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Antiepileptic Effect of *Musa paradisiaca* Stem Juice on Pentylenetetrazole (PTZ)-induced Seizures in Albino Rats

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ABSTRACT: *Musa paradisiaca* stem juice (MPSJ) found an extensive use in folklore medicine, but its antiepileptic properties are yet to be scientifically proven, hence, the aim of this study. Pentylenetetrazole (PTZ)-induced seizure model was adopted, involving twenty-four (n=24) adult male albino rats, randomly distributed into six (6) groups of four animals each (n=4). Group 1 received saline (p.o); group 2 was untreated; group 3 received 4 mg/kg b.w diazepam (p.o); while groups 4, 5 and 6 received 50, 75 and 100% (v/v) of MPSJ (p.o), respectively. The treatment lasted for 10 days before rats were challenged with PTZ (85 mg/kg b.w, i.p) after 45 min. Group 6 showed a significant ($p < 0.05$) increase in brain GABA level compared to that of the untreated group. Groups 5 and 6 showed a significant ($p < 0.05$) reduction in glutamate level and GABA-T activities compared to that of group 2. There were significant ($p < 0.05$) increases in the levels of CAT and SOD of group 6 rats compared to those of the untreated groups, except the GSH that was not statistically significant ($p > 0.05$). Group 6 showed a significantly ($p < 0.05$) higher SOD concentration when compared to group 2. The MDA level of group 4 was significantly ($p < 0.05$) reduced compared to that of group 2. The neuronal necrosis produced by PTZ was ameliorated in group 4 rats and the normal histo-architecture of the brain was restored. This study suggests MPSJ has protective effects against PTZ-induced seizures in rats.

KEYWORDS: Seizure disorder; convulsion; Plantain (*Musa paradisiaca*); pentylenetetrazole; neurotransmitter; GABA-T; antioxidant.

1. INTRODUCTION

Epilepsy poses a tremendous threat to the global community as it affects about 50 million people globally and about 10 million people in Africa [1]. Only 20 % of this affected population is treated with readily available synthetic drugs, while the rest are inappropriately treated or not treated at all; owing to lack of treatment access, thereby constituting serious treatment gaps [1, 2]. Therefore, epilepsy continues to threaten its victims, impairing their physical, psychological and social functioning.

The brain is a complex organ composed of about 100 billion nerve cells (neurons), forming about a trillion synaptic connections [3, 4]. These neurons communicate signals to one another electrochemically, and the neural network formed is organized and coordinated in a fashionable manner [5]. Any shift or disconnect in the normal neuronal discharge leads to health problems. Seizure results when there is a temporal disconnect in the normal neural or electrical activities and communications in the brain [6]. Several factors such as head injury or trauma, central nervous system (CNS) infections, brain tumours, stroke, certain chemicals or drugs, etc., are responsible for this neural disconnect and seizure triggers in the brain.

Pentylenetetrazole (PTZ) is a non-conventional drug (a convulsant) that induces seizures by disrupting neural activity when administered at a lethal dose (85 mg/kg b.w in rats). The mechanism underlying PTZ-

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induced seizures is thought to involve direct non-competitive antagonism of $GABA_A$ receptors that acts through t-butyl-bicyclophosphorothionate site of the receptor to decrease its activity [7, 8].

Various antiepileptic drugs (AEDs) are currently in use with each possessing diverse mechanisms of actions, for instance, the group of AEDs known as benzodiazepines (BZDs) (e.g., diazepam) acts as agonists to GABA receptors to enhance inhibitory neurotransmission [9]. This is due to an increase in the GABA vesicular release and availability at the synapse. PTZ works in antagonism with BZDs (e.g., diazepam) by acting as a GABA blocker at its receptor, leading to excitation signals, due to rise in glutamate levels at the synapse.

In the past few years, some plants have demonstrated potency against seizure disorders; and from phytochemical and pharmacological studies carried out, various biologically active compounds such as flavonoids and other phenolic compounds possessing the same mechanism of action as their synthetic counterparts have been found responsible, and are more beneficial and tolerable [10]. Plantain (*Musa paradisiaca*) is one of the medicinal plants that have reportedly been used in folklore medicine to manage a number of health problems including epilepsy and hysteria [11]. There have always been issues with the ethno-pharmacological use of Musa species (*Musa paradisiaca.* L and *Musasapientum.* L), where both plants were being used interchangeably and/or differently in different regions for the treat of seizure disorders. In some parts of Southeastern Nigeria such as Agulu in Anambra State, Obowo in Imo State and Nsukka in Enugu State, *Musa paradisiaca* is used to treat seizure disorders/convulsions, and sometimes in combination with locally extracted Palm kernel oil (Elu Aki) for the treatment of convulsions in children (Febrile Convulsions). The fruit from both plants is consumed as food. *M. paradisiaca* leaf juice is used in the treatment of fresh wounds, cuts and insect bites; while the leaves act as an arbortifacient [12]. In addition, the fruit has reportedly been used as antiscorbutic, aphrodisiac and diuretic [13], whereas a cold infusion of the root is used to treat venereal diseases and anaemia [14]. In other regions such as Southeastern Asia countries, it was reported that *M. sapientum* (Banana) is used in place of *M. paradisiaca* (*Plantain*) for the treatment of seizures [15], but this does not negate the reportedly ethno-pharmacological use of *M. paradisiaca* for seizure treatment in the Southeastern Nigeria. Previous studies on the phytochemical constituents of both plants have shown that they possess almost similar phytochemicals, mainly alkaloids, saponins, tannin and flavonoids in closely related amounts [16], which could be the reason for which both plants are used interchangeably in different regions. The other reason might be due to the availability of both plants in various regions. Many plant extracts that have shown anticonvulsant activity in animal seizure models are due to the action of flavonoids, alkaloids, tannin, saponins, furanocoumarins and phenylpropanoids on GABA receptors and voltage-gated ion channels [17]. These phytochemicals help to maintain the normal physiological function of the major inhibitory neurotransmitters.

In spite of the fact that numerous conventional medicines are currently in use for the control of epileptic seizures, they are unable to provide a satisfactory efficacy and/or safety due to their various debilitating adverse effects and intolerance issues [18]. However, the search for a more satisfactorily efficacious and more tolerable or safer medicine for the control of epileptic seizures is a necessity, and hence, the goal of this study.

2. RESULTS AND DISCUSSION

2.1. Effect of MPSJ on GABA and glutamate levels; and GABA transaminase (GABA-T) activities in the brain homogenate of PTZ-challenged rats

Gamma-aminobutyric acid (GABA) and glutamate are the two major neurotransmitters implicated in seizure disorders; while GABA is excitatory, glutamate is inhibitory in actions [19]. GABA is formed from glutamate by glutamic acid decarboxylase (GAD) within GABAergic axon terminals and released into the synapse, where it acts on its receptors, $GABA_A$ and $GABA_B$, which control chloride (Cl^-) and potassium (K^+) ions entries into the neuronal cell, respectively, to inhibit signal transmission which results to hyperpolarization [20].

Glutamate on the other hand, is synthesized from glucose metabolism within the glutamatergic axon terminals are later released from its vesicle into the synapse where it acts on its receptors to trigger over-excitatory neurotransmission due to influx of calcium ion (Ca^{2+}) on the postsynaptic cell [21]. Glutamate, being a principal excitatory neurotransmitter and with its interaction with the specific membrane receptor is responsible for many neurological functions. Excessive glutamate receptor activation can induce oxidative stress increase, described by the term excitotoxicity, and play a critical role in epileptic brain damage [22].

GABA-T is an enzyme that degrades GABA into succinic semialdehyde (SSA) [19]. Diazepam is a conventional antiepileptic drug that acts as an antagonist of PTZ by increasing GABA neurotransmitters [23]. Therefore, the more GABA available in the synapse, the fewer the tendencies for seizures to occur; but for glutamate, it is *vice versa*.

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Based on the results of this study, there were variations in the brain GABA and glutamate levels as well as GABA-T activities in the brains of experimental rats treated with MPSJ (Table 1).

The test groups, i.e., 4, 5 and 6 show significant ($p < 0.05$) increases in GABA levels with values, 3.67 ± 0.20, 3.92 ± 0.46 and 4.39 ± 0.52 U/mg, respectively, compared to that of the group 2 that received PTZ with the value of 2.86 ± 0.22 U/mg. PTZ induces seizures by interfering with the function of inhibitory neurotransmitter GABA in the brain [24]. The group 6 that received high dose (100% v/v) of MPSJ demonstrated similar anticonvulsant activity based on their GABA levels, compared with the group 3 that received 4mg/kg b. w of diazepam, indicating that calculated high dose of stem juice could be as potent as diazepam. Decreased GABAergic activity as seen in group 2 is one of the factors for triggering seizures and drugs facilitating GABA action in brain, such as benzodiazepines (e.g. diazepam) is well known for their roles in the treatment of epilepsy [25].The result of this finding agrees with that of Mahmoudi *et al* [26] that reported how 400 mg/kg b. w of hydro-alcoholic extract of *Curcuma zedoaria* caused a marked increase in GABA concentration in the brain homogenate of experimental rats, owing to the presence of flavonoids. Therefore, the observed increase in GABA levels may probably be due to the presence of certain phenolic compounds or flavonoids in the stem juice.

Increased glutamate level has also been suggested to play a role in seizure induction by PTZ as glutamate receptor antagonists have been demonstrated to decrease the PTZ‐induced activity [27]. The levels of glutamate were significantly ($p < 0.05$) decreased in groups 4, 5 and 6 with values, 202.66 ± 5.81, 196.22 ± 7.03 and 206.15 ± 6.36 U/mg, respectively, compared to that of the group 2 (222.59 ± 10.61 U/mg). This result is in consonant with the findings by Mahmoudi *et al* [26] in which hydro-alcoholic extract of *Curcuma zedoaria* with high contents of flavonoids and other phenolic compounds reduced the glutamate concentration in the brain homogenate of rats.On the other hand, the result of this finding is opposed to the elevated glutamate levels observed in the brain tissues of PTZ-challenged mice treated with the fruit extract of *Amomum tsaoko* due to its low flavonoids content as reported by Wang *et al* [28]. In all, this implies that the MPSJ must have demonstrated its anti-seizure potential due to certain phenolic compounds and/or flavonoids.

Table 1. Effect of MPSJ on GABA and Glutamate Levels; and GABA Transaminase (GABA-T) Activities in the Brain Homogenate of PTZ-Challenged Rats

Groups	**GABA (**U/mg**)**	**GABA-T (**mmol/hr/mg protein**)**	**Glutamate (**U/mg **)**
Group 1	5.65 ± 0.73^{d}	0.24 ± 0.01^{a}	182.52 ± 18.33^{a}
Group 2	2.86 ± 0.22^{a}	0.33 ± 0.03^{c}	222.59 ± 10.61^{c}
Group 3	4.71 ± 0.51^{c}	$0.26 \pm 0.01^{a,b}$	$192.07 \pm 16.67^{a,b}$
Group 4	3.67 ± 0.20^{b}	0.28 ± 0.01^{b}	202.66 ± 5.81^{b}
Group 5	3.92 ± 0.46^{b}	0.27 ± 0.01^{b}	$196.22 \pm 7.03^{a,b}$
Group 6	$4.39 \pm 0.52^{b,c}$	0.28 ± 0.02^{b}	$206.15 \pm 6.36^{a,b}$

Data is expressed as mean ± standard deviation (n = 4). The alphabets a, b, c, d indicates the statistical significance. Means with different alphabet as superscripts down the column are significantly ($p < 0.05$) different.
Group 1: Normal control (Saline only for 10 days)
Group 2: Untreated control (saline for 10 days + PTZ 45 min after, on the last day)
Group 3: Standard control (4 mg/kg Diazepam for 10 days + PTZ 45 min after, on the last day)
Group 4: 50% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)
Group 5: 75% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)
Group 6: 100% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)

There is non-significant ($p > 0.05$) differences in the glutamate levels of these test groups (4, 5 and 6) when compared with that of the group 3 treated with diazepam (192.07 ± 16.67 U/mg), suggesting that the stem juice can serve as a better alternative to diazepam.

The activity of GABA-T was significantly ($p < 0.05$) decreased in test groups, 4, 5 and 6 with values, 0.28 ± 0.01, 0.27± 0.01 and 0.28± 0.02 mmol/hr/mg protein, respectively, compared to that of the untreated (group 2) with the value, 0.33 ± 0.03 mmol/hr/mg protein. This implies that 50, 75, and 100% (v/v) of MPSJ have an inhibitory effect on the activity of the GABA-T. The results of this finding is similar with that reported by Kandeda *et al* [29], in which the aqueous extract of *Canarium schweinfurthii*, at all doses decreased GABA-T activity in mice challenged with PTZ, owing to the presence of certain bioactive molecules such as flavonoids

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and phenolic compounds. The emerging evidence suggests that phenols or flavonoids present in MPSJ might be responsible for the observed marked decrease in GABA-T activity in the test groups.

The test groups showed non-significant ($p > 0.05$) differences in their GABA-T activities when compared with that of the group 3 that received 4 mg/kg diazepam (0.26± 0.01 mmol/hr/mg protein). This suggests that MPSJ, in the appropriate dose, can serve as a preferred replacement for synthetic benzodiazepines such as diazepam.

2.2 Effect of MPSJ on antioxidant parameters in the brain homogenate of PTZ-challenged rats

PTZ has the potentiality of causing neurotoxicity owing to its tendency of generating reactive oxygen species which are very harmful to neuronal cells, a phenomenon referred to as oxidative stress [30]. More so, PTZ administration increases the burden of oxidative stress [31].

Generally, ROS unleash great destructions and mayhem on body cells [32]. Natural antioxidants, including SOD, CAT, and GSH are biomarkers of oxidative stress, in which their low levels have impact on the cell damage; and often lead to a corresponding increase in lipid peroxidation product, MDA [33, 34, 35].

From the result of this study, MPSJ was able to trigger the values of SOD, CAT and GSH, as well as reduced MDA values in the brains of rats challenged with PTZ. The results of increases in the values of SOD, CAT and GSH; and decreases in MDA values (see Table 2).

Table 2. Effect of MPSJ on antioxidant parameters and lipid peroxidation product in the brain homogenate of PTZ-challenged rats

Groups	SOD(u/mg protein)	CAT(u/mg protein)	GSH(u/mg protein)	MDA (Mmol/mg)
Group 1	14.69 ± 1.49^{d}	48.32 ± 5.47^{c}	16.84 ± 0.70^{b}	7.94 ± 1.24^{a}
Group 2	9.94 ± 0.38^{a}	24.68 ± 3.03^{a}	13.24 ± 0.92^{a}	13.53 ± 1.47^{d}
Group 3	$13.87 \pm 0.75^{c,d}$	35.24 ± 8.23^{b}	16.63 ± 0.53^{b}	$8.36 \pm 0.85^{a,b}$
Group 4	$12.44 \pm 2.07^{b,c}$	25.48 ± 4.89^{a}	$14.93 \pm 1.01^{a,b}$	$10.81 \pm 1.67^{b,c}$
Group 5	$10.85 \pm 0.69^{a,b}$	24.59 ± 5.27^{a}	14.21 ± 3.12^{a}	$11.03 \pm 0.78^{c,d}$
Group 6	12.73 ± 0.65^{c}	35.40 ± 3.40^{b}	$14.87 \pm 0.55^{a,b}$	$11.06 \pm 0.65^{c,d}$

Data is expressed as mean ± standard deviation (n = 4). The alphabets a, b, c, d indicates the statistical significance. Means with different alphabet as superscripts down the column are significantly ($p < 0.05$) different.
Group 1: Normal control (Saline only for 10 days)
Group2: Untreated control (saline for 10 days + PTZ 45 min after, on the last day)
Group 3: Standard control (4 mg/kg Diazepam for 10 days + PTZ 45 min after, on the last day)
Group 4: 50% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)
Group 5: 75% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)
Group 6: 100% (v/v) MPSJ for 10 days + PTZ 45 min after, on the last day)

In the present study, the induction of seizure using PTZ brought about significant ($p < 0.05$) decreases in SOD, CAT, GSH levels; and a significant ($p < 0.05$) increase in the MDA levels; of group 2 compared to that of the normal control (group 1). This shows the extent of neurotoxicity as a consequence of the oxidative stress following PTZ administration. Recent studies have shown that oxidative stress and the dysfunction of mitochondrion could make the brain become susceptible to epileptic seizures [36]. In addition, seizures lead to the production of free radicals and oxidative damage to proteins, fatty acids, and nucleic acids in neuronal cells [30]. Such neuronal damage is known to be one of the most important factors responsible for the cause and development of seizure [37].

There was a significant ($p < 0.05$) increase in SOD values of groups 3, 4 and 6 when compared to group 2. For CAT, group 3 and 6 showed a significant ($p < 0.05$) increase in CAT values when compared with that of group 2, with no marked differences between each other. SOD effectively reduces the accumulation of superoxide free radicals [34, 35, 38]. For GSH, only group 3 showed a significant increase in GSH levels when compared to that of the group 2.

In the antioxidants investigated, the group 6 that received 100% v/v of MPSJ was more effective in increasing the antioxidant levels with values 12.73 ± 0.65and 35.40± 3.40 u/mg protein, compared to that of the untreated group with values 9.94 ± 0.38 and 24.68 ± 3.03 u/mg protein, respectively. The only exception to this is that of GSH in which 100% v/v MPSJ was not markedly effective. The result of this finding agrees

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with the marked increase in the antioxidants, SOD, and GSH levels of mice induced with PTZ following the treatment with graded doses of *Amomum tsaoko* fruit juice containing high flavonoid, tannin and phenol contents [27]. However, it is obvious by this results that MPSJ demonstrated its activity in a dose-dependent manner where the concentration of these bioactive compounds in the low (50% v/v) and medium (75% v/v) doses of MPSJ was not enough to produce a significant effect. This could also be the reason why 100% v/v MPSJ could not effect GSH markedly. Maybe, a dose above this would have produced a significant result.

In the present study, the brain MDA level was found to be higher in the untreated group than in other groups, suggesting lipid peroxidation and damage. This result is in agreement with that of [15] who reported a significant increase in the brain MDA levels in PTZ-induced kindled group of mice administered with *Musa sapientum* stem extract as compared to control group. Groups 3 and 4 showed a significant ($p < 0.05$) decrease in MDA values (8.36 ± 0.85 and 10.81 ± 1.67 Mmol/mg, respectively) compared to that of the group 2 (13.53 ± 1.47 Mmol/mg). Therefore, only group 4 which received a low dose (50% v/v) MPSJ recorded a marked decrease in the lipid peroxidation product, MDA, suggesting that only 50% v/v MPSJ was able to clear off the lipid peroxidation products in generated by PTZ. Many plants have demonstrated anticonvulsant activity due to their flavonoid, phenols, tannins, furanocoumerin contents which were found to act on GABA receptors and voltage-gated channels [17]. It was also observed that adding flavonoid (i.e., curcumin) to a culture medium of microglia and cortical neurons reduced oxidative damage by inhibiting lipid peroxidation [27]. Therefore, it could be inferred that MPSJ might contain some of these bioactive compounds with antioxidant potentials that were responsible for the observed anti-seizure effects. In our study, the ability of low dose of MPSJto reduce the levels of MDA in the brain tissues of the experimental rats suggests that it could reduce oxidative damage due to seizures.

2.3 Effect of MPSJ on the brain histology of rat challenged with Pentylenetetrazole (PTZ)

In the figure below, plate A shows sections of rat's brain with normal (White arrows) histo-architecture in group 1. Plate B shows sections of rat's brain with numerous necrotic neurons (Blue arrows) in group 2 in which the necrotic neurons appear shrunken, deeply eosinophilic and lack obvious nuclei. Plate C shows sections of rat's brain with normal histo-architecture in which the sections showed normal neurons (White arrows), glial cells (black arrows) and neuropil (N) in group 3 administered with 4mg/kg diazepam. In addition, areas of haemorrhage (Red stain) were observed in plate C. Plate D shows sections of rat's brain with normal brain histo-architecture (White arrows) in Group 4 that received 50% v/v of MPSJ. Plates E and F show sections of rats' brains with little necrotic neurons (Blue arrows) in groups 5 and 6 that received 50% v/v of MPSJ, respectively.

The histological examination of the brain showed numerous necrotic neurons in group 2 (untreated) unlike that of group 1(normal control). This is an indication of necrosis in neuronal cells or tissues through injury, disease, or the interruption of blood flow following the administration of PTZ. Treatment with the standard drug (4 mg/kg b.w of diazepam, i.p) restored the normal histo-architecture of the rat brain, but areas of haemorrhage (red stain) were observed (plate C). This observed haemorrhage might probably be an aberration or an indication of severe damage in the brain due to prolonged diazepam administration. However, treatment with 50% (v/v) MPSJ restored most of the necrotic neurons produced by PTZ to normalcy, but 75 and 100% (v/v) failed to completely clear off the neuronal necrosis or restore the normal histo-architecture of the rat brain. The result of this study is in consonant with that reported by Eman *et al* [39] after studying the effects of *Stevia rebaudiana* in the brain histology of PTZ- induced rats which was found to show atrophy and necrosis in their hippocampus, unlike the test groups. This suggests that MPSJ could protect the neurons and the glial cells from the damaging effects of a convulsant such as PTZ, though not in a dose-dependent manner.

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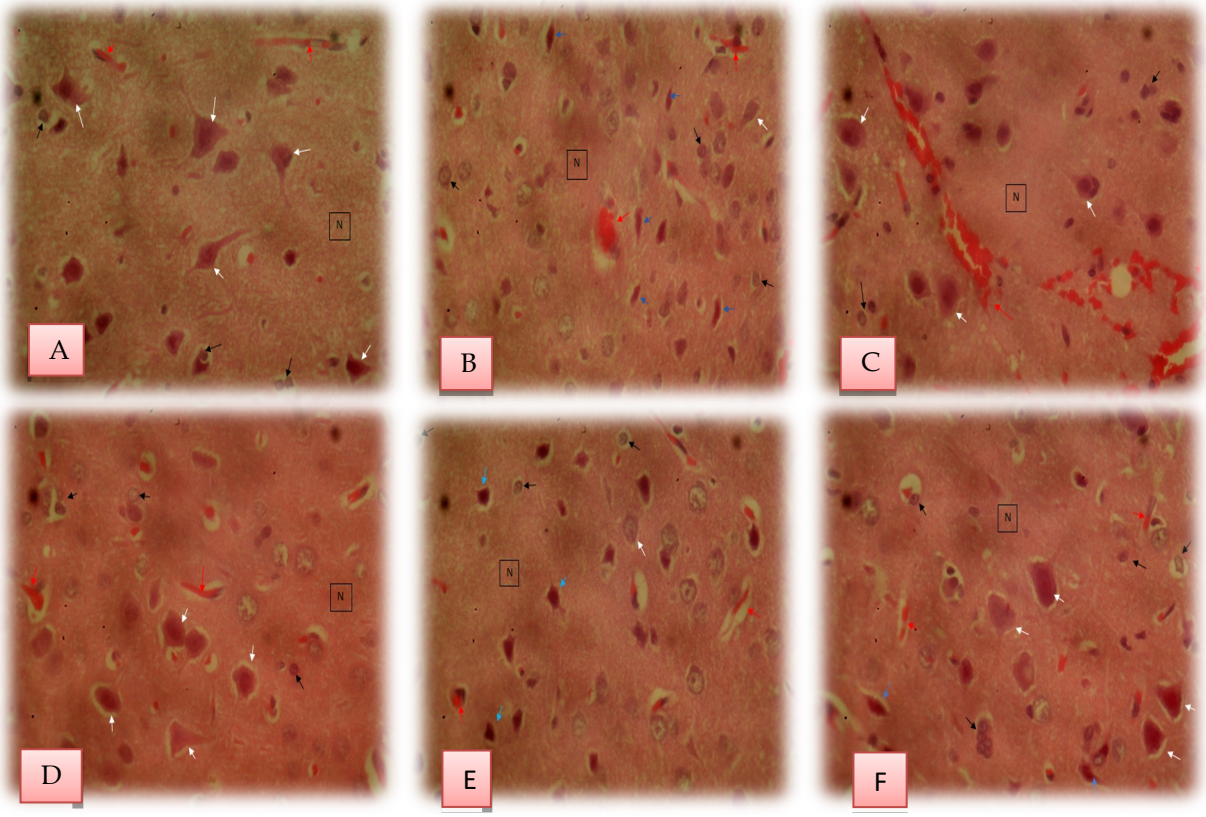

Figure. Effect of MPSJ on the brain histology of rat challenged with pentylenetetrazole (PTZ). Sections of the Brain presented in the various experimental groups showing the histo-architecture of the rats' brains (H&Ex400). White arrow represents normal neurons; Black arrow (glial cells); Red arrow (capillaries); Blue arrow (necrotic neurons); N (Neuropil).

3. CONCLUSION

The results of this study have shown that MPSJ has a significant anti-epileptic effect on PTZ-induced seizures in rats by increasing GABA levels as opposed to glutamate levels, and by decreasing GABA-T activity in the rats' brains. MPSJ was also able to increase SOD, CAT, and GSH levels with a corresponding decrease in MDA levels of PTZ-challenged rats. The neuronal necrosis produced by PTZ in rats that received a low dose of MPSJ was ameliorated and the normal histo-architecture of the brain was also restored to normalcy. This study suggests that MPSJ has a great potential in epilepsy prevention and justifies its use in folklore medicine. However, further studies are required to identify the active ingredients in the juice and ascertain its mechanism of action.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Plant materials

Fresh samples of *M. paradisiaca* stem were used for this study. They were collected on 13th February, 2022 from a plantain plantation located at Obeke village in Uwani Akpotoro Obimo, Nsukka Local Government Area of Enugu State, Nigeria. It was identified by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State Nigeria. Voucher specimen was deposited in the herbarium unit of the Botany Department, University of Nigeria, Nsukka for future use.

4.1.2 Experimental animals

Twenty-four (24) adult male Albino rats (120- 220 g, 10 to 12 weeks) were used for this study. The animals were bred and obtained from the animal farm of the Pharmacology and Toxicology Department, University of Nigeria, Nsukka. Animals were acclimatized for 7 days upon transfer to the work area under standard laboratory conditions with free access to standard pellets (Guinea Feeds Plc, Nigeria) and water prior to the commencement of the experiment. All animal experiments were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals and approved by the

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Ethics and Biosafety Committee, Faculty of Biological Sciences, University of Nigeria, Nsukka, with a Reference Number: UNN/FBS/EC/1090.

4.1.3 Equipment and chemicals/kits

The equipment and chemicals used for this study were of analytical grade. Some of them were obtained from the Biochemistry Department, University of Nigeria, Nsukka, while some were purchased from the Springboard Research Laboratory, Awka, Anambra State, Nigeria. They include but not limited to the following:

*Equipment***:** Mortar and petle (Sevico Plast, Nigeria), Thermostat bath (Corning Ltd., England), Centrifuge and Spectrophotometer (Unico® UV-2102 PC, Texas, USA), Volumetric flask, Beaker, test tubes and measuring cylinder (Pyrex, England), surgical blade and Weighing balance (Mettler Toledo PB 602, Switzerland), Whatman filter paper.

*Chemicals/Kits***:** Pentylenetetrazole (Sigma Aldrich Chem. Co., USA**);** Diazepam (Hoffman-la Roche, Switzerland); Chloroform, Sodium Hydroxide, Ferric Chloride, 90% Ethanol, Potassium Hydroxide, Distilled Water (STC, Nsukka), Thiobarbituric acid (TBA), Pyridoxal phosphate, 10% Trichloroacetic Acid (TCA), Carbon-bicarbonate buffer, phosphate buffer, 14 mM Ninhydrin .Other reagents used for the assays were commercial test Kits and products of Randox, UK, Biovendor, Czech Republic, TECO Diagnostics, USA and Centronic GmbH, Germany.

4.2 Methods

4.2.1 Preparation of stem juice

A fresh sample of *M. paradisiaca* stem was cut, sliced, and washed free of dirt. The juice was then extracted by mechanical crushing using a plastic mortar and pestle, followed by filtration using a Whatman filter paper. Following the method of dosing by Onyenekwe *et al* [11], 100mL of MPSJ was used to make 100% (v/v); while 75% (v/v) was made by measuring 75 mL of MPSJ and made up to 100 mL with distilled water in a volumetric flask; 50% (v/v) was made with 50 mL of *M. paradisiaca* stem juice (MPSJ) made up to 100 mL with water while 25% (v/v) was made with 25 mL of MPSJ made up to 100 mL with water in a volumetric flask.

4.2.2 Experimental design

Pentylenetetrazole (PTZ)-induced seizure model was employed for this study according to Eigyo *et al* [40]. The study was conducted using twenty-four (n = 24) adult male albino rats randomly distributed into six (6) groups of four animals each (n = 4) using a weighing balance. Group 1 received saline (p.o) (control group); group 2 was untreated (positive group); group 3 received 4 mg/kg b. w diazepam (p.o) (standard group); and groups 4, 5 and 6 received 50, 75 and 100% (v/v) of MPSJ(p.o) (test groups). The treatment lasted for 10 days before challenging the rats with PTZ (85 mg/kg b. w, i.p).

4.2.3 Seizure induction procedure

Seizure was induced in the rats with PTZ on the tenth day. The animals in all the experimental groups, except those of group 1 were challenged with PTZ (85mg/kg b.w, i.p) 45 min after treatments with the MPSJ except those in the group 1 [36]

The experimental protocol was summarized as follows;
Group 1: Normal control (Saline, p.o for 10 days)
Group2: Untreated control (Saline, p.o for 10 days+ PTZ 45min after, on the last day)
Group 3: Standard control (4 mg/kg Diazepam, p.o for 10 days + PTZ 45 min after, on the last day)
Group 4: 50% (v/v) MPSJ, p.o for 10 days + PTZ 45 min after, on the last day)
Group 5: 75% (v/v) MPSJ, p.o for 10 days + PTZ 45 min after, on the last day)
Group 6: 100% (v/v) MPSJ, p.o for 10 days + PTZ 45 min after, on the last day)

4.2.4 Brain tissue harvesting and processing

The rats were humanely sacrificed after an exposure to a mild anaesthesia (Chloroform) at 15000 ppm for 5 min. The brain tissues were harvested using a surgical blade. A 10% (w/v) brain tissue homogenate was prepared in ice-cold phosphate buffer (50 mM, pH 7.0) at 4°C using a thermostat bath. An aliquot of the homogenate was immediately deproteinized with an equal volume of 10% TCA, centrifuged at 12,000 × *g* for 20 min at 4°C to obtain a protein-free supernatant, which was used for the determination of the concentration of glutamate, GABA, GABA-T and antioxidant parameters in the brain.

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4.2.5 GABA, glutamate and GABA-T quantification studies

Glutamate levels were determined in rat brain regions by the enzymatic method described by Bernt and Bergmeyer [41]. The γ-aminobutyric acid (GABA) levels were determined in rat brain regions spectrofluorometrically by the method of Lowe *et al* [42] as described by Uchida and O'Brein [43]. The γ-aminobutyric acid-transaminase concentration was measured by the method of Sytinsky *et al* [44]. Lipid peroxidation was estimated by measuring spectrophotometerically the level of the lipid peroxidation product, malondialdehhyde (MDA), as described by Wallin *et al* [45].

4.2.6 Determination of antioxidant parameters and lipid peroxidation product malondialdehyde (MDA)

Superoxide dismutase activity was assayed by the method of Arthur and Boyne [46] as contained in the Randox Kit used. The concentration of catalase was determined by the method of Sinha [47], while glutathione (GSH) was determined according to the method of King and Wootton [48].

4.3 Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison test using Statistical Product and Service Solution (SPSS) version 21. Results were expressed as mean ± SD and a p-value < 0.05 was considered significant.

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