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Antinocieptive activity of the root extracts of *Rhus natalensis* Kraus and *Senna singueana*

Hellen Nyambura Kariuki[1*], Titus Ikusya Kanui[2], Abiy Yenesew[3,] Paul Mungai Mbugua[1] Nilesh Bhailalbhai Patel[1]

[1]Department of Medical Physiology, University of Nairobi, P. O. Box 30197 (00100) Nairobi, Kenya
[2]Department of Veterinary Anatomy and Physiology, University of Nairobi, P. O. Box 30197 (00100) Nairobi, Kenya
[3]Department of Chemistry, University of Nairobi, P. O. Box 30197 (00100) Nairobi, Kenya.

*Corresponding Author: hellen.kariuki@uonbi.ac.ke or hnkariuki@yahoo.com

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Abstract

Rhus natalensis and *Senna singuaenae* are traditional African plants commonly used as medicinal plant in East Africa for the management of pain. The plants are used for management of rheumatism among others. This study investigated the antinociceptive activities of *R. natalensis* and *S. singuaenae* in Swiss albino mice using the tail-flick and hot plate tests. Extract solvent (vehicle), morphine and aspirin were employed as controls. Root extract of *R. natalensis* (100 and 200 mg / kg) and 100 mg /kg of *S. singuaenae* showed no significant antinociceptive activity in the hot plate while the 200mg /kg of *S. singuaenae* showed significant antinociceptive activity ($p<0.05$). In the tail flick tests, root extract of *R. natalensis* (100 and 200 mg / kg) showed highly significant antinociceptive activity ($p<0.01$) while 200mg / kg of *S. singuaenae* showed significant antinociceptive activity ($p<0.05$) compared to the controls. The 100 mg /kg of *S. singuaenae* showed no significant antinociceptive activity in the tail flick. This study lends support to the anecdotal evidence for use of *R. natalensis* and *S. singuaenae* in the management of painful conditions.

Keywords: *Rhus natalensis*; *Senna singuaenae;* analgesic;

Introduction

World Health Organization (W.H.O.) estimates about 80% of the world population to be relying on traditional healers, who use the traditional herbs, for primary health care needs (W.H.O. 2005). The use of herbal medicines in sub Saharan Africa for management of various medical conditions accounts for about 70 % of those with health care needs (W.H.O. 2005). The high cost of acquiring modern medicines and their inadequate supplies in most health care facilities as well as the side effects associated with their use, and the belief that

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plants hold cure for many disease conditions (including painful and inflammatory conditions) have led to a reawakening of interest in the use of plants and plant products in recent years. Medicinal plants are an important and treasured local resource for the people of East Africa. The use of the plants in the indigenous cultures are multiple and diverse (Minja, 1999).

Rhus natalensis and *Senna singuaenae* are plant used in Kenya and Tanzania for the treatment of pain conditions in humans and animals (McCurdy et al., 2005). Most of the folkloric uses of the genus *R. natalensis* and *S. singuaenae* evolve around pain, inflammation and microbial infections. *R. natalensis* and *S. singuaenae* were mentioned as plants used for the treatment of pain and inflammation by the Washamba people of Tanzania (Schlage et al., 2000).

Rhus natalensis leaves and roots are used for the treatment of pain and inflammation. Leaves are boiled and given to cattle as a pain killer (Schlage et al., 2000; Minja, 1999). It has also been found to be potential antiplasmodial agent in combination with other herbs (Gathirwa et al., 2008). *Senna singueana* leaves and roots are used for the treatment of pain and inflammation (Schlage et al., 2000). The alkaloid (-)-cassine isolated from *Senna spectabilis* have been reported to induces anti-inflammatory and anti-hyperalgesics effects in both acute and chronic inflammatory and neuropathic pain models (Da Silva et al., 2011). The aim of this study was to evaluate the analgesic activities of *Rhus natalensis* and *Senna singuaenae* using the tail flick and hot plate tests in mice.

Materials and methods

Plant material and extraction

Plant samples were collected from the Ngong forest area in Nairobi, Kenya, botanically authenticated and a voucher specimen deposited with the University of Nairobi herbarium. The samples were shade dried and powdered. The roots of *Rhus natalensis* and *Senna singuaenae* (250 g) were extracted using CH_2Cl_2 / MeOH (1:1) for 1hour on day one and 24 hours for two sessions on the following two days at room temperature. The three extracts were then combined and the removal of the solvents from the extract done using rotatory evaporation process yielding 40 g of residue of each. The extract was dissolved in 5% dimethylsulfoxide (DMSO) and 95% normal saline to achieve the desired working concentrations. The vehicle constituted of 5% DMSO and 95% normal saline.

Animals

Adult Swiss albino mice of both sexes weighing 20–26 g were used. The animals were maintained under normal laboratory conditions of humidity, temperature and light and allowed access to food and water *ad libitum* for a minimum of 7 days, before the start of the experiments. The “Principle of Laboratory Animal Care” (NIH publication No. 85-23) guidelines and procedures were followed (NIH publication revised 1985).All the tests were carried out during the daytime in a quiet laboratory setting with ambient illumination and temperature similar to those of the animal house. Animals were allowed to acclimatize to the test laboratory setting for 24 hours before the experiments began.

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Standard drugs

The reference drugs used were: [Disprin® (acetylsalicyclic acid – ASA)]-(Reckitt Benckiser), Morphine hydrochloride (Martindale Pharma.).

Administration

Administration of extracts, standard drugs and vehicle (5% DMSO and 95% normal saline) was done intraperitoneally (i. p.) using a 17-gauge needle. Each animal was injected 2 ml / kg 1 hour prior to the tests. Two dose levels of the extract, (100, and 200 mg/kg), were selected from the pilot study carried out in mice based on the information obtained from traditional healers.

Sensorimotor test

To evaluate possible nonspecific muscle relaxant or sedative effects of the extracts of *Rhus natalensis* and *Senna singuaenae*, animals were tested on an apparatus that consisted of three rods, diameter 2.5 cm, with the height of 20, 32, and 64 cm. Mice were placed on top of each rod for 20 seconds to test their sensorimotor function. The animals were selected 24 hours previously by eliminating those mice which did not remain or had no firm grip on the rods for two consecutive periods of 20 s. Mice were treated with root bark extract of *Rhus natalensis* or *Senna singuaenae* (100 and 200 mg/kg), 1 hour prior to the test. Control animals received the same volume of vehicle (5% DMSO in 95% (0.9%NaCl) solution 2 ml/kg i.p.1 h before being tested. The cut-off time used was 20s.

Antinociceptive activity

Hot plate test

An IITC Inc. Model 35D analgesiometer was used**.** Control group of mice (n = 8) were treated with vehicle (2 ml/kg i.p.) The test group mice (n =8) were treated with *Rhus natalensis* or *Senna singuaenae* root extract (100 and 200 mg / kg i.p.), morphine (5 mg /kg) or ASA (100 mg / kg i.p.), or Indomethacin (50mg /kg). One hour following the plant extract or drug administration, the animals was placed in a perspex box on the hot plate maintained at 50 ± 1°C. For both the control and treated animals, the reaction time (in seconds) was taken as the time when the animals licked the hind paw or jumped in an attempt to escape from the box (Hunskaar et al., 1996b).The test mean reaction time (in seconds) was also determined for each plant extract dose, drug and vehicle.

Tail flick test

A radiant heat tail-flick IITC Inc. Model 33 analgesiometer was used to measure response latencies according to the method described previously (Corrêa et al., 1996). Mice responded to a focused heat-stimulus by flicking or removing their tail exposed to a photocell in the apparatus immediately below the tail. The reaction time was recorded for the mice pretreated with vehicle, morphine (5mg /kg), aspirin (100 mg /kg), or root extract of *Rhus natalensis* or *Senna singuaenae* (100 and 200 mg/kg) given i.p. 1 hr before testing. Cut-off

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time of 20s was used to minimize tissue damage (Bannon and Malmberg, 2001). Each animal was tested twice before the administration of drugs or extract to determine the baseline.

Statistical analysis

Data obtained for each set of experiments / tests were pooled and analysis was done using one-way ANOVA followed by Shaffes *post-hoc* test. The differences in the test- versus control-values were considered to be statistically significant at $P < 0.05$. Data is expressed as mean ± S.E.M.

Results

Sensorimotor test

The extract of *Rhus natalensis* and *Senna singuaenae* (100 and 200 mg / kg, i. p.), given 1 h prior to sensorimotor testing, did not affect the motor performance of animals

Hot plate test

R. natalensis 100 mg / kg dose (4.8 ± 0.3sec) and 200 mg / kg (4.0 ± 0.3sec) showed no significant antinociceptive activity in the hot plate test compared to the vehicle treated mice (3.4 ± 0.1sec). *S. singuaenae* 100 mg/kg dose (4.7 ± 0.3sec) also showed no significant antinociceptive activity in the hot plate test compared to the vehicle treated mice (3.4 ± 0.1sec). However *S. singuaenae* 200 mg / kg dose (6.3 ± 0.5 sec) showed a highly significant antinociceptive effect ($p<0.01$) compared to the controls (3.4 ± 0.1sec). The standard drug morphine 5 mg/kg with a latency of 5.9 ± 0.3 showed a very highly significant effects ($p<0.001$) while acetyl salicylic acid (ASA) 100 mg/kg with a latency of 4.9 ± 0.2 sec. showed significant effects ($p < 0.05$) compared to the controls (3.4 ± 0.1sec) (table 1).

Tail flick test

Administration of the root extracts (100 and 200 mg / Kg i. p.) of *R. natalensis* and *S. singuaenae* given 1 hour prior to the test, elicited an increase in the tail flick response latency

Table 1 antinociceptive effect of the *Rhus natalensis* and root extract in the hot plate test in mice.

Group	Dose	Latency (s)
Vehicle	0 mg /kg	3.4 ± 0.1
Rhus natalensis root bark extract	100 mg /kg	4.8 ± 0.3
	200 mg /kg	4.0 ± 0.3
S. singuaenae root bark extract	100 mg /kg	4.7 ± 0.3
	200 mg /kg	5.5 ± 0.2**
Morphine	5mg /kg	5.9 ± 0.3***
Acetyl salicylic acid	100mg /kg	4.9 ± 0.2*

Each group represents the mean ± SEM of 8 animals. *p<0.05, **p<0.01 and ***p<0.001 when compared with the control value subsequent to ANOVA

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Table 2 Antinociceptive effects of the *Rhus natalensis* and *Senna singuaenae* root extract in the tail flick test using mice.

Group	Dose	Latency (s)
Vehicle	0 mg /kg	6.0 ± 0.3
R. natalensis root bark extract	100	7.3 ± 0.3**
	200	7.4 ± 0.3**
S. singuaenae root bark extract	100	6.8 ± 0.1
	200	7.1 ± 0.2*
Morphine	5mg /kg	8.0 ± 0.3***
Acetyl salicylic acid	100mg /kg	8.6 ± 0.3***

Each group represents the mean ± SEM of 8 mice. *p<0.05, **p<0.01, ***p<0.001when compared to the control value subsequent to ANOVA

(Table 2). *R. natalensis* 100 mg / kg dose with a latency of 7.3 ± 0.3 and 200 mg / kg dose with a latency of 7.4 ± 0.3 seconds showed highly significant antinociceptive effects ($p<0.01$) compared to the vehicle treated mice (6.0 ± 0.3sec). *S. singuaenae* 100 mg / kg dose (6.8 ± 0.1sec) showed no significant effect while the 200 mg / kg dose (7.1 ± 0.2sec) showed significant antinociceptive activity ($p < 0.05$) compared to the control (6.0 ± 0.3sec). Morphine 5mg/kg dose (8.0 ± 0.3 sec) and ASA 100 mg / kg (8.6 ± 0.3sec) showed very highly significant effect ($p<0.001$) compared to the vehicle treated mice (6.0 ± 0.3sec) (Table 2).

Discussion

The hot plate test is used in evaluating analgesics effects of pharmacological agents in rodents and especially thermal nociception (Le Bars et al., 2001).The response is centrally integrated (Le Bars et al., 2001; Bannon and Malmberg 2001). In this study, the root extract of *Rhus natalensis* (100 and 200mg / kg) and *Senna singuaenae* 100mg / kg showed no significant antinociceptive in the hot plate test. The *Senna singuaenae* 200mg / kg dose showed significant ($p<0.05$) antinociceptive effect compared to the vehicle treated mice. The antinociceptive effects of *Senna singuaenae* were comparable to those of ASA (100mg/ kg).This indicates that the *Senna singuaenae* extract may have some antinociceptive activities that are supraspinally mediated.

The Tail flick test is a popular and convenient method used to evaluate the antinociceptive activity of different pharmacological agents (King et al., 1997). The test does not require the use of highly sophisticated equipment and results may be obtained rapidly without conditioning effects and without causing undue stress to the experimental animal (King et al., 1997; Bannon and Malmberg, 2001).The tail flick response is a reflex that is spinally integrated, although the response latencies have also been shown to be sensitive to pharmacol-ogical manipulation with analgesics acting at supraspinal levels (Le Bars et al., 2001). The root extract (100 mg/kg) of *Senna singuaenae* showed no significant antinocicep-tive activity while the 200 mg/kg dose showed significant antinociceptive activity in the tail flick test compared to the vehicle treated mice.

The root extract (100 and 200 mg / kg) of *Rhus natalensis* showed highly significant ($p<0.01$) antinociceptive activity in the tail flick test compared to the vehicle treated mice. hese results from the tail flick test suggest that *Rhus natalensis* and *Senna singuaenae* root

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extract may be acting at the spinal level as well as supra spinal. The data therefore supports the traditional / folkloric use of the plants as well as validates their use as analgesics (Kanui, 2006; Njoroge and Bussman, 2007). The root bark extracts of *Rhus natalensis* and *Senna singuaenae* possess significant antinociceptive activities in mice. This supports the anecdotal use of *Rhus natalensis* or *Senna singuaenae* in the management of pain.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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