

# Evaluation of the Anti-inflammatory and Sedative Effects of Leaf Aqueous Extract of *Withania somnifera* (L.) Dunal (Solanaceae) in Rats and Mice

George Jimboyeka Amabeoku, Oluchi Nneka Mbamalu, Munira Ismail, Nabeel Dudhia, Nadeem Noordien, Zukiswa Mabuya and Oatthostse Kakutsi

*Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa*

**Abstract:** The use of medicinal plants in South Africa is cultural. *Withania somnifera* is one of the medicinal plants used to treat various ailments in the country. The plant species has been used by traditional medicine practitioners to treat inflammation and painful conditions like rheumatism. It is also known to be used as a sedative and hypnotic drug. Despite the claims, there is no information in literature to corroborate the therapeutic success of *Withania somnifera* in the treatment of inflammation and insomnia. The study, therefore, investigated the anti-inflammatory and central nervous system depressant activities of the leaf aqueous extract of the plant species in mice and rats. Fresh leaves of *W. somnifera* were collected from Kirstenbosch Botanical Gardens, South Africa, authenticated by a taxonomist and a voucher specimen (UWC 005) deposited in the University's Herbarium. Leaf aqueous extract was prepared using standard extraction methods. The carrageenan-induced rat paw oedema test was used to determine the anti-inflammatory effects while pentobarbitone-induced sleep and locomotor activity tests were used to evaluate the sedative effect of the plant species. Phytochemical qualitative analysis, acute toxicity and HPLC studies of the plant species were also carried out using standard methods. The phytochemical qualitative analysis carried out on the dried powdered leaves of *W. somnifera* showed the presence of saponins, tannins and triterpene steroids. Leaf aqueous extract of *W. somnifera* (100-200 mg/kg IP) significantly prolonged pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice in a dose dependant manner. Diazepam (0.5 mg/kg, i.p.) significantly prolonged pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice. The doses of 100 and 200 mg/kg (i.p.) of the plant species and 0.5 mg/kg (i.p.) of diazepam significantly reduced the locomotor activity of mice. Leaf aqueous extract of the plant species (50-200 mg/kg, i.p.) significantly reduced the oedema produced by carrageenan (1%) in rats over 90 min period of testing. Indomethacin (20 mg/kg, i.p.) significantly reduced carrageenan (1%)-induced oedema in rats over 120 min period of testing. The LD<sub>50</sub> value obtained for the leaf aqueous extract of the plant species following inter-peritoneal injection was 1,600 mg/kg while that following oral administration was probably over 4,000 mg/kg. The HPLC finger-print of the aqueous extract showed distinct peaks at the following retention times 2.977, 3.594, 4.154, 4.406, 4.660 and 15.267 min. The results obtained show that leaf aqueous extract of *W. somnifera* has both sedative and anti-inflammatory effects.

**Key words:** *Withania somnifera*, solanaceae, leaf aqueous extract, anti-inflammatory activity, Pentobarbitone-induced sleep test, locomotor activity, phytochemical analysis, acute toxicity, HPLC study, mice, rats.

## 1. Introduction

Medicinal plants are part of the cultural heritage of South Africa. There are about 3,000 species of plants used as medicines in South Africa. *Withania somnifera* (L.) Dunal is one of the medicinal plants used to treat

various ailments in the country. The plant species belongs to the family of Solanaceae. It is a short shrub with a central stem from which branches extend radially in a star pattern. The leaves are velvety, oblong and pale green and covered with short, fine, silver grey dense hairs. The flowers are small and white or yellow in colour, while the ripe fruit or berry is orange-red [1]. It is widespread and found in most of the provinces in South Africa and also in other parts of Africa, southern

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**Corresponding author:** George Jimboyeka Amabeoku, Ph.D., professor, research fields: pharmacology and ethnopharmacology. E-mail: gamabeoku@uwc.ac.za.

Europe and Asia [2, 3]. *W. somnifera* is locally known as “ubuvimbha” in Zulu, “ubuvuma” in Xhosa, “bofepha” in Sotho and “geneesblaarbossie” in Afrikaans. It is also known in English as Winter cherry [1]. Infusion of the leaves has been used to treat abscesses, inflammation, haemorrhoids, rheumatism and syphilis. The plant species has been used as a sedative and hypnotic to treat people experiencing intermittent periods of stress, a general and inherent tendency towards anxiety and nervousness and bouts of insomnia [1, 2, 4, 5]. Even though traditional medicine practitioners have made claims of effectiveness of the plant species in the treatment and/or management of inflammatory conditions and insomnia, no evidence of any scientific scrutiny has been found in any literature to verify the claims. The study therefore, intended to evaluate the anti-inflammatory and sedative effects of leaf aqueous extract of *Withania somnifera* in rats and mice. The aim of the study also included the phytochemical quantitative and HPLC analyses, and acute toxicity study of the plant species.

## 2. Materials and Methods

### 2.1 Plant Material

Fresh leaves of *Withania somnifera* were collected from Kirstenbosch Botanical Gardens, Republic of South Africa and authenticated by the curator of Kirstenbosch Botanical Gardens and a taxonomist in the Department of Biodiversity and Conservation Biology, University of the Western Cape. A voucher specimen of the plant (Amabeoku 005(UWC)) was then deposited in the University’s Herbarium.

### 2.2 Preparation of the Aqueous Extract of *Withania somnifera*

The fresh leaves were stripped from the branches and weighed (660 g). They were dried in a ventilated oven at 35°C for 5 days. The dried leaves were grounded using a hammer mill and the fine powder obtained was weighed (108 g). Weighed quantity (60g) of the powder was put in to a 1 litre beaker, and 1 litre

of boiling water was then added and allowed to cool. Once cooled, the mixture was filtered using a suction filter. The filtrate collected was frozen at -80°C and freeze dried (FREEZONE, Labconco Co., KansasCity, Missouri, USA). A yield of 15.35 g of dried extract was obtained and stored properly for further use. Fresh plant extract solution was prepared each day of the experiment by dissolving weighed quantity in an appropriate volume of physiological saline. The solutions were administered to the animals intraperitoneally (i.p.) in a volume of 1 ml/100g of body weight of animal.

### 2.3 Animals

Male and female albino mice weighing 18-30 g and male Wistar rats weighing 160-260 g, both bred in the Animal house of the Discipline of Pharmacology, UWC were used. The mice and rats were housed in perspex mouse and rat cages respectively. They had free access to food and water *ad libitum*. The animals were fasted for 16 hours (h) before the experiment, with free access to water. Laboratory conditions of temperature (25 ±1 °C), humidity and alternate 12 h light and 12 h dark cycle were maintained at all times during the experiments. Each mouse or rat was used for one experiment only.

### 2.3 Drugs and Chemicals

Carrageenan (Sigma Chemical Co.) and pentobarbitone (Kyron Lab Ltd, RSA) were dissolved in physiological saline to appropriate volumes. Indomethacin (Sigma Chemical Co.) was dissolved in minimum amounts of dimethylsulfoxide and made up 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. All the drugs were injected into the animals intraperitoneally (i.p.) in a volume of 1 ml/100g of body weight of animals. Constant volume of carrageenan (1%) was

injected into the sub-plantar surface of the right hind paws of rats. Control animals received equal volume injections of physiological saline. The doses and pre-treatment times of the leaf aqueous extract of *W. somnifera* and the standard drugs used were obtained from preliminary studies in our laboratory. The pre-treatment times, following the administration of carrageenan or pentobarbitone, were 15 min (plant extract), 30 min (indomethacin), 15 min (diazepam) and 15 min (physiological saline).

#### 2.4 Phytochemical Analysis

Phytochemical qualitative analysis was performed on the dried powdered leaf of *Withania somnifera* using the methods of Harborne [6] and Ikhiri et al. [7] to determine some of the chemical compounds present in the plant species.

#### 2.5 Pharmacological Activity Assessments, Sedative Effect

##### 2.5.1 Pentobarbitone-Induced Sleep Test

The method of Kitano et al. [8] and Williamson et al. [9] were used to assess the effect of *W. somnifera* on pentobarbitone-induced sleep. Male and female mice were used in groups of eight per dose of plant extract, standard drug or physiological saline. Pentobarbitone, a barbiturate [10] was used to induce sleep in the control mice pre-treated with physiological saline (0.25 ml, i.p.) for 15 min. The onset and duration of sleep were noted by means of a stop clock. Mice are said to be asleep when they lose their righting reflex. The time between the loss and recovery of righting reflex was noted as duration of sleep [11, 12]. Experiments were repeated with animals pre-treated for 15 min with either the plant extract or standard sedative-hypnotic drug, diazepam [10], before the administration of pentobarbitone. The interval between loss and recovery of righting reflex has been used as the index of hypnotic effect [13].

##### 2.5.2 Locomotor Activity Test

Modified method of Marazioti et al. [14] was used to

assess the effect of *W. somnifera* on the locomotor activity of both male and female mice. Spontaneous motor activity was measured using Ugo Basile Activity Cage, Model 7420 comprising of I.R. Beam Array Perspex animal cage and an electronic unit incorporating a counter and a printer. The perspex animal cage (41 cm x 41 cm x 33 cm) has horizontal and vertical sensors on its floor for measuring horizontal and vertical activities of animals respectively. The total photocell interruptions by animals at a given time are picked up by the counter and the results are shown by the printer. Eight mice were used per dose of physiological saline, plant extract or standard drug. Mice were placed individually in the cage for 20 min to acclimatize them to their new environment. The animal was then taken out, pretreated for 15 min with either physiological saline (i.p.), plant extract (i.p.) or standard drug (i.p.) and returned to the cage where the activity was immediately counted for 15 min. The ability of plant extract to significantly decrease the number of spontaneous locomotor activity in mice was taken as an indication of a sedative effect [15].

#### 2.6 Anti-inflammatory Activity

##### 2.6.1 Rat Paw Oedema Test

Modified method of Williamson et al. [9] was used to assess the anti-inflammatory activity of *W. somnifera*. Rats were used in groups of 6 per dose of plant extract, standard drug or physiological saline. 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 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 or standard drug. 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 [9].

### 2.7 Acute Toxicity

The method of Lorke [16] was modified and used for the acute toxicity study. Male and female albino mice were used in groups of four per dose of the plant extract. Animals were fasted for 16 h prior to commencement of the administration of the plant extract. They only had access to water during the fasting period. Graded doses, 100, 200, 400, 800, 1,200, 1,600, 2,000, 2,400, 2,800, 3,200, 3,600 and 4,000 mg/kg, of leaf aqueous extract of *W. somnifera* were given to mice orally by means of a bulbed steel needle and another set of animals received the same graded doses intraperitoneally. Another group of four mice was used as the control, being given 0.25 ml of distilled water orally. Animals were observed for five days for any acute toxicity symptoms such as salivation, piloerection, immobility and/or respiratory distress including deaths. Both tests and control mice had access to food and water during the 5 days observation period. 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, LD<sub>50</sub>, would be calculated.

### 2.8 HPLC Analysis

Chromatographic system: Agilent 1200 system

consisting of degassing system, quaternary pump, auto loading sampler, thermostatted column compartment, diode array detector, fluorescence detector, analyte fraction collector and Agilent ChemStation software; column: Phenomenex Luna (C18) 5µm and dimensions (250 cm x 4.6 mm). Chromatographic conditions: Mobile phase degassed with helium, solvent A: water containing 0.1% formic acid; solvent B: Acetonitrile containing 0.1% formic acid, Mode: flow rate, 0.8 ml/min; injection volume, 50 µl, detector, UV at 370 nm. The eluent was monitored at several wavelengths ranging from 210 to 370 nm, the specific wavelength of interest being 350 nm. The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 18%; 15 min, solvent B: 25%; 20 min, solvent B: 35%; 30 min, solvent B: 90%. The run rate was 30 min.

### 2.9 Statistical Analysis

The results of the anti-inflammatory and sedative effects of *W. somnifera* were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (GraphPad Prism, version 5.0, Graph Pad software, Inc., San Diego Cap 2130, USA). Results obtained were expressed as mean (± S.E.M). P-values less than 5% ( $P < 0.05$ ) were considered statistically significant.

### 2.10 Ethical Consideration

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

## 3. Results

### 3.1 Phytochemical Analysis

The phytochemical qualitative analysis of the dried powdered leaf of *W. somnifera* showed the presence of triterpene steroids, saponins and tannins.

### 3.2 Pharmacological Activity Assessments, Sedative Effect

#### 3.2.1 Effect of Leaf Aqueous Extract of *W. somnifera* on Pentobarbitone-Induced Sleep

Pentobarbitone (40 mg/kg, i.p.) elicited sleep in 100% of mice pretreated with physiological saline (0.25 ml, i.p.) within  $16.88 \pm 2.64$  min. Diazepam (0.5 mg/kg, i.p.) significantly prolonged the sleep produced by pentobarbitone (40 mg/kg, i.p.). The animals slept about three times longer than those given pentobarbitone (40 mg/kg, i.p.) alone. Leaf aqueous extract of *W. somnifera* (50-200 mg/kg, i.p.) dose dependently prolonged the sleep produced by pentobarbitone (40 mg/kg, i.p.). Like diazepam, 100-200 mg/kg (i.p.) of the leaf aqueous extract of the plant species significantly prolonged pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice. The animals slept about four to six times longer than those given pentobarbitone (40 mg/kg, i.p.) alone. *W. somnifera* (50 mg/kg, i.p.) did not significantly affect pentobarbitone (40 mg/kg, i.p.)-induced sleep in mice (Table 1).

#### 3.2.2 Effect of Leaf Aqueous Extract of *W. somnifera* on Locomotor Activity

Locomotor activity readings were obtained before and after treatment with plant extract or standard drug. Leaf aqueous extract of *W. somnifera* (50 mg/kg, i.p.) had little or no effect on locomotor activity. At doses of 100 and 200 mg/kg (i.p.), leaf aqueous extract of the plant species significantly and dose dependently decreased the locomotor activity by 57.26% and 59.94% respectively. Similarly, diazepam (0.5 mg/kg, i.p.) significantly decreased locomotor activity by 62.53% (Table 2).

### 3.3 Anti-inflammatory Activity

#### 3.3.1 Effect of *W. somnifera* on Carrageenan-Induced Oedema

Carrageenan (1%) injected into the subplantar of the right hand paws of the rats pretreated with physiological saline induced acute inflammation or oedema in the paws within 30 min and peaked at 90-120 min. Leaf aqueous extract of *W. somnifera* (25 mg/kg, i.p.) had no effect on carrageenan (1%)-induced oedema over the 4 h period of testing. At doses of 50,

**Table 1** Effect of leaf aqueous extract of *W. somnifera* on pentobarbitone-induced sleep in mice.

Dose (mg/kg) Pentobarbitone	<i>W. somnifera</i>	Diazepam	PS (ml)	Duration of sleep (min) Mean $\pm$ SEM
40	-	-	0.25	16.88 $\pm$ 2.64
40	-	0.5	-	50.00* $\pm$ 6.36
40	50	-	-	24.00 $\pm$ 4.15
40	100	-	-	63.13* $\pm$ 8.76
40	200	-	-	97.00* $\pm$ 15.61

\* $P < 0.001$  compared to pentobarbitone (40 mg/kg, i.p.) control, ANOVA (n = 8).

PS: Physiological saline.

**Table 2** Effect of leaf aqueous extract of *W. somnifera* on locomotor activity in mice.

Treatment group	Dose (mg/kg)	Number of locomotor activity		Percentage decrease in activity (%)
		Before treatment Mean $\pm$ SEM	After treatment Mean $\pm$ SEM	
PS	0.25 ml	204.10 $\pm$ 15.32	206.80 $\pm$ 21.93	
<i>W. somnifera</i>	50	215.20 $\pm$ 25.80	204.90 $\pm$ 30.20	4.79
	100	209.00 $\pm$ 11.16	89.32* $\pm$ 16.46	57.26
	200	214.93 $\pm$ 10.35	86.11* $\pm$ 19.67	59.94
Diazepam	0.5	211.08 $\pm$ 12.74	79.09* $\pm$ 13.40	62.53

\* $P < 0.001$  compared to physiological saline (0.25 ml) + control, ANOVA (n = 8).

PS: Physiological saline. Locomotor activity was counted for 15 min.

**Table 3** Effect of *W. somnifera* on carrageenan-induced oedema in the right hind paw of rat.

Treatment group	Dose (mg/kg)	Paw volume (ml)								
		0(min)	30(min)	60(min)	90(min)	120(min)	150(min)	180(min)	210(min)	240 (min)
UR	-	0.61 ± 0.08	0.58 ± 0.03	0.61 ± 0.04	0.66 ± 0.05	0.57 ± 0.02	0.59 ± 0.06	0.62 ± 0.01	0.6 ± 0.05	0.58 ± 0.03
PS (ml)	0.25	0.65 ± 0.06	0.88 ± 0.06	0.92 ± 0.06	0.97 ± 0.07	0.92 ± 0.07	0.65 ± 0.09	0.67 ± 0.05	0.65 ± 0.07	0.63 ± 0.06
Indo-methacin	5	0.61 ± 0.03	0.84 ± 0.02	1.01 ± 0.06	0.99 ± 0.03	1.08 ± 0.01	0.72 ± 0.06	0.67 ± 0.04	0.68 ± 0.04	0.61 ± 0.08
	20	0.55 ± 0.04	0.63* ± 0.02	0.62* ± 0.05	0.58* ± 0.03	0.58* ± 0.02	0.55 ± 0.04	0.57 ± 0.02	0.52 ± 0.02	0.53 ± 0.03
<i>W. somnifera</i>	25	0.63 ± 0.05	0.90 ± 0.03	0.87 ± 0.01	1.01 ± 0.08	0.94 ± 0.06	0.65 ± 0.02	0.66 ± 0.05	0.62 ± 0.04	0.65 ± 0.02
	50	0.57 ± 0.03	0.62* ± 0.04	0.62* ± 0.04	0.68* ± 0.07	0.73 ± 0.06	0.80 ± 0.04	0.68 ± 0.09	0.77 ± 0.09	0.72 ± 0.07
	100	0.53 ± 0.03	0.70* ± 0.04	0.60* ± 0.04	0.70* ± 0.04	0.72 ± 0.07	0.60 ± 0.06	0.53 ± 0.04	0.78 ± 0.07	0.68 ± 0.05
	200	0.40 ± 0.02	0.60* ± 0.04	0.58* ± 0.03	0.77* ± 0.03	0.77 ± 0.06	0.63 ± 0.07	0.58 ± 0.03	0.76 ± 0.03	0.77 ± 0.06
Indo-methacin + <i>W. somnifera</i>	5 + 25	0.60 ± 0.05	0.64* ± 0.02	0.61* ± 0.04	0.66* ± 0.01	0.61* ± 0.04	0.68 ± 0.04	0.59 ± 0.05	0.66 ± 0.03	0.64 ± 0.02

\* $P < 0.05$  compared to carrageenan (1%) control, ANOVA (n=6).

UR: Untreated rats.

PS: Physiological saline.

100 and 200 mg/kg (i.p.), the plant extract significantly reduced oedema produced by carrageenan (1%) over 90 min period of testing. Indomethacin (20 mg/kg, i.p.) significantly reduced carrageenan (1%)-induced oedema over 120 min of testing while the dose of 5 mg/kg (i.p.) did not affect the oedema over the 4 hr testing period. Combined administration of the lowest and sub-effective doses of leaf aqueous extract of *W. somnifera* (25 mg/kg, i.p.) and indomethacin (5 mg/kg, i.p.) produced a significant reduction in carrageenan (1%)-induced oedema over 120 min of testing period (Table 3).

### 3.4 Acute Toxicity Test

There were no deaths or signs of acute toxicity observed after oral administration of 100-4,000 mg/kg of the leaf aqueous extract of *Withania somnifera* with the highest dose (4,000 mg/kg, p.o.) tested being the NOAEL (no-adverse-effect-level), suggesting that the LD<sub>50</sub> was probably greater than 4,000 mg/kg (p.o.) in mice. On the other, leaf aqueous extract of the plant species at the dose range of 100-4,000 mg/kg injected into mice intraperitoneally, produced some acute toxicity symptoms such as hypolocomotor activity, piloerection, salivation and respiratory distress from the dose of 1,200 mg/kg (i.p.). Death was also observed at this dose where one mouse died. All the animals died

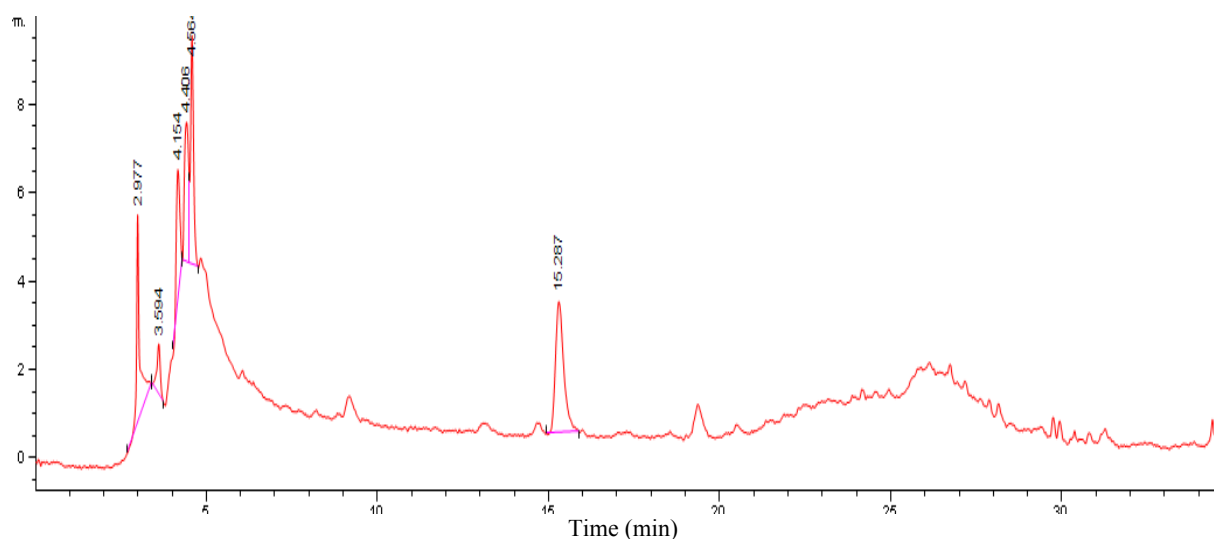
from the dose of 2,000 mg/kg (i.p.). The no adverse effect level (NOAEL) for the intraperitoneal injection was 800 mg/kg while the lowest-observed-adverse-effect level (LOAEL) was 1,200 mg/kg. The LD<sub>50</sub> value of the leaf aqueous extract of the plant species after the intraperitoneal injection was calculated to be 1,600 mg/kg.

### 3.5 HPLC Analysis

The HPLC fingerprint of the leaf aqueous extract of *W. somnifera* showed distinct peaks at the following retention times, 2.977, 3.594, 4.154, 4.406, 4.660 and 15.267 min (Fig. 1).

## 4. Discussion

In the present study, indomethacin antagonised the oedema or acute inflammation produced by carrageenan in the right hind paw of rats over 120 min of the 4 h observation period. Similarly, leaf aqueous extract of *W. somnifera* attenuated carrageenan-induced oedema but over 90 min of the 4 h observation period. Combined administration of both sub-effective doses of plant extract and indomethacin antagonised the oedema or acute inflammation produced by carrageenan in the right hind paw of rats over 120 min of the 4 h observation period. Vinegar et al. [17] reported that chemical mediators such as serotonin,



**Fig. 1** HPLC fingerprint of aqueous extract of *Withania somnifera*.

bradykinin, histamine and prostaglandin-like substance are involved in carrageenan-induced oedema. Other reports by Adedapo et al. [18], Perianayagam et al. [19] and Swingle [20] also implicated histamine, 5-hydroxytryptamine, bradykinin and prostaglandin at different phases of carrageenan-induced rat paw oedema. Rao et al. [21] have shown that carrageenan-induced rat paw oedema test is sensitive to cyclooxygenase inhibitors and may be used to evaluate the NSAIDs (non-steroidal anti-inflammatory drugs). Indomethacin, a NSAID, is known to produce its anti-inflammatory effect by blocking cyclooxygenase activity resulting in inhibition of prostaglandin synthesis [10]. Collier et al. [22] reported that the NSAIDs may antagonise mediators such as serotonin, bradykinin and capsaicin some of which have found to be involved in carrageenan-induced rat paw oedema. It is not a coincidence; therefore, that indomethacin antagonised carrageenan-induced rat right hind paw oedema in this study. Considering the similar result obtained with both the leaf aqueous extract of the plant species and the combined treatment with both sub-effective doses of plant extract and indomethacin, it is probable that inhibition of mediators of inflammation may underpin the anti-inflammatory effect of *W. somnifera*. Pentobarbitone (a barbiturate)-induced sleep has been widely used to evaluate sedative effect of drugs [13,

15]. It has been shown that activation of GABA<sub>A</sub> receptors mediates sleep [23]. Sedative and hypnotic effects of barbiturates have been attributed to the involvement of GABAergic mechanism [23]. In the present study, diazepam significantly prolonged sleep induced by pentobarbitone. Diazepam, a benzodiazepine, acts by enhancing GABA neurotransmission [10] and also known to promote sleep by the same mechanism [23]. It is, therefore, not surprising that diazepam prolonged pentobarbitone-induced sleep. Similarly, leaf aqueous extract of *W. somnifera* significantly prolonged pentobarbitone-induced sleep. It is probable, therefore, that GABAergic mechanism may be involved in the sedative effect of the leaf aqueous extract of the plant species. Locomotor activity is considered as an index of alertness and its decrease is said to be an indication of sedation [24]. Maj et al. [25] showed that stimulation of central dopamine receptors caused increase in motility. Austin and Kalivas [26] have shown that central dopaminergic mechanism is involved in locomotor activity and that an increase in dopamine neurotransmission would increase locomotor activity. Enhancement of GABAergic neurotransmission has been shown to decrease central dopaminergic neurotransmission [26-28]. Mogenson and Jones [29] have reported that picrotoxin-induced locomotor

activity was antagonised by GABA injected into the globus pallidus emanating from the ventral tegmental area of the brain. In the present study, diazepam significantly decreased locomotor activity in mice. Diazepam, a benzodiazepine [10], known to act by enhancement of GABAergic neurotransmission may be modulating central dopaminergic neurotransmission resulting in the observed decrease in locomotor activity. Leaf aqueous extract of the plant species, like diazepam, significantly decreased locomotor activity. It is tempting therefore, to suggest that the plant extract may be modulating central dopaminergic neurotransmission via GABA mechanism. The results of the phytochemical quantitative analysis done in this study showed that the plant species contains triterpene steroids, saponins and tannins. Bruneton [30] reported that saponins have both analgesic and anti-inflammatory properties. Pendota et al. [31], in their study on the anti-inflammatory, analgesic and antipyretic activities of *H. pauciflorus* showed the presence of tannins, flavonoids, steroids and/or terpenes and suggested that these activities may be due to these chemical components in the plant species. Since *W. somnifera* contains secondary metabolites such as triterpene steroids, saponins and tannins, it is possible that these metabolites may be contributing to the anti-inflammatory activity of the plant species. Even though the secondary metabolites contained in *W. somnifera* have also been mentioned in various studies as being present in many plant species which have sedative effects, none has shown any direct link between the secondary metabolites and sedation. The HPLC fingerprint of the plant species obtained revealed major characteristic peaks at the following retention times, 2.977, 3.594, 4.154, 4.406, 4.660 and 15.267 min. The acute toxicity test carried out showed that the LD<sub>50</sub> value obtained for *W. somnifera* could be greater than 4,000 mg/kg (p.o.). The LD<sub>50</sub> value of the leaf aqueous extract of the plant species after the intraperitoneal injection was 1,600 mg/kg.

## 5. Conclusion

The results obtained show that *W. somnifera* has both anti-inflammatory and sedative activities. The anti-inflammatory activity may be produced by the plant species inhibiting various chemical mediators including prostaglandins and bradykinin. GABAergic mechanism may underpin the sedative effect of *W. somnifera*. The LD<sub>50</sub> values of 4,000 mg/kg (p.o.) and 1,600 mg (i.p.) obtained for the plant species are relatively high and show that it may be safe in or nontoxic to mice. The result obtained justifies the use of the plant species by traditional medicine practitioners in South Africa for the treatment of inflammatory conditions and insomnia. However, more studies are needed to further elucidate the mechanism of the anti-inflammatory and sedative effects of *W. somnifera*.

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