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Involvement of gamma aminobutyric acid in the anticonvulsant effect of the leaf methanol extract of *Ruta graveolens* L. (Rutaceae) in mice

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Abstract: The possible involvement of gamma aminobutyric acid (GABA), in the anticonvulsant effect of *Ruta graveolens* L. was investigated by studying the effect of the leaf methanol extract against seizures elicited by either pentylenetetrazole (PTZ), bicuculline, picrotoxin or N-Methyl-DL-Aspartic acid (NlvIDLA) in mice. Leaf methanol extract of *Ruta graveolens,* phenobarbitone, diazepam and muscimol significantly antagonized seizures induced by PTZ, bicuculline or picrotoxin. Combined treatment of sub- effective doses of *R. graveolens* and muscimol significantly antagonized seizures induced by PTZ, bicuculline or picrotoxin. Dimethylsulfoxide (DMSO) or phenytoin did not significantly affect the seizures produced by PTZ, bicuculline or picrotoxin. *Ruta graveolens,* phenobarbitone, diazepam, phenytoin or DMSO did not significantly affect seizures produced by NlvIDLA. LY233053 significantly antagonized seizures produced by NlvIDLA. Combined treatment of sub-effective doses of LY233053 and *Ruta graveolens* did not significantly alter NlvIDLA-induced seizures. The phytochemical qualitative analysis of the plant species showed the presence of tannins, cardiac glycosides, saponins, flavonoids, triterpene steroids and alkaloids. The LD50 value obtained following oral administration of the leaf methanol extract of *R. graveolens* was above 4000 mg kg^{-1}. The HPLC fingerprint of the plant species revealed certain characteristic peaks at 350 run. The data obtained in this study, indicate that the leaf methanol extract of *R. graveolens* has anticonvulsant activity. The data obtained also indicate that GABA mechanism may probably be involved in the anticonvulsant effect of the plant extract. The relatively high LD50 obtained for the plant species, given orally, indicates that it is safe in mice.

Introduction

Epilepsy is a worldwide neurological disorder which 1s also prevalent in South Africa. Besides the use of standard medicines to manage and treat the condition effectively, medicinal plants have been used especially in rural commmrities by traditional medicine practitioners to manage and treat epilepsy (Van Wyk *et al.,* 1997; Wat 1967). One of such medicinal plants is *Ruta graveolens* L.

The plant species originated from the Mediterranean region and is now well distributed worldwide (Watt and Breyer-Brandwijk, 1962). In South Africa, *R. graveolens* which belongs to the family of Rutaceae, is commonly grown as herbs in gardens and has become well established in some parts of the country such as the Calvinia District where it iswell known as a medicinal plant (Van Wyk *et al.,* 1997; Rood, 1994; Palmer and Pitman, 1972). Locally, *R. graveolens* is known as "wynruit or binnewortel" in Afrikaans and rue or herb of grace in English (Van Wyk *et al.,* 1997). *Ruta gravealens* L. is a small evergreen, aromatic, woody perennial shrub of about a meter in height. The leaves are irregularly divided into several leaflets which are variable in size and shape. Clusters of small yellow flowers are found at or near the tips of the branches. Each flower has four yellow petals with irregularly toothed margins. The fruits of the plant species are small four lobed capsules which are heavily covered with glands (!auk *et al.,* 2004; Van Wyk *et al.,* 1997). Rue has been used as a medicinal herb for centuries. Leaf infusions are taken for fever, convulsions and fits in children, and for epilepsy and hysteria (Van Wyk *et al.,* 1997; Watt, 1967). Alcoholic tinctures have been used to treat, respiratory problems and heart diseases (Ojala *et al.,* 2000; Rood, 1994; Watt and Breyer-Brandwijk, 1962). Decoctions have been used to ease child birth (Browner, 1985). Toothache and earache have been treated with briused leaves (Rood, 1994; Wat 1967; Watt and Breyer-Brandwijk, 1962). The plant is traditionally used in Europe for a wide range of aihnents, ranging from hysteria to rhewnatism (Atta and Alkofahi, 1998; Grieve, 1967). Ithas been used as a contraceptive (Browner, 1985) and also to relieve symptoms of hang over (Atta and Alkofahi, 1998). Despite the wide use of *Ruta graveolens* by traditional medicine practitioners and homeopaths in the treatment and management of epilepsy (Van Wyk *et al.,* 1997; Grieve, 1967; Watt and Breyer-Brandwijk, 1962), little or no information exists in any literature about studies on the possible mechanism of the anticonvulsant effect of the plant species. The main aim of this study was, therefore, to verify the anticonvulsant effect and also to investigate the possible mechanism involved of the leaf methanol extract of *Ruta graveolens* m mice. Phytochemical qualitative analysis, acute toxicity and HPLC studies of the plant species were also carried out.

Materials and methods

Plant material: Fresh leaves of *Ruta graveolens* L. were bought from the Nursery, Kirstenbosch Botanical Garden, South Africa, in February 2011. A sample of the collected leaves was identified by 1.1r F. Weitz, a taxonomist, in the Department of Biodiversity and Consenration Biology, University of the Western Cape and a voucher specimen (No. 6974) was deposited in the University's Herbarium.

Preparation of the methanol plant extracts: Fresh leaves were separated from branches of the plant and weighed (222.5 g). They were then washed with distilled water, air dried for an hour and dried in an oven at 40°C for 2 days. The dried leaves were milled into coarse powder (177.6 g) using the hammer mill. For the preparation of the leaf methanol extract, 25 g of the dried powder of *Ruta graveolens* was

extracted in a Soxhlet extractor with 500 mL of methanol for 5 h. The resultant methanol filtrate was evaporated to dryness using a Buchi RE 11 rotavapor and Buchi 461 water bath. A yield of 6.3 g extract of crude methanol was obtained and presenred in a refrigerator. Fresh solution of the crude leaf methanol extract was prepared on each day of the experiment by dissolving a weighed quantity of the methanol extract in a small volume of dimethylsulfoxide (DMSO) and made up to the appropriate volwne with physiological saline. The solutions were administered intraperitoneally (i.p.) to mice in a volume of 1 mL/l 00 g of animals.

Animals: 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 g 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*. All animals were fasted for 16 h. during which they had access to water prior to the commencement of the experiments. Laboratory condition of temperature (25±1°C) humidity and a 12 h. light/12 h dark cycle were maintained at all times during the experiments. Each mouse was used only once in the experiments.

Drugs and chemicals: Pentylenetetrazole (PTZ, Sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), N-methyl-DL-aspartic acid (NMDLA, Sigma Chemical Co.), phenobarbitone sodium (BDH Chemicals Ltd), 5,5 diphenylhydantoin sodium salt (Phenytoin, Sigma Chemical Co.), muscimol (Sigma Chemical Co.) and LY233053 (Sigma Chemical Co.) were all dissolved in physiological saline to appropriate volwnes. (+) Bicuculline (Sigma Chemical Co.) was suspended in a small volume of Tween 80 and adjusted to the appropriate volume with physiological saline. Diazepam (Valiwn, Roche, South Africa) was dissolved in a minimwn amount of propylene glycol and made up to the appropriate volwne with physiological saline. Dimethylsulfoxide (DMSO, Sigma Chemical Co.) solution was prepared by dissolving equal volwne, used to dilute the plant extract, in an appropriate volume of physiological saline. All drugs were injected intraperitoneally (i.p.) in a volwne of 1 mL/l 00 g of animal. Equal volume injections of the appropriate vehicles such as physiological saline and DMSO were given to the control animals. The plant extract and drug solutions were prepared fresh on the days of the experiment. The doses and pre-treatment times of the leaf methanol extract and the standard antiepileptic drugs used were obtained from preliminary studies in laboratory. The pre-treatment times following the administration of pentylenetetrazole, bicuculline, picrotoxin or NlvIDLA were 15 min (plant extract), 10 min (phenobarbitone), 20 min (diazepam), 20 min (phenytoin), 30 min (LY233053), 1 h (muscimol) and 15 min (DMSO solution).

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

HPLC analysis

Chromatographic system: Beckman HPLC consisting of double pump Programmable system Solvent Module model 126; Diode Array detector module model 160; Samstmg computer 386 with management System Gold (Gold V 601) software applied by Beckman; column. Cl 8 Bondapak 5 µrn and dimentions (250x 4.6 mm').

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

Anticonvulsant activity assessment: The method described by Vellucci and Webster (1984) and modified by Arnabeoku and Chikuni (1993), was used to assess the anticonvulsant activity of the leaf methanol extract of *Ruta graveolens*. The mice were kept singly in transparent perspex mice cages 30 min before the start of the experiment to get used to their new environment. Control animals were pretreated for 15 min with physiological saline (0.25 mL, i.p.) and then standard convulsant agents, such as, PTZ (95 mg kg-', i.p.), bicuculline (40 mg kg-', i.p.), picrotoxin (12 mg kg-', i.p.) or NMDLA (400 mg kg-^{1}, i.p.) was administered to induce convulsion in the mice. The animals ere obsenred for 30 min for tonic convulsion. 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 obsenration. Test animals, eight per group, were pre-treated with either the leaf methanol extract of the plant species (25-100 mg kg-', i.p.), phenobarbitone (l2 mg kg-', i.p.), diaz.eparn (0.5 mg kg-', i.p.), phenytoin (30 mg kg-', i.p.), muscirnol (0.6-2 mg kg-', i.p) or LY233053 (1-5 mg kg-', i.p.) prior to the adininistration of any of the convulsant agents. The animals were also obsenred for 30 min for tonic convulsion. The time of the onset of seizures and proportion of animals convulsing or not convulsing were obtained during the 30 min period of obsenration. The experiment was repeated with another group of eight mice pretreated for 15 min with DMSO (0.25 mL, i.p.) prior to the adininistration 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). All the experiments were carried out in a quiet laboratory at an ambient temperature of 25±1°C and between 8.30 arn-17.00 pm on each a day of the experiment.

Acute toxicity study: The modified methods of Lorke (1983) by Ojewole (2006) and El Hilaly *et al.* (2004) were used for the acute toxicity study of *R. graveolens*. The acute toxicity study was carried out to establish the median lethal dose (LD_{50}) of the plant extract. Mice were fasted for 16 hours and then randomLy divided into groups of eight mice per cage . Graded doses of the plant extract (l 00, 200, 400, 800,

1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg kg-') were separately administered orally by means of a bulbed steel needle to mice in each test group. The control group received 0.25 mL (p.o.) of physiological saline by means of a bulbed steel needle. The mice were then allowed free access to food and water and obsenred for 5 days for signs of acute toxicity including death. If necessary, at the end of the 5 days obsenration period, log dose-response cwves would be constructed for the plant extract from which the median lethal dose would be calculated.

Statistical Analysis: The data on the onset of tonic convulsion were analysed using one way analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test (Graph Pad Prism, version 5.0, Graph Pad software, Inc., San Diego Cap 2130, USA). The number of animals convulsing was analysed using the chi-squared test (Bienvenu *et al.,* 2002). The data obtained were expressed as Mean±SEM. P values of less than 5% ($p<0.05$) were considered statistically significant .

Ethical consideration: The experimental protocol used in this study was approved (07/04/31) by the Uinversity of the Western Cape Ethics Committee, Bellville 7535, South Africa and conforms with the University's Regulations Act concerning animal experiments.

Results

Phytochemical analysis: The qualitative phytochemical analysis of the dried powdered leaf of *Ruta graveolens* showed that the chemical constituents in the plant species include tannins, saponins, cardiac glycosides, alkaloids, flavonoids and triterpene steroids.

Anticonvulsant activity

Effect of leaf methanol extract of *Ruta graveolens* on pentylenetetrazole (ptz)-induced seizures: Pentylenetetrazole (PTZ, 95 mg kg-', i.p.) produced tonic convulsion in 100% of the animals tested. Leaf methanol extract of *Ruta graveolens* (25-50 mg kg-', i.p.) did not significantly affect the onset or incidence of the tonic convulsion elicited by PTZ (95 mg kg-', i.p.). Doses of 25 and 50 mg kg-' (i.p.) of *R. graveolens* protected 25 and 37.5% of the mice against PTZ (95 mg kg^{-1}, i.p.)-induced tonic convulsion, respectively.

The onset of the tonic convulsion produced by PTZ (95 mg kg-', i.p.) was significantly prolonged by *R. graveolens* (100 mg kg-', i.p.). The number of animals convulsing as a result of PTZ (95 mg kg^{-1}, i.p.) was also significantly reduced by *R. graveolens* (100 mg kg-', i.p.) which protected 62.5% of the animals against the seizures. Phenobarbitone (12 mg kg^{-1}, i.p.) and muscimol (2 mg kg^{-1}, i.p.) significantly delayed the onset of PTZ (95 mg kg-', i.p.)-elicited tonic convulsion and also significantly reduced the number of animals convulsing as a result of the tonic convulsion. Phenobarbitone (12 mg kg-', i.p.) and muscimol (2 mg kg-', i.p.)

protected 75 and 87.5% of mice against the tonic convulsion, respectively. Diazeparu (0.5 mg kg-', i.p.) completely protected all the animals used against PTZ (95 mg kg-', i.p.)-elicited tonic convulsion. Like leaf methanol extract of *R. graveolens* (25 mg kg-', i.p.), muscimol (0.6 mg kg-', i.p.) did not significantly affect the onset or incidence of the 10 tonic convulsion elicited by PTZ (95 mg kg-', i.p.). Muscimol (0.6 mg kg-^{1}, i.p.) protected 25 of mice against the tonic convulsion. However, combined therapy of leaf methanol extract of *R. graveolens* (25 mg kg-^{1}, i.p.) and muscimol (0.6 mg kg-', i.p.) significantly prolonged the onset of the tonic convulsion produced by PTZ (95 mg kg-', i.p.) and significantly reduced the number of animals convulsing. The combined therapy protected 87 .5% of the animals against the tonic convulsion. DMSO (0.25 mL, i.p.) or phenytoin (30 mg kg-', i.p.) did not significantly affect the onset or incidence of the tonic convulsion elicited by PTZ (95 mg kg-', i.p.) (Table 1).

Effect of leaf methanol extract of *Ruta graveolens* on bicuculline-induced seizures: Bicuculline (40 mg kg-^{1} , i.p.) induced tonic convulsion in all the eight mice used for the experiment. Leaf methanol extract of *R. graveolens* (100 mg kg-', i.p.) significantly delayed the onset of bicuculline (40 mg kg-^{1}, i.p.)-induced tonic convulsion and also significantly reduced the nwnber of animals convulsing. *R. graveolens* (100 mg kg-^{1}, i.p.) protected 75% of the animals against the convulsion produced by bicuculline (40 mg kg-', i.p.). Similarly, 50 mg kg-' (i.p.) of *R. graveolens* significantly delayed the onset of the tonic convulsion elicited by bicuculline (40 mg kg-^{1}, i.p.). However, the incidence of the tonic convulsion was not significantly affected by *R.graveolens* (50 mg kg-', i.p.) which protected 50% of m ice against the convulsion.

R. graveolens (25 mg kg-^{1}, i.p.) neither significantly altered the onset nor the incidence of bicuculline (40 mg kg-^{1}, i.p.)-elicited tonic convulsion. Phenobarbitone (12 mg kg-', i.p.), diazeparu (0.5 mg kg-', i.p.) or muscimol (2 mg kg-', i.p.) significantly delayed the onset of tonic convulsion produced by bicuculline (40 mg kg-', i.p.) and also significantly reduced the nwnber of animals convulsing. Phenobarbitone (12 mg kg-', i.p.), diazeparu (0.5 mg kg-', i.p.) or muscimol (2 mg kg-', i.p.) protected 87.5% of mice against bicuculline (40 mg kg- , i.p.)-elicited tonic convulsion.

Neither phenytoin (30 mg kg-', i.p.), DMSO (0.25 rnL, i.p.) nor muscimol (0.6 mg kg-^{1} , i.p.) significantly altered the onset or incidence of bicuculline (40 mg kg-^{1} , i.p.)-induced tonic convulsion. The combined therapy of leaf methanol extract of *R. graveolens* (25 mg kg-', i.p.) and muscimol (0.6 mg kg-', i.p.) significantly delayed the onset of the tonic convulsion produced by bicuculline (40 mg kg-', i.p.) and significantly reduced the number of animals convulsing. The combined therapy protected 62.5% of the animals against the tonic convulsion (Table 2).

Effect of leaf methanol extract of *Ruta Graveolens* (RG) on picrotoxin (PIC)-induced seizures: Picrotoxin (15 mg kg-[1], i.p.) produced tonic convulsion within 10.75 min in all the mice used. Leaf methanol extract of *Ruta graveolens* (50-100 mg kg-', i.p.) significantly prolonged the onset of the tonic convulsion produced by picrotoxin (15 mg kg-', i.p.).

Table 1: Effect of leaf methanol extract of *Ruta graveolens* (RG) on pentylenetetrazole (PTZ)-induced seizures in mice

Dose (mg kg^{-1})									
PTZ	RG	Pheno barbitone	Diazepam	Phenytoin	DMSO	Muscimol	No convulsed/ no used	Percentage protection (%)	Onset of tonic convulsion (min) Mean±SEM
95	-	-	-	-	-	-	8/8	0.0	5.25±0.70
95	25	-	-	-	-	-	6/8	25.0	5.67±0.70
95	50	-	-	-	-		5/8	37.5	16.13±4.52
95	100	-	-	-	-	-	3/8^{+}	62.5	26.13±3.00*
95	-	12	-	-	-	-	2/8^{++}	75.0	25.63±2.99*
95	-	-	0.5	-	-	-	0/8^{++++}	100.0	0*
95	-	-	-	30	-	-	8/8	0.0	4.25±0.35
95	-	-	-	-	0.25 mL	-	8/8	0.0	4.88±0.55
95	-	-	-	-	-	2	1/8^{+++}	87.5	29.10±4.80*
95	-	-	-	-	-	0.6	6/8	25.0	11.25±1.72
95	25	-	-	-	-	0.6	1/8^{+++}	87.5	27.78±3.62*

*p<0.001 compared to PTZ (95 mg kg^{-1}, i.p.) control, ANOVA (n = 8) $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.005, $^{++++}$p<0.001 compared to PTZ (95 mg kg^{-1}. i.p.) control, Chi-squared test (n = 8) DMSO: Dimethylsulfoxide

Table 2: Effect of leaf methanol extract of *Ruta graveolens* (RG) on bicuculline (BIC)-induced seizures in mice

Dose (mg kg^{-1})									
BIC	RG	Pheno barbitone	Diazepam	Phenytoin	DMSO	Muscimol	No convulsed/ no used	Percentage protection (%)	Onset of tonic convulsion (min) Mean±SEM
40	-	-	-	-	-	-	8/8	0.0	4.13±0.52
40	25	-	-	-	-	-	8/8	0.0	7.75±0.37
40	50	-	-	-	-		4/8	50.0	19.13±4.12*
40	100	-	-	-	-	-	2/8^{++}	75.0	25.75±2.79**
40	-	12	-	-	-	-	1/8^{+++}	87.5	27.75±2.25**
40	-	-	0.5	-	-	-	1/8^{+++}	87.5	25.63±1.38**
40	-	-	-	30	-	-	8/8	0.0	4.00±0.68
40	-	-	-	-	0.25 mL	-	8/8	0.0	4.25±0.59
40	-	-	-	-	-	2	1/8^{+++}	87.5	27.50±2.50*
40	-	-	-	-	-	0.6	8/8	0.0	8.63±0.65
40	25	-	-	-	-	0.6	3/8^{+}	62.5	21.75±4.03**

*p<0.005, **p<0.001, compared to bicuculline (40 mg kg^{-1}, i.p.) control, ANOVA (n = 8) $^{+}$p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.005 compared to bicuculline (40 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8) DMSO: Dimethylsulfoxide

Table 3: Effect of leaf methanol extract of *Ruta Graveolens* (RG) on picrotoxin (PIC)-induced seizures in mice

Dose (mg kg^{-1})									
BIC	RG	DMSO	Pheno-barbitone	Diaze-pam	Pheny-toin	Musci-mol	No convulsed/ no used	Percentage protection (%)	Onset of tonic convulsion (min) Mean±SEM
15	-	-	-	-	-	-	8/8	0.0	10.75±0.75
15	25	-	-	-	-	-	8/8	0.0	10.88±0.44
15	50	-	-	-	-		3/8^{+}	62.0	23.50±3.17*
15	100	-	-	-	-	-	2/8^{++}	75.0	26.88±2.29**
15	-	0.25 mL	-	-	-	-	8/8	0.0	11.13±0.88
15	-	-	12	-	-	-	0/8^{+++}	100.0	0**
15	-	-	-	0.5	-	-	0/8^{+++}	100.0	0**
15	-	-	-	-	30	-	8/8	0.0	11.00±0.65
15	-	-	-	-	-	2	1/8^{+++}	87.5	29.13±0.88**
15	-	-	-	-	-	0.6	5/8	37.5	18.35±3.98
15	25	-	-	-	-	0.6	2/8^{++}	75.0	22.60±3.98*

*p<0.005, **p<0.001 compared to picrotoxin (15 mg kg^{-1}, i.p.) control, ANOVA (n = 8). $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.005, $^{++++}$p<0.001 compared to picrotoxin (15 mg kg^{-1}, i.p.) control, Chi-squared, test (n = 8) DMSO: Dimethylsulfoxide

These doses of the plant extract also significantly reduced the number of animals convulsing. *R*. graveolens (50 mg kg-^{1}, i.p.) and (J OO mg kg-', i.p.) protected 62.5 and 100% of mice against picrotoxin-induced tonic convulsion, respectively. Dose of 25 mg kg-', i.p.) of the leaf methanol extract of *R*. *graveolens* did not significantly affect the onset or incidence of picrotoxin (15 mg kg-^{1}, i.p.)-induced tonic convulsion. Phenobarbitone (12 mg kg-^{1}, i.p.), diazeparu (0.5 mg kg-', i.p.) or muscimol (2 mg kg- ,' i.p.) significantly delayed the onset of tonic convulsion produced by picrotoxin (15 mg kg-', i.p.) and also significantly reduced the number of animals convulsing. Muscimol (0.6 mg kg-', i.p.) did significantly alter the onset or incidence of picrotoxin (15 mg kg-^{1}, i.p.)-induced tonic convulsion. but protected 37 .5% of mice against the tonic convulsion. However, the combined therapy of leaf methanol extract of *R*. *graveolens* (25 mg kg-', i.p.) and muscimol (0.6 mg kg-', i.p.) significantly delayed the onset of the tonic convulsion produced by picrotoxin (15 mg kg-', i.p.) and significantly reduced the number of animals convulsing. The combined therapy protected 75% of the animals against the tonic convulsion. DMSO (0. 25 mL, i.p.) or phenytoin (30 mg kg-', i.p.) did not significantly affect the onset or incidence of picrotoxin (15 mg kg-', i.p.)-induced tonic convulsion (Table 3).

Effect of leaf methanol extract of *Ruta graveolens* (RG) On N-methyl-DL aspartic acid (NMDLA)-induced **seizures:** NlvIDLA (400 mg kg-^{1}, i.p.) produced tonic convulsion within 2.63 min in 100% of mice used. In all the doses (25-100 mg kg-', i.p.) used, the leaf methanol extract of *R*. *graveolens* did not significantly affect the onset of NlvIDLA (400 mg kg-^{1} , i.p.)-induced tonic convulsion or the nwnber of animals convulsing. Phenobarbitone (12 mg kg-', i.p.), diazeparu (0.5 mg kg-', i.p.), phenytoin (30 mg kg-', i.p.) or DMSO (0.25 mL, i.p.) also did not significantly affect the onset of NMDLA (400 mg kg-', i.p.)-induced tonic convulsion or the number of animals convulsing. LY 233053 (5 mg kg-', i.p.) significantly prolonged the onset of the tonic convulsion produced by NMDLA (400 mg kg-', i.p.) and significantly reduced the number of animals convulsing. Dose of 5 mg kg-^{1} (i.p.) of LY 233053 protected 87.5% of mice against NMDLA (400 mg kg-', i.p.)-induced tonic convulsion. LY 233053 (I mg kg-', i.p.) did not significantly affect the onset or incidence of NMDLA (400 mg kg-', i.p.)-induced tonic convulsion.

Table 4: Effect of leaf methanol extract of *Ruta graveolens* (RG) on N-methyl-DL aspartic acid (NMDLA)-induced seizures in mice

Dose (mg kg^{-1})									
NMDLA	RG	Pheno-barbitone	Diazepam	Phenytoin	DMSO	LY23-3053	No convulsed/ no used	Percentage protection (%)	Onset of tonic convulsion (min) Mean±SEM
400	-	-	-	-	-	-	8/8	0.0	2.63±0.38
400	25	-	-	-	-	-	8/8	0.0	2.50±0.35
400	50	-	-	-	-		8/8	0.0	2.63±0.50
400	100	-	-	-	-	-	8/8	0.0	2.75±0.41
400	-	12	-	-	-	-	8/8	0.0	2.50±0.37
400	-	-	0.5	-	-	-	8/8	0.0	2.75±0.80
400	-	-	-	30	-	-	8/8	0.0	27.50±0.63
400	-	-	-	-	0.25 mL	-	8/8	0.0	2.64±0.57
400	-	-	-	-	-	5	1/8*	87.5	27.25±2.75*
400	-	-	-	-	-	1	8/8	0.0	3.50±0.73
400	25	-	-	-	-	1	8/8	0.0	3.88±0.93

*p<0.001 compared to NMDLA (400 mg kg^{-1}, i.p.) control, ANOVA (n = 8) +p<0.005 compared to NMDLA (400 mg kg^{-1}, i.p.) control, Chi-squared test (n = 8) DMSO: Dimethylsulfoxide

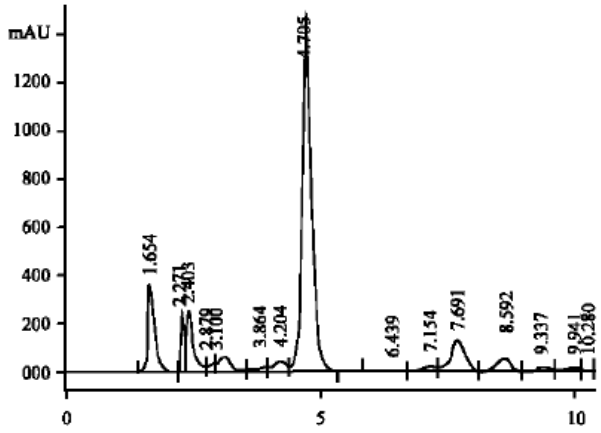

Fig. 1: HPLC chromatogram of leaf methanol extract of *Ruta. graveolens*

Furthermore, the combined treatment of the leaf methanol extract of *R. graveolens* (I00 mg kg-', i.p.) and LY233053 (I mg kg-', i.p.) also did not significantly affect the onset or incidence of the tonic convulsion produced by NMDLA (400 mg kg-', i.p.) (Table 4).

Acute toxicity: The doses of 100-4000 mg kg-' (p.o.) of the leaf methanol extract of *Ruta graveolens* caused no deaths and there were no signs of acute toxicity. Since the highest dose tested was 4000 mg kg-' (p.o.), this is taken as the no-adverse-effect-level (NOAEL). The LD50 obtained for *R. graveolens* following oral administration was probably greater than 4000 mg kg-^{1} in mice.

HPLC analysis: The chromatographic spectrum of the leaf methanol extract of *Ruta graveolens* obtained revealed major characteristic peaks at the following retention times (mni): 1.654, 2.271, 2.403, 4.705 and 7.691 min (Fig. 1). 139

Discussion

The study investigated the anticonvulsant effect of the leaf methanol extract of *R. graveolens* inmice and the possible involvement of GABA.

In this study, PTZ (95 mg kg-', i.p.), bicucullnie (40 mg kg-', i.p.), picrotoxni (15 mg kg-', i.p.) and NMDLA (400 mg kg-', i.p.) all produced tonic convulsions inthe mice used. The leaf methanol extract of *Ruta graveolens* (50-100 mg kg-', i.p.) antagonized the tonic convulsions produced by PTZ, bicuculline and picrotoxin but did not affect NlvIDLA-induced tonic convulsion. Similarly, phenobarbitone (12 mg kg-^{1}, i.p.), diazepam (0.5 mg kg-', i.p) and muscimol (2 mg kg-',i.p.) antagonized the tonic convulsions produced by PTZ, bicuculline and picrotoxin but did not affect the tonic convulsion produced by NlvIDLA. Phenytoin (30 mg kg-^{1}, i.p.) did not affect the tonic convulsions produced by either of the four convulsant agents. LY 23350 (5 mg kg-', i.p.) antagonized NMDLA-niduced tonic convulsion.

According to Meldrum (1975), Olsen (1981), Czuczwar and Patsalos (2001), Waller *et al.* (2005) and Rang *et al.* (2012), gamma amniobutyric acid (GABA), a major inhibitory neurotransmitter, with its receptors, GABAA receptors and glutamic acid, an excitatory neurotransmitter with its receptors, NlvIDA receptors in the brain may be implicated in epilepsy. They reported that the enhancement and inhibition of GABA mediated inhibition at GABAA receptors in the brain antagonizes and causes convulsion, respectively. They also reported that the enhancement and inhibition of glutamic acid neurotransmission at NlvIDA receptors in the brain enhances and antagonizes convulsion, respectively. According to Rang *et al.* (2012) and De Sano *et al.* (1999), pentylenetetrazole acts by blocking GABAA receptors thus inhibiting GABA mediated inhibition. Phenobarbitone and diazepam, standard antiepileptic drugs (Rang *et al.,* 2012; Waller *et al.,* 2005) are known to act by enhancing GABA neurotransmission in the brain through the GABAA receptor-chloride ionophore complex. In the present study, phenobarbitone and diazepam antagonized PTZ-induced tonic convulsion and this may probably be as a result of their enhancing GABA neurotransmission. Phenytoin, another standard anticonvulsant, did not antagonize the tonic convulsion produced by PTZ. According to Rang *et al.* (2012) and Waller *et al.* (2005), phenytoin produces its antiepileptic effect by blocking the entry of sodium ions into brain cells and thus, inhibiting the generation of repetitive action potential. Muscimol effectively antagonized PTZ-induced tonic convulsion in mice. Muscimol, a selective and powerful GABAA receptor agonist, acts by interacting with GABAA receptors inthe brain to mimic the effects of GABA (Rang *et al.,* 2012; Lloyd, 1985). The leaf methanol extract of *R. graveolens* was shown to antagonize the tonic convulsion produced by PTZ. It is therefore, possible to suggest that *R. graveolens* may be affecting GABA mechanism to produce its anticonvulsant activity. The combined therapy of very low doses of *R. graveolens* and muscimol which were in themselves, ineffective, significantly antagonized PTZ-induced tonic convulsion. This supports the assertion that GABA mechanism may be involved in the anticonvulsant activity of *R. graveolens*.

In the present study, bicuculline induced tonic convulsion in mice. According to Rang *et al.* (2012), Nicoll (2001) and Lloyd (1985), bicuculline, a potent GABAA receptor antagonist, produces its convulsant activity by blocking GABAA receptors and this inhibits GABA neurotransmission in the brain. Phenobarbitone and diazeparn, both known to enhance GABA neurotransmission 1n the brain, antagonized bicuculline-induced tonic convulsion. Similarly, muscimol, a specific GABAA receptor agonist, antagonized the tonic convulsion produced by bicuculline. The leaf methanol extract of *R. graveolens* also antagonized bicuculline- induced tonic convulsion. Phenytoin, known to exert its antiepileptic effects by blocking sodiwn ion entry into the brain, did not antagonize bicuculline-induced tonic convulsion. The combined therapy of very low doses of muscimol and leaf methanol extract of the plant species, which when given separately, were ineffective against bicuculline-induced convulsion, effectively antagonized the tonic convulsion. These findings further support the claim that

activation of GABA mechanisms may underpin the anticonvulsant activity of *R. graveolens.*

According to Rang *et al.* (2012), picrotoxin produces convulsion by blocking GABAA receptor-linked chloride ion channel to prevent the entry of chloride ions into the brain and thus inhibit GABA neurotransmission. In this study, picrotoxin produced tonic convulsion in mice which was antagonized by phenobarbitone and diazepam, both of which are known to enhance GABA neurotransmission in the brain. Muscimol, a specific GABAA receptor agonist, known to mimic the effect of GABA at GABAA receptors, also effectively antagonized picrotoxin-induced tonic convulsion. The leaf methanol extract of *R. graveolens* also antagonized picrotoxin- induced tonic convulsion. However, phenytoin whose anticonvulsant effect depended on its blocking of sodium ion entry into the brain, did not affect picrotoxin-induced tonic convulsion. The combined therapy of low doses of muscimol and leaf methanol extract of *R. graveolens,* known to be ineffective when given separately, antagonized the tonic convulsion produced by picrotoxin. These findings also show that the leaf methanol extract of *R. graveolens* may be enhancing GABA neurotransmission to produce its anticonvulsant activity. N-Methyl-DL-aspartic acid (NMDLA) produces its anticonvulsant effects by acting as a specific agonist at NlvIDA receptors to mimic the action of glutamate, the excitatory neurotransmitter which also acts at the same receptors in the brain (Rang *et al.,* 2012; Besancon *et al.,* 2008; Chapman and Meldium, 1993). In the present study, phenobarbitone and diazepam, both of \Vhich are known to enhance GABA neurotransmission in the brain, did not affect NlvIDLA-Induced tonic convulsion in mice. Muscimol, a specific GABAA receptor agonist known to mimic the effects of GABA at this receptor, also did not affect the tonic convulsion produced by NlvIDLA. The leaf methanol extract of *R. graveolens,* in all the doses used, did not alter Nlv1DLA-induced tonic convulsion. Phenytoin known to exert its anticonvulsant effect by blocking sodiwn ion entry into the brain also did not alter the tonic convulsion produced by NMDLA. LY233053, a competitive NlvIDA receptor antagonist, which acts by blocking the effect of glutamate at NlvIDA receptor (Borowicz *et al.,* 1996; Madden *et al.,* 1992) effectively antagonized NlvIDLA-induced tonic convulsion. The combined therapy of low doses of muscimol and LY 233053 which were in themselves ineffective against NlvIDLA-induced tonic convulsion, did not affect the convulsion produced by NlvIDLA. This shows that glutamate mechanisms may not be involved in the anticonvulsant activity of *R. graveolens.* In the present study, the phytochemical qualitative analysis of the dried leaf extract of *R. graveolens* revealed the presence of the following chemical components: saponins, tannins, cardiac glycosides, alkaloids, triterpene steroids and flavonoids. Abu Safieh *et al.* (1986), Mimaki *et al.* (1997) and Muazu and Kaita (2008) from their various studies, have shown the anticonvulsant effect of alkaloids. Fwthermore, flavonoids have also been shown to have anticonvulsant effect (Ibrahim *et al.,* 2008; Van Heerden *et al.,* 2002). According to the studies of Singh *et al.* (2012) and Chauhan *et al.* (1988), saponin and triterpene steroids also have anticonvulsant effects. Since *R. graveolens* has been shown to

contain alkaloids, flavonoids, saponins and triterpene steroids a1110ngst other chemical components, it is possible, therefore, that these secondary metabolites may be contributing to the anticonvulsant activity of the plant species.

The data obtained from the acute toxicity study carried out showed that following oral administration of the leaf methanol extract to mice, the LD_{50} may be greater than 4000 mg kg^{-1}. The leaves of *R. graveolens* are given orally as an infusion by traditional medicine practitioners (Van Wyk *et al.,* 1997). Therefore, the high LD_{50} obtained for the plant species following oral administration indicates that it 1s safe in and non-toxic to the animals. The HPLC fingerprint obtained for *R. graveolens* revealed the presence of characteristics peaks at 350 nm which may be used to identify the exact species of the plant.

Conclusion

The data obtained from this study indicate that the leaf methanol extract of *R. graveolens* has anticonvulsant activity which may be underpinned by enhancement of GABA neurotransmission. Secondary metabolites such as saponins, triterpene steroids, alkaloids and flavonoids found in the leaves of the plant species may also in part be contributing to the anticonvulsant activity. These findings justify the use of *R. graveolens* by traditional medicine practitioners in the management and treatment of epilepsy. However, fwther studies are needed to elucidate the full mechanism(s) of the anticonvulsant activity of the plant species. Also additional toxicological studies are needed to determine the safety profile of *R. graveolens*.

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