

ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF THE LEAF METHANOL EXTRACT OF *RUTA GRAVEOLENS* L. (RUTACEAE) IN MICE AND RATS

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Abstract

Background: *Ruta graveolens* has been used to treat toothache, earache, rheumatism and fever with little scientific evidence corroborating these uses.**Materials and Methods:** The leaf methanol extract of *Ruta graveolens* was evaluated for antinociceptive activity using the acetic acid writhing and hot-plate tests in mice, also anti-inflammatory and antipyretic activities using the carrageenan-induced oedema and *E. coli*-induced pyrexia tests in rats, respectively.**Results:** *R. graveolens* (100 mg/kg, i.p.), significantly reduced the number of acetic acid-induced writhes by 54 %. *R. graveolens* (400 mg/kg, i.p.), significantly delayed the reaction time in mice to thermal stimulation 15, 30, 45, and 60 min after treatment. Combined treatment of the lowest and sub-effective doses of the leaf methanol extract (25 mg/kg, i.p.), and indomethacin (10 mg/kg, i.p.) significantly reduced the number of acetic acid-induced writhes in mice. The leaf methanol extract of *R. graveolens* (50 - 400 mg/kg, i.p.), significantly reduced carrageenan-induced oedema over the 4 h period of testing. Combined treatment of the lowest doses of *R. graveolens* (25 mg/kg, i.p.) and indomethacin (2 mg/kg, i.p.) produced a significant reduction in carrageenan-induced oedema over the 4 h period of testing. *R. graveolens* (100 - 400 mg/kg, i.p.) significantly reduced *E. coli*-induced pyrexia over the 5 h period of testing. Given together, the lowest dose of *R. graveolens* (25 mg/kg, i.p.) and pentoxifylline (10 mg/kg, i.p.) produced a significant reduction in pyrexia induced by *E. coli* (50 µg/kg, i.m.) over the 5 h period of measurement. The LD₅₀ value obtained for *R. graveolens* was greater than 4000mg/kg (p.o), suggesting that the plant species may be safe in or nontoxic to mice.**Conclusion:** The data obtained indicate that *R. graveolens* has antinociceptive, anti-inflammatory and antipyretic activities, justifying the use of the plant species by traditional medicine practitioners in the management and treatment of pain, inflammation and fever.**Key words:** *Ruta graveolens*, Rutaceae, antinociceptive activity, anti-inflammatory activity, Antipyretic activity, leaf methanol extract

Introduction

Pain, inflammation and fever are essential events that occur in response to underlying conditions such as toothache and earache, autoimmune diseases and infections. These events have been well managed and treated with the numerous effective orthodox medicines available. However, an estimated 70% of the South African population in both rural and urban areas rely on the use of plants for medicinal purposes (Light et al., 2005; Tabuti et al., 2003; Amabeoku et al., 1998). One of such plants is *Ruta graveolens* L. *Ruta graveolens* belongs to the Rutaceae family. Native to Europe and commonly grown in South Africa, it is locally known as Rue or the Herb of Grace in English, and “wynruit” or “binnewortel” in Afrikaans. *R. graveolens* is a woody, evergreen shrub of up to a metre in height with a characteristic aromatic scent. The plant has yellow flowers that are made up of 4 petals each. The leaves are irregularly divided into hairless leaflets that have translucent glands (van Wyk et al., 1997). The leaves can be used fresh or dried for medicinal purposes. Leaf infusions are taken for fever, epilepsy and hysteria. Alcoholic tinctures have been used for respiratory problems and heart diseases. Bruised leaves are used for toothache and earache, while decoctions have been used to ease childbirth (van Wyk et al., 1997; Watt and Breyer-Brandwijk, 1962).

Ratheesh and Helen (2007), investigated the anti-inflammatory activity of ethanolic and methanolic extracts of *R. graveolens* Linn. against carrageenan-induced paw oedema in wistar male rats. They reported that the two extracts of the plant species have anti-inflammatory activity and suggested that mediators of inflammation such as histamine, serotonin and prostaglandins may be involved. Park et al. (2010), in their study, investigated the antinociception effect and mechanism of *R. graveolens* L. in mice and reported that the plant species has antinociception property which may be underpinned by opioidergic and α_2 -adrenergic receptors. Asgarpanah and Khoshkam (2012) in their paper reviewed various studies on the phytochemistry and pharmacological properties of *R. graveolens* L. and showed that the plant species has been used in the treatment of painful and inflammatory conditions. Despite all these reports, little information exists on studies on the mechanism of antinociception activity of *R. graveolens* as shown by Park et al. (2010), and none was found regarding possible mechanism (s) of the anti-inflammatory and antipyretic activities of the plant species especially using the drug interaction methods. Therefore, this study was intended to verify the antinociceptive, anti-inflammatory and antipyretic activities of *R. graveolens* and to investigate the possible mechanisms of the antinociceptive, anti-inflammatory and antipyretic activities using interaction studies. Phytochemical analysis, acute toxicity and HPLC studies of the plant species were also carried out.

Materials and Methods

Collection and identification of plant material

Freshly picked leaves and twigs of *R. graveolens* were bought from Herb Organic Gardens, Robertson, Western Cape, South Africa. The plant material was authenticated by a taxonomist in the Department of Biodiversity and Conservative Biology at the University of the Western Cape. A voucher specimen (UWC 8968), was deposited in the University's Herbarium.

Preparation of the methanol extract of *Ruta graveolens*

The fresh leaves and twigs of the plant species (2.5885 kg), were dried in the oven at 35°C to 40°C for 2 days. The dried leaves and twigs

<http://dx.doi.org/10.4314/ajtcam.v11i3.25>

were then weighed (1.0163 kg) and ground to a fine powder using a laboratory miller. The fine powder obtained weighed 968.2 g. A weighed quantity, 50 g, of the powder was extracted in a soxhlet extractor using 500 ml of methanol as a solvent for 24 h. The methanol filtrate was evaporated to dryness using a Buchi RE II rotavapor and Buchi 461 water bath. The resultant semi-solid substrate was then frozen at -80°C and freeze-dried (LSL Secfroid SR, Model 3021, Switzerland) for 5 days. A yield of 9.4 g of dried leaf methanol extract was obtained and stored in a desiccator until 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 and made up to the appropriate volume with physiological saline. The solution was administered intra-peritoneally to mice or rats in a volume of 1 ml/100 g of body weight of the animals.

Animals

Male and female albino mice and rats bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, Bellville, South Africa were used in this study. Mice weighing between 18 – 30 g, and rats between 180 – 210 g were used. Mice were used in groups of eight and rats in groups of six for the experiments. All animals had access to food and water *ad libitum*. Prior to commencement of the experiments, the animals were fasted for 16 h but still had access to water. The laboratory in which experiments were performed was maintained at an ambient temperature of 22 ± 2°C. Alternate 12 h light and 12 h dark cycle was also maintained. Each animal was used for one experiment only.

Drugs and Chemicals

Acetic acid (Merck), was dissolved in physiological saline to an appropriate volume. Indomethacin (Sigma Chemical Co.), was dissolved in a minimum amount of dimethylsulfoxide (DMSO, Sigma Chemical Co.) and made up to an appropriate volume with physiological saline. Carrageenan (Sigma Chemical Co.) was dissolved in physiological saline to an appropriate volume. Paracetamol (4-acetamidophenol, Sigma Chemical Co.) was dissolved in a minimum volume of propylene glycol and made up to an appropriate volume with physiological saline. *Escherichia coli* (*E. coli*, Sigma Chemical Co.) and pentoxifylline (Sigma Chemical Co.) were dissolved in physiological saline to appropriate volumes. DMSO solution was prepared by dissolving the same volume used to dilute the plant material in physiological saline to an appropriate volume. Acetic acid, indomethacin, paracetamol and DMSO were administered intra-peritoneally (i.p.) to all mice. Acetic acid was administered in a volume of 0.25 ml to mice. Indomethacin, paracetamol, pentoxifylline and DMSO were administered (i.p.) to all rats. Carrageenan was injected into the subplantar surface of the right hind paws of the rats. *E. coli*, lipopolysaccharide, was administered intramuscularly (i.m.) into the thighs of rats. Fresh drug solutions were prepared each morning of the experiment. All drugs were administered in a volume of 1 ml/100 g of body weight of the animals. Control animals received equal volume injections of the appropriate vehicle. The doses and pre-treatment times of the plant extract and standard drugs were obtained from preliminary studies conducted in our laboratory. The pre-treatment times prior to the administration of 3 % acetic acid were 15 min (plant extract), 15 min (paracetamol), and 30 min (indomethacin). The pre-treatment times prior to the administration of *E. coli* were 15 min (plant extract), 15 min (paracetamol) and 15 min (pentoxifylline). The pre-treatment time prior to the administration of acetic acid, carrageenan or *E. coli* was 15 min (DMSO).

Assessment of pharmacological activities

Antinociceptive activity of *Ruta graveolens*

Acetic Acid Writhing Test

The methods of Garcia et al. (2004); Williamson et al. (1996) and Koster et al. (1959) were used to assess the antinociceptive activity of *R. graveolens*. Mice were used in groups of eight per dose of plant extract, standard drugs, physiological saline or DMSO. The animals were kept individually in transparent perspex mouse cages before the commencement of the experiment. Control mice were pre-treated with physiological saline and after 15 min, each mouse was injected (i.p.) with 0.25 ml of 3 % acetic acid. The mice were left for 5 min, and the writhes were counted for 30 min. The experiment was repeated using other groups of animals which were pre-treated for 15 min with graded doses of plant extract and paracetamol, and for 30 min with indomethacin prior to injecting them with 0.25 ml of 3 % acetic acid. The experiment was also done using combined treatment of the lowest and sub-effective doses of the plant extract and indomethacin. All the experiments were performed in the pharmacology laboratory between 09h00 and 17h00. The ability of the plant extract to significantly reduce the number of acetic acid-induced writhes was taken as antinociceptive activity (Garcia et al., 2004; Williamson et al., 1996; Koster et al., 1959).

Hot-Plate Test

The methods of Williamson et al. (1996), and Eddy and Leimback (1953), were used. Mice were used in groups of eight per dose of plant extract, physiological saline or DMSO. The animals were kept individually in transparent perspex mouse cages before the commencement of the experiment. Control mice were pre-treated with physiological saline and after 15 min, each mouse was placed in an analgysimeter (TIIC, USA), maintained at 55°C. The pain threshold is considered to be reached when the animals lift and/or lick their hind paws or attempt to jump out of the animal enclosure. The time taken for animals to exhibit these characteristics, known as the reaction time, was noted by means of the 'STOP' button on the analgysimeter. The animals were tested before and then 15 min, 30 min, 45 min and 60 min after administration (i.p.), of physiological saline. The experiment was repeated using other groups of animals which were tested before and then 15 min, 30 min, 45 min and 60 min after the administration of graded doses of plant extract. All experiments were performed in the pharmacology laboratory between 09h00 and 17h00. The ability of the plant extract to delay the reaction time was taken as antinociceptive activity.

Anti-inflammatory Activity of *Ruta graveolens*

Rat Paw Oedema Test

The modified method of Williamson et al. (1996) was used to assess the anti-inflammatory activity of *R. graveolens*. Rats were used in groups of 6 per dose of plant extract, standard drug, physiological saline or DMSO. The animals were kept individually in transparent perspex rat cages before the commencement of the experiment. Control animals were pre-treated for 15 min with 0.25 ml (i.p.) of physiological saline. Acute

<http://dx.doi.org/10.4314/ajtcam.v11i3.25>

inflammation or oedema was induced by injecting 0.1 ml of 1 % carrageenan into the subplantar surface of the right hind paw of the rat. Inflammation or oedema following carrageenan injection was noticeable within 30 min. The volume of the right hind paw was measured before and then after the injection of carrageenan at 30 min intervals for 4 h by volume displacement method using plethysmometer (TIIC, USA). The experiment was repeated using other groups of animals which were pre-treated with graded doses of plant extract, standard drug or DMSO. The volumes of the untreated rats' right hind paws were also measured at 30 min intervals for 4 h. Acute inflammation or oedema was expressed as a mean increase in paw volume with respect to physiological saline control. All the experiments were performed in the pharmacology laboratory between 09h00 and 17h00. The ability of the plant extract to attenuate the paw oedema was taken as an indication of an anti-inflammatory activity. Inhibition was expressed as a percentage increase or decrease in oedema volume (Williamson et al., 1996).

Antipyretic Activity of *Ruta graveolens*

E. coli-induced Pyrexia Test

The method of Santos and Rao (1998), was modified for the antipyretic activity assessment of *R. graveolens*. Rats were used in groups of six per dose of plant extract, standard drug, physiological saline or DMSO. The animals were kept individually in transparent perspex rat cages before the commencement of the experiment. Control animals were pre-treated for 15 min with 0.25 ml (i.p.) of physiological saline. Pyrexia was induced with 50 µg/kg of *E. coli* administered intra-muscularly (i.m.), into the thighs of the animals. The rectal temperature was measured before and after the administration of *E. coli* at 1 h interval for a period of 5 h with a digital thermometer (TIIC, USA), inserted 2.3 cm into the rectum of the rat. The experiment was repeated using other groups of animals which were pre-treated with graded doses of plant extract, standard drugs or DMSO. The rectal temperatures of the untreated rats were also measured at 1 h interval for 5 h. The increase of at least 1°C above the normal temperature after the administration of *E. coli* was taken as pyrexia (Amabeoku et al., 2010; Santos and Rao, 1998). The rectal temperatures of normothermic rats were also measured at 1 h interval for 5 h. The effects of the different doses of plant extracts used were also evaluated alone on the rectal temperature of normothermic rats. Experiments were also carried out to study the effect of the combined treatment of the lowest doses of the plant extract and pentoxifylline against the *E.coli*-induced pyrexia. All the experiments were performed in the pharmacology laboratory between 09h00 and 17h00. The ability of the plant extract to attenuate *E.coli*-induced pyrexia was taken as an indication of antipyretic activity.

Phytochemical Analysis

The dried powdered leaf of the plant species was analysed for various chemical compounds using standard protocols of Ikhiri et al. (1992), and Harborne (1984). The chemical compounds tested for included alkaloids, cardiac glycosides, flavonoids, saponins, tannins, triterpene steroids and quinones.

HPLC Analysis

HPLC analysis of the leaf methanol extract of *R. graveolens* was carried out to characterize the plant species.

Chromatographic system: Beckman HPLC system consisting of a double pump Programmable Solvent Module model 126; Diode Array detector module model 160; Samsung computer 386 with management System Gold (V601) software applied by Beckman; column. C18 Bondapak 5 µm and dimensions (250 x 4.6 mm2).

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. The run rate was 30 min.

Acute Toxicity Testing

The methods described by Lorke (1983) and modified by Ojewole (2006) were used to determine the median (LD₅₀) of the plant extract. Mice were fasted for 16 h and then randomly divided into groups of eight per cage. Graded doses of the plant extract (100, 400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600, 4000 mg/kg) were separately administered orally to mice in each test group by means of a bulbed steel needle. Control mice were pre-treated orally with 0.25 ml of physiological saline by means of a bulbed steel needle. The mice were allowed free access to food and water and observed for 5 days for signs of acute toxicity or death. If death was recorded within the period of observation, log dose-response curves would be constructed for the plant extract from which the median lethal dose would be calculated.

Statistical Analysis

The data obtained for the antinociceptive, anti-inflammatory and antipyretic activities were analysed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (GraphPad Prism, version 5.0, GraphPad Software, Inc., San Diego CA 92130, USA) and presented as Mean ± Standard Error Mean (SEM). P values of less than 5 % (P < 0.05) were considered to be significant.

Ethical Considerations

All experimental protocol used in this study were approved (12/2/23), by the University of the Western Cape Ethics Committee, Bellville 7535, South Africa and conformed to the University's Regulation Act concerning animal experiments.

Results

Pharmacological Activities: Antinociceptive Activity of *Ruta graveolens*

Acetic Acid Writhing Test

Acetic acid (0.25 ml) produced a significant number of writhes in control mice that were pre-treated with physiological saline (0.25 ml, i.p.). The leaf methanol extract of *R. graveolens* (100 mg/kg, i.p.) significantly reduced the number of acetic acid-induced writhes by 54 %. Similarly, indomethacin (20 mg/kg, i.p.) and paracetamol (500 mg/kg, i.p.) profoundly reduced the number of writhes induced by 0.25 ml (i.p.) of 3 % acetic acid by 57 % and 80 %, respectively. The plant extract (25, 50, 200 and 400 mg/kg, i.p.), indomethacin (10 mg/kg, i.p.) and DMSO (0.25 ml, i.p.) did not significantly alter the writhes induced by 0.25 ml (i.p.) of 3 % acetic acid. *R. graveolens* (25 mg/kg, i.p.) produced 39 % inhibition of 3 % acetic acid-induced writhes whereas 50, 200 and 400 mg/kg (i.p.) of the plant extract, indomethacin (10 mg/kg, i.p.) and DMSO (0.25 ml, i.p.) produced 40 %, 31 %, 32 %, 20 % and 1 % inhibition of the acetic acid-induced writhes respectively. When combined, the lowest and sub-effective dose of the leaf methanol extract (25 mg/kg, i.p.) and the lowest and sub-effective dose of indomethacin (10 mg/kg, i.p.) significantly reduced the writhes induced by 0.25 ml of 3 % acetic acid in mice despite the individual doses having no significant effect. Together, both sub-effective doses produced 59 % inhibition of 3 % acetic acid-induced writhes in mice (Table 1).

Table 1: Effect of *R. graveolens* on acetic acid-induced writhing in mice

Treatment Groups	Dose (mg/kg)	Mean	Number of Writhes ± SEM	Percentage inhibition (%)
PS	-		26.0	1.94
<i>R. graveolens</i>	25		15.75	3.16
	50		15.5	0.5
	100		12.0*	1.94
	200		18.0	2.58
	400		17.75	3.20
Indomethacin	10		20.75	2.29
	20		11.25*	2.94
<i>R. graveolens</i> + Indomethacin	25			
	10		10.75*	2.48
Paracetamol	500		5.13**	2.45
DMSO	-		25.63	4.80
				1

* $p < 0.05$, ** $p < 0.001$ compared to 3% acetic acid control (0.25 ml, i.p.), ANOVA ($n = 8$).

Writhes are expressed as number of counts per 30 minutes.

PS: Physiological saline

DMSO: Dimethylsulfoxide

Hot-Plate Test

Animals reacting to hot-plate thermal stimulation at 55°C were observed using parameters such as licking and/or lifting of hind paws, or attempting to jump out of the animal enclosure. Mice pre-treated with physiological saline reacted to hot-plate thermal stimulation. This occurred within 8.58 ± 0.73 sec in the first 15 min after administration of physiological saline (0.25 ml, i.p.) and within 6.85 ± 1.74 sec, 60 min later. The leaf methanol extract of *R. graveolens* (50 mg/kg, i.p.) significantly delayed the reaction time of mice to hot-plate thermal stimulation 30 min after treatment. *R. graveolens* (100 mg/kg, i.p.), significantly delayed the reaction time of mice to thermal stimulation 15, 30, and 60 min after treatment. *R. graveolens* (200 mg/kg, i.p.), significantly delayed the reaction time of mice 60 min after treatment. *R. graveolens* (400 mg/kg, i.p.), significantly delayed the reaction time in mice to thermal stimulation 15, 30, 45, and 60 min after treatment. The plant extract (25 mg/kg, i.p.) and DMSO (0.25 ml, i.p.), did not significantly affect the reaction time of mice to hot-plate thermal stimulation (Table 2).

Table 2. Effect of *R. graveolens* on hot-plate induced nociception in mice

Treatment groups	Dose (mg/kg)	Reaction time (s)				
		0 min	15 min	30 min	45 min	60 min
PS	0.25 ml	8.48 $\pm$ 0.32	8.58 $\pm$ 0.73	8.48 $\pm$ 0.64	10.50 $\pm$ 1.81	6.85 $\pm$ 1.74
<i>R. graveolens</i>	25	6.85 $\pm$ 0.95	9.70 $\pm$ 0.41	10.39 $\pm$ 0.87	10.89 $\pm$ 0.33	9.19 $\pm$ 0.52
	50	8.85 $\pm$ 0.38	9.76 $\pm$ 0.54	16.18 $\pm$ 0.80**	8.77 $\pm$ 0.67	7.26 $\pm$ 0.51
	100	9.19 $\pm$ 0.72	12.88 $\pm$ 0.62*	14.22 $\pm$ 0.26**	9.94 $\pm$ 0.39	11.25 $\pm$ 0.25*
	200	6.85 $\pm$ 1.00	9.84 $\pm$ 0.78	9.90 $\pm$ 0.69	9.85 $\pm$ 0.44	11.79 $\pm$ 0.66**
	400	8.48 $\pm$ 0.11	13.93 $\pm$ 0.68**	14.50 $\pm$ 0.51**	15.58 $\pm$ 0.35*	12.05 $\pm$ 0.35**
DMSO	0.25 ml	9.77 $\pm$ 1.12	10.54 $\pm$ 1.51	10.06 $\pm$ 1.09	8.16 $\pm$ 1.60	7.66 $\pm$ 0.36

* $p < 0.05$, ** $p < 0.025$ compared to physiological saline (0.25 ml, i.p.) control, ANOVA ($n = 8$).

The reaction time in seconds is expressed as Mean $\pm$ SEM.

PS: Physiological saline

DMSO: Dimethylsulfoxid

Pharmacological Activity: Anti-inflammatory Activity of *Ruta graveolens* Rat Paw Oedema Test

Carrageenan (1%) injected into the subplantar of the right hind paws of the rats pre-treated with physiological saline induced acute inflammation or oedema in the paws within 30 min which was also the peak time of the oedema. The leaf methanol extract of *R. graveolens* (50 - 400 mg/kg, i.p.), significantly reduced the carrageenan-induced oedema over the 4 h period of testing. Indomethacin (10 mg/kg, i.p.), significantly reduced the carrageenan-induced oedema over the 4 h period of testing. The plant extract (25 mg/kg (i.p.), and indomethacin (2 mg/kg, i.p.) did not significantly affect the oedema elicited by 1 % carrageenan in the rats' right hind paws. However, when combined, the lowest and subeffective dose of *R. graveolens* (25 mg/kg, i.p.), and the lowest and subeffective dose of indomethacin (2 mg/kg, i.p.), produced a significant reduction in carrageenan-induced oedema over the 4 h period of testing. DMSO (0.25 ml, i.p.), did not significantly affect the carrageenan-induced oedema in rats (Table 3).

Table 3: Effect of *R. graveolens* (RG) on carrageenan-induced oedema in the right hind paw of rat

Treatment Group	Dose (mg/kg)	Paw volume (ml)	0 (min)	30	60	90	120	150	180	210	240
UR	-	0.65±0.02	0.52±0.02	0.58±0.05	0.62±0.03	0.62±0.03	0.68±0.05	0.72±0.06	0.72±0.10	0.75±0.03	
PS (ml)	0.25	0.72±0.10	1.53±0.27	1.28±0.18	1.52±0.16	1.35±0.10	1.27±0.11	1.28±0.15	1.32±0.80	1.30±0.65	
RG	25	0.75±0.07	1.47±0.11	1.12±0.20	1.45±0.08	1.13±0.16	1.23±0.10	1.31±0.14	1.28±0.16	1.25±0.08	
	50	0.68±0.05	0.92±0.06*	0.80±0.07	0.90±0.10*	0.73±0.05*	0.73±0.04*	0.77±0.07*	0.68±0.07*	0.63±0.06*	
	100	0.62±0.03	0.72±0.06*	0.75±0.03*	0.70±0.05*	0.70±0.06*	0.63±0.02*	0.67±0.07*	0.53±0.04*	0.57±0.03*	
	200	0.58±0.05	0.68±0.09*	0.72±0.03*	0.70±0.07*	0.63±0.04*	0.60±0.02*	0.63±0.08*	0.63±0.09*	0.57±0.05*	
	400	0.33±0.02	0.42±0.03*	0.45±0.03*	0.42±0.03*	0.35±0.02*	0.35±0.02*	0.37±0.03*	0.33±0.06*	0.33±0.03*	
IND	2	0.62±0.03	1.28±0.03	1.15±0.09	1.90±0.04	1.25±0.08	1.28±0.08	1.21±0.07	1.25±0.05	1.30±0.07	
	10	0.53±0.02	0.58±0.05*	0.68±0.05*	0.68±0.06*	0.62±0.03*	0.60±0*	0.57±0.03*	0.53±0.05*	0.52±0.04*	
RG + IND	25										
	2	0.63±0.02	0.88±0.04*	0.87±0.02*	0.70±0.05*	0.55±0.06*	0.45±0.03*	0.47±0.05*	0.47±0.07*	0.40±0.03*	
DMSO (ml)	0.25	0.62±0.10	1.52±0.12	1.30±0.07	1.53±0.09	1.35±0.08	1.28±0.09	1.27±0.11	1.27±0.13	1.35±0.03	

*P<0.05 compared to physiological saline (0.25 ml, i.p.) control, ANOVA (n=6)

UR: Untreated rats

PS: Physiological saline

IND: Indomethacin

Pharmacological Activity: Antipyretic Activity of *Ruta graveolens* *E. coli*-induced Pyrexia Test

Normothermic rats showed mean rectal temperatures ranging from 35.2 ± 0.35 °C within the 1st hour of measurement up to 36.0 ± 0.43 °C at the 5th hour of measurement. *E. coli* (50 µg/kg, i.m.), produced a time-dependent increase in the mean rectal temperature in rats pre-treated with physiological saline. *R. graveolens* (100 - 400 mg/kg, i.p.) significantly reduced *E. coli*-induced pyrexia over the 5 h period of testing. Pentoxifylline (50 mg/kg, i.p.) also significantly reduced *E. coli*-induced pyrexia over the 5 h period of testing. *R. graveolens* (25 mg/kg, i.p.) and pentoxifylline (10 mg/kg, i.p.) did not significantly alter the mean rectal temperatures of rats over the 5 h period of measurement. When combined, the lowest and sub-effective dose of *R. graveolens* (25 mg/kg, i.p.) and the lowest and sub-effective dose of pentoxifylline (10 mg/kg, i.p.) produced a significant reduction in pyrexia induced by *E. coli* (50 µg/kg, i.m.) over the 5 h period of measurement. Paracetamol (500 mg/kg, i.p.) and DMSO (0.25 ml, i.p.) did not significantly affect *E. coli*-induced pyrexia in rats over the 5 h period of measurement (Figure 1). The leaf methanol extract of *R. graveolens* (400 mg/kg, i.p.) significantly reduced the mean rectal temperature in normothermic rats over the 5 h period of measurement. *R. graveolens* (25 - 200 mg/kg, i.p.) did not affect the mean rectal temperatures of normothermic rats over the 5 h period of measurement (Figure 2).

Phytochemical analysis

Data obtained from the phytochemical qualitative analysis of *R. graveolens* indicated the presence of the following chemical components: alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and triterpene steroids. However, the test performed showed that the leaves of *R. graveolens* did not contain quinones (Table 4).

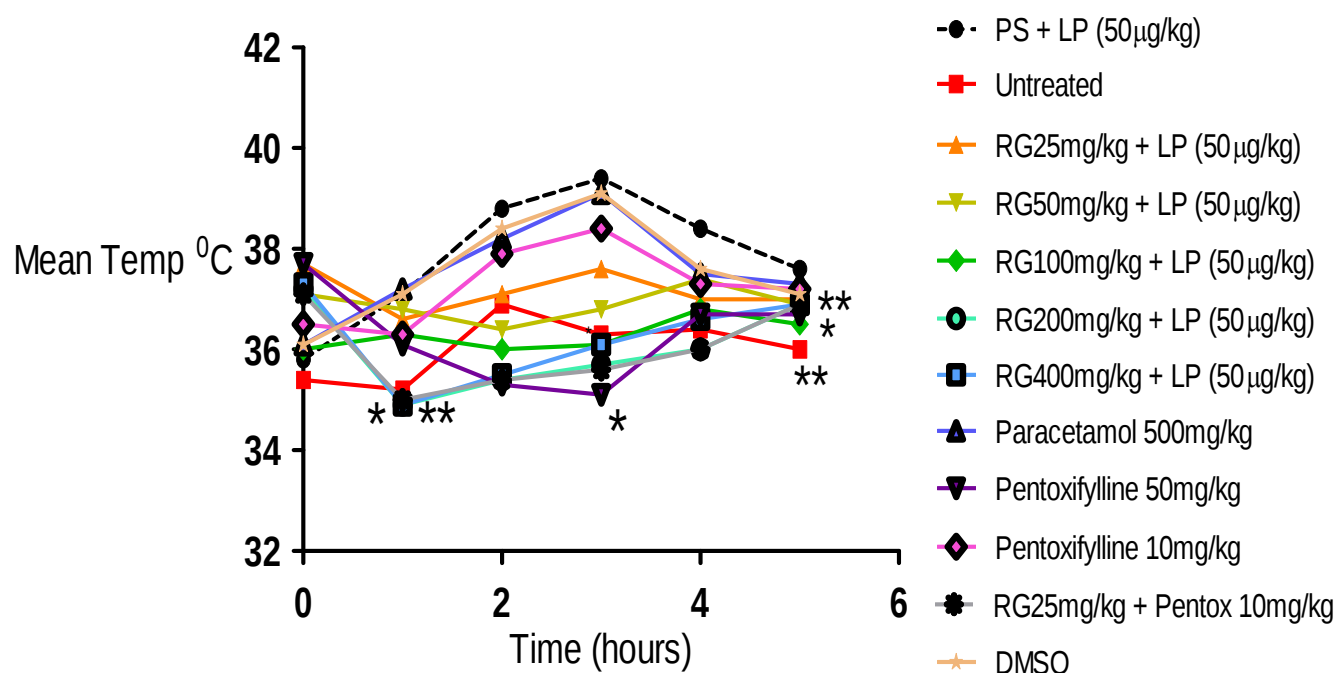


Figure 1: Effect of *R. graveolens*, paracetamol, pentoxifylline on lipopolysaccharide (*E. coli*)-induced pyrexia in rats
PS: Physiological saline; LP: Lipopolysaccharide; RG: *R. graveolens*; DMSO: Dimethylsulfoxide

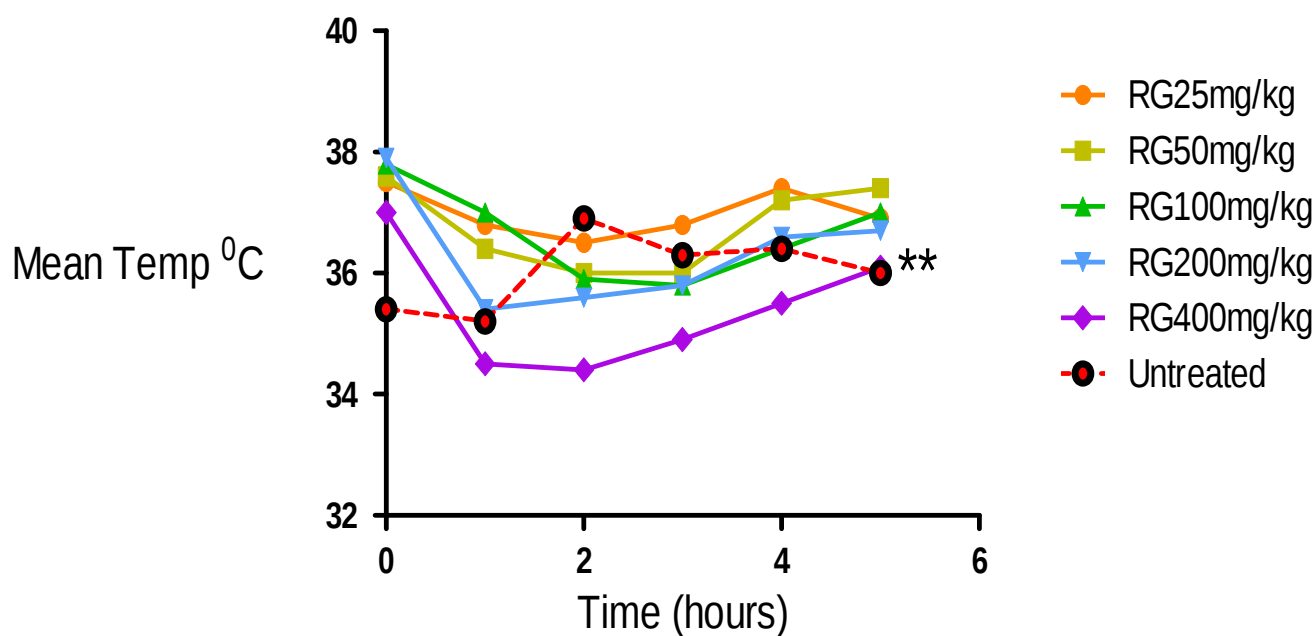


Figure. 2: Effect of *R. graveolens* on the rectal temperature of normothermic rats
RG: *R. graveolens*

Table 4: Phytochemical analysis of *R. graveolens*

Chemical Components	Results
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Quinones	-
Saponins	+
Tannins	+
Triterpene steroids	+

Key

+ (Positive) means present

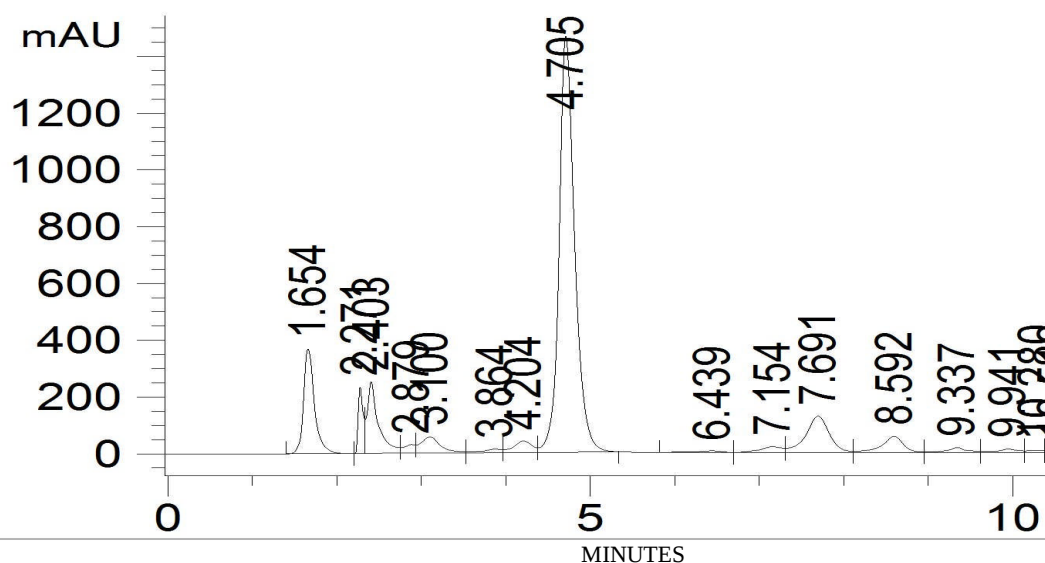
- (Negative) means absent

Acute Toxicity Test

The acute toxicity study did not show any deaths or signs of acute toxicity in mice following oral administration of 100 - 4000 mg/kg of the leaf extract of *R. graveolens*. The 4000 mg/kg (p.o.) dose of the plant extract was therefore, the no-adverse-effect-level (NOAEL). The LD₅₀ obtained for the plant species may probably be greater than 4000 mg/kg (p.o.) in mice.

HPLC Analysis

The HPLC fingerprint of the plant species obtained revealed major characteristic peaks at the following retention times (minutes): 1.654, 2.271, 2.403, 4.705 and 7.691 (Figure 3).

Figure 3: HPLC fingerprint of *R. graveolens***Discussion**

In this study, the leaf methanol extract of *R. graveolens* significantly antagonised the acetic acid-induced writhing in mice. Similarly, indomethacin and paracetamol significantly antagonised acetic acid writhing in mice. In addition, sub-effective doses of *R. graveolens* and indomethacin combined significantly antagonised acetic acid-induced writhing in mice. Satyanaraya et al. (2004) has shown that acetic acid induces writhing by stimulating the production of prostaglandins. Standard analgesic drugs indomethacin and paracetamol, have been shown to inhibit prostaglandin synthesis in the brain (Rang et al., 2012; Flower and Vane, 1972). Therefore, it is not surprising that both indomethacin and paracetamol significantly antagonised acetic acid-induced writhing. Since *R. graveolens* and also the combined therapy of sub-effective doses of *R. graveolens* and indomethacin antagonised acetic acid-induced writhes, it is possible to suggest that the plant extract may be producing antinociceptive activity through the manipulation of the prostaglandin system.

The leaf methanol extract of *R. graveolens* significantly delayed the reaction time of mice to thermal stimulation produced by the hot-plate. Morphine, a standard centrally acting analgesic drug, is known to attenuate nociception produced by the hot-plate (Amabeoku et al., 2012). It is therefore, possible that *R. graveolens* may be acting through certain central pain receptors to delay nociception produced by hot-plate in the study.

<http://dx.doi.org/10.4314/ajtcam.v11i3.25>

Williamson et al. (1996), Koster et al. (1959), and Eddy and Leimback (1953), stipulated that the acetic acid writhing and hot-plate tests are used to evaluate peripherally and centrally acting analgesic drugs respectively. Since *R. graveolens* significantly antagonised both acetic acid-induced writhing and thermal stimulation produced by hot-plate, it is tempting to suggest that the plant species has both peripheral and central antinociceptive activities.

In the present study, the leaf methanol extract of *R. graveolens* profoundly reduced carrageenan-induced rat paw oedema. Indomethacin also antagonised carrageenan-induced rat paw oedema. In addition, sub-effective doses of *R. graveolens* and indomethacin combined significantly antagonised carrageenan-induced rat paw oedema. Carrageenan induces rat paw oedema due to histamine, serotonin, bradykinin and prostaglandin like substances (Vinegar et al., 1969). Perianayagam et al. (2006), Adedapo et al. (2008) and Swingle (1974), have shown that mediators such as prostaglandins, bradykinin, histamine and serotonin are implicated at different phases of carrageenan-induced rat paw oedema. Capasso et al. (1975) and Di Rosa et al. (1971) have also reported the implication of histamine, 5-hydroxytryptamine, bradykinin, nitric acid, and prostaglandins in carrageenan-induced rat paw oedema. The carrageenan-induced rat paw oedema test is known to be sensitive to cyclo-oxygenase (COX) inhibitors, and may also be used to evaluate the effect of NSAIDs (Rao et al., 2005). COX - 1 and COX - 2 enzymes interact to form prostaglandins. In inflamed tissue, COX - 2 is unregulated. This may be responsible for the enhanced production of prostaglandins (Ratheesh et al., 2009). In a study conducted by Ratheesh et al. (2009), the reduction of rat paw oedema and the reduced activity of COX - 2 indicated anti-inflammatory effects. Amabeoku et al. (2012), reported that *C. orbiculata* reduced carrageenan-induced rat paw oedema, and suggested that the plant may have affected inflammatory mediators to produce its anti-inflammatory activity. Also, indomethacin produces anti-inflammatory effects by inhibiting COX, thus inhibiting prostaglandin synthesis (Rang et al., 2012). The data on the anti-inflammatory activity of *R. graveolens* in the present study are in agreement with those of Amabeoku et al. (2012).

In the present study, the leaf methanol extract of *R. graveolens* alone significantly reduced the rectal temperature in normothermic rats. *R. graveolens* also significantly reduced pyrexia induced by the lipopolysaccharide (LPS) or bacterial endotoxin, *E. coli*. In addition, pentoxifylline significantly reduced *E. coli*-induced pyrexia in rats. Furthermore, combined therapy of sub-effective doses of *R. graveolens* and pentoxifylline antagonised *E. coli*-induced pyrexia. According to Kluger (1991), lipopolysaccharide can induce fever in rats. This may be due to prostaglandins and cytokines such as interleukin (IL) - 1 β , IL - 6 and tumor necrosis factor alpha (TNF - α). Flower and Vane (1972), found that the antipyretic activity of paracetamol is produced by the inhibition of prostaglandin synthetase. This results in the blockade of prostaglandin synthesis in the brain. In this study, paracetamol, an analgesic and antipyretic drug, did not significantly affect *E. coli*-induced pyrexia in rats. While it is believed that prostaglandins regulate body temperature (Dascombe, 1985), studies suggest that pyrexia produced by LPS in rats, guinea-pigs and rabbits implicated the stimulation of TNF - α (Roth and Zeisberger, 1995; Kluger, 1991; LeMay et al., 1990). This may explain why paracetamol did not significantly affect *E. coli*-induced pyrexia in rats. Pentoxifylline is a TNF - α antagonist (Marcinkiewicz et al., 2000; LeMay et al., 1990). It is not surprising therefore, that pentoxifylline antagonised pyrexia induced by LPS in this study. This is in agreement with the findings of Santos and Rao (1998), which show that pentoxifylline reduced pyrexia induced by LPS. Since *R. graveolens* and also the combined therapy of sub-effective doses of *R. graveolens* and pentoxifylline antagonised *E. coli*-induced pyrexia, it is probable that TNF- α inhibition may underpin the antipyretic activity of the plant species.

The phytochemical tests carried out on the powdered leaf of *R. graveolens* indicated the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and triterpine steroids. Israili (2009) has reported that saponins and flavonoids have analgesic and antipyretic activity. Bruneton (1999) reported that saponins have analgesic and anti-inflammatory activities. Pendota et al. (2009), conducted a study on the use of *H. pauciflorus* in inflammation, pain and fever and suggested that these activities may be due to the presence of tannins, flavonoids, steroids and/or terpenes. Another study by Ojewole (2005), speculated that the presence of flavonoids and triterpenoids in *B. pinnatum* may account for the plant species' antinociceptive and anti-inflammatory activities. According to Kotb (1985) and Benazir et al. (2011), *R. graveolens* contains volatile oils, resins and flavonoids. Flavonoids isolated from other plant extracts have proven to possess analgesic activity. Since *R. graveolens* contains secondary metabolites such as flavonoids, tannins, saponins and triterpene steroids, it is possible that these metabolites may be contributing to the antinociceptive, anti-inflammatory and antipyretic activities of the plant species in this study.

The HPLC fingerprint obtained for *R. graveolens* revealed characteristic peaks at the following retention times (minutes): 1.654 min, 2.271 min, 2.403 min, 4.705 min and 7.691 min. The acute toxicity test carried out revealed that the LD₅₀ value obtained for the plant species after oral administration may be greater than 4000mg/kg.

The data obtained in this study indicate that *R. graveolens* has antinociceptive, anti-inflammatory and antipyretic activity which may be due to the plant species inhibiting mediators implicated in pain, inflammation and fever. The role of saponins, flavonoids and triterpene steroids suggest that these secondary metabolites may contribute to the antinociceptive, anti-inflammatory and antipyretic activities of *R. graveolens*. The relatively high LD₅₀ value of 4000 mg/kg (p.o.) obtained for the plant species indicates that it may be non-toxic to/or safe in mice. The results obtained substantiate the use of the plant species by traditional medicine practitioners in South Africa for the management and treatment of essential events such as pain, inflammation and fever. Further studies are needed to elucidate the full mechanisms of antinociceptive, anti-inflammatory and antipyretic activities of *R. graveolens*. In addition, further toxicological studies are needed to determine the safety profile of *R. graveolens*.

Acknowledgements

The National Research Foundation, South Africa funded the study (67983). The authors are grateful to Mr V. Javeen, senior academic support staff, Discipline of Pharmacology, for his technical assistance.

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