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ANTIEPILEPTOGENIC AND MEMORY ENHANCING POTENTIALS OF METHANOL LEAF EXTRACT OF *PARQUETINA NIGRESCENS* (AFZEL) BULLOCK IN PENTYLENETETRAZOLE-KINDLED MICE

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ABSTRACT

Parquetina nigrescens is an important ethnomedicinal plant in Nigeria, use in the treatment of epilepsy and enhancement of memory. The aim of the study is to evaluate the antiepileptogenic and memory enhancing potential of methanol leaf extract of *P. nigrescens.* Antiepileptogenic and memory enhancing potentials were investigated in mice following pentylenetetrazole (PTZ)-induced-kindling 48 hourly (35 mg/kg, *i.p*) and daily oral treatment with the extract at doses of 250, 500 and 1000 mg/kg for a period of 22 days. Seizure behaviour was observed using the Racine's scale for 30 minutes after PTZ administration. On the 22nd day, 1 h after 11th PTZ injection; animals were assessed for cognitive performance in Y-maze and elevated plus maze (EPM) and as well in EPM on day 23. The extract at all doses and sodium valproate significantly ($p<0.05$) decreased seizure scores across treatment days. In the EPM, the extract non-significantly decreased transfer latency on day 22 at all doses tested and significantly ($p<0.05$) decreased transfer latency at 1000 mg/kg dose on day 23. Sodium valproate only significantly ($p<0.05$) decreased transfer latency on day 22. In the Y-maze test, the extract only at 1000 mg/kg and sodium valproate significantly ($p<0.05$) increased % spontaneous alternation and no significant increase in the number of arm entry was observed with the extract. The methanol leaf extract of *P. nigrescens* possesses antiepileptogenic property and improved learning and memory hence, may potentially improve epilepsy-related learning and memory impairment.

Keywords: Antiepileptogenesis; kindling; learning, memory; pentylenetetrazole; *Parquetina nigrescens*

INTRODUCTION

Epilepsy is a chronic neurological disorder invoked by the excessive abnormal discharge of brain neurons characterized by recurrent seizures (Wang *et al.,* 2012). Epileptogenesis is the development of epilepsy or its progression. It is usually a long-term process of converting a previously normal brain into one capable of generating spontaneous recurrent seizures following a brain insult such as head trauma, stroke, *status epilepticus*, etc (Williams *et al.,* 2007; Koepp *et al.*, 2017). Antiepileptogenesis is a process that prevents the development or progression of epilepsy and sometimes modify the severity of the disease by reducing frequency and duration of seizures (Pitkänen 2010; Kaminski *et al.,* 2014).

Treatment of epilepsy involves the use of anticonvulsant drugs. These drugs are effective in appreciable number of patients with epilepsy but do not control seizures in about 30 % of people with the disease. Clinical outcome of conventional anticonvulsant drugs used to prevent epilepsy in post-traumatic brain injury have not been promising (Temkin 2009; Mani *et*

al., 2011; Pitkänen and Lukasiuk 2011). Moreover, they are associated with varying degrees of adverse events of which all having central nervous system related side effects due to their mechanism of action (Schmidt 2009; Löscher and Schmidt 2011).

Patients with epilepsy often suffer from substantial cognitive impairment and other psychiatric comorbidities which can significantly increase mortality (Tellez-Zenteno *et al*., 2007). Epilepsy-related cognitive dysfunction such as memory, attention and information processing difficulties are sometimes more burdensome than the seizures and is due to the underlying aetiology of the disease. Cognitive impairment in epilepsy is a consequence of complex interactions among the etiologies of the disease, seizures and antiepileptic drugs (Raspall-Chaure *et al.,* 2008). During inter-ictal state, glutamatergic overactivation mediates excitotoxicity which may result to memory deficit, while exhaustion of glutamatergic input during the ictal phase leads to progressive memory impairment (Choudhary *et al.,* 2013). Furthermore, seizures directly injure neural networks that are the normal substrate for cognitive function (Kanner, 2008; LaFrance *et al.,* 2008).

Temporal lobe is mostly implicated in focal epilepsy and plays a critical role in speech, learning, memory and affective behaviour. Thus, structural damage within the lobe would disrupt these functions (Vrinda *et al.,* 2019). Also anticonvulsant drugs impact negatively on cognitive function (Holmes 2015). For example, drugs that potentiate GABAergic or block glutamatergic transmissions are anticonvulsant candidates. These mechanisms potentially lead to cognitive impairment; as glutamatergic transmission and dis-inhibition of GABAergic system increases cognitive function (Löscher *et al.,* 2013). Thus, antiepileptic drug discovery may focus on mechanisms that suppress seizures and reduce comorbidities (Rogawski and Löscher 2004) or targeting a single mechanism which is involved in seizure generation and comorbidities (Löscher *et al.,* 2013). Antiepileptogenic therapies may not only modify seizure properties or cure epilepsy but may prevent or ameliorate epilepsy-related psychiatric disorders (Schmidt et al., 2014).

The use of medicinal plants in the treatment of epilepsy is centuries-old practice among different cultures (Sirven *et al.,* 2003). Herbal medications are commonly used to control epileptic seizures when antiepileptic drugs fail most especially among the low-income earners (Schachter 2009; Liu *et al.*, 2017) *Parquetina nigrescens* is a perennial plant which grows in secondary forest and around villages in Nigeria as well as in Senegal (Oluwafemi and Debiri, 2008). The plant is used ethnomedicinally to treat epilepsy, memory loss etc among the Yoruba tribe of Nigeria (Wahab 2015; Elufioye 2012). The methanol stem extract was found to improve cognitive function (Mahmud *et al., 2019*; Mahmud *et al.,* 2020). This study therefore aimed at investigating the antiepileptogenic and cognitive effect of methanol leaf extract of *P. nigrescens* in pentylenetetrazole kindled mice.

MATERIALS AND METHODS

Drugs and Chemicals

Methanol (Sigma Aldrich, Germany), pentylenetetrazole (Sigma Aldrich, Germany), Sodium valproate (Sanofi Synthelabo, USA), distilled water (Juhel Pharmaceuticals, Nigeria)

Experimental Animals

Fifty (50) male Swiss Albino mice of body weight 18-22 g were obtained from the

Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in a well-ventilated room in the Animal House, fed on standard laboratory animal feeds and water *ad libitum*.

The experimental protocol was approved by Ahmadu Bello University Committee on Animal Use and Care with an Approval NO: ABUCAUC/2022/009 and all experiments were performed in accordance with the National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals (1986). The experiment was carried out between 8:00 - 18:00 hours of the day. At the end of the experiment, animals were returned to the Animal House Facility of the Department of Pharmacology and Therapeutics for proper disposal by humane killing.

Plant Collection and Identification

The leaves, stems and roots of plant were collected in the month of June, 2016 in the bush within Samaru, Zaria Sabo Geri Local Government Area, Kaduna State, Nigeria. The freshly harvested plant material was identified by Mallam Umar Gallah of the National Institute for Chemical Research Technology (NARICT) Zaria, Kaduna State. A voucher specimen was prepared (voucher number: 01624) and deposited in the herbarium unit of NARICT.

Extract Preparation

The leaves were collected, washed and air-dried under shade for 3 weeks. The dried leaves were crushed into coarse powder with the aid of a pestle and mortal. Two-hundred gram (200 g) of the powdered leaf material was extracted with 1.5 L of 70 % methanol for 7 days with occasional shaking using cold maceration. The extract was collected in a round bottom flask where it was decanted into an evaporating dish through a filter paper. This was concentrated using a rotary evaporator and then further dried over a water bath at a temperature of 40 ^{0}C to obtain a dark-green solid residue subsequently referred to as methanol leaf extract of *P. nigrescens* (MPN) and then stored in a desiccator until required for use.

Phytochemical Screening

The preliminary phytochemical screening of the extract was carried out according to the methods described by Trease and Evans (2002).

Acute Toxicity Study

The oral median lethal dose (LD_{50}) was estimated according to the Organization for Economic Cooperation and Development (OECD) 425 guideline for limit test (OECD 2008). Briefly, 5 mice were used for the test. A mouse was fasted for 3 h and administered 5000 mg/kg extract orally and then observed for sign of toxicity and death for 48 hours with special attention within the first 4 h after dosing. Food was further with-held for 2 h. The same procedure was repeated for another mouse when death did not occur. Finally, the remaining 3 mice were administered 5000 mg/kg extract and observed for 2 weeks for death.

Antiepileptogenic and Cognitive Studies

Experimental design

A total of fifty mice were randomly divided into five (5) groups of 10 mice each. Group 1 (negative control) and 2 (positive) were pre-treated with 10 mL/kg distilled water and 200 mg/kg sodium valproate per oral respectively. Group 3, 4 and 5 received 250, 500 and 1000 mg/kg of the extract per oral respectively. All the mice were pretreated daily for 22 days. Immediately after seizure observations on day 22, mice were assessed for behavioural cognitive performance in Y-maze and elevated plus maze tests.

Pentylenetetrazole-induced kindling

The method of Piredda *et al.,* (1986) was employed. Thirty minutes post-drug treatment, PTZ (35 mg/kg) was administered intraperitoneally to all the mice every 48 hours and observed for 30 minutes for seizure activity which was scored using Racine's scale described as follows: Stage 0: No response; Stage 1: Ear and facial twitching; Stage 2: Myoclonic body jerks without upright position; Stage 3: Myoclonic jerks, upright position with clonic forelimb convulsions; Stage 4: Tonic-clonic seizures; Stage 5: Generalized tonic-clonic seizures, loss of postural control (Racine, 1972).

Y-Maze test

The method described for cognitive assessment and locomotor activity by Hodges (1996); D'Mello and Steckler, (1996); Kraeuter and Guest (2019) were adopted with slight modification. Briefly, the Y-maze apparatus consisted of three identical arms of 15 x 15 x 10 cm with the connector (10 x 6 x 10 cm) radiating from the center. Mice were placed at the end of one of the arms (starting arm) with their head pointing away from the center of the maze, and allowed to explore for 3 min (pretesting) with one of the arms closed. Entry into any of the arms was considered complete when the hind paws of mice entirely entered the arm. Four hours later, the same procedure was repeated and mice were allowed to explore for 5 min (testing) (D'Mello and Steckler 1996; Hodges 1996). The number of spontaneous alternation and arm entries were taken. Spontaneous alternation behavior defined as three consecutive entries in three different arms (i.e. A, B, C or B, C, A, etc.).

The percentage alternation score was calculated using the following formula:
(Total alternation number/Total number of entries - 2) x 100

% alternation and arm entries were taken as indices of working memory and locomotive activity respectively.

Elevated plus maze test

The procedure for testing learning and memory were followed as described by Itoh *et al.* (1991) and Dhingra *et al.* (2004). The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze elevated to a height of 50 cm from the floor. Mice were placed at the end of an open arm, facing away from the central platform and transfer latency (TL1) on day 22 was taken for learning. Transfer latency is the time taken for the animal to move from the open arm into one of the closed arms with all its four legs. Animals that failed to enter into the closed arm within 60 sec, were gently pushed in and TL assigned 60 sec. Mice were allowed to explore the maze for another 20 sec and then returned to home cage. Twenty-four hours later, day 23 transfer latency (TL2) was also taken to assess retention of the learned-task (memory). The maze was cleaned with 70 % alcohol to avoid olfactory cues after each trial.

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20. Nonparametric data were presented as median and analyzed using Kruskal Wallis test followed by Dunn's post hoc test. Parametric data were presented as mean ± standard error of mean (SEM) and analyzed using One-way ANOVA followed by Dunnett's post hoc test for multiple comparison. P-value < 0.05 was considered statistically significant.

RESULTS

Phytochemical constituents

The methanol leaf extract of *P. nigrescens* contain carbohydrate, flavonoids, saponins, alkaloids, phenolics, steroids/triterpenes.

Acute toxicity

The oral median lethal dose of the extract was found to be ≥ 5000 mg/kg.

Effect of Methanol Leaf Extract of *Parquetina nigrescens* on Seizure Scores of Pentylenetetrazole-kindled Mice

The PTZ group progressively increased seizure score throughout the administration. The extract at all doses tested and sodium valproate significantly ($p<0.05$) decreased seizure score compared to the negative control (PTZ+DW) group. The extract decreased seizure score at dose of 250 mg/kg all through administration except on day 5 and 7 (figure 1).

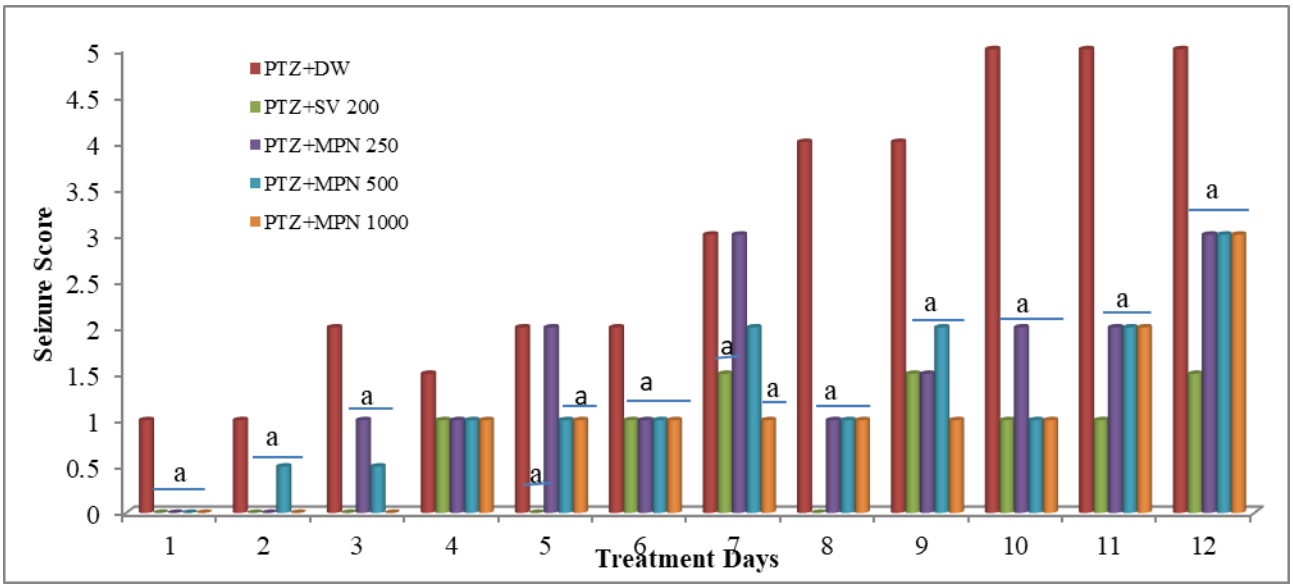

Figure 1: Effect of Methanol Leaf Extract of *Parquetina nigrescens* on Seizure Scores of Pentylenetetrazole-kindled Mice

Values are presented as median scores, analyzed using Kruskal Wallis, followed by Dunn's post hoc test for multiple comparison, $^{a}p<0.05$; as compared to PTZ+D/W control group; n=10; D/W = Distilled Water; PTZ= Pentylenetetrazole; SV = Sodium valproate; MPN = Methanol Extract of *Parquetina nigrescens*

Effect of Methanol Leaf Extract of *Parquetina nigrescens* on Transfer Latency in Elevated Plus Maze Following Pentylenetetrazole-induced Kindling in Mice

The extract at all doses non-significantly decreased transfer latency on days 22 and 23, at 1000 mg/kg dose, it significantly ($p<0.05$) decreased the transfer latency compared with the negative control. Sodium valproate significantly ($p<0.05$) decreased the TL on day 22 and non-significantly on day 23 compared with the negative control (Figure 2).

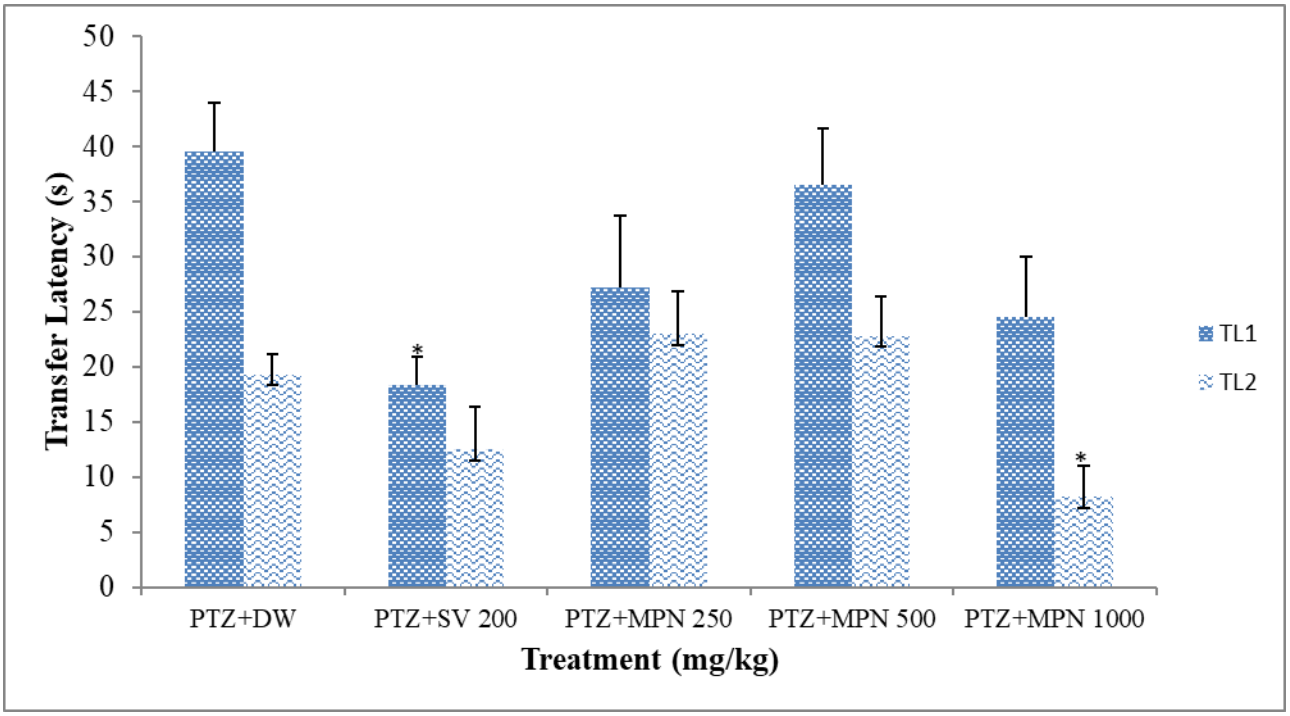

Figure 2: Effect of Methanol Leaf Extract of *Parquetina nigrescens* on Transfer Latencies in Elevated Plus Maze Following Pentylenetetrazole-induced Kindling in Mice

Values are Mean ± S.E.M., analyzed using One-way ANOVA, followed by Dunnette post hoc test, *$p<0.05$ as compared to PTZ+DW group, n=6, D/W = Distilled Water; PTZ= Pentylenetetrazole; SV = Sodium valproate; MPN = Methanol Extract of *Parquetina nigrescens*

Effect of Methanol Leaf Extract of *Parquetina nigrescens* on % Alternation in Y-Maze Following Pentylenetetrazole-induced Kindling in Mice

The extract at all doses increased % spontaneous alternation which was only significant ($p<0.01$) at 1000 mg/kg compared to the PTZ+DW group. Sodium valproate significantly ($p<0.05$) increased % spontaneous alternation compared to the PTZ+DW group. (Figure 3).

Effect of Methanol Leaf Extract of *Parquetina nigrescens* on Number of ArmEntryinY-Maze Following Pentylenetetrazole- induced Kindling in Mice

The extract at all doses tested did not significantly alter the number of arm entry compared to the PTZ+DW group. Sodium valproate significantly ($p<0.05$) increased the number of arm entry compared with the PTZ+DW group (Figure 4).

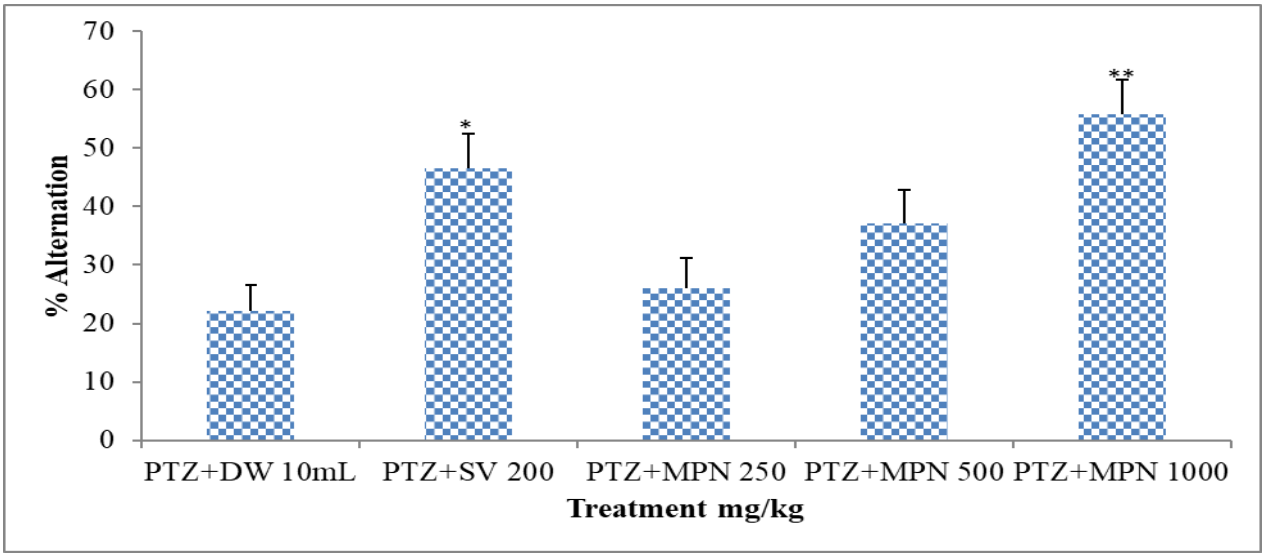

Fig. 3: Effect of Methanol Leaf Extract of *Parquetina nigrescens* on % Alternation in Y-Maze Following Pentylenetetrazole- induced Kindling in Mice

Values are Mean ± S.E.M., analyzed using One-way ANOVA followed by Dunnett post hoc test, *$p<0.05$, **$p<0.01$ as compared to PTZ+DW group n=6, D/W = Distilled Water; SV = Sodium valproate; PTZ = Pentylenetetrazole; MPN = Methanol Extract of *Parquetina nigrescens*

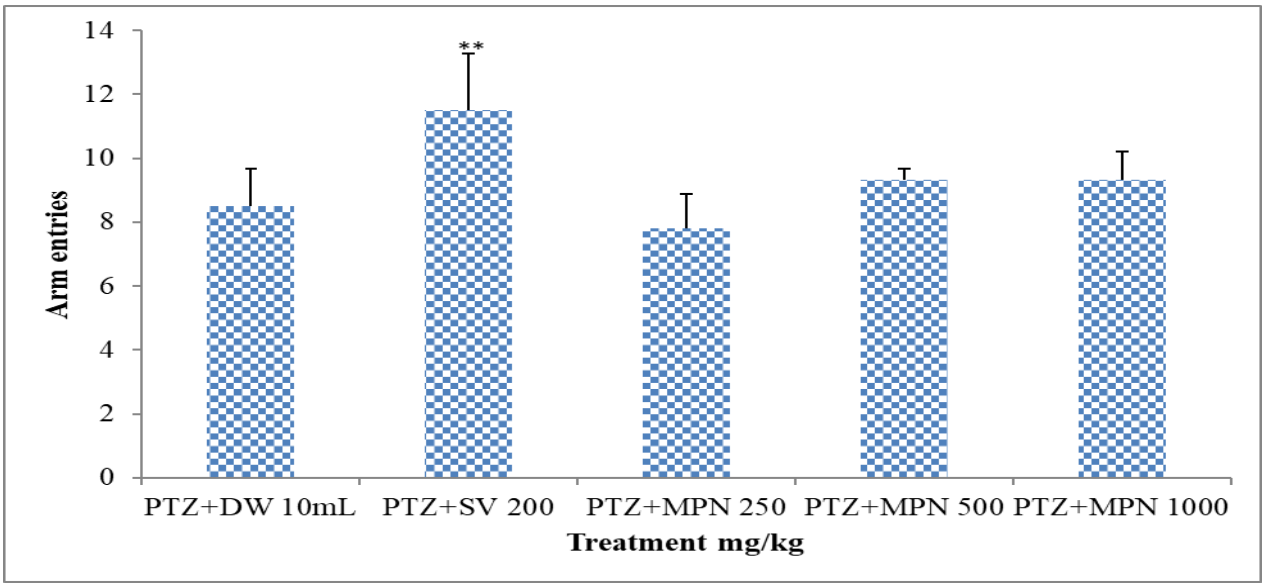

Figure 4: Effect of Methanol Leaf Extract of *Parquetina Nigrescens* on Arm Entries in Y-Maze Following Pentylenetetrazole- induced Kindling in Mice

Values are Mean ± S.E.M., analyzed using One-way ANOVA followed by Dunnett post hoc test, *$p<0.05$, **$p<0.01$ as compared to PTZ+DW group, n=6, D/W = Distilled Water; SV = Sodium valproate; PTZ = Pentylenetetrazole; MPN = Methanol Extract of *Parquetina nigrescens*

DISCUSSION

Early antiepileptic drug discovery focused on seizure suppression (Temkin *et al.,* 2001). These drugs do not delay the onset or prevent the occurrence of epilepsy and are often associated with varying degrees of central nervous system-related side effects that can worsen cognitive function (Schmidt 2009; Löscher and Schmidt 2011). Epilepsy on the other hand is associated with substantial cognitive impairment and other psychiatriccomorbiditieswhich significantly increase morbidity and mortality (Tellez-Zenteno *et al.*, 2007). Therefore, antiepileptic drug search that do not only focus on seizures suppression but also reduce cognitive impairment in epilepsy may significantly reduce the burden of the disease. Antiepileptogenic therapies may not only modify seizure properties or cure epilepsy but may prevent or ameliorate epilepsy-related psychiatric disorders (Schmidt *et al.,* 2014).

In the PTZ- induced kindling, the extract possesses antiepileptogenic activity by being able to decrease seizure scores. PTZ-mediated chemical kindling is a common reproducible and inexpensive method to produce an animal model of epilepsy (Shimada and Yamagata 2018). PTZ is a gamma aminobutyric acid ($GABA_A$) receptor antagonist that suppresses the function of inhibitory synapses in the central nervous system, leading to increased neuronal activity. This regulation causes generalized seizures in animals (Squiresl *et al.,* 1984; DeLaney 2018). Chronic administration of PTZ induces kindling phenomena through suppression of $GABA_A$, benzodiazepine receptors and $GABA_A$-stimulated Cl^- influx through the membrane of cortical neurons (Rocha *et al.,* 1996; Rocha *et al.,* 1996). Seizures do not develop while these mechanisms are actively functioning. Aside the dis-inhibitory effect of PTZ on GABAergic transmission, it causes up-regulation of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding as well as gradual and long-lasting increase in N-methyl D-aspartate (NMDA) receptor binding by glutamate in the dentate gyrus and CA3 area of the hippocampus which are important mechanism of epilepsy induction in PTZ- kindling model (Ekonomou *et al.,* 2001; Schünzel *et al.*, 1992). Therefore, the ability of methanol leaf extract of *P. nigrescens* to reduced seizure scores in the PTZ kindling model may possibly be mediated through potentiation of GABAergic inhibitory transmission or blockade of glutamatergic transmission in the brain probably at the dentate and CA3 regions of the hippocampus.

Experimental and clinical evidences suggest cognitive dysfunction in epilepsy (Genkova-Papazova and Lazarova-Bakarova 1995). In PTZ-induced memory deficit, generation of free radicals and the subsequent neuronal damage may play a crucial role (Kumar *et al.,* 2013). In the elevated plus maze; sodium valproate improved learning and memory by being able to decrease transfer latencies on day 22 and 23 possibly due to antagonistic effect on the mechanism(s) of PTZ kindling-induced cognitive impairment. The extract improved epilepsy-related learning and memory deficit by reducing transfer latencies on day 22 and 23 respectively. Elevated plus maze is a model commonly used to evaluate effect of test compound on learning and memory *(Itoh et al.,* 1991). In the Y maze, the extract and sodium valproate improve acquisition and retention memory by increasing % spontaneous alternation which implies its potential improvement in epilepsy-related spatial learning and memory impairment (Kaure *et al.,* 2013). PTZ induces neuronal loss and glial activation in specific brain regions such as hippocampus implicated in spatial

learning and memory (Kaur *et al.,* 2013). Y-maze is commonly employed for testing compounds that improve spatial learning and memory which are hippocampal dependent. Working memory is the ability to remember and process information at the same time (Davelaar 1998). Working memory, assessed by Y-maze, is a form of short-term memory considered to be a core cognitive process that underpins a range of behaviors, from perception to problem solving and action control; and is closely related to measures of intelligence (Sarnyai *et al.,* 2000; Ma *et al.,* 2014). The improvement in cognitive function of the extract maybe due to its ability to suppress seizure severity which would otherwise damage neural network responsible for learning and memory. Arm entries and transfer latencies are strongly influenced by locomotor behavior which may result from local events within the muscle itself (Yarube *et al.,* 2016). Therefore, compounds that have a stimulating or sedating effect may significantly affect these cognitive parameters consequently giving false positive or negative result respectively. The inability of the extract to increase number of arm entry implies a true reflection of the extract in improving learning and memory deficit in PTZ-kindled mice.

In the preliminary study, the oral median LD_{50} was estimated to be ≥ 5000 mg/kg which suggests that the extract is relatively non-toxic (Matsumura *et al.,* 1985). However, the doses used for the study were lower than 30 % of the LD50 which have been shown to be relatively safe for ethnopharmacological research (Vongtau *et al.,* 2004). Preliminary phytochemical screening revealed the presence of carbohydrate, saponins, flavonoids, cardiac glycoside, phenolics, and triterpenes which may be responsible for the observed pharmacological effects. Triterpenes and steroids, among other phytochemicals have been reported to possess anticonvulsant activity (Barua *et al.,* 2013). Flavonoids, carbohydrates are known to increased cognitive functions (Balkrishna *et al.,* 2020). Flavonoids have been reported to inhibit voltage gated Na^+channels and activate inhibitory GABAergic receptors. Antioxidant effects of flavonoids lead to increased seizure threshold; inhibition of NMDA receptor and ameliorating epilepsy related psychiatry disorder (Singh *et al.,* 2014).

CONCLUSIONS

The methanol leaf extract of *Parquetina nigrescens* possesses antiepileptogenic potentials and reversed epilepsy-related learning and memory impairment. The findings of the study further lent pharmacological credence for the ethnomedicinal use of the plant in the treatment of epilepsy.

Conflict of interests

The authors declare no conflict of interests.

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