragrance and eaten as a condiment (Praditvarn *et al*., 1950); hot water extract of dried entire plant is taken orally as a stomachic (Wasuwat, 1967); hot water extract of dried root is taken orally for diabetes in Thailand (Ngamwathana *et al* 1971). Hot water extract of entire plant is used externally by Laotian Hmong in Minnesota for healing wounds and bone fractures in USA (Spring, 1989). Lemongrass is used to treat fever, jaundice, hypertension, diabetes mellitus and obesity (Viana *et al.,* 2000; Adeneye and Agbaje, 2007). It is also used to treat malaria in Nigeria.

II. MATERIALS AND METHODS

Fresh leaves of *Cymbopogon citratus* were collected in June 2012, from the of Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The leaves were collected in the morning between the hours of 7am and 9am.

Essential oil and aqueous extract extraction

Essential oil (EO) was obtained by hydrodistillation of fresh leaves using a Clavenger Apparatus (AOAC,1990). The total distillation time was approximately 4hrs. The collected oil was dried over magnesium sulphate to remove extra water, stored in an amber bottle and kept in a refrigerator, until behavioural assays were carried out.

The aqueous extract was obtained using a reflux apparatus from 500 g fresh leaf samples and 1,000 ml of distilled water. The extract was concentrated using a rotary evaporator at 50^{o}C and freeze dried and dissolved in distilled water to obtain the graded dose levels used in the study.

Preparation of the essential oil and aqueous extract for administration

An emulsion of the essential oil of*Cymbopogon citratus* was prepared fresh on each day of the experiment, using Tween 80 and distilled water as vehicle. While the freeze dried aqueous extract was dissolved in distilled water.

Animals

Male and female mice weighing 18-25 g were selected for the study. They were purchased from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. They were housed in the animal house before they were transferred to the laboratory following which they were allowed to acclimatize within the laboratory environment for a period of one week before the commencement of the experiments. They were randomly divided into groups of six animals and were housed in clean cages laden with fresh wood shavings. They were fed with growers mash (manufactured by UAC Foods Nigeria Plc.) and water was provided *ad libitum.*

Behavioural studies

Novelty induced and exploratory behaviour studies

The open field apparatus consist of a wooden box (60 x 60 x 30 cm) the floor of the box was divided into 16 squares (15 x 15 cm). The method described by Nyeem *et al*., (2006) was used for these experiments. Mice were treated with the essential oil(Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline,0.1 ml/10g), or the standard (1 mg/kg diazepam i.p.).Each mouse after one hour was placed in one of the corner squares and observed over a 30 minutes period, the spontaneous exploratory activity of the mouse were measured. Three parameters namely locomotion (line crossing), rearing and grooming were recorded. 1 mg/kg diazepam (Nyeem *et al*., 2006) served as the positive control. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

Anxiolytic tests

(a) Elevated plus-maze studies

The elevated plus-maze is a modification of the apparatus validated to measure anxiety in rodents by Lister (Lister, 1987, 1990). The apparatus consists of a plus-shaped maze formed by two opposite open arms (30 x 5 x 0.25 cm); crossed with two arms enclosed by walls(30 x 5 x15 cm). The open and enclosed arms converge into a central platform (5.0 cm×5.0 cm). The maze is elevated to a height of 40.0 cm from ground level. Each mouse after one hour of the administration of the oil,(Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline, 0.1 ml/10g), or the standard (1 mg/kg diazepam i.p.), was placed at the centre of the elevated plus maze with its head facing the open arm. During the 5-minute experiment the behaviour of each mouse was recorded as:

(i) the number of entries into the open or closed arms; and
(ii) time spent by the mouse in each arm

Every precaution was taken to ensure that no external stimuli other than the height of the maze could invoke anxiety. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

(b) Hole board test (Bossier & Simon 1960)

The Hole board consist of a wooden board with 16 holes evenly distributed on the floor and elevated to a height of 25 cm. Each mouse after one hour of the administration of the oil, (Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline, 0.1 ml/10g), or the standard (1 mg/kg diazepam i.p.), was placed at the centre of the hole board for a period of five minutes. The number of times the animal dipped its head in the holes were noted and recorded. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

2.2.4 Learning and memory test – (Y-maze)

The Y-maze apparatus consist of three equally spaced arms (120^0: 41 cm long x 15 cm high). The floor of each arm is made of wood (5 cm wide). Each mouse after one hour of the administration of the oil (Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline ,0.1 ml/10g), or the standard (1 mg/kg diazepam i.p.), was placed at the centre of the Y- maze and was allowed to roam the different arms of the maze for six minutes, records of the arms visited and the sequence of the arms the animal entered were manually recorded and noted in the record book. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

Anti-depressant test - Forced swimming test

Each mouse after one hour of the administration of the oil (Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline ,0.1 ml/10g), or the standard (fluoxetine 0.2 mg/10 g, p.o.), was placed inside a cylinder filled half-way with water for a period of six minutes. The mouse was allowed to acclimatize for a period of two minutes after which the scoring of active (swimming and climbing) or passive (immobility) behaviour of the mouse were noted and recorded in the record book. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

Analgesic test - Hot plate test

Six groups of six mice each were used for this experiment. Group 1-4 received 50, 100, 150, and 200 mg/kg of the oil, p.o., respectively. The fifth and the sixth groups were administered 1% Tween 80 in normal saline, 0.1 ml / 10g (Ayoka *et al*., 2006) and morphine (0.1 mg/10 g) orally and intraperitoreally respectively. Hot plate analgesia meter (ANALGESIA HOTPLATE manufactured by Columbus Instruments of 950 North Hague Avenue, Columbus, Ohio 43204, USA) was used in this study.The hot plate was maintained at a constant 55ºC surface temperature. Each mouse after one hour of the administration of the oil(Selected doses include 50, 100, 150 and 200 mg/Kg), the vehicle (1% Tween 80 in normal saline, 0.1 ml/10g), or the standard (morphine, 0.1 mg/10 g i.p.), was placed on the hot surface of the plate, following which, Start/Stop button, was pushed to

activate the timer. Time was displayed in tenths of a second until a temperature discomfort (paw-lick response) was observed and the Start/Stop button was pushed again to deactivate the timer. The timer reading was recorded manually from the front panel display. This procedure was repeated using the aqueous extract (250, 500, 1000 and 2000 mg/kg).

Statistical analysis

The data were analyzed using the GraphPad® primism 4.0 statistical software. The test doses were compared with control by one way analysis of variance (ANOVA) followed by post hoc analysis using Student Newman-Kuels multiple comparison tests. Test doses were also compared with the standard drug (diazepam). All results were expressed as mean ± standard error of mean (SEM). P values less than 0.05 were taken as significant (i.e. $p < 0.05$). Appropriate tables and figures were used to display the data.

The level of significance for all tests was set at $p<0.05$.

III. Results

Novelty- Induced Behavior (NIB) (Locomotion, Rearing and Grooming)

Locomotor activity, (locomotion, rearing and grooming) were investigated for the behavioral studies. All the test doses of the essential oil did not significantly increase not only the locomotion, but also the number of rearing and grooming when compared to vehicle-treated group.

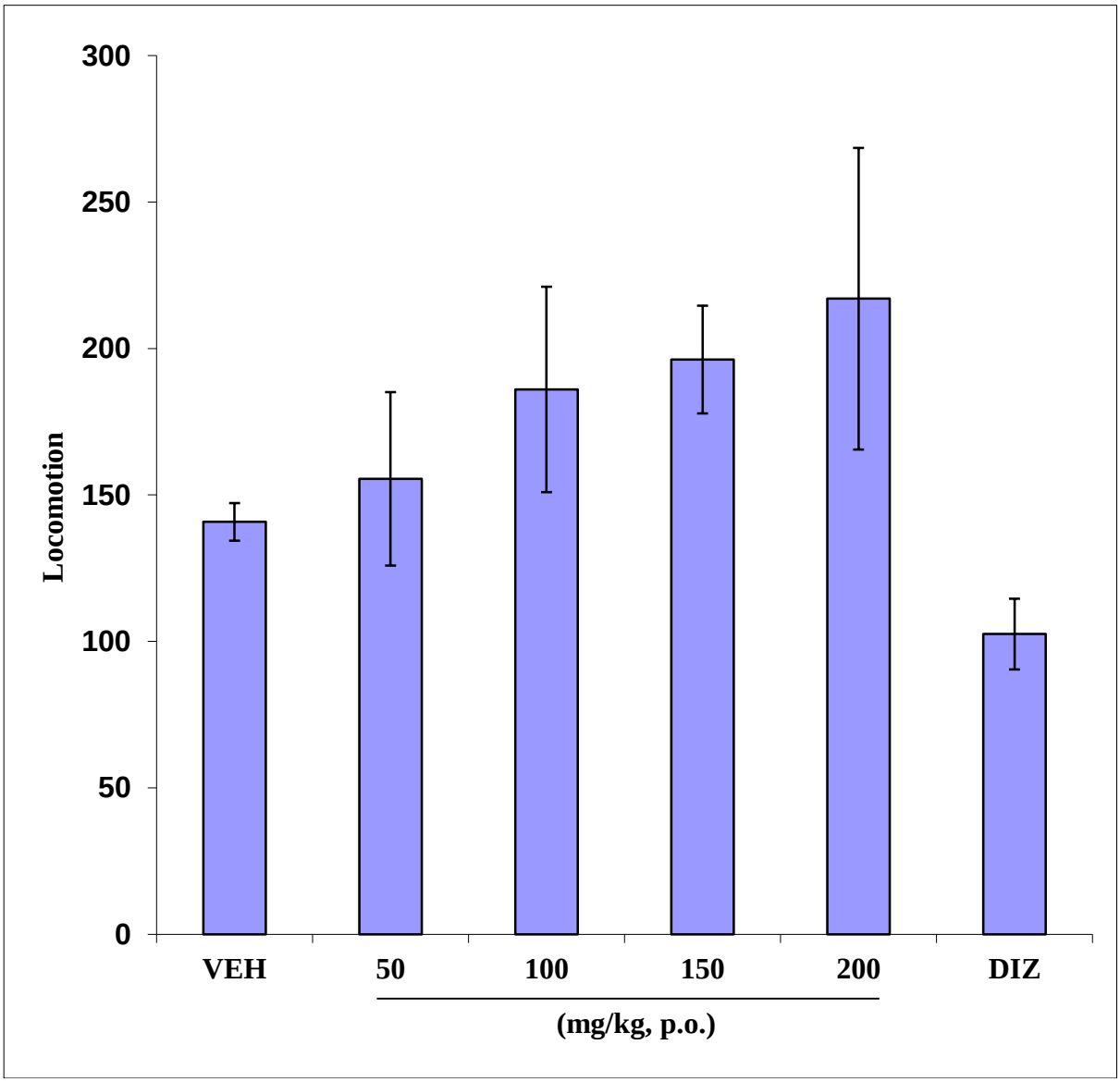

Figure 4a: Effects of the essential oil (EO) of *Cymbopogon citratus* on locomotion. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p).

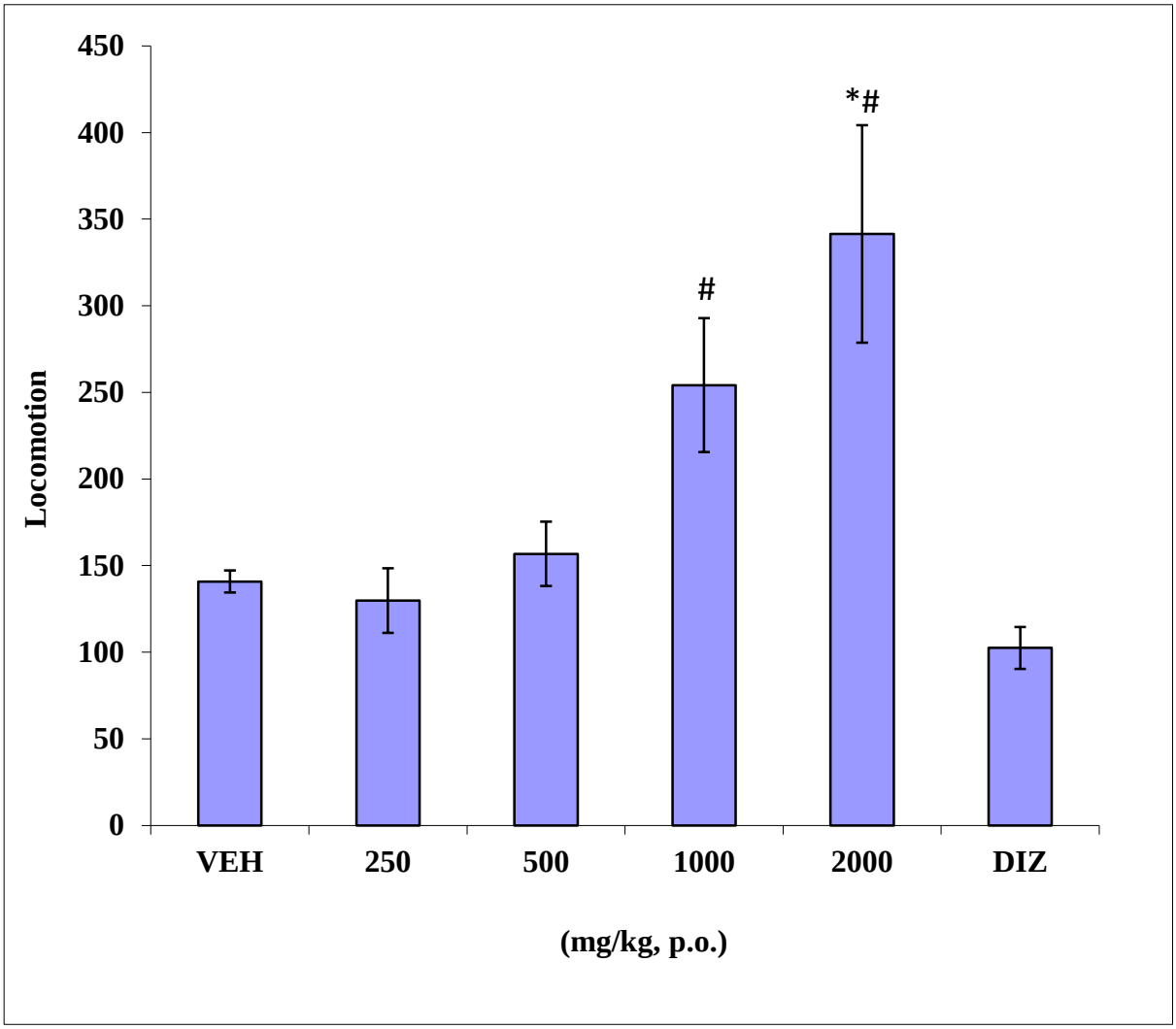

Figure 4b: Effects of the Aqueous extract (AQ) of *Cymbopogon citratus* on locomotion. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1 mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with diazepam).

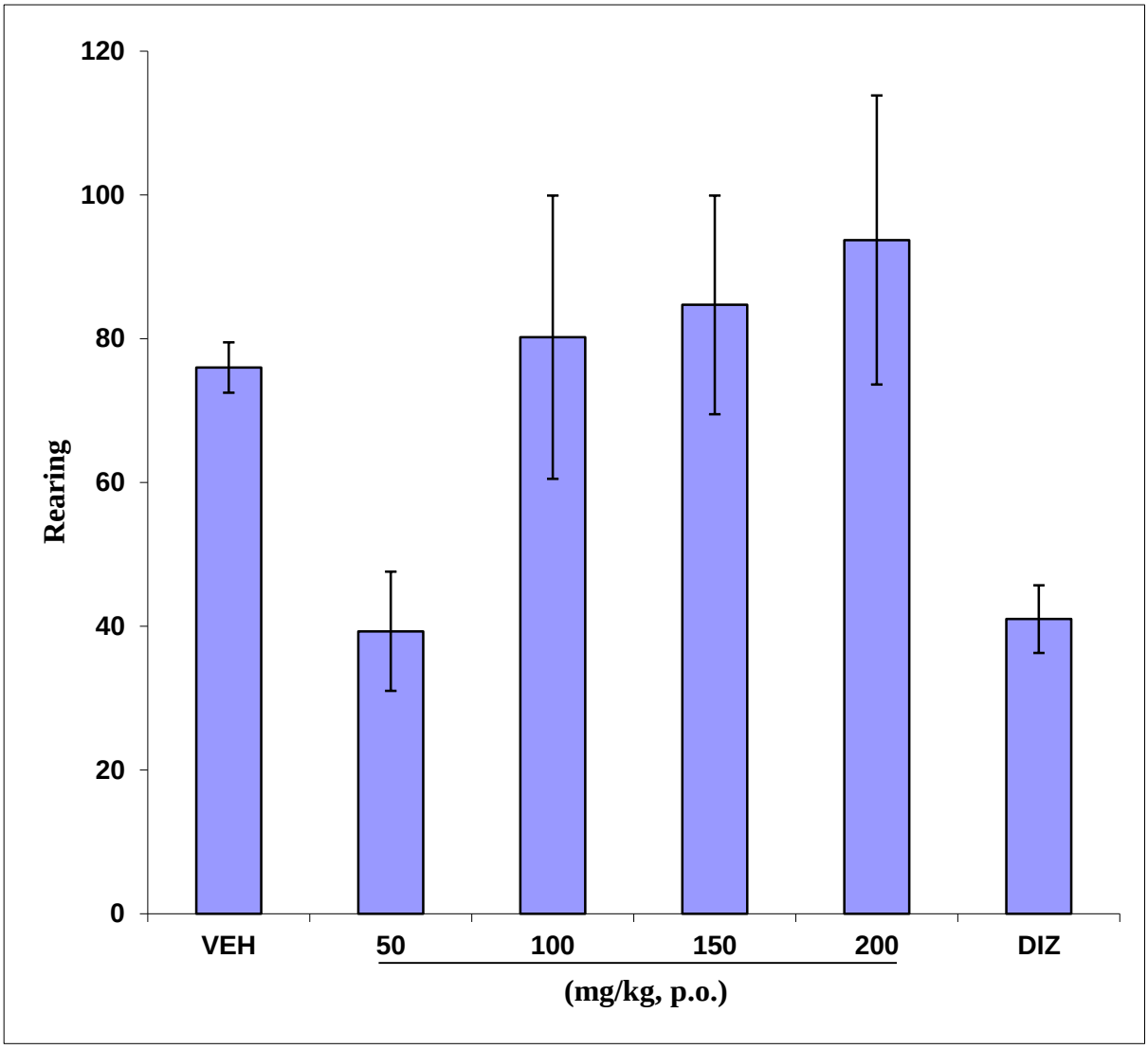

Figure 5a: Effects of the essential oil (EO) of *Cymbopogon citratus* on Rearing. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.).

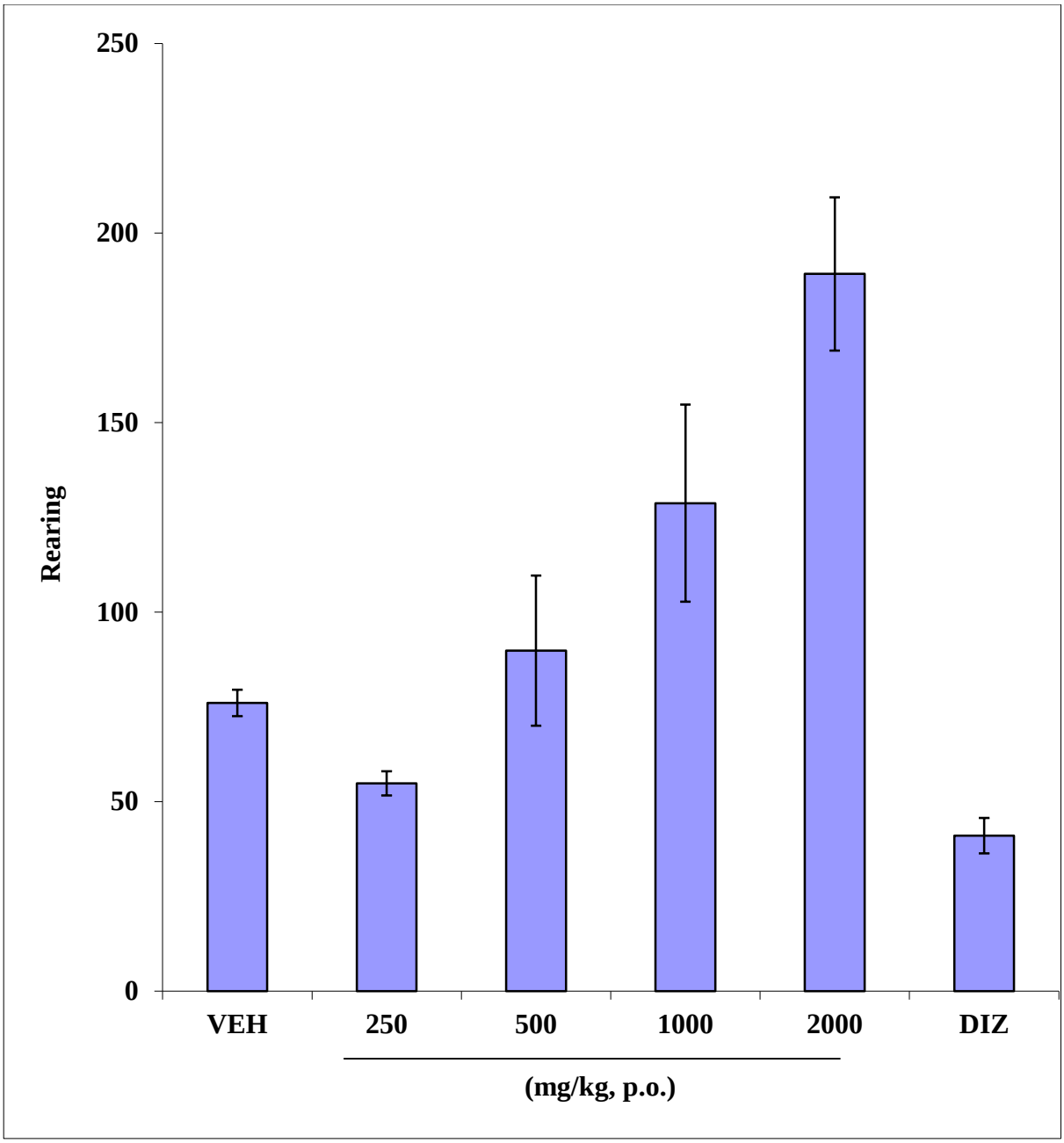

Figure 5b: Effects of the Aqueous extract (AQ) of *Cymbopogon citratus* on Rearing.Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with diazepam).

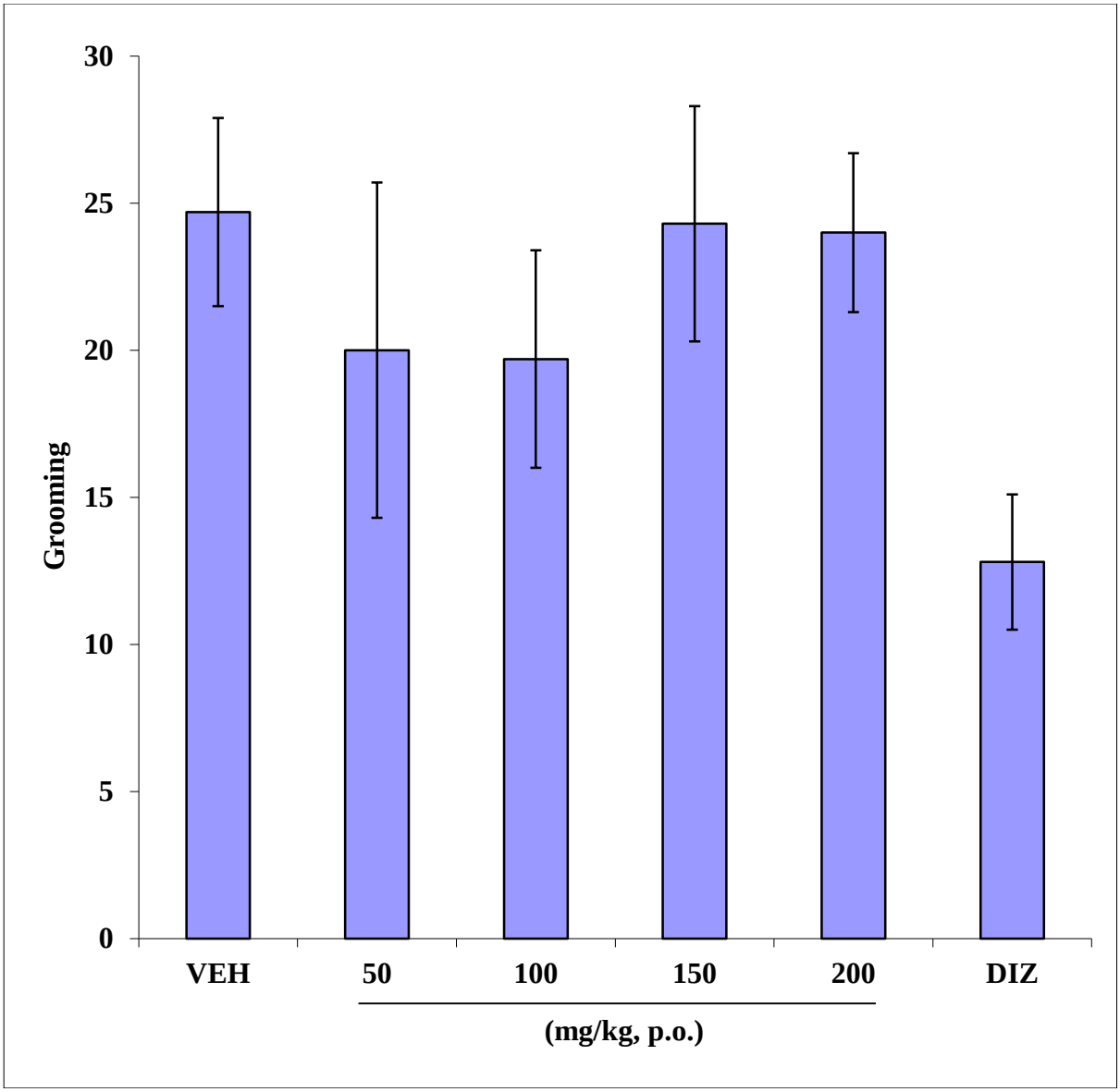

Figure 6a: Effects of the essential oil (EO) of *Cymbopogon citratus* on Grooming Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.).

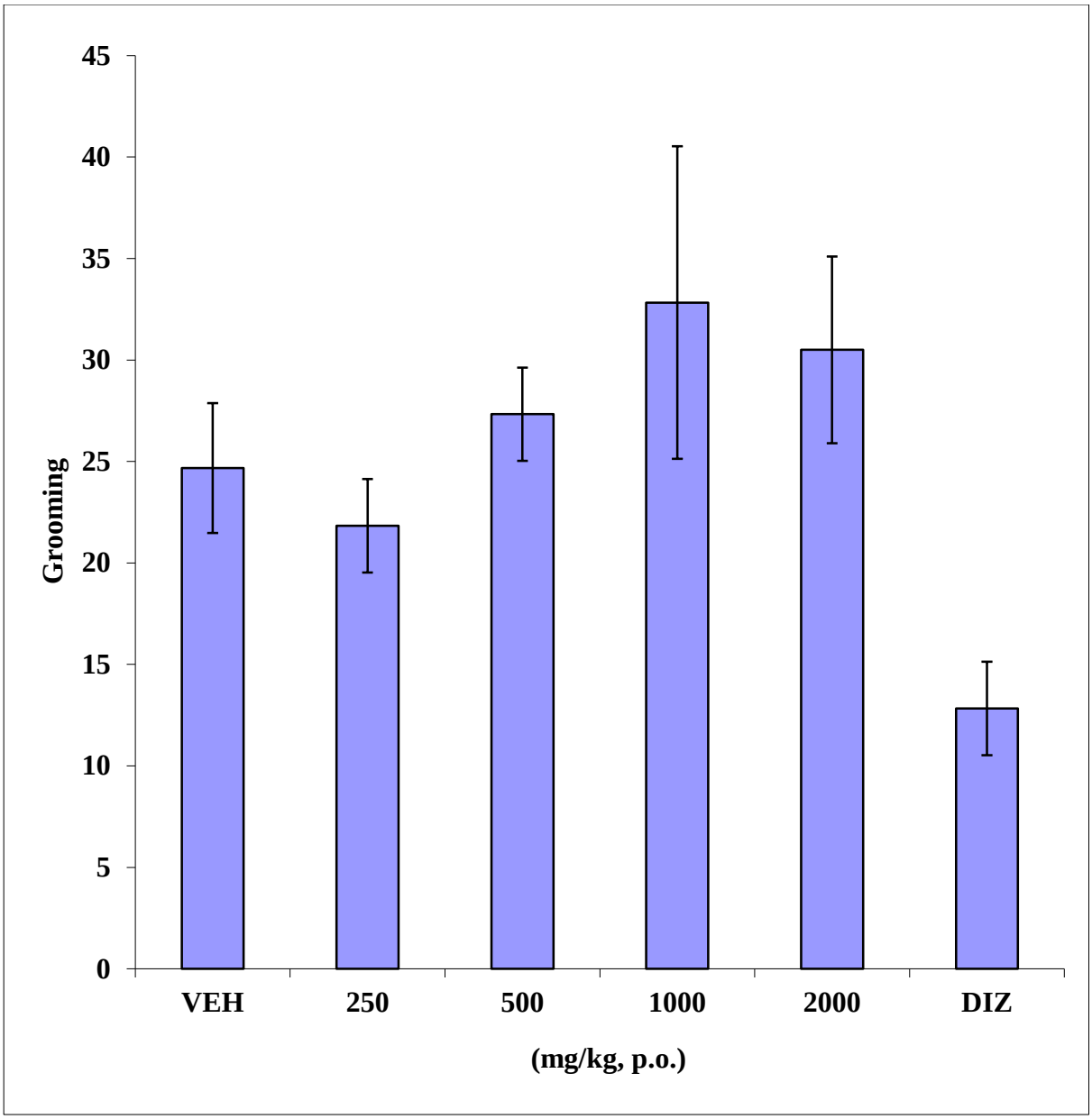

Figure 6b: Effects of aqueous extract (AQ) of *Cymbopogon citratus* on Grooming Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). $^{\#}p < 0.05$ (when compared with diazepam).

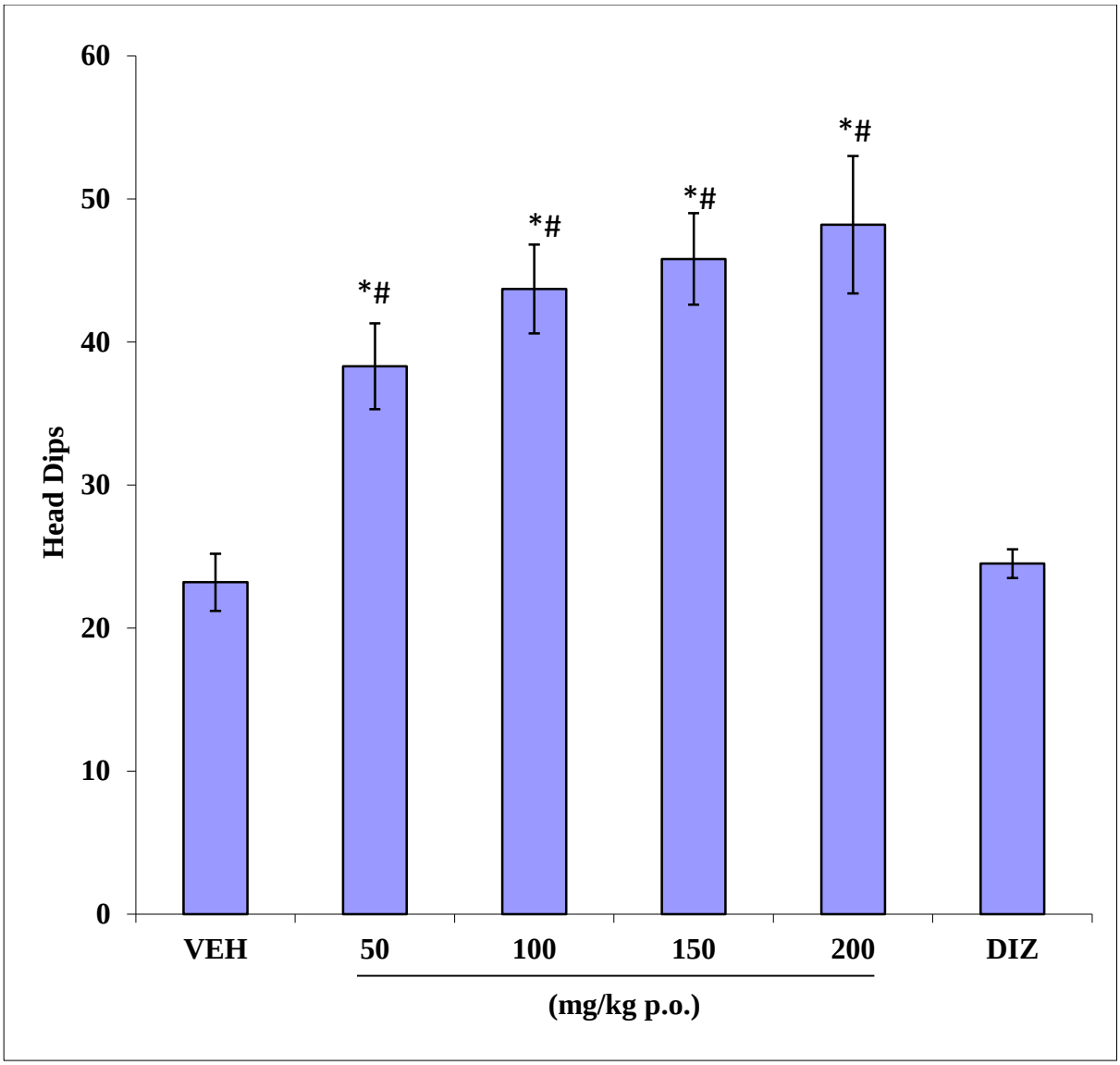

Figure 7a: Effects of the essential oil (EO) of *Cymbopogon citratus* on Head dips. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with diazepam).

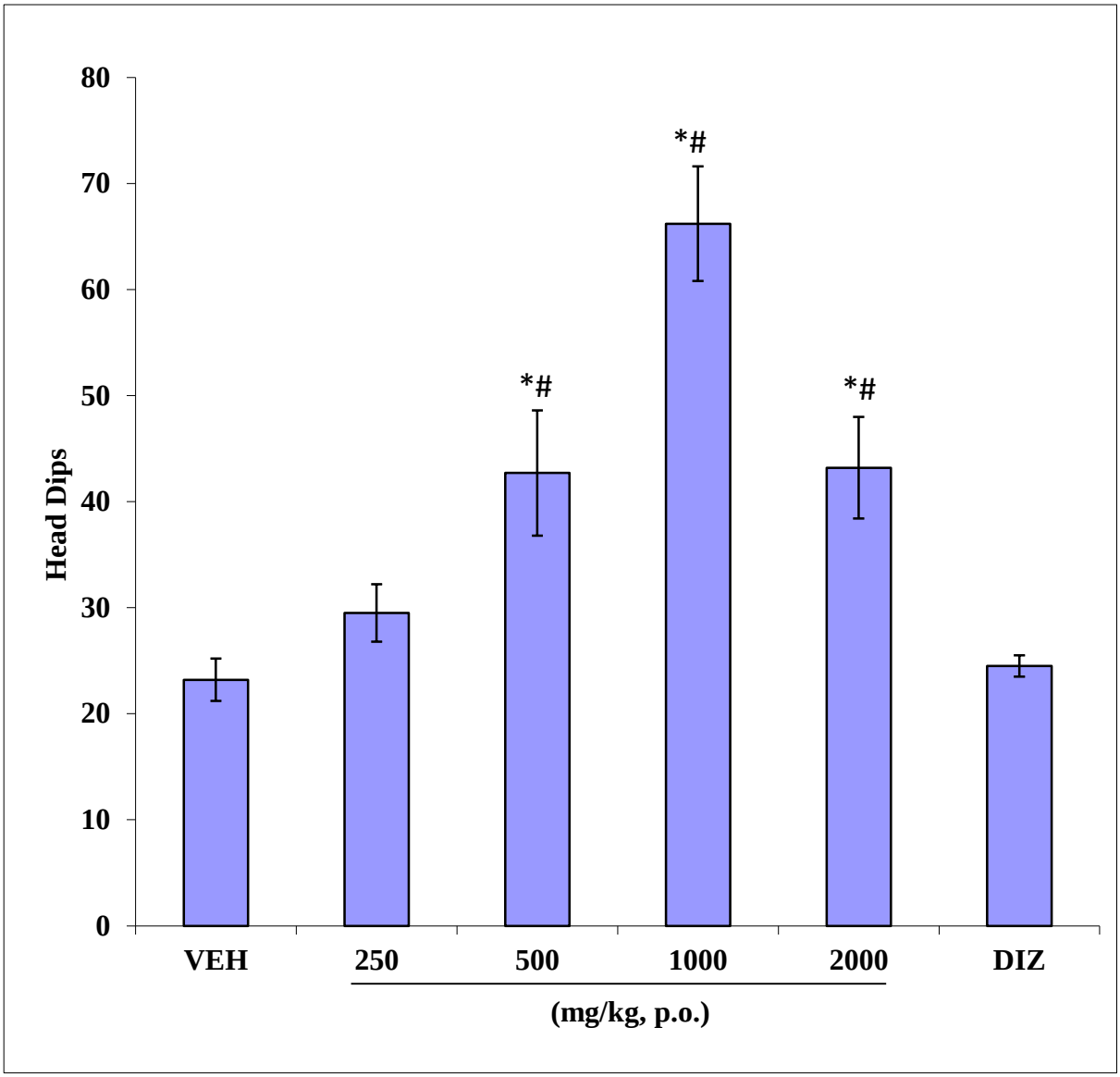

Figure 7b: Effects of the aqueous extract (AQ) of *Cymbopogon citratus* on Head dips. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with diazepam).

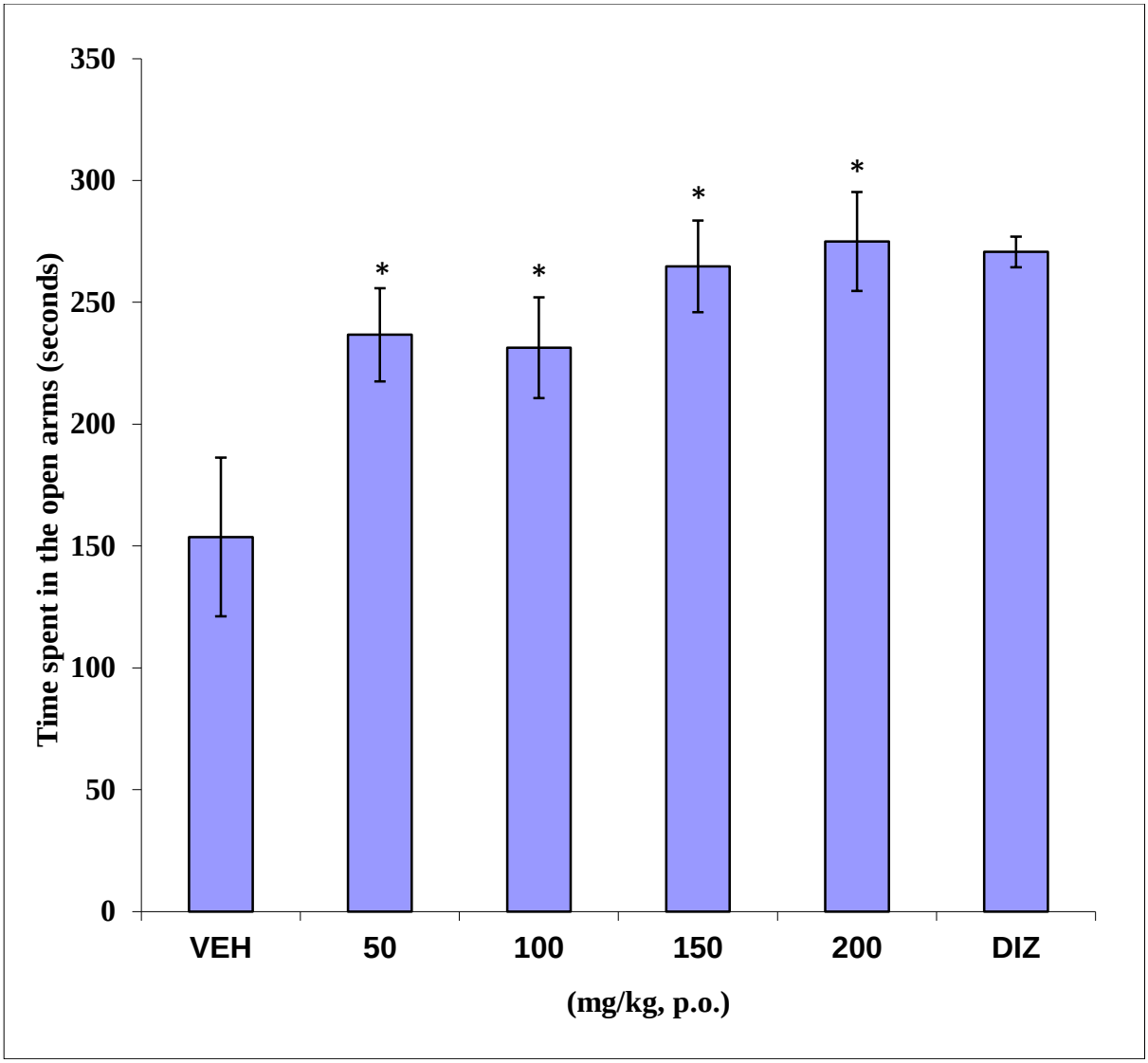

Figure 8a: Effects of the essential oil (EO) of *Cymbopogon citratus* on the time spent in the open arms of the elevated plus maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *p < 0.05 (when compared with vehicle).

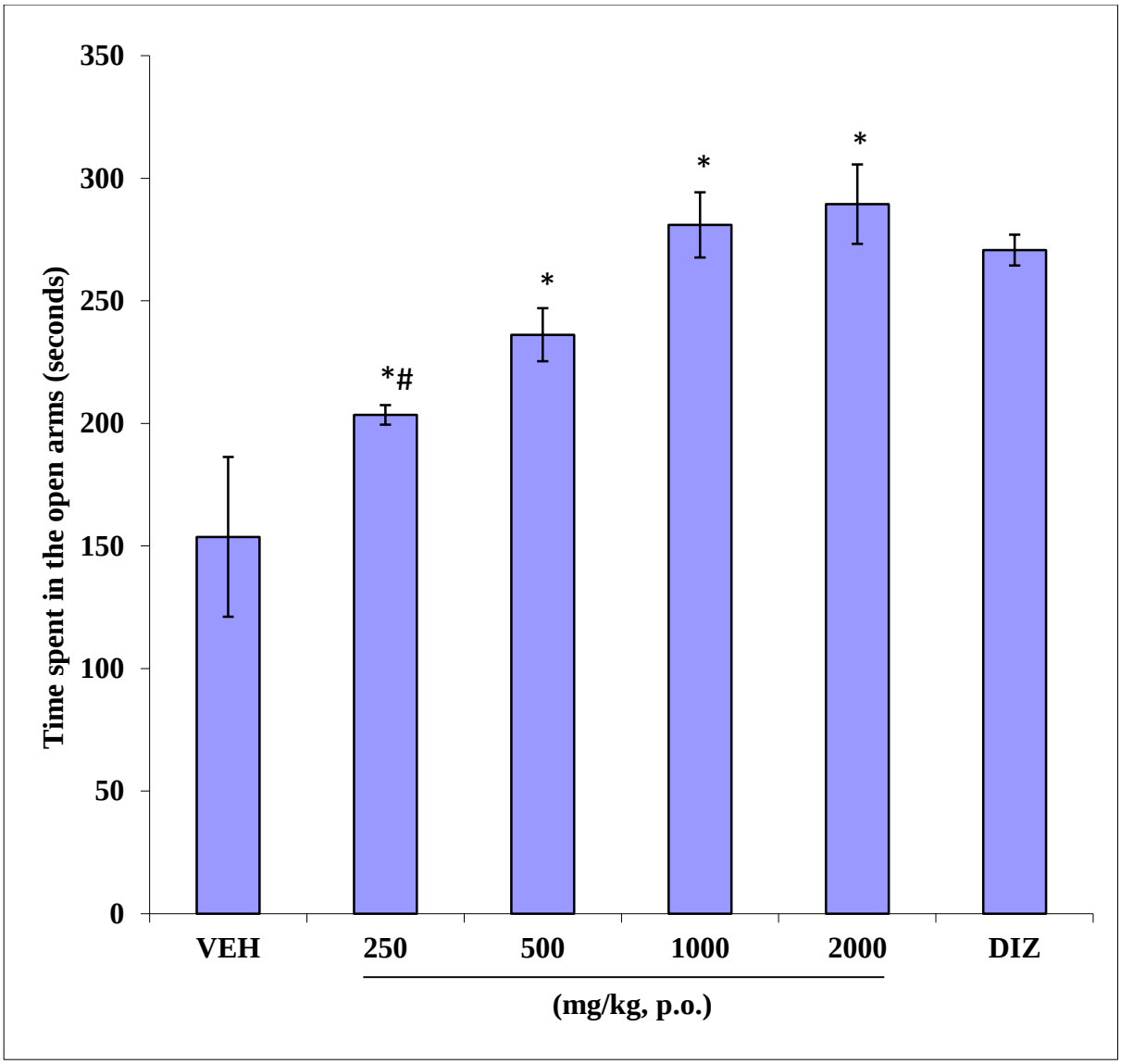

Figure 8b: Effects of the aqueous extract (AQ) of *Cymbopogon citratus* on the time spent in the open arms of the elevated plus maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *p < 0.05 (when compared with vehicle); #p < 0.05 (when compared with diazepam).

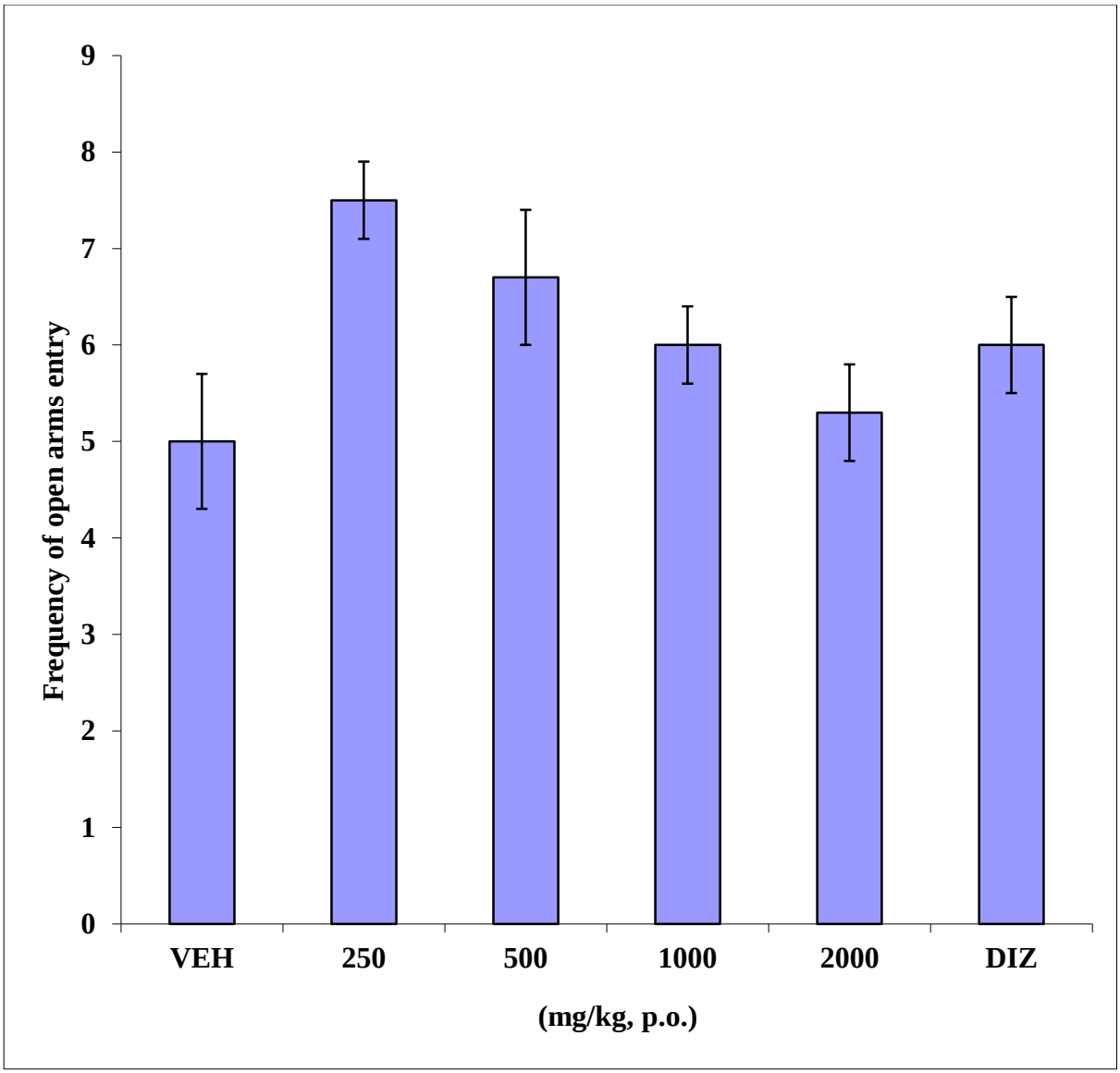

Figure 9a: Effects of the essential oil (EO) of *Cymbopogon citratus* on the frequency of open arms entry in the elevated plus maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.).

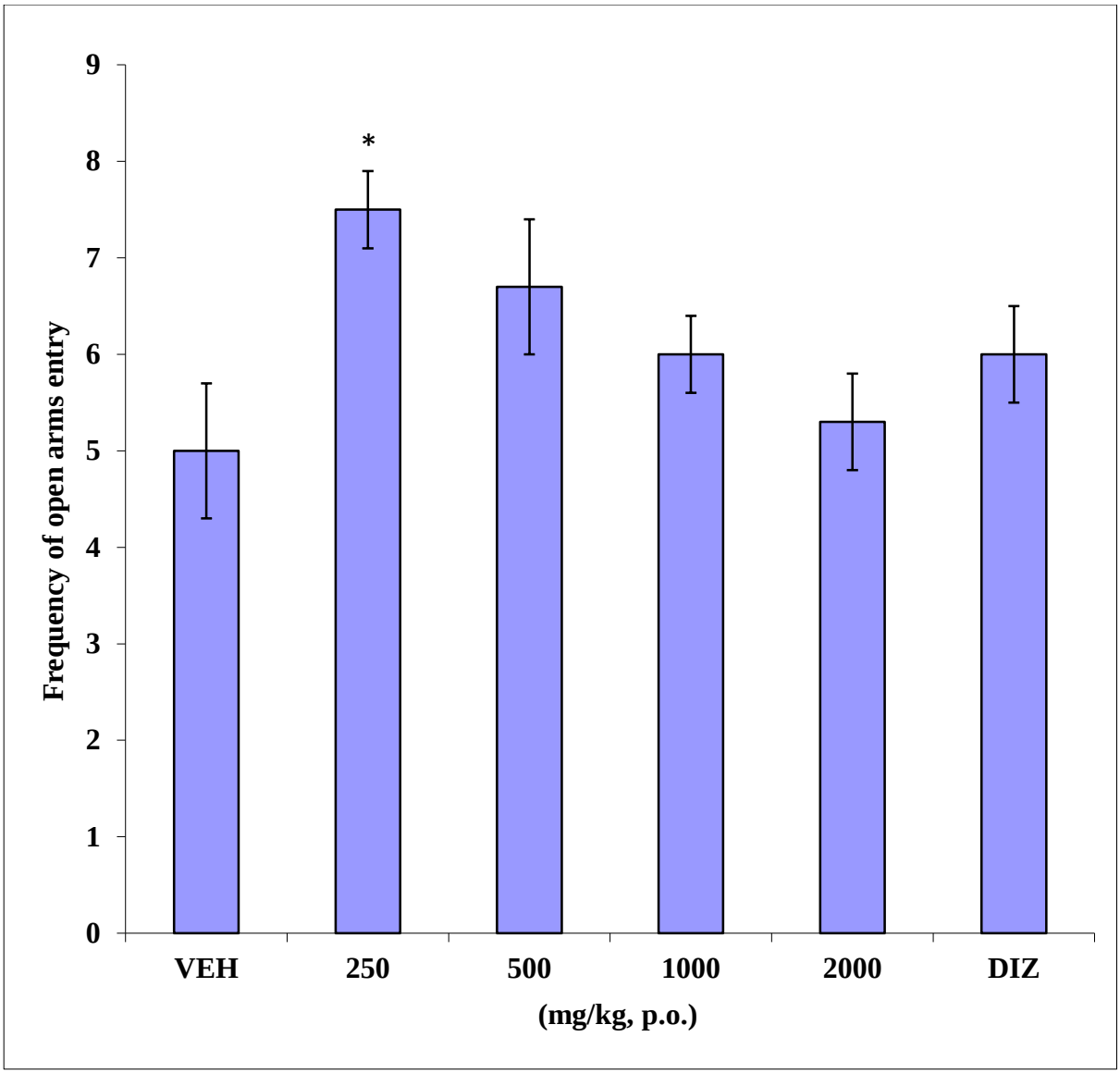

Figure 9b: Effects of the aqueous extract (AQ of *Cymbopogon citratus* on the frequency of open arms entry in the elevated plus maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg), DIZ-diazepam (1mg/kg, i.p.). *p < 0.05 (when compared with vehicle).

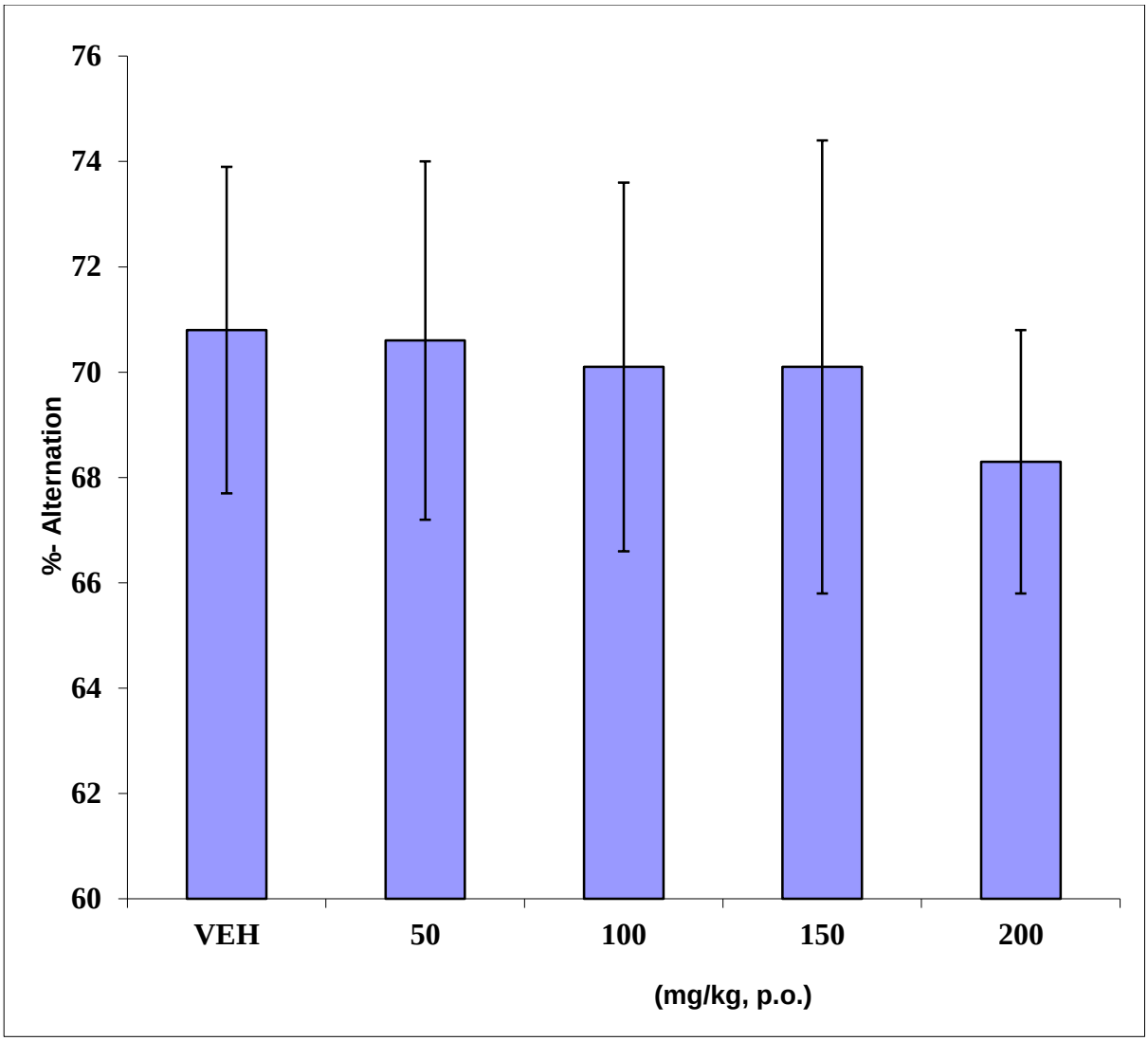

Figure 10a: Effects of the essential oil (EO) of *Cymbopogon citratus* on alternation in the Y-Maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline (10 ml/kg),

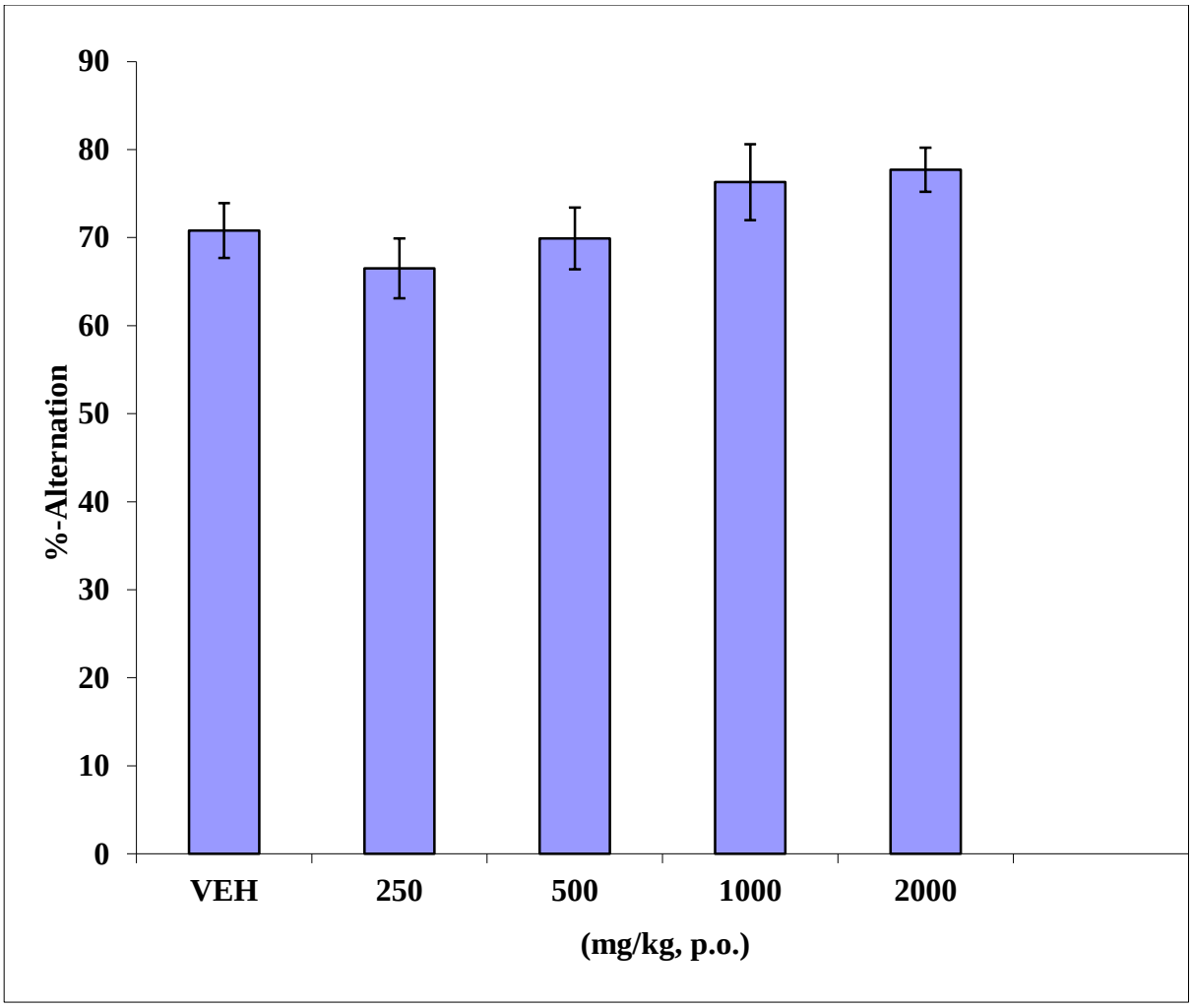

Figure 10b: Effects of the aqueous extract (AQ) of *Cymbopogon citratus* on alternation in the Y-Maze. Each bar represents Mean ±SEM (n=6). VEH - normal saline [10 ml/kg).

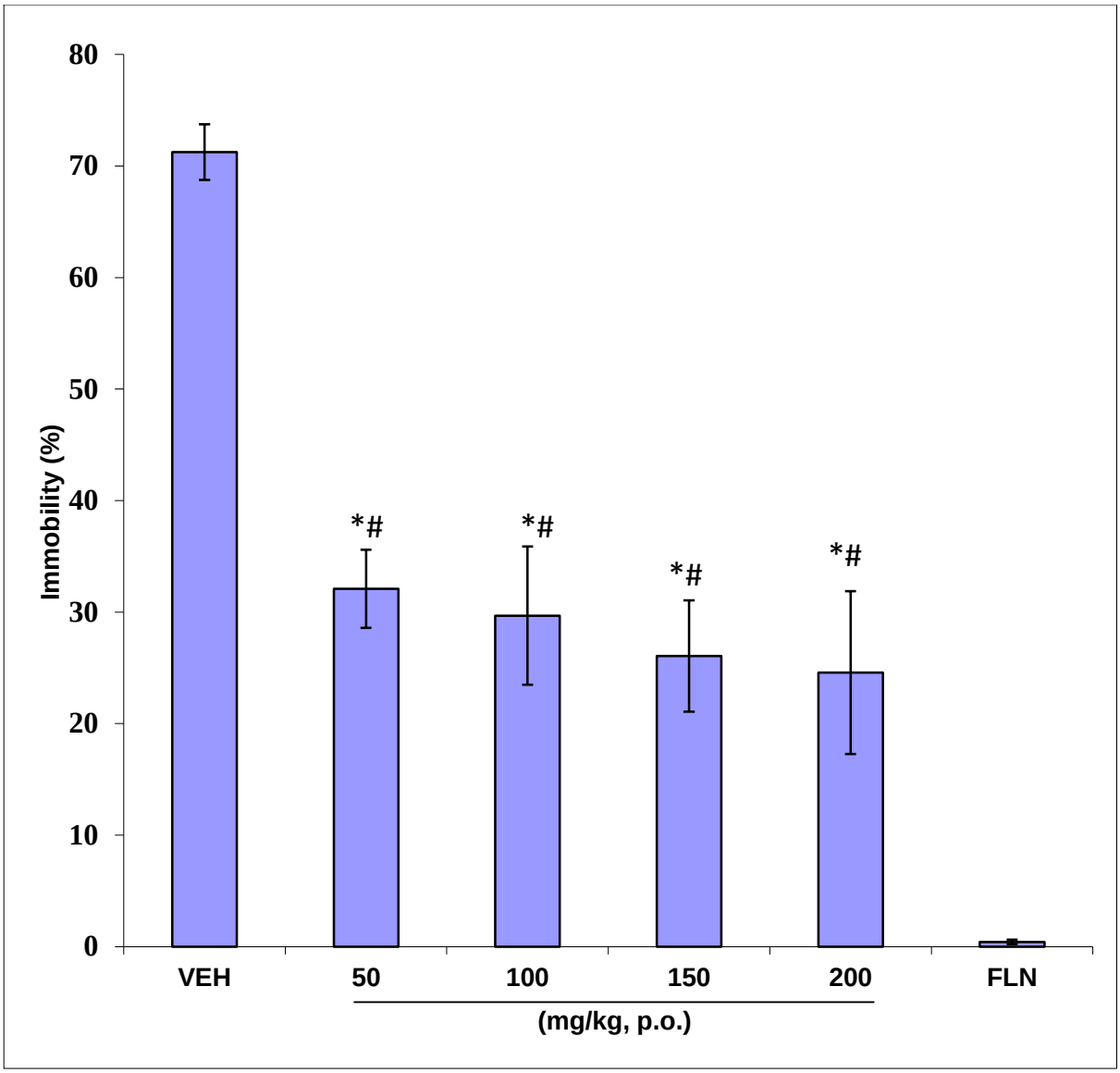

Figure 11a: Effects of the essential oil (EO) of *C. citratus* on immobility in the forced swimming test (FST). Each bar represents mean ±SEM (n=6). VEH - normal saline (10 ml/kg), FLN – fluoxetine (20 mg/kg, o.p.). *p < 0.05 (when compared with vehicle); $^{\#}p < 0.05$ (when compared with fluoxetine).

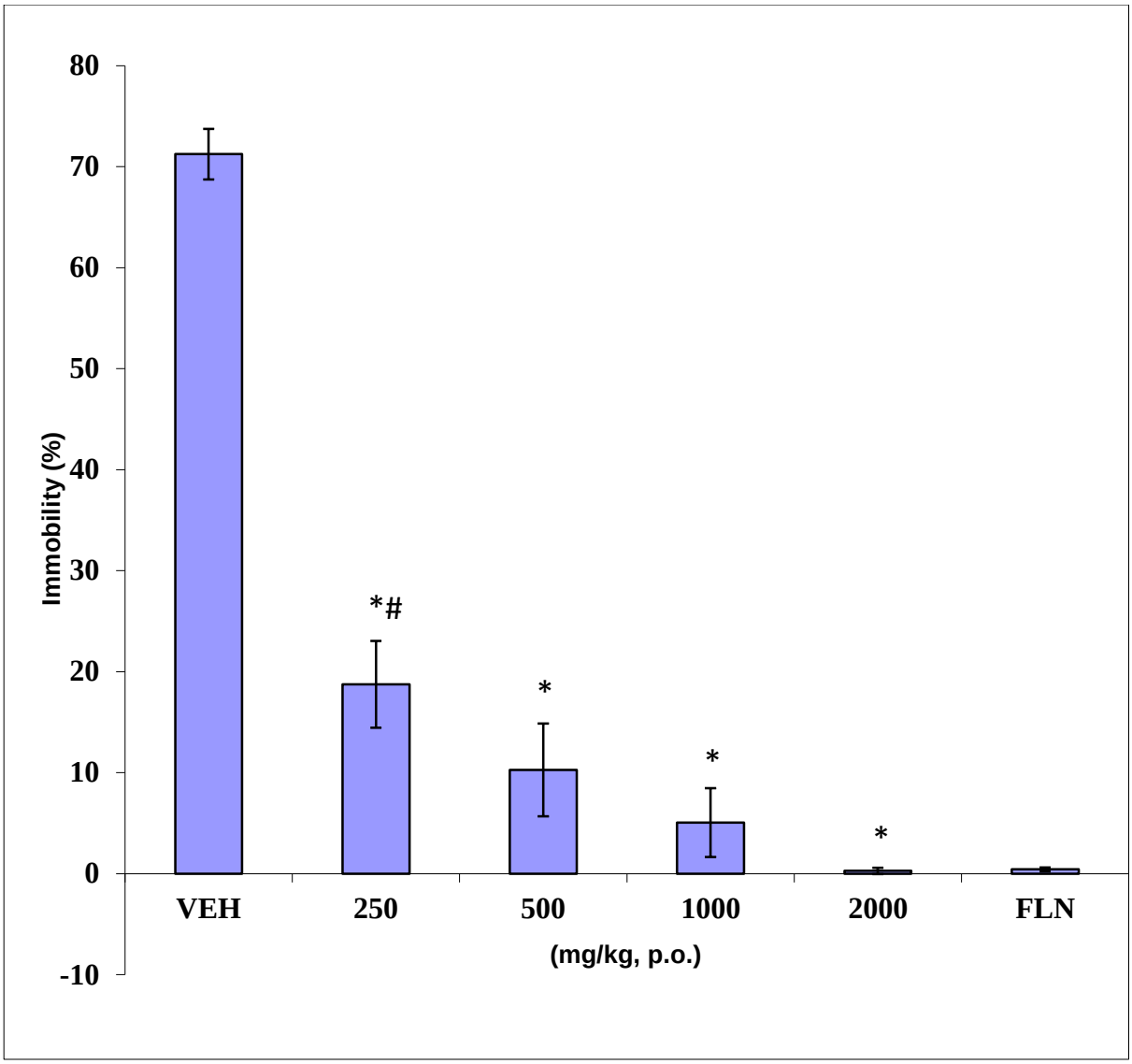

Figure 11b: Effects of the aqueous extract (AQ) of *C. citratus* on immobility in the forced swimming test (FST). Each bar represents mean ±SEM (n=6). VEH - normal saline (10ml/kg), FLN – fluoxetine (20mg/kg, o.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with fluoxetine).

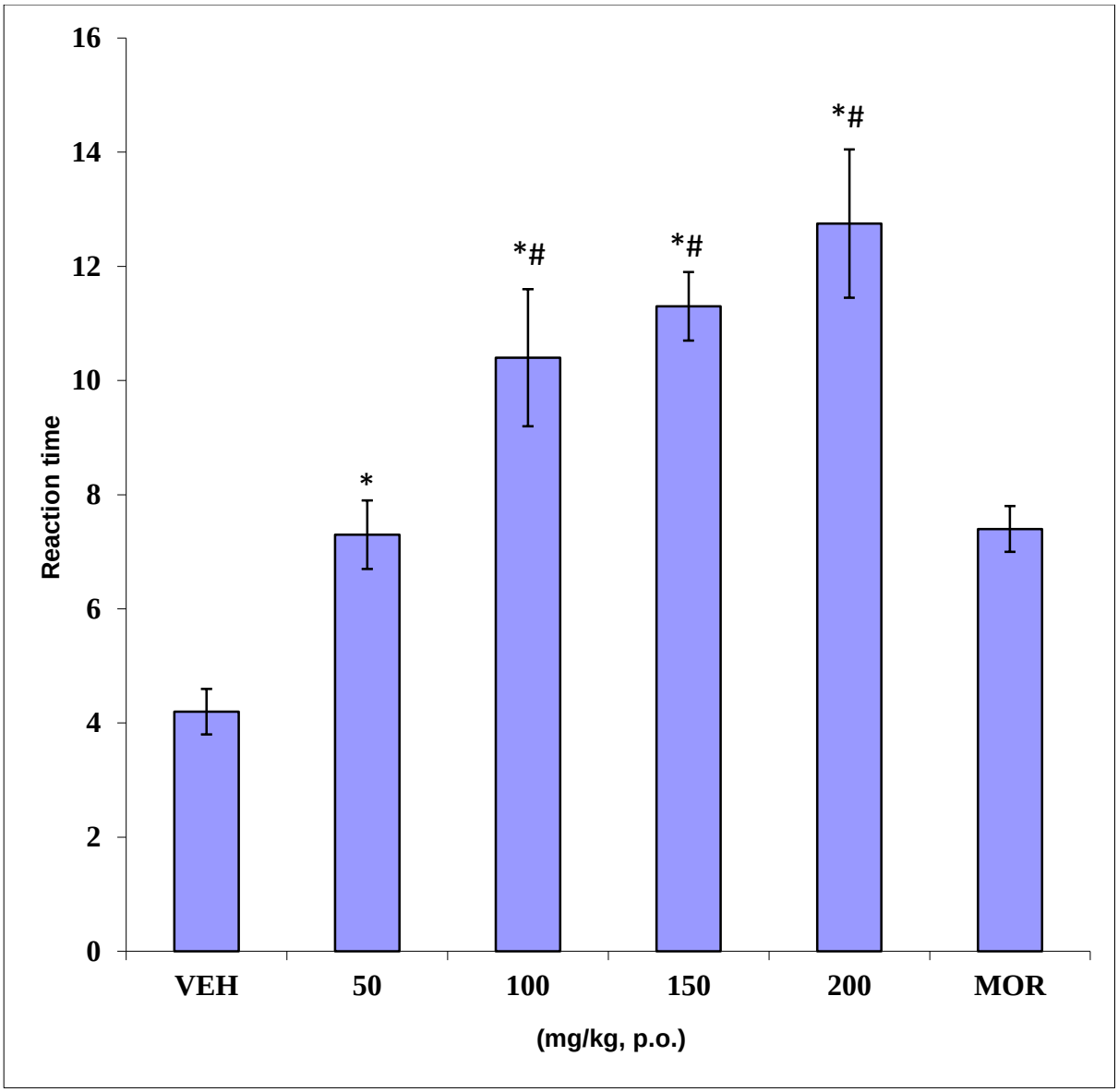

Figure 12a: Effects of the essential oil (EO) of *Cymbopogon citratus* on the reaction time in the hot plate test. Each bar represents mean ±SEM (n=6). VEH - normal saline (10 ml/kg), MOR - morphine (10 mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); $^{\#}p < 0.05$ (when compared with morphine).

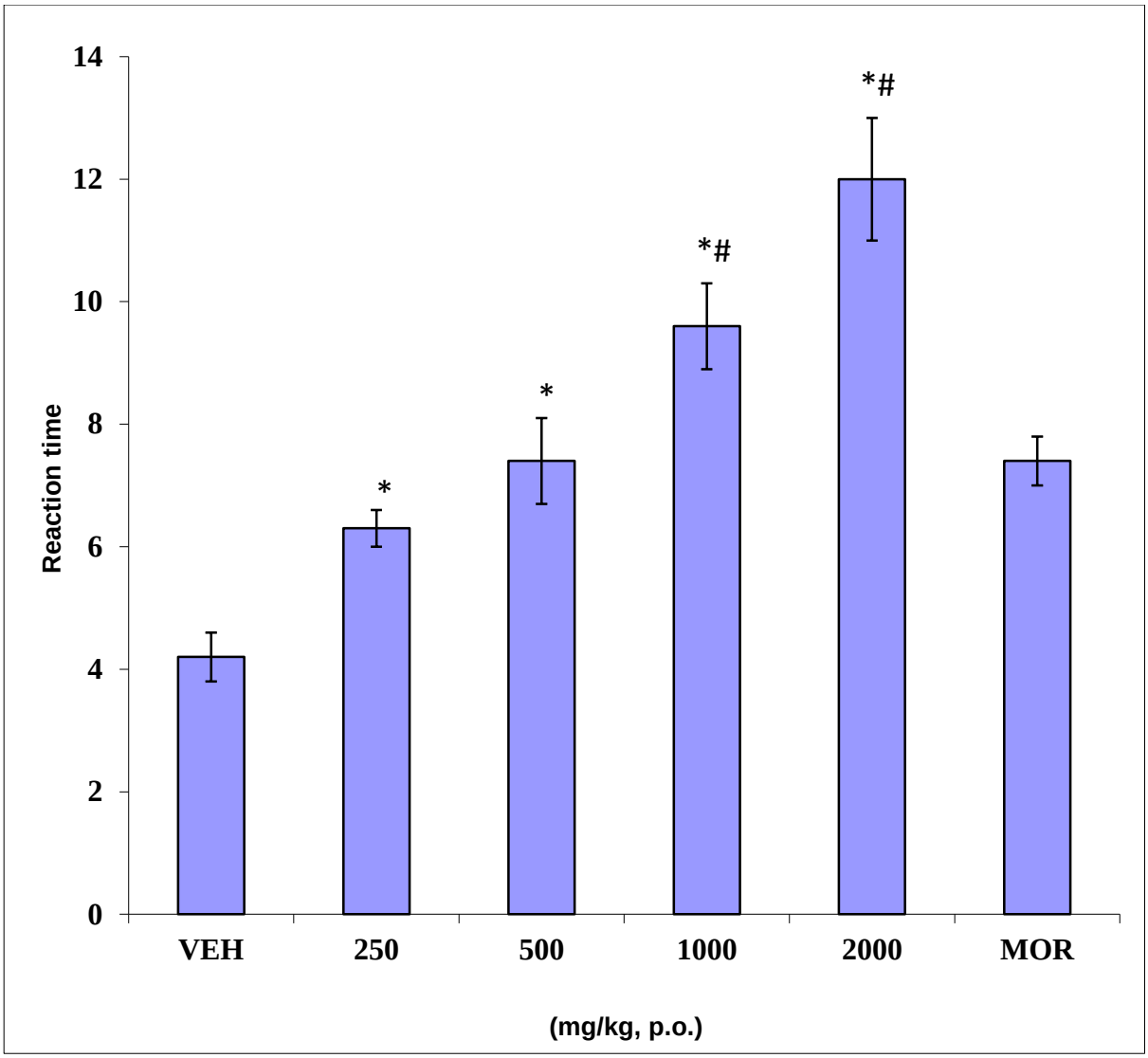

Figure 12b: Effects of the aqueous extract (AQ) of *Cymbopogon citratus* on the reaction time in the hot plate test.Each bar represents mean ±SEM (n=6). VEH - normal saline (10 ml/kg), MOR-morphine (10 mg/kg, i.p.). *$p < 0.05$ (when compared with vehicle); #$p < 0.05$ (when compared with morphine).

IV. Discussion

This study investigated the neuropharmacological effects of the essential oil and aqueous leaf extract of C*ymbopogon citratus* (lemongrass) in mice. This was with a view to elucidating the ethnomedicinal claims for its effectiveness in treating several central nervous system disorders. This study included the determination of acute toxicity, novelty induced behaviour (locomotion, rearing and grooming), anxiolytic (hole board and elevated plus maze tests), test on learning and memory (Y-maze), antidepressant test (forced swimming test) and analgesic test (hot plate).

The aqueous extract and essential oil of *Cymbopogon citrates* were examined for effects on locomotion, rearing and grooming in mice. The open-field test, among other uses is a method used to assess locomotor activity (Ennaceur *et al*., 2006). In this study, the essential oil had no significant effect on the open field test. However, the aqueous extract at the higher doses of 1000 mg/kg and 2000 mg/kg showed significant increase in novelty induced behaviours of locomotion, rearing and grooming, suggesting a stimulant activity at higher doses. Locomotion is mediated mainly through dopaminergic pathway (Rang *et al.*, 1999), but other neurochemical pathways have been reported to modulate locomotor activities in animals. Generally, CNS depressants have inhibitory effects on locomotor activities and other exploratory or inquisitiveness of animals (Haque *et al.*, 2001). A decrease in locomotor activity in rodents is suggestive of a possible CNS – depressant activity (Cooper *et al*., 1996).

The result of the anxiolytic test on hole board showed that the EO and the AQ caused significant ($p<0.05$) increase in the number of head-dips produced by all the test doses of both the aqueous extract and the essential oil. This result suggests, that the EO and AQ of *C. citratus* have anxiolytic properties and it is corroborated by the work of File and Pellow in which they concluded that, an increase of the hole-dipping response reveals a positive anxiolytic-like effect (File and Pellow, 1985).

The results of the elevated plus-maze test showed that, the extract and the essential oil significantly ($p<0.05$) increased the time spent in the open arms (i.e., anxiolytic-like action) in a non-dose-related way. The number of entries into the arms were not significantly affected by both aqueous extract and the essential oil in comparison to the control values, this result is corroborated by the work done by Lister (1990) and File (2001) in which they reported that absence of effect on motor activity was also observed in elevated plus maze, where treated mice performed similarly to the control mice in relation to total arm entries, a mixed measure that reflects changes in motor activity more than in anxiety. In this way, absence of deleterious effect on coordination and motor activity due to EO can be accepted. Drugs that increase the open arms exploration are considered anxiolytics and the reverse holds true for anxiogenic compounds (Handley and McBlane,1993). In this study, diazepam was used as a positive control and, as expected, it increased the time spent in the open arms of the elevated plus-maze apparatus, confirming its anxiolytic actions (Stock *et al*., 2000). Similarly the essential oil and the aqueous extract significantly ($p<0.05$) increased the time spent in the open arms suggesting anxiolytic effects.

The extracts had no effect on working memory as the percentage alternation produced was not different from that of the control.

The forced swimming test demonstrated that the essential oil and aqueous extract of C*ymbopogon citratus* clearly acted as an antidepressant in the mice. All the doses were able to reduce immobility time. Reduction of immobility was comparable to that observed after the p.o. administration of the reference antidepressant drug fluoxetine.

In thehot plate test, both the EO and the extract increased the time taken for the discomfort of paw-licking to be observed, this increase was dose dependant. For the EO this increase was significantly ($p<0.05$) different from that of the reference drug, morphine at all the dose levels except for the 50 mg/kg dose level. While for the aqueous extract it was only at the 1000 mg/kg and 2000 mg/kg. The hot plate method has been found to be suitable for evaluation of centrally acting analgesics (Woolfe and MacDonald, 1944).

V. Conclusion

It is hereby, concluded that the essential oil and aqueous leaf extract of *Cymbopogon citratus* possess significant ($p<0.05$) anxiolytic, anti-depressant and analgelsic properties, but did not have significant effect on novelty induced behviours (locomotion, rearing and grooming) and on learning and memory.

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