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Evaluation of the Anticonvulsant Activity of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) in Mice

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Abstract: The anticonvulsant activity of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) was investigated by studying the effects of the leaf methanol and aqueous extracts on seizures induced by pentylenetetrazole, bicuculline, picrotoxin, N-methyl-DL-aspartic acid and strychnine in mice. Both methanol and aqueous extracts of *Z. capense* significantly antagonized ($p<0.05$-0.005) seizures induced by pentylenetrazole (PTZ), picrotoxin and strychnine. Methanol extract of *Z. capense* significantly antagonized ($p<0.05$) bicuculline-induced seizures while the aqueous extract significantly delayed ($p<0.001$) the onset of the seizures. Both methanol and aqueous extracts of the plant species significantly delayed ($p<0.05$-0.005) the onset of N-methyl-DL-aspartic acid (NMDLA)-induced seizures. Phenobarbitone and diazepam significantly antagonized ($p<0.001$) seizures induced by PTZ, bicuculline and picrotoxin but did not alter NMDLA-induced seizures. Phenobarbitone significantly attenuated ($p<0.01$) strychnine-induced seizures. Phenytoin or dimethylsulfoxide did not alter the seizures induced either by PTZ, bicuculline, picrotoxin, NMDLA or strychnine to any extent. The LD_{50} value obtained following oral administration of both the leaf aqueous and methanol extracts of *Z. capense* was above 3200 mg kg^{-1} and that obtained after intraperitoneal administration was 283.6 mg kg^{-1}. The phytochemical analysis of the plant species revealed the presence of alkaloids, triterpene steroids, reducing sugars, saponins, tannins and quinones. The data obtained indicate that the leaf methanol and aqueous extracts of *Z. capense* have anticonvulsant activity which may probably involve both GABAergic, glutaminergic and glycinergic mechanisms. The relatively high LD_{50} value obtained following oral administration of the plant extract shows that it is non-toxic and /or safe in mice.

Key words: *Zanthoxylum capense*, leaf methanol and aqueous extracts, anticonvulsant activity, acute toxicity, phytochemical qualitative analysis, rutaceae

INTRODUCTION

South Africa has a rich biodiversity and cultural diversity. About 3000 plant species are used in the country for medicinal purposes (Van Wyk *et al*., 1997). One of such plants used to treat various ailments by traditional medicine practitioners especially among the rural dwellers in South Africa is *Zanthoxylum capense* (Thunb.) Harv. It belongs to the Rutaceae family. It is a small multi-branched tree growing up to 10 m with thick thorn on the bark and stem. The leaves contain several pairs of leaflets and the flowers are greenish-white in colour. *Zanthoxylum capense* is widely distributed in the Eastern and Northen parts of South Africa (Palmer and Pitman, 1972; Van Wyk *et al*., 1997). It is known locally as Umnungamabele in Zulu, Umlungumabele in Sotho, small knobwood in English and Kleinperdepram in Afrikaans (Van Wyk *et al*., 1997). Infusions of the leaves have been used to treat fever, stomach ache, flatulent colic, toothache, epilepsy and so on (Watt and Breyer-Brandwijk, 1962; Watt, 1967; Forbes, 1986; Pujol, 1990; Hutchings, 1996). According to the information in the Dictionary of Natural Products (1996), no detailed study has been done on the plant species. Furthermore, no information exists in any literatures to corroborate the claims of therapeutic successes of the plant species in the treatment of various ailments including epilepsy. The core aim of the study was, therefore, to evaluate the anticonvulsant activity of the leaf methanol and aqueous extracts of *Z. capense* in mice. Acute toxicity study, phytochemical analysis of various chemical compounds in the leaves of the plant species and HPLC characterization of the plant were also carried out.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Zanthoxylum capense* (Thunb.) Harv., were collected from Kirstenbosch National Botanical Garden, Cape Town, Republic of South Africa on the 17 September, 2009. A sample of the

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collected leaves was identified by a taxonomist, Mr. Frank Weitz, in the Department of Biodiversity and Conservation Biology, University of the Western Cape and a voucher specimen (Voucher No. 6865) deposited in the University's Herbarium.

Preparation of methanol and aqueous plant extracts: The fresh leaves were separated from the thorny branches, stems and flowers of the plant and weighed (782 g). They were then washed with distilled water, air dried for an hour and dried in an oven at 35°C for 3 days. The dried leaves (669 g) were milled into coarse powder (402 g) using the hammer mill. For the preparation of the leaf methanol extract, 80 g of the dried powder of *Z. capense* was extracted in a soxhlet extractor with 500 mL of methanol for 72 h. The methanol filtrate was evaporated to dryness using a Buchi RE11 rotavapor and Buchi 461 water bath. A yield of 25 g of crude methanol extract was obtained and preserved in a dessicator for further use. The leaf aqueous extract was prepared by adding 1 L of boiled distilled water to 100 g of dried coarse powder placed in a 2 l beaker. The infusion was allowed to percolate and cool for 2 h and then filtered. The resultant filtrate was frozen at -80°C and freeze-dried (LSL Secfroid SR, Model 3021, Switzerland) for 4 days to obtain a yield of 37 g of dried leaf aqueous extract which was stored in a dessicator for further use. Fresh solution of the crude methanol extract was prepared on each day of the experiment by dissolving a given quantity of the methanol extract in a small volume of dimethylsulfoxide (DMSO) and made up to the appropriate volume with physiological saline while that of the aqueous crude extract was prepared by dissolving a given quantity of the dried extract in an appropriate volume of physiological saline. Both the leaf methanol and aqueous solutions were administered intraperitoneally (i.p.) to mice in a volume of 1 mL/100 g of animal.

Animals: In this study, male albino mice bred in the Animal House of the Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, South Africa, weighing between 18 and 30 g, were used in groups of eight per dose of plant extract or drug. They had free access to food and water *ad libitum,* except during the short fasting period prior to commencement of experiments when they had access only to water. Laboratory conditions of temperature (25±1°C), humidity and light (alternate 12 h light and 12 h dark) were maintained at all times during the experiments. Each mouse was used for one experiment only.

Drugs and chemicals: Pentylenetetrazole (PTZ, Sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), N-methyl-DL-aspartic acid (NMDLA, Sigma Chemical Co.), strychnine (Sigma Chemical Co.), phenobarbitone (Gardenyl®, Rhone-poulenc Rorer, South Africa) and 5,5 diphenylhydantoin sodium salt (Phenytoin, Sigma Chemical Co.) were all dissolved in physiological saline to appropriate volumes. Bicuculline (Sigma Chemical Co.) was suspended in 0.5 mL of Tween 80 and adjusted to an appropriate volume with physiological saline. Diazepam (Valium®, Roche, South Africa) was suspended in a minimum volume of polyethylene glycol 400 (Fluka AG, Buchs) and adjusted to an appropriate volume with physiological saline. Fresh drug solutions were prepared on each day of the experiments. All drugs were injected intraperitoneally (i.p.,) in a volume of 1 mL/100 g of animal. Control animals received equal volume injections of the appropriate vehicle. The doses and pre-treatment times of the leaf methanol or aqueous extract and the standard antiepileptic drugs used were obtained from preliminary studies in our laboratory. The pre-treatment times following the administration of pentylenetetrazole, bicuculline, picrotoxin, NMDLA or strychnine were 15 min (plant extract), 10 min (phenobarbitone), 20 min (diazepam), 20 min (phenytoin) and 15 min (DMSO solution).

Phytochemical analysis: The dried powdered leaf of the plant species was analysed for various chemical compounds using standard protocols and well established methods (Harborne, 1984; Ikhiri *et al*., 1992), (Table 1).

HPLC analysis: Chromatographic system: Beckman HPLC system consisting of double pump Programmable Solvent

Table 1: Phytochemical screening of leaf extract of *Z. capense*

Compounds	Tests/Reagents
Alkaloids	Dragendorf's reagent/Meyer's reagent
Saponins	Frothing test
Cardiac glycosides	Lieberman's test/keller-killiani test
Tannins	Ferric chloride reagent
Reducing sugars	Fehling's reagent
Triterpene steroids	Sulphuric acid reagent
Flavonoids	Acid-alcohol/solid magnesium/amy-alcohol
Quinones	Hydrochloric acid/Ether:Chloroform/Sodium hydroxide

Module model 126; Diode Array detector Module model 168; Samsung computer 386 with management System Gold (Gold V601) software supplied by Beckman; Column, C18 Bondapak 5 μm and dimensions (250×4.6 mm).

Chromatographic conditions: Mobile phase: solvent A: 1% acetic acid; solvent B: methanol; Mode: gradient; flow rate, 1 min/min; injection volume, 10 μL; detector, UV at 350 nm. The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 20%; 5 min, solvent B: 40%; 15 min, solvent B: 60%; 20 min, solvent B: 80% and 27 min, solvent B: 20%. The run rate was 30 min.

Assessment of anticonvulsant activity: The method used to assess the anticonvulsant effect of the leaf methanol or aqueous extract of *Z. capense*, was as described by Vellucci and Webster (1984) and modified by Amabeoku and Chikuni (1993). The mice were housed singly in transparent perspex mice cages half an hour prior to commencement of the experiment to acclimatize to their new environment. Standard convulsant agents, PTZ (100 mg kg^{-1}), bicuculline (40 mg kg^{-1}), picrotoxin (12 mg kg^{-1}), NMDLA (400 mg kg^{-1}) and strychnine (2 mg kg^{-1}) were separately administered intraperitoneally to induce convulsions in mice. The animals were observed for 30 min for tonic convulsions. Seizures were manifested as tonic hind-limb extensions. The time of the onset of seizures and proportion of animals convulsing or not convulsing were obtained during the 30 min period of observation.

Experiments were repeated with other groups of animals pre-treated with either the leaf methanol or aqueous plant extract, phenobarbitone (12 mg kg^{-1}, i.p.) diazepam (0.5 mg kg^{-1}, i.p.) phenytoin (30 mg kg^{-1}, i.p.) or DMSO (0.3 mL i.p.) before the administration of any of the convulsant agents. The ability of the plant extract to prevent or prolong the latency or onset of the tonic hind limb extensions was taken as an indication of anticonvulsant activity (Amabeoku and Chikuni, 1993; Amabeoku *et al*., 1998). Animals that did not convulse during the 30 min period of observation were considered as not having convulsed.

Acute toxicity testing: The method described by Dietrich (1983) and modified by Ojewole (2006) and Hilaly *et al*. (2004) were used to determine the median lethal dose (LD_{50}) of the leaf aqueous or methanol extract. Mice were fasted for 16 h and then randomly divided into groups of eight mice per cage. Graded doses of the plant extract (100, 200, 300, 400, 600, 800, 1600 and 3200 mg kg^{-1}) were separately administered orally by means of a bulbed steel needle to mice in each test group. The control group was administered with 0.25 mL (p.o.) of normal saline by means of a bulbed steel needle. The acute toxicity experiment was repeated by administering the graded doses of the leaf aqueous or methanol extract of *Zanthoxylum capense* or control vehicle intraperitoneally to other groups of animals. The mice in both the test and control groups were then allowed free access to food and water and observed for over 48 h for signs of acute toxicity including death. Log dose-response curves were then constructed for the plant extract from which the median lethal dose was calculated where applicable.

Statistical analysis: The data on the onset or latency of tonic convulsions were analysed using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison. The proportion of animals convulsing was analysed using the Chi-squared test (Bienvenu *et al*., 2002). Data obtained were expressed as mean (±SEM). p-values less than 5% ($p<0.05$) were considered statistically significant.

Ethical considerations: The experimental protocol used in this study was approved by the Ethics Committee of the University of the Western Cape, Bellville 7535, South Africa and conforms with the University's Regulations Act concerning animal experiments.

RESULTS

Phytochemical analysis: The phytochemical qualitative analysis of the dried powdered leaf of *Z. capense* revealed the presence of the following chemical constituents: alkaloids, triterpene steroids, reducing sugars, saponins, tannins and quinones. Cardiac glycosides and flavonoids were absent.

HPLC analysis: The chromatographic fingerprint of the leaf methanol extract of *Zanthoxylum capense* showed major peaks at the following retention times (minutes): 1.227; 1.441 and 1.900 and 5.213 (Fig. 1).

Anticonvulsant assessment: Effect of leaf methanol extract of *Zanthoxylum capense* on pentylenetetrazole (PTZ)-induced seizures Pentylenetetrazole (PTZ, 100 mg kg^{-1}, i.p.) induced tonic seizures in all the eight mice used. 50-200 mg kg^{-1} (i.p.) of leaf methanol extract of *Zanthoxylum capense*, in a dose-dependent manner, significantly delayed the onset of PTZ (100 mg kg^{-1}, i.p.)-induced tonic seizures and also significantly reduced the incidence of the seizures. *Z. capense* (50 mg kg^{-1}, i.p.) protected 62.5% of mice against PTZ-induced seizures while 100-200 mg kg^{-1} (i.p.) protected 87.5% of mice.

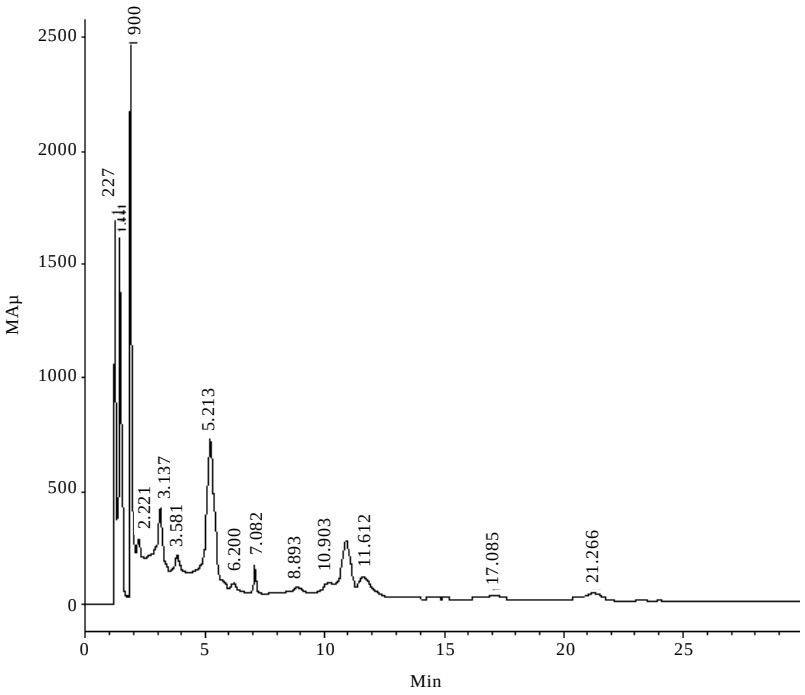

Fig. 1: Chromatographic fingerprint of *Zanthoxylum capense*

Table 2: Effect of leaf methanol extract of *Zanthoxylum capense* (ZC) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg kg^{-1})								
PTZ	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	DMSO	No. convulsed/ No. used	Protection (%)	Onset of tonic convulsions (min) Mean±SEM
100	-	-	-	-	-	8/8	0	3.25±0.25
100	50	-	-	-	-	3/8^{+}	62.5	6.33±0.20*
100	100	-	-	-	-	1/8^{++}	87.5	10.00±0*
100	200	-	-	-	-	1/8^{++}	87.5	13.00±0
100	-	12	-	-	-	0/8^{+++}	100	0*
100	-	-	0.5	-	-	0/8^{+++}	100	0*
100	-	-	-	30	-	8/8	0	3.63±0.32
100	-	-	-	-	0.3 mL	8/8	0	3.38±0.33

*p<0.001 vs. pentylenetetrazole (100 mg kg^{-1}, i.p.) control, ANOVA (n = 8).$^{+}$p<0.05, $^{++}$p<0.005, $^{+++}$p<0.001 vs. pentylenetetrazole (100 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8). DMSO: Dimethylsulfoxide

Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepam (0.5 mg kg^{-1}, i.p.) completely protected mice against PTZ (100 mg kg^{-1}, i.p.)-induced seizures. Phenytoin (30 mg kg^{-1}, i.p.) and DMSO (0.3 mL) did not alter the onset or incidence of seizures induced by PTZ (100 mg kg^{-1} i.p.) to any significant extent (Table 2).

Effect of leaf methanol extract of *Zanthoxylum capense* on bicuculline-induced seizures: Bicuculline (40 mg kg^{-1}, i.p.) induced tonic convulsions in all the animals used. Leaf methanol extract of *Z. capense* (50-200 mg kg^{-1}, i.p.) dose dependently and significantly delayed the onset of bicuculline (40 mg kg^{-1}, i.p.)-elicited seizures. 100-200 mg kg^{-1} (i.p.) of *Z. capense* significantly reduced the incidence of bicuculline-induced seizures by protecting 62.5% of mice against the seizures. *Z. capense* (50 mg kg^{-1}, i.p.) protected only 50% of the animals against bicuculline-induced seizures. Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepam (0.5 mg kg^{-1}, i.p.) completely antagonized bicuculline (40 mg kg^{-1}, i.p.)-induced tonic convulsions. Phenytoin (30 mg kg^{-1}, i.p.) and DMSO (0.3 mL) did not alter the onset or incidence of seizures induced by bicuculline (40 mg kg^{-1}, i.p.) to any significant extent (Table 3).

Effect of leaf methanol extract of *Zanthoxylum capense* on picrotoxin-induced seizures: Twelve milligram per kilogram (i.p.) of picrotoxin induced tonic convulsions in all the mice used. *Z. capense* (50-200 mg kg^{-1}, i.p.) dose dependently and significantly delayed the onset of tonic

Table 3: Effect of leaf methanol extract of *Zanthoxylum capense* (ZC) on bicuculline (BIC) – induced seizures in mice

Dose (mg kg^{-1})								Onset of tonic
BIC	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	DMSO	No. convulsed/ No. used	Protection (%)	convulsions (min) Mean±SEM
40	-	-	-	-	-	8/8	0	3.50±0.27
40	50	-	-	-	-	4/8	50	7.25±0.34*
40	100	-	-	-	-	3/8^{+}	62.5	12.67±0.20*
40	200	-	-	-	-	3/8^{+}	62.5	13.00±0.07*
40	-	12	-	-	-	0/8^{++}	100	0*
40	-	-	0.5	-	-	0/8^{++}	100	0*
40	-	-	-	30	-	8/8	0	3.63±0.38
40	-	-	-	-	0.3 mL	8/8	0	3.25±0.37

*p<0.001 vs. bicuculline (40 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.001 vs. bicuculline (40 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8). DMSO: Dimethylsulfoxide

Table 4: Effect of leaf methanol extract of *Zanthoxylum capense* (ZC) on picrotoxin (PCT) - induced seizures in mice

Dose (mg kg^{-1})								Onset of tonic
PTC	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	DMSO	No. convulsed/ No. used	Protection (%)	convulsions (min) Mean±SEM
12	-	-	-	-	-	8/8	0	6.75±0.31
12	50	-	-	-	-	4/8	50	12.75±0.60*
12	100	-	-	-	-	3/8^{+}	62.5	14.67±0.54*
12	200	-	-	-	-	2/8^{++}	75	15.00±0.50*
12	-	12	-	-	-	0/8^{+++}	100	0*
12	-	-	0.5	-	-	0/8^{+++}	100	0*
12	-	-	-	30	-	8/8	0	6.88±0.44
12	-	-	-	-	0.3 mL	8/8	0	6.13±0.58

*p<0.001 vs. picrotoxin (12 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 vs. picrotoxin (12 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8). DMSO: Dimethylsulfoxide

Table 5: Effect of leaf methanol extract of *Zanthoxylum capense* (ZC) on N-methyl-DL-aspartic acid (NMDLA) - induced seizures in mice

Dose (mg kg^{-1})								Onset of tonic
NMDLA	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	DMSO	No. convulsed/ No. used	Protection (%)	convulsions (min) Mean±SEM
400	-	-	-	-	-	8/8	0	2.25±0.25
400	50	-	-	-	-	8/8	0	2.38±0.32
400	100	-	-	-	-	8/8	0	3.38±0.38*
400	200	-	-	-	-	8/8	0	3.43±0.55*
400	-	12	-	-	-	8/8	0	2.38±0.42
400	-	-	0.5	-	-	8/8	0	2.25±0.37
400	-	-	-	30	-	8/8	0	2.63±0.26
400	-	-	-	-	0.3 mL	8/8	0	2.38±0.50

p<0.05 vs. NMDLA (400 mg kg^{-1}, i.p.) control, ANOVA (n = 8). Leaf methanol extract of *Zanthoxylum capense* and the standard anticonvulsants, in all the doses used, did not significantly affect the incidence of NMDLA (400 mg kg^{-1}, i.p.)-induced seizures, Chi-squared test (n = 8). DMSO: Dimethylsulfoxide

convulsions. 50 mg kg^{-1} (i.p.) of *Z. capense* protected 50% of the animals against picrotoxin (12 mg kg^{-1}, i.p.). However, 100 mg kg^{-1} (i.p.) and 200 mg kg^{-1} (i.p.) of *Zanthoxylum capense* significantly antagonized the incidence of the seizures induced by picrotoxin (12 mg kg^{-1}, i.p.) by protecting 62.5% and 75% of mice against the seizures, respectively. Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepanı (0.5 mg kg^{-1}, i.p.) completely protected the animals against picrotoxin (12 mg kg^{-1}, i.p.)-induced seizures while phenytoin (30 mg kg^{-1}, i.p.) and DMSO (0.3 mL) neither affected the onset nor the incidence of the seizures to any significant extent (Table 4).

Effect of leaf methanol extract of *Zanthoxylum capense* on N-methyl-DL-aspartic acid (NMDLA)-induced seizures: NMDLA (400 mg kg^{-1}, i.p.) elicited tonic seizures in all the animals used. *Zanthoxylum capense* (100-200 mg kg^{-1}, i.p.) significantly delayed the onset of NMDLA (400 mg kg^{-1}, i.p.)-elicited tonic seizures but did not alter the incidence of the seizures. *Zanthoxylum capense* (50 mg kg^{-1}, i.p.), phenobarbitone (12 mg kg^{-1}, i.p.), diazepanı (0.5 mg kg^{-1}, i.p.), phenytoin (30 mg kg^{-1}, i.p.) and DMSO (0.3 mL) neither affected the onset nor the incidence of NMDLA (400 mg kg^{-1}, i.p.)-induced seizures to any significant extent (Table 5).

Effect of leaf methanol extract of *Zanthoxylum capense* on strychnine-induced seizures: Strychnine (2 mg kg^{-1}, i.p.) produced tonic convulsions in 100% of mice used. *Z. capense* (50-200 mg kg^{-1}, i.p.), in a dose related manner significantly delayed the onset of strychnine (2 mg kg^{-1}, i.p.)-induced tonic convulsions. *Z. capense* (100 and 200 mg kg^{-1}, i.p.) significantly reduced the

incidence of strychnine (2 mg kg^{-1}, i.p.)-induced seizures by protecting 62.5% and 75% of mice against the seizures while 50 mg kg^{-1}, (i.p.) of the plant extract protected 25% of the animals against the seizures. Phenobarbitone (12 mg kg^{-1}, i.p.) significantly delayed the onset of strychnine (2 mg kg^{-1}, i.p.)-induced tonic seizures and also significantly reduced the incidence of the seizures by protecting 75% of mice against strychnine-induced seizures. Diazepam (0.5 mg kg^{-1}, i.p.) and phenytoin (30 mg kg^{-1}, i.p.) did not alter the onset or incidence of strychnine (2 mg kg^{-1}, i.p.)-induced seizures to any significant extent but protected 25% and 12.5% of the animals against the seizures, respectively. DMSO (0.3 mL) neither affected the onset nor the incidence of strychnine (2 mg kg^{-1}, i.p.)-induced seizures to any significant extent (Table 6).

Effect of leaf aqueous extract of *Zanthoxylum capense* on pentylenetetrazole (PTZ)-induced seizures: Pentylenetetrazole (100 mg kg^{-1}, i.p.) elicited tonic convulsions in 100% of the animals used. Leaf aqueous extract of *Zanthoxylum capense* at a dose of 200 mg kg^{-1} (i.p.) significantly reduced the incidence of tonic convulsions induced by PTZ (100 mg kg^{-1}, i.p.) by protecting 87.5% of mice against the seizures and also significantly delayed the onset of the tonic convulsions. The other two doses of *Z. capense* (50 and 100 mg kg^{-1}, i.p.) also significantly reduced the incidence of tonic seizures induced by PTZ (100 mg kg^{-1}, i.p.) by protecting 62.5% of the mice against PTZ (100 mg kg^{-1}, i.p.)-induced seizures. However, the doses did not affect the onset of the seizures to any significant extent. Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepam (0.5 mg kg^{-1}, i.p.), completely antagonized the seizures produced by PTZ (100 mg kg^{-1}, i.p.). Phenytoin (30 mg kg^{-1}, i.p.) did not have any significant effect on PTZ (100 mg kg^{-1}, i.p.)-induced seizures (Table 7).

Effect of leaf aqueous extract of *Zanthoxylum capense* on bicuculline-induced seizures: Bicuculline (40 mg $kg,^{-1}$ i.p.) induced tonic seizures in all the animals used. *Zanthoxylum capense* (50-200 mg kg^{-1}, i.p.) significantly prolonged the onset of bicuculline (40 mg kg^{-1}, i.p.) induced tonic convulsions in a dose dependent manner. The doses of 50-100 mg kg^{-1} (i.p.) protected 25% of mice against the seizures. However, the leaf aqueous extract of *Z. capense* (200 mg kg^{-1}, i.p.) did not affect the incidence of bicuculline (40 mg kg^{-1}, i.p.)-induced tonic convulsions to any extent. Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepam (0.5 mg kg^{-1}, i.p.) profoundly antagonized the seizures elicited by bicuculline (40 mg kg^{-1}, i.p.) by protecting all the animals against the seizures. Phenytoin (30 mg kg^{-1}, i.p.) did not have any significant effect on the onset or the incidence of bicuculline (40 mg kg^{-1}, i.p.)-induced tonic convulsions (Table 8).

Effect of leaf aqueous extract of *Zanthoxylum capense* on picrotoxin-induced seizures: Picrotoxin (12 mg kg^{-1}, i.p.) elicited tonic seizures in all the eight mice used. Leaf aqueous extract of *Zanthoxylum capense* (50-100 mg kg^{-1},

Table 6: Effect of leaf methanol extract of *Zanthoxylum capense* (ZC) on strychnine (SCN) – induced seizures in mice

Dose (mg kg^{-1})								
SCN	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	DMSO	No. convulsed/ No. used	Protection (%)	Onset of tonic convulsions (min) Mean±SEM
2	-	-	-	-	-	8/8	0	3.38±0.32
2	50	-	-	-	-	6/8	25	7.00±0.32*
2	100	-	-	-	-	$3/8^{+}$	62.5	10.67±0.41*
2	200	-	-	-	-	$2/8^{++}$	75	12.50±0.25
2	-	12	-	-	-	$2/8^{++}$	75	15.00±0.50*
2	-	-	0.5	-	-	6/8	25	4.00±0.22
2	-	-	-	30	-	7/8	12.5	3.86±0.24
100	-	-	-	-	0.3 mL	8/8	0	3.50±0.33

*p<0.001 vs. strychnine (2 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.01 vs. strychnine (2 mg kg^{-} ,^{1} i.p.) control, Chi-squared test (n = 8)
DMSO: Dimethylsulfoxide

Table 7: Effect of leaf aqueous extract of *Zanthoxylum capense* (ZC) on pentylenetetrazole (PTZ) - induced seizures in mice

Dose (mg kg^{-1})							
PTZ	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	No. convulsed/ No. used	Protection (%)	Onset of tonic convulsions (min) Mean±SEM
100	-	-	-	-	8/8	0	3.75±0.59
100	50	-	-	-	$3/8^{+}$	62.5	5.00±0.00
100	100	-	-	-	$3/8^{+}$	62.5	7.00±1.54
100	200	-	-	-	$1/8^{++}$	87.5	10.00±0*
100	-	12	-	-	$0/8^{+++}$	100	0*
100	-	-	0.5	-	$0/8^{+++}$	100	0*
100	-	-	-	30	8/8	0	3.88±0.44

*p<0.001 vs. PTZ (100 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.005, $^{+++}$p<0.001 vs. PTZ (100 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8)

Table 8: Effect of leaf aqueous extract of *Zanthoxylum capense* (ZC) on bicuculline (BIC)-induced seizures in mice

Dose (mg kg^{-1})							
BIC	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	No.convulsed/ No. used	Protection (%)	Onset of tonic convulsions (min) Mean±SEM
40	-	-	-	-	8/8	0	3.50±0.33
40	50	-	-	-	6/8	25	6.50±0.58*
40	100	-	-	-	6/8	25	11.67±1.64*
40	200	-	-	-	8/8	0	14.13±0.58*
40	-	12	-	-	0/8^{+}	100	0*
40	-	-	0.5	-	0/8^{+}	100	0*
40	-	-	-	30	8/8	0	3.75±1.47

*p<0.001 vs. bicuculline (40 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.001 vs. bicuculline (40 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8).

Table 9: Effect of leaf aqueous extract of *Zanthoxylum capense* (ZC) on picrotoxin (PCT)-induced seizures in mice

Dose (mg kg^{-1})							
PCT	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	No.convulsed/ No. used	Protection (%)	Onset of tonic convulsions (min) Mean±SEM
12	-	-	-	-	8/8	0	7.50±0.73
12	50	-	-	-	6/8	25	11.17±2.57
12	100	-	-	-	5/8	37.5	8.80±1.65
12	200	-	-	-	3/8^{+}	62.5	8.67±0.41
12	-	12	-	-	0/8^{++}	100	0*
12	-	-	0.5	-	0/8^{++}	100	0*
12	-	-	-	30	8/8	0	7.88±1.13

*p<0.001 vs. picrotoxin (12 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.001 vs. picrotoxin (12 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8)

i.p.) did not have any significant effect on the onset or incidence of picrotoxin (12 mg kg^{-1}, i.p.)-induced seizures in mice 50 and 100 mg kg^{-1}, (i.p.) protected 25 and 37.5% of the animals against the seizures, respectively. *Z. capense* (200 mg kg^{-1}, i.p.) significantly reduce the incidence of picrotoxin (12 mg kg^{-1} i.p.) induced seizures but did not affect the onset of the seizures. It protected 62.5% of the animals against picrotoxin (12 mg kg^{-1}, i.p.)-induced seizures. Phenobarbitone (12 mg kg^{-1}, i.p.) and diazepam (0.5 mg kg^{-1}, i.p.), completely antagonized picrotoxin (12 mg kg^{-1}, i.p.)-induced seizures. They protected 100% of the animals against the seizures. Phenytoin (30 mg kg^{-1}, i.p.) did not have any significant effect on picrotoxin (12 mg kg^{-1}, i.p.)-induced seizures (Table 9).

Effect of leaf aqueous extract of *Zanthoxylum capense* on N-methyl-DL-aspartic acid (NMDLA)-induced seizures: N-methyl-DL-aspartic acid (400 mg kg^{-1}, i.p.) elicited tonic seizures in all the animals used. *Zanthoxylum capense* (50-100 mg kg^{-1}, i.p.) did not significantly affect the incidence or onset of NMDLA (400 mg kg^{-1}, i.p.)-induced seizures. However, the dose of 200 mg kg^{-1}, (i.p.) of the leaf aqueous extract of *Z. capense* protected 50% of the mice against NMDLA (400 mg kg^{-1}, i.p.)-induced seizures and significantly delayed the onset of the seizures. Phenobarbitone (12 mg kg^{-1}, i.p.), diazepam (0.5 mg kg^{-1}, i.p.) and phenytoin (30 mg kg^{-1}, i.p.), did not significantly affect the incidence or the onset of NMDLA (400 mg kg^{-1}, i.p.)-induced seizures (Table 10).

Effect of leaf aqueous extract of *Zanthoxylum capense* on strychnine-inducedseizures: Strychnine (2 mg kg^{-1}, i.p.) elicited tonic convulsions in 100% of the mice used. Leaf aqueous extract of *Zanthoxylum capense* (50 mg kg^{-1}, i.p.) did not significantly affect the onset or incidence of the tonic convulsions induced by strychnine (2 mg kg^{-1}, i.p.) but protected 25% of the animals against the seizures. *Z. capense* (100-200 mg kg^{-1}, i.p.) significantly reduced the incidence of seizures elicited by strychnine (2 mg kg^{-1}, i.p.) by protecting 62.5% of animals against the seizures and also significantly delayed the onset of the seizures. Similarly, phenobarbitone (12 mg kg^{-1}, i.p.) significantly delayed the onset of strychnine (2 mg kg^{-1}, i.p.)-induced convulsions and significantly reduced the incidence of the seizures by protecting 62.5 % of the animals. Diazepam (0.5 mg kg^{-1}, i.p.) and phenytoin (30 mg kg^{-1} i.p.), did not affect the onset or incidence of strychnine (2 mg kg^{-1}, i.p.)-induced convulsions to any significant extent but protected 12.5% of the animals against the seizures (Table 11).

Acute toxicity test: There were no deaths or signs of toxicity observed after oral administration of 100-3200 mg kg^{-1} of the leaf aqueous or methanol extract of *Zanthoxylum capense* with the highest dose tested (3200 mg kg^{-1}) being the no-adverse-effect-level (NOAEL). That is, the LD_{50} was obviously greater than 3200 mg kg^{-1} (p.o.) in mice. However, toxic effects as well as the mortality rate increased significantly with the intraperitoneal injection of either the leaf aqueous or methanol extract of *Zanthoxylum capense* at a relatively

Table 10: Effect of leaf aqueous extract of *Zanthoxylum capense* (ZC) on N-methyl-DL-aspartic acid (NMDLA)-induced seizures in mice

Dose (mg kg^{-1})					No.convulsed/	Protection	Onset of tonic convulsions (min)
NMDLA	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	No. used	(%)	Mean±SEM
400	-	-	-	-	8/8	0	2.00±0.33
400	50	-	-	-	8/8	0	2.25±0.65
400	100	-	-	-	8/8	0	4.38±1.98
400	200	-	-	-	4/8	50	3.25±0.18*
400	-	12	-	-	8/8	0	2.38±0.50
400	-	-	0.5	-	8/8	0	2.00±0.87
400	-	-	-	30	8/8	0	2.13±0.02

*$p<0.005$ vs. NMDLA (400 mg kg^{-1}, i.p.) control, ANOVA (n = 8). Leaf aqueous extract of *Zanthoxylum capense* and the standard anticonvulsants, in all the doses used, did not significantly affect the incidence of NMDLA (400 mg kg^{-1}, i.p.)–induced seizures, Chi-square test (n = 8)

Table 11: Effect of leaf aqueous extract of *Zanthoxylum capense* (ZC) on strychnine (SCN) -induced seizures in mice

Dose (mg kg^{-1})					No.convulsed/	Protection	Onset of tonic convulsions (min)
SCN	ZC	Pheno-barbitone	Diaze-pam	Pheny-toin	No. used	(%)	Mean±SEM
2	-	-	-	-	8/8	0	3.00±0.19
2	50	-	-	-	6/8	25	3.17±0.35
2	100	-	-	-	3/8^{+}	62.5	5.67±0.41*
2	200	-	-	-	3/8^{+}	62.5	9.33±0.20*
2	-	12	-	-	3/8^{+}	62.5	13.00±0.35*
2	-	-	0.5	-	7/8	12.5	3.29±0.44
2	-	-	-	30	7/8	12.5	3.57±0.49

*$p<0.001$ vs. strychnine (2 mg kg^{-1}, i.p) control, ANOVA (n = 8). $^{+}p<0.05$ vs. strychnine (2 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8)

low dose (300 mg kg^{-1}) where five out of the eight animals used died, six animals died at 400 mg kg^{-1} and all the animals died at 600-3200 mg kg^{-1}.

The NOAEL for the intraperitoneal injection was 200 mg kg^{-1} while, the Lowest-Observed-Adverse-Effect Level (LOAEL) was 300 mg kg^{-1}. Some toxic effects like hypoactivity and salivation were evident five minutes after intraperitoneal injection of 300 mg kg^{-1} of the extract, while other effects like piloerection, tremor and diarrhoea manifested themselves gradually, becoming more noticeable at higher doses and persisting till death. The LD_{50} value of the leaf aqueous or methanol extract of *Zanthoxylum capense* after intraperitoneal injection was found to be 283.6 mg kg^{-1}.

DISCUSSION

The results of the present study show that the leaf methanol and aqueous extracts of *Zanthoxylum capense* L. attenuated the seizures produced by PTZ, bicuculline, picrotoxin, NMDLA and strychnine. Furthermore, phenobarbitone, diazepam and not phenytoin attenuated the seizures produced by PTZ, bicuculline and picrotoxin but the three anticonvulsants did not affect NMDLA seizures. Phenobarbitone attenuated strychnine seizures.

The aetiology of epilepsy may probably be due to an imbalance in the brain between inhibitory neurotransmission mediated by the major inhibitory neurotransmitter, gamma aminobutyric acid (GABA) and excitatory neurotransmission mediated mainly by glutamate (Czuczwar and Patsalos, 2001; Waller *et al*., 2005; Rang *et al*., 2007). The enhancement and inhibition of GABA neurotransmission at $GABA_A$ receptors attenuates and enhances convulsion, respectively (Czuczwar and Patsalos, 2001; Waller *et al*., 2005; Rang *et al*., 2007). Pentylenetetrazole is thought to produce seizures by inhibiting GABA neurotransmission at $GABA_A$ receptors in the brain (De Sarro *et al*., 1999; Rang *et al*., 2007). The standard antiepileptic drugs, phenobarbitone and diazepam, have been shown to produce their antiepileptic effect by enhancing GABA neurotransmission in the brain (Rang *et al*., 2007). The attenuation of PTZ-induced seizures by phenobarbitone and diazepam in this study, may probably be due to their enhancement of GABA neurotransmission. Phenytoin, on the other hand, did not affect PTZ-induced seizures because it is thought to exert its antiepileptic activity by blocking the entry of sodium ions into brain cells and hence, inhibiting generation of repetitive action potential (Rang *et al*., 2007). The leaf methanol and aqueous extracts of *Z. capense* were shown to attenuate PTZ-induced seizures, therefore, it is possible that GABA mechanisms may be involved in its anticonvulsant activity.

Bicuculline, a selective $GABA_A$ receptor antagonist, has been shown to elicit its convulsant activity by blocking $GABA_A$ receptors and thereby, inhibiting GABA neurotransmission in the brain (Rang *et al*., 2007). In this study, bicuculline produced seizures that were attenuated by both phenobarbitone, diazepam and the leaf methanol and aqueous extracts of *Z. capense*. Phenytoin did not

affect bicuculline seizures. This further shows that GABA mechanisms may be involved in the anticonvulsant activity of *Z. capense*.

In the present study, picrotoxin produced seizures in mice that were antagonized by phenobarbitone, diazepam and the leaf methanol and aqueous extracts of *Z. capense* but not by phenytoin. Picrotoxin is thought to produce convulsion by blocking $GABA_A$ receptor-linked chloride ion channel thus, preventing influx of chloride ions into the brain cells despite activation of $GABA_A$ receptors by GABA (Rang *et al*., 2007). It is probable therefore, that the plant species may be attenuating picrotoxin-induced seizures by enhancing GABA-mediated inhibition in the brain.

According to Rang *et al*. (2007) and Chapman and Meldrum (1993), NMDLA, a specific agonist at NMDA receptors, produces effects similar to glutamic acid at NMDA receptors and exerts its convulsant effect by activating the receptors to enhance glutamic acid-mediated excitation in the brain. In this study therefore, it is not surprising that the NMDLA- produced seizures in mice were not affected by either phenobarbitone, diazepam or phenytoin. However, the leaf methanol and aqueous extracts of *Z. capense* significantly delayed the onset of NMDLA-induced seizures thus, indicating that glutaminergic mechanism may be involved in the anticonvulsant activity of the plant species.

Data obtained from this study show that strychnine produced convulsion in mice. Strychnine, a convulsant agent, produces its effect by blocking the receptors for glycine, which is the main inhibitory transmitter acting on motor neurons (Rang *et al*., 2007). In the present study, phenobarbitone and the leaf methanol and aqueous extracts of *Z. capense* but not diazepam and phenytoin, significantly antagonized strychnine-induced seizures. It is probable that the plant species may also be exerting its anticonvulsant activity by enhancing glycinergic mechanisms. Rang *et al*. (2007) have reported that the anticonvulsant activity of phenobarbitone may not be only due to its interaction with GABA. It is therefore, possible that phenobarbitone may also be enhancing glycinergic mechanisms to exert its anticonvulsant activity. Benzodiazepines have been shown not to affect strychnine-induced convulsions in experimental animals (Rang *et al*., 2007). However, phenobarbitone in moderate to high doses and even, diazepam (2.5 mg) given IV every 1 h for 4 h and phenytoin (10 mg kg^{-1}) given IV at 50 mg min^{-1} have all been used to prevent strychnine seizures as a result of strychnine poisoning in humans (Lambert *et al*., 1981; Boyd *et al*., 1983).

Traditionally, the leaves of *Z. capense* are used as infusion and given orally for the treatment of epilepsy and other ailments (Van Wyk *et al*., 1997). The high LD_{50} value of probably greaterthan 3200 mg kg^{-1} obtained for the leaf aqueous extract of the plant species when given orally to mice, shows that the extract is non-toxic and/or safe in mice. On the other hand, the LD_{50} value of 283.6 mg kg^{-1} obtained with intraperitoneal administration of the leaf aqueous extract is very low. However, it is very unlikely that traditional medicine practitioners will use the parenteral route to administer the plant material to their patients.

The HPLC analysis of the plant extract in our study showed the presence of distinct peaks at the wavelength of 350 nm which may characterize the plant. The present study also shows that the leaves of *Z. capense* contain alkaloids, triterpene steroids, reducing sugars, saponins, tannins and quinones.. It is important to note that, in other studies, plant triterpenoids evaluated for anticonvulsant activity against PTZ-induced convulsion in mice protected 10-40% of the animals (Chauhan *et al*., 1988). It is possible, therefore, that saponin, which may be of triterpenoid type, and the triterpene steroid present in *Z. capense* might contribute to the anticonvulsant activity of the plant species.

In conclusion, the results obtained in the present study suggest that *Z. capense* has anticonvulsant activity and this justifies the use of the plant species by traditional medicine practitioners in the treatment of epilepsy. It may also be possible to suggest that the anticonvulsant activity may be exerted via more than one mechanism especially since the leaf methanol and aqueous extracts of the plant species are thought to be affecting both GABAergic, glutaminergic and glycinergic neurotransmissions. The data also show that the plant species given orally is non toxic and/or safe in mice.

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