Journal of Advances in Medicine and Medical Research

24(4): 1-10, 2017; Article no.JAMMR.36654
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Comparative Effects of Antiepileptic Agents *Dichrostachys glomerata* Ethanol Extract and Carbamazepine on Seizures and Anxiety in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author UEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PPE managed the literature searches. Author EEO managed the analysis of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/36654
Editor(s):
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(2) Philippe E. Spiess, Department of Genitourinary Oncology, Moffitt Cancer Center, USA and Department of Urology and Department of Oncologic Sciences (Joint Appointment), College of Medicine, University of South Florida, Tampa, FL, USA.
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Complete Peer review History: http://www.sciencedomain.org/review-history/21622

Original Research Article

Received 6th September 2017
Accepted 23rd October 2017
Published 28th October 2017

ABSTRACT

Aim: Treatment for neurological disorders have been sought through both herbal and orthodox medicine, therefore this present study compared the antiepileptic effects of *Dichrostachys glomerata* (*D. glomerata*) and Carbamazepine (CBZ) on Pentylenetetrazole (PTZ) induced seizures in mice.
Materials and Methods: Twenty-four Swiss white mice of 20 – 30 g body weight were randomly divided into four groups of six mice. They were treated with normal saline (0.1 ml/10 g body weight i.p) for control, PTZ (65 mg/kg body weight i.p), *D. glomerata* (4.5 mg/kg body weight i.p) and CBZ (40 mg/kg body weight i,p). The seizure assay was carried out and the light-dark box and elevated

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plus maze were used to test for anxiety. Treatment was for seven days. On the experimental day, tests were conducted 15 minutes after PTZ administration. CBZ served as the reference drug.
Results: Both CBZ and *D. glomerata* increased the latency of seizure ($p<0.001$) but that of CBZ group was significantly higher ($p<0.001$) compared to DG group. CBZ significantly lowered ($p<0.01$) the duration of seizure compared to the D.G group. In the light-dark box, CBZ lowered the duration the mice spent in the dark chamber compared to the control and DG groups ($p<0.05$). In the elevated plus maze, CBZ and *D. glomerata* significantly decreased ($p<0.05$) the grooming duration compared to control. CBZ also significantly increased ($p<0.05$) the open arm entry frequency.
Conclusion: *D. glomerata* exhibited anxiolytic and anticonvulsant effects in comparison to placebo. Although *D. glomerata* was effective in reducing seizures in mice, CBZ was observed to be more efficient in alleviating anxiety.

Keywords: Antiepileptic effects; Carbamazepine; Dichrostachys glomerata; Pentylenetetrazole; seizure.

ABBREVIATIONS

CBZ: Carbamazepine; DG: Dichrostachys glomerata; PTZ: Pentylenetetrazole; ANOVA: Analysis of variance; SEM: Standard error mean; SAP: Stretch attend posture; WHO: World Health Organization; GABA: Gamma-Aminobutyric acid

1. INTRODUCTION

About 50 million people worldwide have epilepsy and only two out of every three cases are discovered in developing countries [1]. Epilepsy is a neurological disorder characterized by recurring attacks of motor, sensory or psychological malfunction with or without unconsciousness and characterized by seizures [2,3]. It is not a single disorder but rather a syndrome with vastly divergent symptoms; all involving episodic abnormal electrical activity in the brain and numerous seizures [4]. These seizures are transient signs and/or symptoms of excessive or hyper synchronous neuronal activity in the brain [5]. Epilepsy becomes more common in people as they age [6]. According to Vyawahare et al. [7], it accounts for 1% of the world's burden of diseases, and the prevalence rate is reported at 2%.

Carbamazepine (CBZ) is an anticonvulsant and mood- stabilizing drug. It is a synthetic compound primarily used in the treatment of epilepsy and bipolar disorder [8], as well as trigeminal neuralgia [9]. It acts by potentiating GABA receptors [10].

The herb *Dichrostachys glomerata* (synonym: *Dichrostachys cinerea*) with the common name " Sickle bush" is a spiny deciduous shrub that is up to 7 meters high, with a rounded crown 3 m wide. The bark is rough, yellow to grey-brown and frequently fissured. This specie of herb is widely spread in Senegal to Western Cameroon, and extending across Africa and could also be found in Asia and Australia [11,12]. It is called *Anyiawu Nwoke* (Ebonyi state, Nigeria). The bark of this herb is locally used to treat dysentery, headaches, syphilis, as well as cough and it is also used as purgative and strong diuretic [11, 12]. Powdered roots and leaves of this herb are used in traditional medicine to treat epilepsy [11, 12,13]. *D. glomerata* might have acted by enhancing, or in some ways interfering with GABAergic neurotransmission and its anticonvulsant activity may be attributed to the presence of tannins, flavonoids and saponins [14-16].

This research was therefore aimed at scientifically validating the efficacy of *D. glomerata* and comparing its antiepileptic effects with those of an orthodox drug (Carbamazepine) on seizures and anxiety in mice.

2. MATERIALS AND METHODS

2.1 Animals

Twenty four male Swiss mice weighing between 20 – 30 g were used for this study. They were obtained from the animal house of the Department of Physiology of the College of Medical Sciences, University of Calabar, Nigeria. The animals were kept in well ventilated cages at room temperature 25 ± 2°C and exposed to a normal 12/12 hours light/dark cycle. The mice

had access to rodent chow from Vital Feeds Nigeria Limited and clean tap water *ad libitum*. The mice were randomly assigned to four groups of six animals each with group 1 serving as control. While group 2 was for Pentylenetetrazole (PTZ) only, group 3 was PTZ with *D. glomerata* and group 4 was PTZ with Carbamazepine.

2.2 Preparation of *Dichrostachys glomerata* Ethanol Extract

The plant *Dichrostachys glomerata* was identified in the botanical garden of the University of Calabar, Nigeria and a sample deposited in the University of Calabar herbarium with the voucher number MIA 2005. The leaves of the plant were harvested and air-dried at room temperature for about four days and then processed into powder using electric blender in Pharmacology Laboratory of Pharmacology Department, University of Calabar, Nigeria. The powdered sample thus weighing 706.3 g was then subjected into maceration chamber using 250 mls of ethanol. The mixture in the maceration chamber was left to ferment for about 48 hours after which it was filtered off using Whatmann paper 1. The filtrate was then evaporated to dryness in the oven (Fan convection laboratory oven – PF 200, purchased from Carbolite Gero Neuhausen, Germany) at temperature of about 40°C. A gel-like paste of crude extract was obtained. The paste was then scooped into a sample bottle using spatula and stored in the desiccator prior to its use.

2.3 Preliminary Phytochemical Analysis of *Dichrostachys glomerata*

The extract obtained was subjected to various chemical tests to detect the chemical constituents present in them [17].

2.4 Determination of the LD_{50} of Ethanol Extract of *Dichrostachys glomerata in Mice*

Thirty (30) mice were used to determine the lethal dose of the ethanol extract of *Dichrostachys glomerata* [18].

2.5 Inducement of Seizure

Seizures were induced in the mice when Pentylenetetrazole was intraperitoneally administered at the dose of 65 mg/kg body weight. Seizures were assessed in terms of onset of seizure (latency), duration of seizures and frequencies of jerk. The latency of seizure was the time it took for onset of seizure to occur after Pentylenetetrazole was being administered while the duration of seizure was how long the seizure lasted.

2.6 Drug Preparation and Treatment

All the drugs used in this study were prepared by dissolving in 0.9% saline and were intraperitoneally administered at the rate of 0.1 ml/10 g body weight. The ethanol extract of *D. glomerata* with LD_{50} of 13.5 mg/kg body weight was reconstituted to a stock concentration of 1 mg/ml from which 4.5 mg/kg body weight was administered i.p. Carbamazepine grounded powder with LD_{50} of 114 mg/kg body weight [19] was constituted to a stock concentration of 1 mg/ml from which 40 mg/kg body weight was administered i.p. Groups 3 & 4 were treated with *D. glomerata* and CBZ respectively for seven days and on the experimental day tests were conducted 15 minutes after administration of PTZ. For this present study, CBZ was the reference drug.

2.7 Light and Dark Transition Box

The light and dark transition box is a test of unconditioned anxiety. It is based on the clash between exploring in a novel environment and aversion of rodents to bright light [20]. This box is made of plywood and consists of two compartments of unequal size as described by Costal [21]. The small compartment (18 x 27 cm) is painted black and constitutes 2/5 of the box. The larger compartment (27 x 27cm) is painted white – making up 3/5 of the box [20]. These compartments are connected by a door (7.5 x 7.5 cm) that is located at floor level in the center of the wall between the two compartments. The floor is divided into 9 x 9 cm squares and is covered with Plexiglas. Both the compartments are covered with lids of clear Plexiglas. The apparatus was located in a room (2 m x 5 m) and lit by a 60- watt red lamp for background lighting. The mice were scooped into the apparatus and allowed to explore it for 5 minutes and the behaviours of the mice in the box were recorded. Behaviors scored included: grooming duration, frequency of stretch attend postures, line crossing, transition as well as dark and light chambers duration.

2.8 The Elevated Plus Maze

This apparatus is used to assess the anxiety and fear levels of the mice. The test is based on the inborn aversion of rodents to open, bright illuminated spaces [22]. The Elevated plus maze was built according to the description of Lister [23]. The maze has two open arms (45 x 5 cm^2) with 0.25 cm high edges and two closed arms (40 x 5 cm^2) with 15 cm high walls radiating from a central square (5 x 5 cm). The open arms contain a slight edge (4 mm high) to prevent the mice from slipping and falling off the edge [24]. Prior to the test, the plus maze arms, surfaces and closed sides were cleaned with methylated spirit to eliminate olfactory clues and to remove fecal boll and urine. The mice were placed in the central square of the plus maze such that the mice faced an open arm away from the experimenter upon placement. Immediately after placement, a stop watch was started and the mice were allowed to explore the apparatus for 5 minutes. The test sessions were recorded and videotaped. Behaviors scored included open arm entry, open arm duration, close arm duration, grooming duration, stretch attend posture and rearing.

2.9 Statistical Analysis

The data derived from the tests were analyzed by one way analysis of variance (ANOVA) followed by post hoc student's Neuma-Keuls test using the SPSS program. Data were presented as mean ± SEM (Standard error of mean) and *p* value less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Preliminary Phytochemical Screening

Results of phytochemical analysis of ethanol extract of *Dichrostachys glomerata* is given in Table 1.

3.2 Lethal dose of Dichrostachys Glomerata

The LD_{50} of *Dichrostachys glomerata* was determined. From the analysis, it was found to be 13.5 mg/kg (Fig. 1).

Table 1. Phytochemical analysis of ethanol extract of *Dichrostachys glomerata*

Phytochemical constituents	Relative abundance
Cardiac Glycosides	-
Alkaloids	-
Flavonoids	+++
Reducing sugar	+
Saponins	++
Steroids	-
Anthraquinone	-
Triterpenoids	+
Tannins	+++
Fat (Crude)	++
Protein (Crude)	++

Key: +++ = present in large quantity
++ = present in moderate quantity
+ = present in traces
- = absent

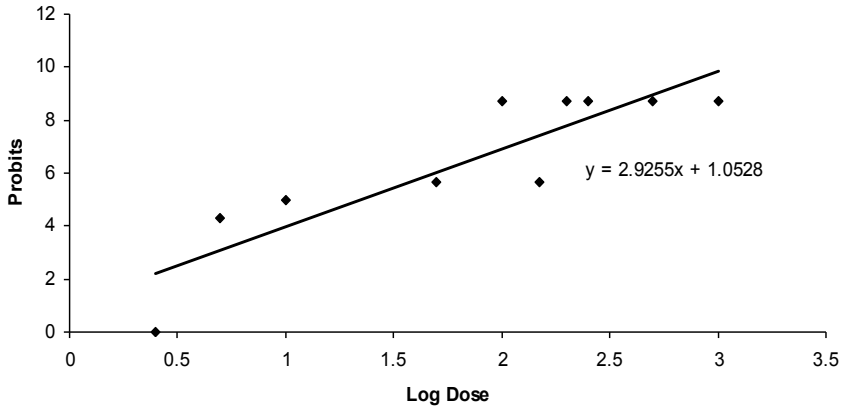


Fig. 1. Chat showing probits plotted against Log dose for the ethanol extract of *Dichrostachys glomerata* during the lethality studies in mice
LD_{50} = 13.5 mg/kg;
ED_{50} = 4.5 mg/kg.

3.3 Assessment of Seizures

The latency of seizures for the PTZ + *D. glomerata* with that of PTZ + Carbamazepine groups were significantly higher ($p<0.001$) compared to PTZ induced epileptic group (Fig. 2A) but the latency of seizure for PTZ + Carbamazepine group was significantly higher ($p<0.001$) compared to PTZ+*D. glomerata.*

The jerk frequency of both PTZ + *D. glomerata* group together with PTZ + Carbamazepine group were significantly lower ($p<0.001$) compared to the PTZ group (Fig. 2B).

The duration of seizure for the PTZ + *D. glomerata* group was significantly lower ($p<0.05$) compared to PTZ induced epileptic group (Fig. 2C) while that of PTZ + Carbamazepine group was significantly lower ($p<0.01$) compared to PTZ group. This indicates that Carbamazepine was able to significantly reduce the time the seizure lasted as compared to *D. glomerata.*

3.4 Light-dark Transition Box

In the test for unconditioned fear using the light-dark transition box, the dark chamber duration of the mice treated with *Dichrostachys glomerata* and Carbamazepine were significantly lower ($p<0.001$) compared to PTZ group (Fig. 3A). The dark chamber duration of the PTZ + CBZ group was significantly lower ($p<0.05$) compared to control and slightly lower compared to PTZ+ DG group.

The frequency of stretch attend posture (SAP), a strong behavioural score for fear and anxiety was significantly lower ($p<0.001$) for the mice treated with CBZ compared to the control. The frequency of SAP for the *D. glomerata* group did not differ from the control (Fig. 3B) but was higher than the PTZ+CBZ group.

The grooming durations of the epileptic mice treated with both *D. glomerata* and Carbamazepine were significantly lower ($p<0.05$) compared to the PTZ group. The value was significantly increased ($p<0.05$) in the PTZ group compared to control. (Fig. 3C). The grooming durations for both the PTZ + DG and PTZ + CBZ groups did not significantly differ amongst themselves.

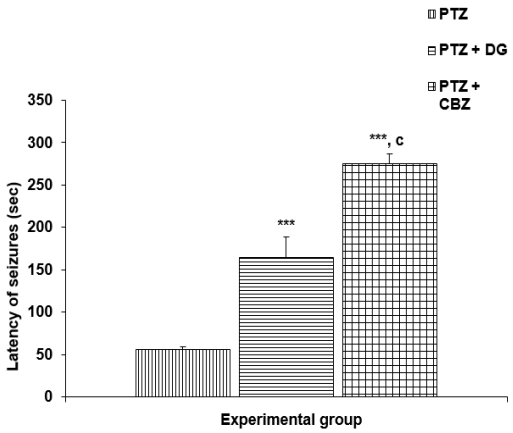
The line crossing frequencies of all experimental groups were not significantly different (Fig. 3D).

The transitions made by PTZ + DG group was significantly lower ($p<0.01$) compared to control (Fig. 3E) and higher ($p<0.05$) compared to PTZ group. The value for DG + CBZ group was not significantly different compared to PTZ group.

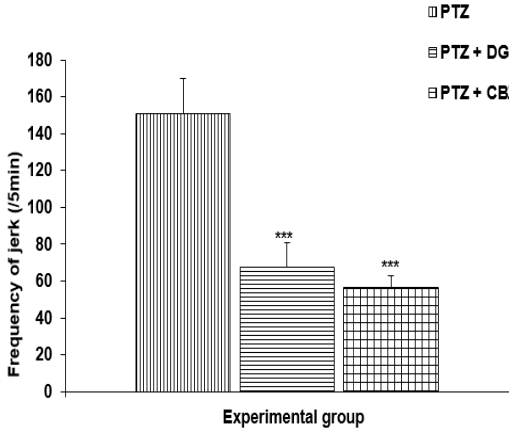
3.5 Elevated Plus Maze

The open arm entry frequency for PTZ + CBZ was significantly higher ($p<0.05$) compared to the PTZ group (Fig. 4A), whereas that of both PTZ and PTZ + DG were not significantly different.

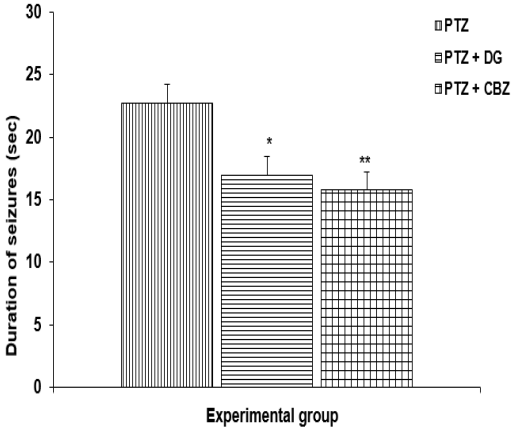
The close arm duration for the PTZ and PTZ + DG groups were not significantly different compared to the control (Fig. 4B). The duration of close arm for the PTZ + CBZ group was significantly lower ($p<0.05$) compared to the control group and slightly lower compared to the PTZ + DG group.



A



B



C

Fig. 2. Comparison of (A) Latency of seizures (B) Frequency of jerks and (C) Duration of seizures between the different experimental groups. Values are mean ± SEM, n=6.

**p<0.05, **p<0.01, ***p<0.001 vs PTZ; c=p<0.001 vs PTZ + DG.*

The stretch attend posture for PTZ + CBZ group was significantly lowered ($p<0.05$) compared to the control (Fig. 4C). The value for both PTZ + DG and PTZ + CBZ groups were significantly reduced ($p<0.001$) compared to the PTZ group. The value for PTZ + CBZ group was slightly lower compared to PTZ + DG group.

The grooming durations of both PTZ + DG and PTZ + CBZ groups were significantly reduced ($p<0.05$) compared to control and PTZ groups (Fig. 4D). There was no significance difference between the two groups.

The rearing frequencies for the PTZ, PTZ + DG and PTZ + CBZ groups were significantly lower ($p>0.001$) compared to the control. The value for PTZ + CBZ group was observed to be slightly lower compared to the PTZ + DG group (Fig. 4E).

4. DISCUSSION

Phytochemical analysis of the ethanol extract of *D. glomerata* showed the presence of tannins, saponins, flavonoids, reducing sugar, crude protein, crude fat and triterpenoids. Steroids, anthraquinones and alkaloids were absent.

In PTZ test, generalized myoclonic and clonic seizures were induced by systemic administrations of convulsant doses of PTZ and this is thought to represent a valid model for generalized absence and/or myoclonic seizure in humans [25,26]. The latency of seizure, following administration of Carbamazepine and *Dichrostachys glomerata* was high compared to the PTZ group. This means that both Carbamazepine and *D. glomerata* were able to delay the onset of seizure. The duration of seizure in the PTZ + D.G and PTZ + CBZ groups were lower compared to the PTZ group, signifying that Carbamazepine and *D. glomerata* were able to reduce the time epilepsy lasted. This is in line with the belief of traditional herbal practitioners that *D. glomerata* is used in treating epilepsy [11-13].

Carbamazepine and *D. glmerata* both reduced the dark chamber duration of the mice. Carbamazepine further showed anxiolytic actions by reducing the stretch attend postures (SAP) of the mice. The herb *D. glomerata* also reduced SAP but this was not significant. Although *D. glomerata* and CBZ did not affect the line crossing (distance travelled), *D. glomerata* slightly increased the transitions of the mice, showing that it possesses anxiolytic effects or tendencies because increased transitions highlight increased exploratory behavior and occurs as a result of decrease in anxiety [27, 28]. Both agents CBZ and *D. glomerata* lowered the grooming duration of the mice. Grooming duration reduction was found to be as a result of anxiolytic experience. Thus, *D. glomerata* exhibited anxiolytic tendencies.

For the elevated plus maze tests, while *D. glomerata* did not affect the open arm entries of the mice, CBZ increased the open arm entry of the mice and also decreased the time the mice spent in the close arm of the apparatus. This increase in open arm entry frequency and decrease in close arm duration of rodents are actions typically observed after administration of

anxiolytics. *D. glomerata* reduced the SAP of the mice. This reduction was also observed following CBZ administration. *D. glomerata* together with CBZ reduced the time the mice spent grooming themselves. This confirms the anxiolytic actions of *D. glomerata.*

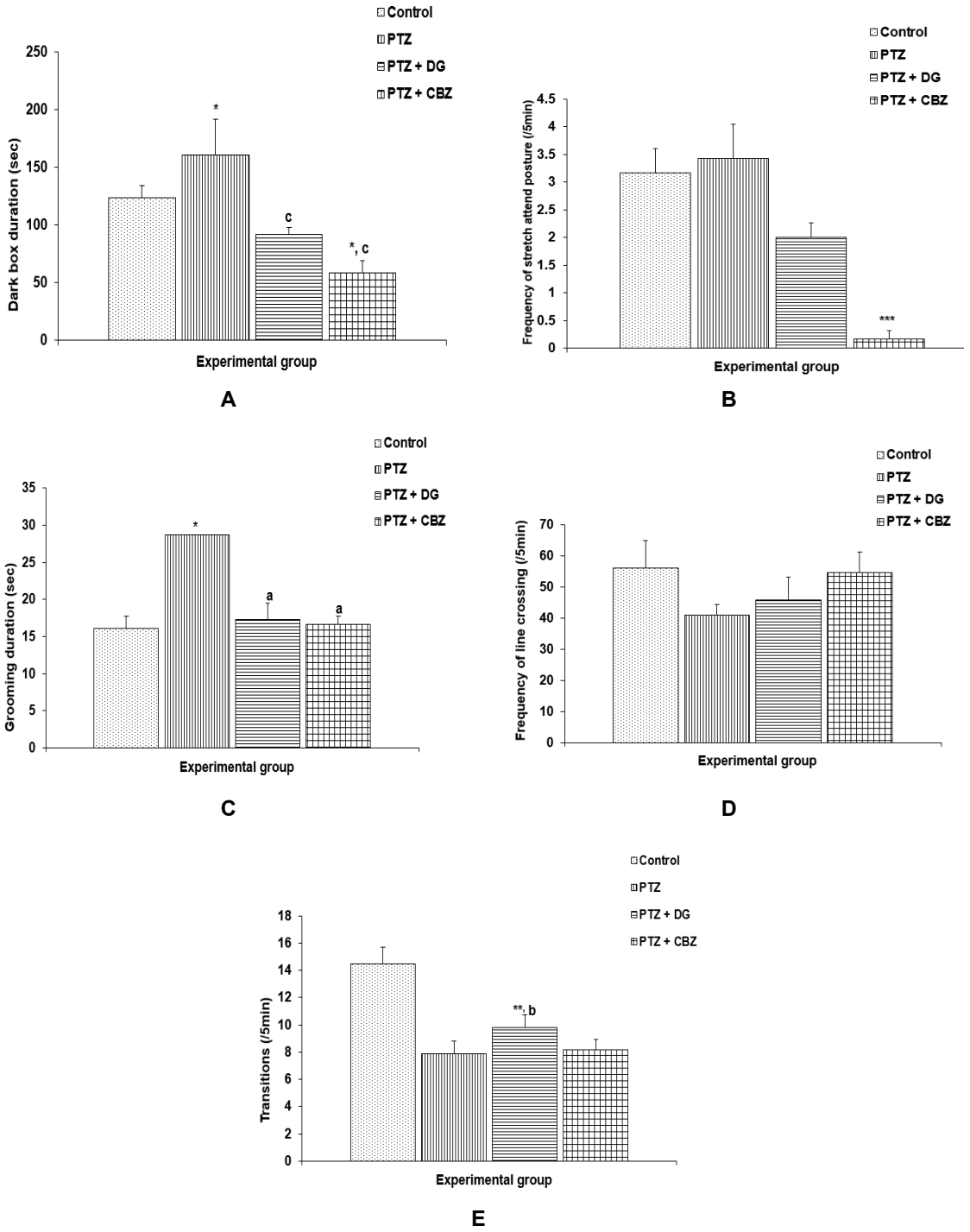


Fig. 3. Comparison of (A) Dark box duration (B) Stretch attend posture frequency (C) Grooming duration (D) Line crossing frequency and (E) Transition frequency in the light-dark box test of the different experimental groups. Values are mean ± SEM, n=6.

**p<0.05, **p<0.01, ***p<0.001 vs control; a=p>0.05 vs PTZ; b=p<0.05 vs PTZ; c=p<0.001 vs PTZ.*

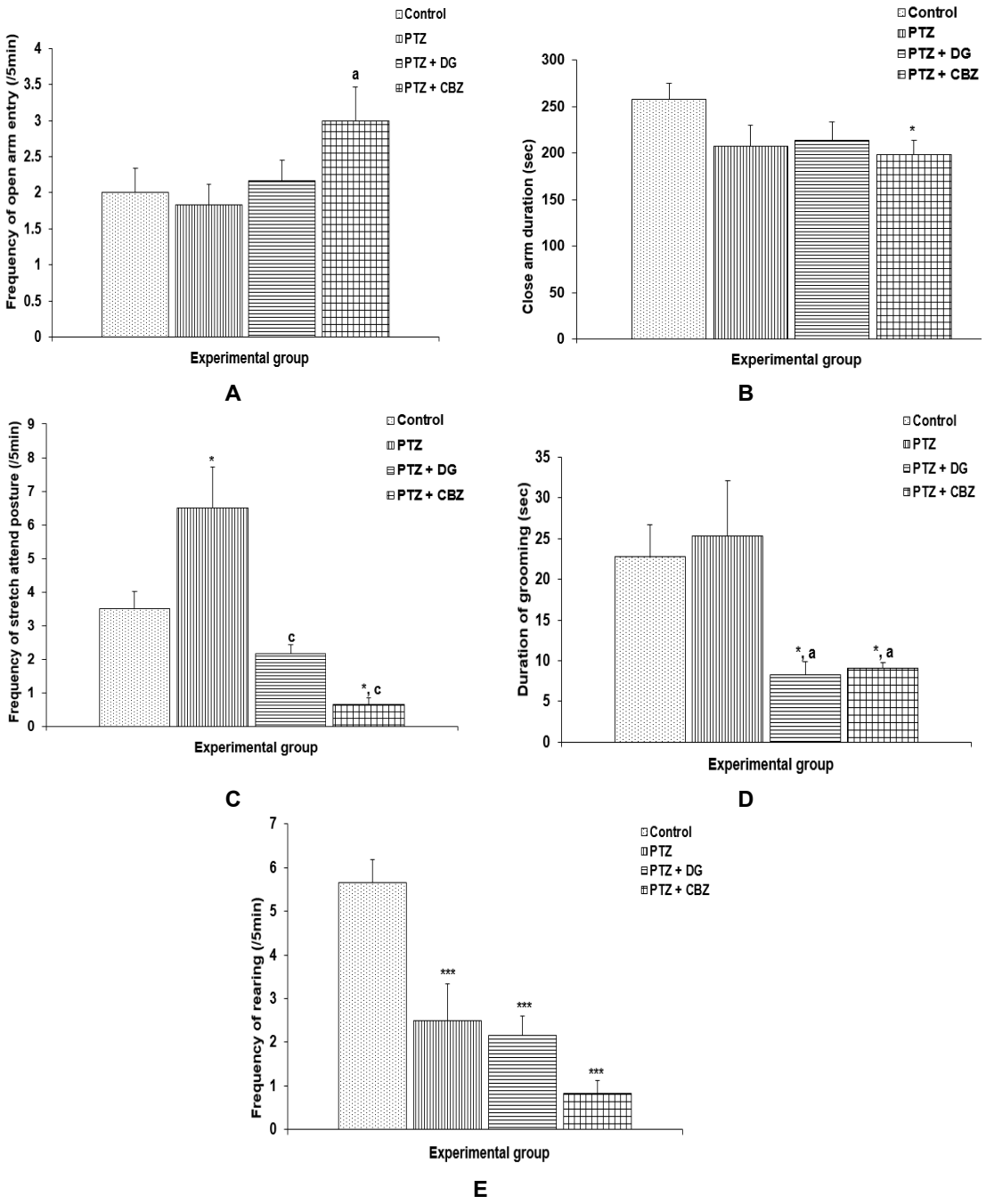


Fig. 4. Comparison of (A) Open arm entry frequency (B) Close arm duration (C) Stretch attend posture frequency (D) Grooming duration and (E) Rearing frequency in the elevated plus maze test of the different experimental groups. Values are mean ± SEM, n=6.

**p<0.05, ***p<0.001 vs control; a=p<0.05 vs PTZ; c=p<0.001 vs PTZ.*

The findings of this study depict that both Carbamazepine and *D. glomerata* inhibited and/or attenuated PTZ-induced seizures. As reported by Mahomed and Ojewole [29], CBZ (just like other anticonvulsants) exerted its anticonvulsant activity by mediating GABA inhibition.

Anxiety disorder is caused by excessive neurological activity in the area of the brain that

is responsible for emotional arousal [30]. This increased level of arousal is experienced as anxiety. This excessive neurological activity is thought to arise from the fact that certain inhibitory neurons are not functioning properly by not releasing GABA [31]. This reduced levels of GABA leads to increase in anxiety. Anxiety can also occur when there is inability of GABA to bind to its receptors. However, the ability to bind to these receptors is influenced by the presence of benzodiazepines produced invivo. The binding of these substances to the sites on receptive neurons increases the ability of GABA binding to its own site [32]. Hence, the effectiveness of GABA is increased, thereby reducing neural activity, which accordingly decreases one's level of anxiety. It is believed that Carbamazepine exerted its anxiolytic effects by potentiating GABA receptors [10] and thus facilitating the binding of GABA to them. This present study agrees with the study on Carbamazepine by Zangrossi et al. [33]. *D. glomerata* may have reduced anxiety in the mice by also mediating these GABA receptors due to the presence of flavonoids in them [16]. These phytochemicals may have increased GABA's sensitivity to its receptors.

5. CONCLUSION

D. glomerata (D.G) and Carbamazepine (CBZ) reduced the epileptic effects of Pentylenetetrazole (PTZ). *D. glomerata* was able to significantly reduce the onset, duration of seizure and reverse anxiety related behaviours compared to Carbamazepine (the reference drug for this study). This implies that not only is *Dichrostachys glomerata* an antiepileptic, it is also an anxiolytic agent. However, the anxiolytic effect of CBZ was more potent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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