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Effect of *Peristrophe bicalyculata* Leaf Extract on Oxidative Stress Enzymes and Haematological Indices of Pentylenetetrazole-induced Kindled Rats

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ABSTRACT

Peristrophe bicalyculata extract is used in the management of convulsions and snake bites among other uses in northern Nigeria. In the present study, the effect of methanol leaf extract of *P. bicalyculata* (200 and 400 mg/kg) was evaluated on the course of kindling, oxidative stress markers and haematologic indices using pentylenetetrazole (PTZ)-induced kindled rats. Preliminary phytochemical screening of the extract was also carried out. Phytochemical screening showed the presence of alkaloids, glycosides, cardiac glycosides, saponins, steroids, triterpenes, tannins and flavonoids. *P. bicalyculata* extract at all doses tested reduced the severity of seizure episodes induced by PTZ. Evaluation of oxidative stress biomarkers showed no significant differences ($p>0.05$) in the levels of malondialdehyde, however, the glutathione levels were significantly ($p<0.01$) increased at 200 and 400 mg/kg of the extract treated groups. Haematological findings revealed a significant ($p<0.01$) increase in lymphocyte levels at 200 mg/kg of the extract as well as the standard drug (valproic acid) used. The results revealed that, the methanol leaf extract of *Peristrophe bicalyculata* possesses anticonvulsant activity in chronic seizure model and thus, posseses antiepileptogenic properties with no antioxidant activity.

Keywords: *Peristrophe bicalyculata*, Oxidative stress, Haematological, Kindling

INTRODUCTION

Epilepsy is a serious neurologic condition associated with psychiatric comorbidity and stigma (Fiest *et al.,* 2014), and is considered one of the most burdensome neurologic disorders worldwide (Gooch *et al.,* 2017). It is characterized by recurrent seizures that range from briefest losses of attention or muscle jerks to severe and prolonged convulsions. Kindling is the most widely employed model for studying seizure mechanisms and is considered as a useful experimental model for human epilepsy (Mason and Cooper, 1972). Pentylenetetrazole (PTZ)-induced kindling is a well-known model for epilepsy. It is a process whereby repeated administration of sub-convulsive doses of PTZ causes gradual seizure development culminating in generalized tonic-clonic seizures (Becker *et al.,* 1992). It has been reported that, free radical generation due to the increase in activity of the glutamatergic transmitter plays a crucial role in neuronal cell death of the PTZ kindling in rats (Becker *et al*., 1997, Sejima *et al.,* 1997 and Rauca *et al.,* 1999).

Peristrophe bicalyculata (Retz) Nees belongs to the family Acanthaceae. It is native to warm tropical regions of Africa, including Mauritania, Niger, Nigeria, India, Burma and Thailand (Burkill, 1985). In Nigeria, the plant is called "Tubanin Dawaki" which means "Flower of the horse" by the Hausas. The leaves of *P. bicalyculata* were used traditionally in the management of eye and ear problems, bacterial infections and as antidote to venomous stings and bites (Burkill, 1985). According to Rashmi *et al*., (2010), *P. bicalyculata* is used as a traditional remedy for tuberculosis, snake bites, hysteria and psychomotor disorders. *P. bicalyculata* extract has been reported to possess antihypertensive (Abdulazeez *et al*., 2011), antibacterial (Janakiraman *et al*., 2012) and anti-cancer activities (Tanavade *et al*., 2012). Abimbola *et al.,* (2013) showed that the whole plant has anti-trypanosomal activity on *Trypanosma brucei-brucei* infected rats.

The plant was also reported to be used as memory enhancer and in the management of convulsions among traditional medical practitioners especially in Zaria, northern part of Nigeria (Malam Rabi'u Salihu, Personal Communication, 2016). The aim of the study is to establish the anticonvulsant and antioxidant properties of methanol leaf extract of *Peristrophe bicalyculata* in rats.

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MATERIALS AND METHODS

Drugs, chemicals and equipment

Methanol, pentylenetetrazole (Sigma chemical CO., St. Louis, USA) Germany), sodium valproate (Sanofi-aventis, UK), Soxhlet apparatus (Sigma Aldrich, USA), analytical balance AE240 (Mettler Instrument Corporation, U.S.A.),

Collection and preparation of plant material

Peristrophe bicalyculata leaves were collected from Lere Local Government area, Kaduna State, Nigeria, in the month of August, 2016. It was identified and authenticated by Mallam Namadi Sanusi, a taxonomist with the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria by comparing with an already deposited voucher specimen (Number 2863) as reference at the Herbarium Section of the department. The leaves of the plant were air dried at room temperature for three weeks until constant weight was attained. The dried leaves were then milled into coarse powdered form using pestle and mortar. The powdered leaf (500 g) was extracted with 2.5 L of 90% $^{v}/_{v}$ methanol (90% Methanol: 10% water) for 72 hours exhaustedly using the soxhlet extraction method. The extract was evaporated in a thermostat oven at 50 ºC. The dried extract was weighed and then stored in a desiccator until needed for the main experiments.

Animals

Wistar rats (150-220 g) of either sex were obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept in a well ventilated condition at ambient temperature and fed with a standard animal feeds with adequate access to water *ad-libitum* under laboratory conditions in accordance with National Academy of Science, Guide for the Care and Use of Laboratory Animals (1996).

Phytochemical screening

Phytochemical screening was conducted on the methanol leaf extract of *P. bicalyculata* to validate the presence or otherwise of secondary metabolites such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and others using standard methods as described by Evans (2002).

Pentylenetetrazole-induced kindling test

The method described by Gupta *et al*., (2001) and Dhir *et al*., (2007) were adopted. A sub-convulsive dose of PTZ (35 mg/kg) was injected intraperitoneally (*i.p.*) every 48 hours for 21 days. Thirty six (36) rats were divided into four (4) groups of nine (9) rats per group. Group1 served as negative control and were pre-treated with normal saline 1 mL/kg *i.p*. Groups 2 and 3 were pre-treated with 200 and 400 mg/kg (*i.p)* of *P. bicalyculata* extract respectively. Group 4 was treated with 100 mg/kg of valproic acid. Thirty minutes post treatment, all the groups received 35 mg/kg of PTZ subcutaneously and then observed for a period of 20 minutes. Seizure intensities were scored on every treatment day as follows:

Stage 1: Ear and facial twitching
Stage 2: Convulsive waves throughout the body
Stage 3: Myoclonic jerks, rearing.
Stage 4: Turning over onto one side
Stage 5: Turning over onto the back, generalized tonic-clonic seizures.

The PTZ administration was stopped when animals in the first group (Normal saline + PTZ treated group) showed adequate kindling, i.e. seizure score of 4-5 (injection 8) and the subsequent high death rate that occurred in the group.

Blood sample collection

Blood samples were collected from the rats through retro-orbital puncture (from optic vein) into plain sterile tubes without anticoagulant. The blood was left to clot at room temperature and then centrifuged at 3500 rpm for 10 min. The serum was separated and used to evaluate the oxidative stress markers. Blood samples were also collected into sample bottles containing ethylene diamine tetra-acetic acid (EDTA) and used for haematological evaluation.

Oxidative stress markers and haematological evaluation

Oxidative stress enzyme makers and haematological analyses were performed at Kayomeg Medical Diagnostic Ltd, Kaduna state. Malondialdehyde was analyzed using GenAsia ELISA Kit (Catalogue No. GA-E0164RT) and Glutathione was also analyzed using GenAsia ELISA Kit (Catalogue No. GA-E3957RT), samples were analyzed with Rayto ELISA reader (RT 2150c). The haematological indices evaluated were red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), lymphocytes and mean corpuscular haemoglobin concentration. This was carried out using automated haematology machine (Biobase, Model BK610, China).

Statistical Analysis

Data obtained were statistically analyzed using SPSS (Version 20). The difference between means was analyzed by one way analysis of variance (ANOVA) followed by Dunnett post hoc test, while data on seizure scores were analyzed using Kruskal-Wallis test. Values of $p<0.05$ were considered significant.

RESULTS

Phytochemical screening

The methanol leaf extract of the plant showed the presence of glycosides, cardiac glycosides, alkaloids, saponins, triterpenes, tannins and flavanoids (Table 1).

Effect of methanol leaf extract of *P. bicalyculata* on PTZ-induced kindling in rats

P. bicalyculata extract (200 and 400 mg/kg) reduced the severity of seizure episodes induced by sub-convulsive dose of PTZ when compared to normal saline group. The reduction was significant ($p<0.05$) at 400 mg/kg after the second and fourth injections. The standard drug (valproic acid, 100 mg/kg) also significantly ($p<0.01$) reduced the severity of the seizure episodes induced by PTZ throughout the injection period (Fig. 1).

Effect of methanol leaf extract of *P. bicalyculata* on oxidative stress biomarkers of PTZ kindled rats

The administration of *P. bicalyculata* extract (200 and 400 mg/kg) showed no significant ($p>0.05$) difference in the levels of malondialdehyde when compared to normal saline groups. However, the glutathione levels were significantly ($p<0.01$ and $p<0.001$) higher at 200 and 400 mg/kg respectively when compared to normal saline group (Table 2).

Effect of methanol leaf extract of *P. bicalyculata* on haematological parameters of pentylenetetrazole-induced kindled rats

P. bicalyculata extract did not produce significant changes in the levels of most of the haematological indices tested when compared to normal saline group. However, the lymphocytes were significantly ($p<0.05$) decreased at 200 mg/kg. Similar result was obtained with the valproic acid-treated group (Table 3).

Table 1: Phytochemical constituents of methanol leaf extract of *P. bicalyculata*

Constituents	Inference
Alkaloids	+
Cardiac glycosides	+
Saponins	+
Flavonoids	+
Triterpenes	+
Tannins	+
Steroids	-
Anthraquinones	-

Key: Present = (+), Absent= (–)

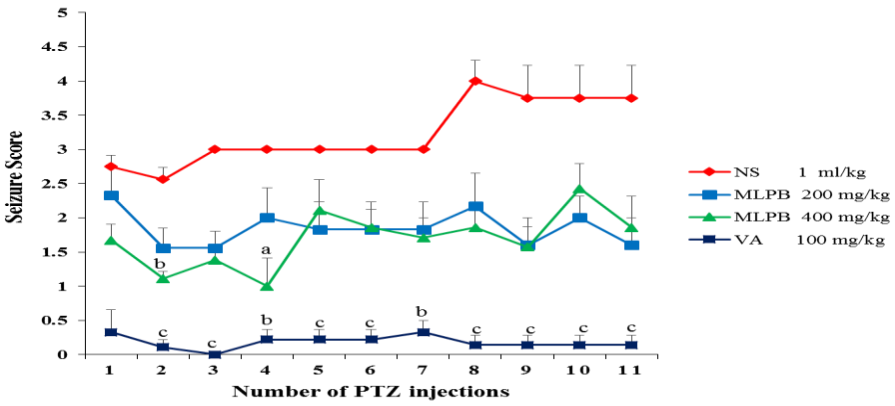

Fig. 1: Effect of methanol leaf extract of *Peristrophe bicalyculata* on PTZ-induced kindling in rats. Seizure score presented as Mean ± S.E.M., the superscripts a, b and c represent $p<0.05$, $p<0.01$ and $p<0.001$ respectively compared to normal saline (NS) control group. n=6, NS = Normal Saline, MLPB = Methanol leaf extract of *Peristrophe bicalyculata*, VA = Valproic acid.

Table 2: Effect of methanol leaf extract of *P. bicalyculata* on oxidative stress biomarkers of PTZ-induced kindled rats

Treatment (mg/kg)	Malondialdehyde (nmols/mg protein)	Glutathione (µg/ml)
N/saline 1 ml/kg	353.36 ± 25.02	4.10 ± 0.48
PTZ 35	425.67 ± 40.46	8.00 ± 0.29**
MLPB 200	385.80 ± 10.07	7.00 ± 0.59**
MLPB 400	370.73 ± 20.49	8.42 ± 1.01***
VA 100	510.70 ± 52.25*	3.91 ± 0.50

Values are presented as Mean ± S.E.M., * p<0.05, ** p<0.01 and *** p<0.001 compared to normal saline group – One way ANOVA followed Dunnett's test, n=5, N/Saline = Normal saline, VA = Valproic acid, MLPB = Methanol leaf extract of *Peristrophe bicalyculata*

Table 3: Effect of methanol leaf extract of *P. bicalyculata* on haematological parameters of PTZ-induced kindled rats

Parameter	Units	Treatment (mg/kg)				
		NS (1 ml/kg)	PTZ 35	MLPB 200	MLPB 400	VA 100
Haemoglobin	g/dL	13.76 ± 0.22	9.12 ± 0.91	11.14 ± 1.16	11.22 ± 0.59	9.04 ± 2.61
RBC	× 10^6/ µL	4.90 ± 0.14	5.00 ± 0.46	6.34 ± 0.84	6.27 ± 0.33	4.99 ± 1.47
WBC	× 10^3/ µL	3.58 ± 0.32	10.34 ± 1.85*	9.10 ± 1.28	8.16 ± 1.52	6.16 ± 1.91
Lymph	%	72.54 ± 2.14	57.30 ± 3.14	44.52 ± 7.49**	58.88 ± 1.24	52.26 ± 5.37*
PCV	%	42.88 ± 0.48	30.94 ± 3.08	34.98 ± 4.17	34.32 ± 1.82	28.44 ± 8.35
MCHC	g/dL	32.06 ± 0.27	29.58 ± 0.80	32.30 ± 1.40	32.72 ± 0.45	32.22 ± 0.42

Values are presented as Mean ± S.E.M., * p<0.05, ** p<0.01 compared to normal saline group - One way ANOVA followed by Dunnett's test, n=5, N/Saline = Normal saline, VA=Valproic acid, MLPB = Methanol leaf extract of *Peristrophe bicalyculata*, RBC=Red blood cells, WBC=White blood cells, Lymph=Lymphocytes, PCV=Packed cell volume, MCHC=Mean Corpuscular Haemoglobin Concentration.

DISCUSSION

Phytochemical screening of methanol leaf extract of *P. bicalyculata* revealed the presence of some important secondary metabolites including alkaloids, cardiac glycosides, saponins, flavonoids and tannins. Some of these phyto-constituents have been reported to possess anticonvulsant activity (Faggion *et al.*, 2011). In this study, the anticonvulsant activities of *P. bicalyculata* leaf extract may also be attributed to the presence of some of these bioactive principles.

Kindling is a widely accepted and established experimental model for human epilepsy. It is characterized by repeated administration of sub convulsive doses of PTZ to produce a progressive increase convulsion, which consequently culminate to generalized seizure in animals (Ono *et al.,* 1990; Rossi, 1996). PTZ induced kindling is employed for understanding the neurobiology of the epilepsy and also utilized for evaluating the effectiveness of antiepileptic drugs (Choudhary *et al.*, 2013). In PTZ-induced seizure following kindling, the hippocampus area of the brain is mostly affected (Takemiya *et al.*, 2003). The methanol leaf extract of *P. bicalyculata* at all the doses tested was able to reduce the intensity of the seizure. This is evident by not allowing the seizure to reach the classical seizure stage (stage 3-5) suggesting that, the extract may have antiepileptogenic activity.

Oxidative stress occurs when the steady state balance of pro-oxidant to antioxidant is shifted to the direction of the former creating potential for organic damage. Pro-oxidants include free radicals, atoms or cluster of atoms with single unpaired electrons (Stamler *et al*., 1992). Production of free radicals is associated with damage caused to cell structures and the pathogenesis of central nervous system (CNS) condition such as epilepsy, Parkinson's disease, stroke and dementia (Kong and Lin, 2010; Malinska *et al.*, 2010). The CNS is highly sensitive to oxidative stress due to its high oxygen consumption and low activity of antioxidant defenses (Halliwell, 1996; Estevez *et al*., 2011). When physiological levels of ROS are generated, it can be scavenged by enzymatic antioxidant defense system such as glutathione peroxide (GPX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) and non-enzymatic antioxidant defense system such as Vitamin C and Vitamin E. Excessive ROS levels due

to increased ROS production, decreased antioxidant defense ability or both leads to oxidative stress (Winyard *et al.*, 2005).

Several experimental models of seizures have demonstrated increased levels of oxidative stress markers in various regions of the brain after seizures (Dal-Pizzol *et al.*, 2000). Increased lipid peroxidation has been demonstrated in PTZ-induced seizures in the entire cortex in rats, electroshock-induced seizure in mice in the entire brain and pilocarpine-induced status epilepticus in rat hippocampus (Bashkatova *et al.*, 2003; Patsoukis *et al.*, 2004) and Qi *et al*. 2001). Antiepileptic drugs (AEDs) may modulate oxidative stress in epileptic patients (Yuksel *et al.*, 2000). Animal studies in which antioxidants were used in addition to anticonvulsants, showed decreased oxidative stress and reduced frequency of seizures (Willmore *et al.*, 1986; Tan *et al.*, 1998; Patsoukis *et al.*, 2004). Our findings revealed that, the methanol leaf extract of *P. bicalyculata* lacks antioxidant and free radical scavenging properties. PTZ-induced kindling causes peroxidation, thus increases the levels of malondialdehyde and glutathione compared to the normal saline group. The plant extract was unable to significantly decrease the level of malondialdehyde (MDA) compared to the PTZ treated group. This shows that, the extract was not able to inhibit lipid peroxidation and scavenge the free radicals generated. Malondialdehyde is the principal and most studied product of polyunsaturated fatty peroxidation. The PTZ-treated group showed an increased glutathione level indicating a high level peroxidation and generation of free radical evidenced by mobilization of glutathione in abundance to scavenge the free radicals generated thereby countering the peroxidation taking place in the tissue. Glutathione is a tripeptide and a major endogenous antioxidant found in all cells which react with the free radicals to protect cells from superoxide radical, hydroxyl radical and singlet oxygen and consequently causes lipid peroxidation (Schulz *et al.*, 2000).

Antiepileptic drugs were observed to have some haematological unwanted side effects (Rang *et al.*, 2016). Haematological side effects reported with clinically useful antiepileptic drugs include thrombocytopenia, megaloblastic anaemia, aplastic anemia, agranulocytosis, increased white blood cells count and increased platelet counts (Dorszewska *et al.*, 2009; Linnebank *et al.*, 2011 and Ozdemir *et al.*, 2011). Phenobarbitone, phenytoin and primidone cause a reduction in folic acid levels predisposing patients to megaloblastic anaemias. The use of antiepipleptic drugs in particular carbamazepine and valproic acid is associated with nine fold increased risk of aplastic anaemias (Acharya and Bussel, 2000; Handoko *et al.*, 2006; Bachmann *et al.*, 2011). The haematological parameters evaluated neither showed significant increase nor decrease in the indices suggesting that, the methanol leaf extract of *P. bicalyculata* has no effect on the haematological indices and the plant did not share the unwanted haematological side effects of some standard antiepileptic drugs. The significant increase in WBC counts in the PTZ-treated group was possibly because of the increase in oxidative stress in the process of seizure induction by PTZ (Kurhaluk *et al.*, 2017).

CONCLUSION: The results showed that, the methanol leaf extract of *Peristrophe bicalyculata* possesses anticonvulsant activity in chronic seizure model and thus, posseses antiepileptogenic activity with no antioxidant property.

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