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ANTICONVULSANT SCREENING OF THE AQUEOUS AND ETHANOL EXTRACTS OF *Mitragyna inermis* BARK IN PENTYLENETETRAZOLE AND STRYCHNINE INDUCED SEIZURES IN ALBINO RATS

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ABSTRACT

Mitragyna inermis is a medicinal plant found in Nigeria and has diverse ethno medical uses including epilepsy. The aim of this study was to evaluate the anti-convulsant activity of aqueous and ethanolic extracts of *Mitragyna inermis* bark. The anticonvulsant activity of the extracts was investigated by studying the effects on seizures induced by pentylenetetrazole and strychnine in albino rats. The aqueous and ethanolic extracts of *Mitragyna inermis* at doses between 62.5 and 500 mg/kg dose dependently increased the onset of clonic convulsion induced by pentylenetetrazol and strychnine. There was a statistical significant difference between the effect of the extracts (at 250 and 500mg/kg) and the negative control ($p<0.05$). The data obtained suggest that *Mitragyna inermis* possesses anticonvulsant property with ethanolic extract having a superior activity than the aqueous extract. The activity of the extracts was found to prolong the onset of pentylenetetrazole induced seizures much higher than the strychnine induced seizures. Therefore, the activity of the extracts may be due to the involvement of Gamma amino butyric acid (GABA).

Key Words:- *Mitragyna inermis*, Convulsion, Pentylenetetrazole, Strychnine.

INTRODUCTION

Medicinal plants have been used as folklore remedies over the years to treat, manage or control man's ailments. They contain a large variety of chemical substances that possess important therapeutic properties used in the treatment of these ailments (World Health organization, 2002). High cost of conventional drugs, particularly in resource poor communities of the African continent together with the development of human resistant pathogens, emergence of new diseases, unsatisfactory efficacy and toxicity of conventional drugs have prompted the search and use of plants as an alternative for the treatment of diseases (Sibanda and Okoh, 2008; Adoum *et al*., 2012). Significant numbers of plants have shown varying degree of anticonvulsant effect as herbal medicines in Sub-Saharan Africa (Azas *et al*., 2002; Mu'azu and Kaita, 2008; Adoum *et al*., 2012). A typical example of such plants is *Mitragyna inermis* which usually exists as a shrub or tree with a dense, wide crown and is well known for its ornamental and medicinal purposes. Its roots, leaves and bark are well known in traditional medicine among the Hausa/Fulani tribe in Northern Nigeria in the treatment of several diseases including epilepsy (Mu'azu and Kaita, 2008). Several findings on the chemotherapeutic potentials of *Mitragyna inermis* have shown that they can be sources of

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antimicrobial, antidiabetic, antimalarial, antihypertensive and anticonvulsant compounds (Rabe and Van Staden, 1997; Adoum *et al*., 2012; Azas *et al*., 2002; Ouedrago *et al*., 2004; Uthman *et al*., 2013). The therapeutic potential of *Mitragyna inermis* in the management of epilepsy by traditional herbalist in most of our communities necessitated this study aimed at screening the anticonvulsants activity of the bark extracts of *Mitragyna inermis* so as to ascertain the validity of its use in the ethnomedical practices in Northern Nigeria.

Materials and Methods

Experimental Site

The experiment was conducted in the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy, University of Maiduguri, Borno state, Nigeria.

Plant Collection and Identification

The bark of *Mitragyna inermis* was collected in May 2014 from out sketch of Maiduguri Metropolitan City, Borno State, Nigeria. The bark was washed on the same day and allowed to dry in a well-ventilated room for two weeks. The bark of the plant was identified and authenticated by a taxonomist Prof. S.S. Sanusi in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. The voucher specimen number was assigned (MI2048) and deposited in herbarium of Faculty of Pharmacy, University of Maiduguri, Nigeria.

Preparation of Plant Extract

The dried bark of *Mitragyna inermis* was pounded using a mortar and pestle in which 500g and 500g were weighed differently into separate containers and each was macerated for 24 hours with 250 ml and 350 ml of aqueous and ethanol (99%) respectively. The plant material for ethanol was macerated twice (the second with the recovered solvent). The filtrates were evaporated in a rotary evaporator at 60°C (water) and 50°C (ethanol) and the resultants were air dried and stored. The extracts were then referred to as aqueous and ethanolic extracts. The aqueous and ethanolic extractive values were 23.5 g and 30.7 g with yields of 4.7% and 6.14% respectively. A freshly prepared solution or suspension of the extracts was used on each day of the practical.

Animals

Swiss albino rats of both sexes were bred in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri. The animal house is a well-ventilated room and the rats were feed with standard feed (grower mess). The ethical clearance was not applied for due to the absence of such committee in the University.

Phytochemical analysis of the bark extracts

The preliminary phytochemical analysis of the bark extracts of *Mitragyna inermis* was conducted based on the standard method of Trease and Evans (1989) and Sofowora (1993).

Administration of the drug solutions and extracts

Pentylenetetrazole and Strychnine (SIGMA-ALDRICH, Co., 3050 Spruce street, St. Louis, MO 63103 USA 314-771-5765) were obtained from Pharmacology Laboratory of Faculty of Pharmacy, University of Maiduguri. Diazepam (Valium® by Roche) was purchased from the University of Maiduguri Teaching Hospital (UMTH). Water for injection B.P. was purchased from Juhel Pharmaceuticals. Each solution of the drugs was prepared fresh on each day of the experiment and the remainder discarded after the day's work. The extracts, pentylenetetrazole (PTZ), strychnine (STC), diazepam (DZ) and the vehicle (water for injection B.P.) were administered intra-peritoneally (IP).

Pharmacological Studies

Acute toxicity

The method used was a modification of that described by Lorke (1983). In phase 1 eight (8) rats (110-150g) were grouped into four (4) with two (2) rats per group. The rats were administered the aqueous extract 10, 100, 1000 and 2000 mg/kg body weight (IP) respectively. Similarly, the same design was also carried out with ethanol bark extract administered instead of aqueous bark extract. The rats were observed for signs of toxicity and death within 24 hours at which the phase II was appropriately determined based on the results of phase I.

Pilot Studies

Two (2) rats each (110-150 g) were administered Pentylenetetrazole (PTZ) (SIGMA-ALDRICH, Co., 3050 Spruce street, St. Louis, MO 63103 USA 314-771-5765) 50 mg/kg, PTZ 100 mg/kg, PTZ 200 mg/kg, Strychnine (STC) (SIGMA-ALDRICH, Co., 3050 Spruce street, St. Louis, MO 63103 USA 314-771-5765) 2.5 mg/kg, STC 5 mg/kg, STC 10 mg/kg and 10 ml/kg of distilled water (IP) respectively and observation was made for onset of jerk, onset of convulsion and death. The appropriate convulsive doses of the PTZ and STC were determined which were used for the study

Pentylenetetrazole Induced Seizure in Rat

Sixty (60) rats (110-150 g) were grouped into 10 groups of 6 rats each. The first group received water for injection B.P. 2 ml/kg, the second group received diazepam (by Roche) 10 mg/kg, the third, fourth, fifth and sixth groups received the aqueous extract 500, 250, 125 and 62.5 mg/kg respectively. The seventh, eighth, ninth and tenth groups received ethanolic extract 500, 250, 125 and 62.5 mg/kg respectively. Thirty (30) minutes later all the rats were administered PTZ 100 mg/kg and observed for onset of jerk, onset of convulsion and death. Absence of a convulsion of at least 5 seconds duration indicates a compounds ability to abolish the effect of pentylenetetrazole induced seizure threshold (Swinyard *et al*., 1989).

Strychnine Induced Seizure in Rat

Sixty (60) rats (110-150 g) were grouped into 10 groups of 6 rats each. The first group received water for injection B.P. 2 ml/kg, the second group received diazepam 10 mg/kg, the third, fourth, fifth and sixth groups received the aqueous extract 500, 250, 125 and 62.5 mg/kg respectively. The seventh, eighth, ninth and tenth groups received ethanolic extract 500, 250, 125 and 62.5 mg/kg respectively. Thirty (30) minutes later all the rats were administered STC 5 mg/kg and observations were made for onset of jerk, onset of convulsion and death.

Statistical Analysis

The results were analyzed with Graph Pad Instat software using pooled student T-test. A $p<0.05$ was considered significant, $p<0.01$ was considered highly significant and $p<0.001$ was considered extremely significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of the aqueous and ethanolic extracts of *Mitragyna inermis* bark showed the presence of alkaloids, anthraquinones, flavonoids, glycosides, reducing sugar, saponins, tannins and terpenoids. However, phlabotannins and steroids were not detected in aqueous extract. Alkaloids and glycosides were in moderate quantities in aqueous extract, while alkaloids, flavonoids and anthraquinones were present in high quantities in the ethanol extract (Table 1).

Acute toxicity

The acute toxicity of aqueous bark extract of *Mitragyna inermis* in rats was found to be greater than 2000 mg/kg, while the ethanolic extract was found to be 1587.5 mg/kg (Table 2).

Anti-seizure activity of *Mitragyna inermis* bark extracts on pentylenetetrazole induced seizure

The aqueous and ethanol extracts of *Mitragyna inermis* bark exhibited significant anticonvulsant activity, shown by a dose dependent increase in the onset time of clonic convulsion in albino rats. However, the aqueous extract at 250 mg/kg and 500 mg/kg showed a statistical significant difference with the negative control ($p<0.05$). The ethanolic extract at 125mg/kg, 250mg/kg and 500 mg/kg showed statistically significant increase in the onset of clonic convulsion ($p<0.05$). The extracts also exhibited a dose dependent significant reduction in various phases of epileptic seizures when compared with the negative control. Diazepam used as positive control protected all the rats from clonic convulsion. The percentage protection of the extracts against the mortality of PTZ induced epileptic seizures was also dose dependent with the ethanol extract having better protection when compared with the aqueous extract (Table 3).

Anti-seizure activity of *Mitragyna inermis* bark extracts on strychnine induced seizure

The aqueous and ethanol extracts of *Mitragyna inermis* bark exhibited significant anticonvulsant activity against strychnine, shown by a dose dependent increase in the onset time of clonic convulsion. The aqueous extract at 250 mg/kg and 500 mg/kg showed a statistical significant difference with the negative control ($p<0.05$). The ethanolic extract at 500 mg/kg had a very significant increase in the onset of clonic convulsion ($p<0.001$). The ethanol extract at 250mg/kg also exhibited a significant reduction in the onset of convulsion when compared with the negative control. Diazepam used as positive control did not protect all the rats from clonic convulsion. However, the diazepam was able to protect all the rats from death, while the extracts did not protect the rats from death due to strychnine intoxication (Table 4).

Table 1. Quantitative phytochemistry of bark extracts of *Mitragyna inermis*

Phytoconstituents	Results	
	Aqueous	Ethanol
Alkaloids	++	+++
Anthraquinones	+	+++

Flavonoids	+	+++
Glycosides	++	++
Phlabotannins	-	+
Reducing sugar	+	+
Saponins	+	++
Steroids	-	+
Tannins	+	++
Terpenoids	+	+

= absent; + = present in low quantity, ++ = present in moderate quantity, ++ + = present in high quantity

Table 2. Acute toxicity study of aqueous and ethanolic bark extracts of *Mitragyna inermis* in albino rats

Phases	***Extract***	
	Aqueous	***Ethanol***
Phase 1	10mg/kg (IP)	10mg/kg (IP)
	100mg/kg (IP)	100mg/kg (IP)
	1000mg/kg(IP)	1000mg/kg (IP)
	2000mg/kg (IP)	2000mg/kg* (IP)
Phase 2	-	1200 mg/kg (IP)
	-	1400 mg/kg (IP)
	-	1800 mg/kg* (IP)
LD_{50}	**>2000mg/kg**	**1587.5 mg/kg (IP)**

*= Death, IP = Intraperitoneal route, LD_{50} = Lethal Dose that can kills 50% of rats

Table 3. Effect of aqueous and ethanol extracts of *Mitragyna inermis* bark on pentylenetetrazole induced seizures in albino rats

Treatment	**Dose (mg/kg)**	**Onset of Clonic Convulsion in Seconds (Mean±SEM)**	**Protection (%)**
Control	Vehicle (10 ml/kg)	184.40±20.35	00
Diazepam	10	A	100
Aqueous extract	500	680.00±78.32**	66.67
	250	420.00±38.46*	33.33
	125	241.00±30.32	00
	62.5	220.00±25.62	00
Ethanol extract	500	1190.00±95.26***	83.33
	250	660.00±396.52**	66.67
	125	450.00±295.32**	33.33
	62.5	285.00±215.32	00

Vehicle = Water for injection B.P., A = absence of convulsion,***= $p<0.001$, **= $p<0.01$, *= $p<0.05$, N = 6

Table 4. Effect of aqueous and ethanol extracts of *Mitragyna inermis* bark on strychnine induced seizures in albino rats

Treatment	**Dose (mg/kg)**	**Onset of Clonic Convulsion in Seconds (Mean±SEM)**	**Quantal Protection**
Control	Vehicle (10 ml/kg)	137.00±24.18	0/6
Diazepam	10	2410.00±300.58	6/6
Aqueous extract	500	449.00±66.52**	0/6
	250	290.00±30.28*	0/6
	125	206.00±27.32	0/6
	62.5	159.00±19.35	0/6
Ethanol extract	500	590.00±65.32***	0/6
	250	417.00±41.37**	0/6

	125	219.00±25.39	0/6
	62.5	138.00±15.21	0/6

Vehicle = Water for injection B.P., A = absence of convulsion, ***= $p<0.001$, **= $p<0.01$, *= $p<0.05$, N = 6

DISCUSSION

The presence of alkaloids, anthraquinones, flavonoids, glycosides, reducing sugar, saponins, tannins and terpenoids in aqueous and ethanol bark extracts of *Mitragyna inermis* agrees with several literature reports in which similar compounds were detected (Uthman *et al*., 2013; Cheng *et al*., 2002). Uthman and his colleaques (2013) did not detected anthraquinone and terpenoid which may be attributable to the differences in the plant part used. Cheng *et al* (2002) detected some phytochemicals of pharmacological interest in the leaf extract has not been tested in the present study which include polyphenols, speciophylline and uncarine. Danjuma *et al* (2009) reported that tannins extracted from the stem bark of *Xeromphis nilotica* may be responsible for the observed behavioural effects in mice and this can easily be linked to anticonvulsant activity.

The acute toxicity of ethanol bark extract of *Mitragyna inermis* in rats obtained in this study agrees with the report of Adoum and his colleagues (2012) in which the extract was found to be very slightly toxic to the test rats. The report of Konkon *et al* (2008) indicates that aqueous leaf extract was relatively less toxic in rats after oral administration. However, the result of the present study contradicts the report of Uthman *et al* (2013) in which the aqueous extract was found to be less toxic in mice after oral administration.

The anti convulsant activity demonstrated by the bark extract of *Mitragyna inermis* in this study agrees with several literature reports on the chemotherapeutic potentials of plants as a good source of anticonvulsants, antimicrobial, antidiabetic, antimalarial and antihypertensive compounds (Rebe and Van Staden, 1997; Azas *et al*., 2002; Quedrago *et al*., 2004; Uthman *et al*., 2013). The presence of phytochemical constituents in the plant such as tannins, saponins, flavonoids and alkaloids may be responsible for the observed effects.

Mitragyna inermis was able to protect the albino rats from PTZ and Strychnine induced convulsion may be attributable to the activity of GABA and glycine receptors (Larson, 1969; Sharma, 2008). Both the extracts (aqueous and ethanol) protected the albino rats from convulsion with ethanol extract having superior activity. This can be explained on the basis of the polarity of the solvent and its ability to extract anticonvulsant principles. The superiority of the extracts on PTZ induced convulsion indicates that the extract might have higher activity for $GABA_A$ receptor than the glycine receptors. Diazepam used as a positive control was able to protect the rats from convulsion and gives 100% protection from death. This confirms that diazepam is a $GABA_A$ agonist, PTZ is a $GABA_A$ antagonist, while the extracts may be a $GABA_A$ agonist with less activity than Diazepam.

The result of this study showed that the aqueous and ethanolic bark extracts of *Mitragyna inermis* had better anticonvulsants activity on PTZ induced convulsion than STC induced convulsion. Strychnine is a competitive antagonist of the inhibitory amino acid glycine (Larson, 1969). The inability of the extracts to protect against strychnine-induced seizure suggests little or no effect on the glycine receptors. The activity of the plant may be due to the presence of some bioactive phytochemical constituents detected such as alkaloid that has GABA potentiating effect.

CONCLUSION

The results of this study suggest that the aqueous and ethanol bark extracts of *Mitragyna inermis* contains bioactive constituents which possess anticonvulsant activity that may be beneficial in the management of epilepsy and supports the traditional use of this plant in the management of epilepsy. Further work is needed to determine the constituent(s) responsible for these observed activity and the possible mechanism of action.

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