

Antinociceptive activity of *Toddalia asiatica* (L) Lam. in models of central and peripheral pain

Hellen N. Kariuki^{1*}, Titus I. Kanui³, Abiy Yenesew², Nilesh B. Patel¹, Paul M. Mbugua⁴

¹Department of Medical Physiology and ²Department of Chemistry, University of Nairobi, Nairobi, Kenya

³School of Agricultural & Veterinary Sciences, South Eastern University College, Kitui, Kenya

⁴The Presbyterian University of East Africa, Kikuyu, Kenya.

*Corresponding Author: hellen.kariuki@uonbi.ac.ke

Received: 3rd April 2012, Revised: 16 April 2012, Accepted: 16 April 2012

Abstract

Toddalia asiatica within the context of traditional African medicine is a commonly used medicinal plant in East Africa for the management of pain and inflammatory conditions. It is used by the Masai in both Kenya and Tanzania for management of rheumatism among others. The present study was undertaken to investigate the antinociceptive activities of *T. asiatica* in Swiss albino mice in acetic acid-induced writhing, tail-flick and hot plate pain tests. The extract solvent (vehicle), morphine and aspirin were employed as negative and positive controls respectively. The acetic acid -induced writhing test was used as the screening test and as the root bark extract was found to be more potent than the leaf extract, the former was investigated using the hot plate and the tail flick tests. The root bark extract (200 mg / kg) showed highly significant ($p < 0.001$) antinociceptive activity in the hot plate and the tail flick tests. The 100mg/kg dose showed significant ($p < 0.05$) activity in the tail flick test but not significant in the hot plate test. The present study, therefore lends support to the anecdotal evidence for use of *T. asiatica* in the management of painful conditions.

Keywords *Toddalia asiatica*; Antinociception; writhing test; tail flick; hot plate

Introduction

Pain as a sensory modality, represents the symptom for the diagnosis of several diseases and conditions. It has a protective function. Pain is widely accepted as one of the most important determinants of quality of life because of its widespread adverse effects, including diminishing mental health and wellbeing and impairing the individual's ability to perform daily activities. Chronic pain impacts upon a large proportion of the adult population, including the working age population, and is strongly associated with markers of social disadvantage (Blyth et al., 2001). For thousands of years medicine and natural products have been closely linked prominently through the use of traditional medicines. Clinical, pharmaco-

logical, and chemical researches of these traditional medicines, which are derived predominantly from plants, are the basis of many drugs. Medicinal plants contain a diversity of biologically active compounds that belong to different natural product chemical classes. Throughout history man has used many different forms of therapy for the relief of pain, among them medicinal herbs among them, medicinal herbs such as *Papaver somniferum* from which morphine was isolated. Developing treatments for pain relief has been the motivating factor behind many studies carried out in response to the demand for powerful analgesics and that exhibit their pharmacological response through new mechanisms of action and with less side effects (De Sousa, 2011).

Toddalia asiatica (L) Lam. (Rutaceae), also known as Wild Orange tree, is a green leafy climber growing in the evergreen forests. It is vastly distributed in the tropical regions of Africa, Asia and Madagascar and contains coumarins, quinoline and benzophenanthridine alkaloids. The alkaloids of the crude extract have been shown to have anti-inflammatory effects in rats using the carragennan test (Balasubramaniam, et al., 2011) and to inhibit the auricle swelling caused by xylol and joint swelling caused by agar in rats (Hao, et al., 2004). It has been shown to have anti-malarial and anti-leukimatic properties (McCurdy C. R. et al., 2005, Schlage et al., 2000). The roots and the leaves are used in Kenya and Tanzania for the treatment of neuropathic and inflammatory pain (McCurdy et al., 2005, Orwa et al., 2008). Most of the folkloric uses of the genus *Toddalia asiatica* evolve around pain, inflammation and microbial infections. *T. asiatica* was the second most mentioned plant for the treatment of pain and inflammation by the Washamba people of Tanzania (Schlage et al., 2000). This study was undertaken to assess the potential analgesic actions of *Toddalia asiatica* using chemical and thermal nociceptive tests.

Materials and Methods

Plant acquisition and preparation of the extracts

Plant samples were collected from the Ngong forest area in Nairobi in February 2008 and were botanically authenticated by the University of Nairobi Herbarium and a voucher specimen number HNK/MPD/001 deposited with the herbarium. The air-dried and powdered roots of *Toddalia asiatica* (250 g) were extracted using CH₂Cl₂ / MeOH (1:1) for 1 hour on day one and 24 hours for two sessions on the following two days at room temperature. The three extracts were combined and the removal of the solvents from the extract was done by rotator evaporation process yielding 40 g of brown residue which 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 at least 7 days, before the commencement of the experiments. The “Principle of Laboratory Animal Care” (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised 1985). All the tests were carried out during the daytime in a quiet laboratory setting with ambient illum-

ination and temperature similar to those of the animal house. Animals were allowed to acclimatize to the test laboratory setting for 1 hour before the experiments began.

Standard drugs

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

Administration

Extracts, standard drugs and vehicle (5% DMSO and 95% normal saline) were injected 2ml/kg intraperitoneally (i. p.) using a microlitre syringe and a 17 gauge needle. Three dose levels of the extract, (50, 100, and 200 mg/kg), were selected from the pilot study carried out earlier in mice based on the information obtained from traditional healers.

Sensorimotor test

To evaluate possible nonspecific muscle relaxant or sedative effects of the extract of *Toddalia asiatica*, animals were tested on an apparatus that consisted of 3 rods, diameter 2.5 cm, with the height of 20, 32, and 64 cm. Animals were placed on top of each rod for 20 seconds to test their sensorimotor function. The animals were selected 24 h previously by eliminating those mice which did not remain or had no firm grip on the rods for two consecutive periods of 60 s. Animals were treated with the standard drugs or extract of *Toddalia asiatica* (50,100 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 2ml / kg, i. p.) 1 h before being tested. The cut-off time used was 20s.

Antinociceptive activity

Acetic acid-induced writhing test

Control group of mice received vehicle (2ml / kg, i. p.). Mice in the test groups ($n = 6$ per group) received *T. asiatica* leaf or root extracts at 50, 100 and 200 mg / kg i. p. One hour following *T. asiatica* extract or vehicle administration, 0.1 ml of a 7% acetic acid solution was intraperitoneally injected into each of the test mice (Koster et al., 1959). The amount of time (seconds) the animal spent in pain behavior (abdominal contractions) within the next 30 min following acetic acid administration was recorded.

Hot plate test

An IITC Inc. Model 35D analgesiometer was used in this test. Control group of mice ($n = 8$) were treated with vehicle (2 ml / kg i. p.) The test group mice ($n = 8$) were treated with *T. asiatica* root bark extract at 50, 100 and 200 mg / kg i. p., morphine (5 mg / kg) or ASA (100 mg / kg i. p.), respectively. One hour following the plant extract or drug administration, the mice were separately placed in a perspex box on a 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 paw or jumped in an attempt to escape from the box (Bannon

and Malmberg, 2001; 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). Animals responded to a focused heat-stimulus by flicking or removing their tail exposing a photo-cell in the apparatus immediately below the tail (Bannon and Malmberg, 2001). The reaction time was recorded for the animals pretreated with vehicle, morphine 5mg /kg, aspirin 100 mg/kg or root bark extract of *T.asiatica*, (50, 100 and 200 mg / kg, i. p.) 1 hr before testing. Cut-off time of 20 s was used to minimize tissue damage. Each animal was tested twice before administration of drugs 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 \pm S.E.M. The dose was the independent variable.

Results

Sensorimotor test

The extract of *T. asiatica* (50, 100 and 200 mg / kg, i. p.), given 1 h prior to sensorimotor testing, did not affect the motor performance of animals when compared with the control group response.

Acetic acid-induced writhing test

Injection of *T. asiatica* (50, 100 and 200 mg / kg, i. p.), inhibited the acetic acid- induced abdominal constriction. Figure 1 shows the latencies of the leaf extract. The 50 mg/kg dose had a latency of 87.3 ± 7.9 seconds and the 100mg/kg had 62.00 ± 4.9 seconds and both of these doses did not show any significant effect on the acetic acid induced abdominal contractions compared to the control (83.7 ± 8.7). The 200mg / kg dose (47.3 ± 9.6) of the same exhibited a highly significant effect ($p < 0.01$) compared to the control (83.7 ± 8.7).

Figure 2 illustrates the latencies of the root bark extracts. The 50mg / kg dose with a latency of 61.8 ± 4.2 seconds did not show any significant effect compared to the controls (83.7 ± 8.7), while the 100mg/kg (36.5 ± 8.2) and the 200 mg / kg (35.0 ± 6.7) showed highly significant effects ($p < 0.01$). A significant reduction in the number of acetic acid-induced abdominal contractions of the extract injected mice compared to the vehicle treated mice was taken as an indication of analgesic activity. The results showed that the root bark extract was more potent than the leaf extract (Figure 1 and 2).

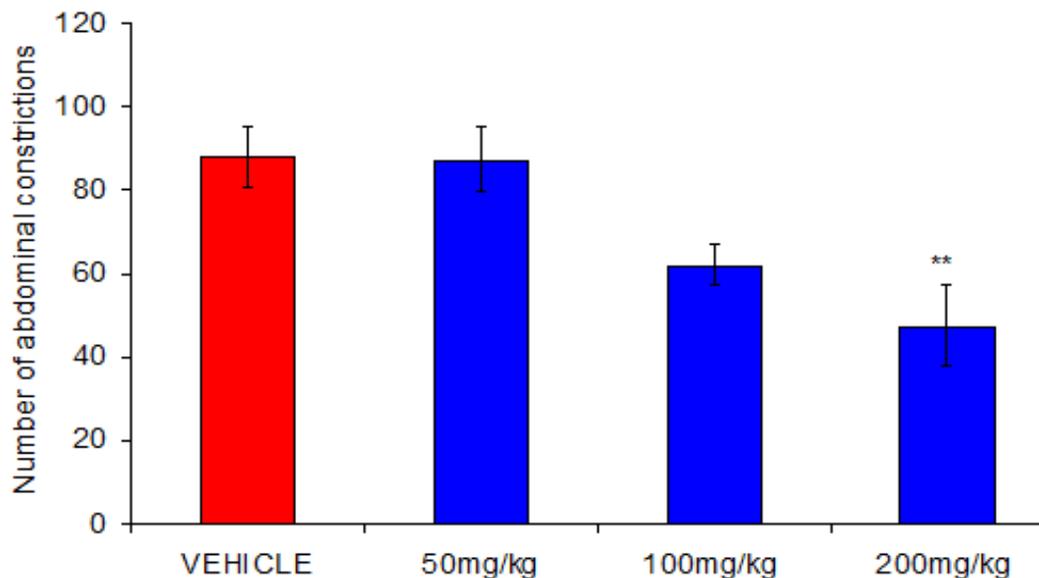


Figure 1. Effect of leaf extract on acetic acid-induced writhings in animals (n=6). ** = $p < 0.01$ as compared to control group.

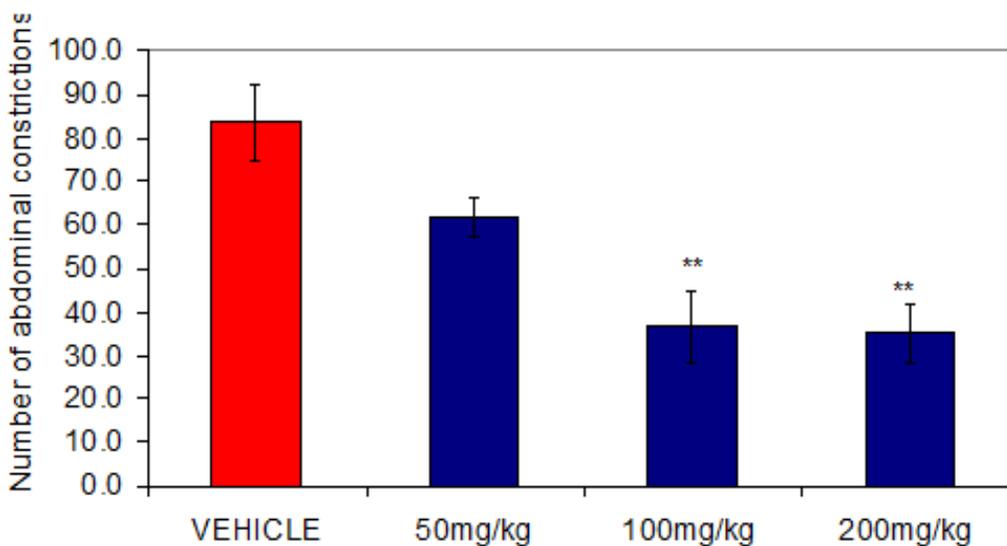


Figure 2. Effect of root bark extract on acetic acid-induced writhings in animals (n=6) ** $p < 0.01$ as compared to control group.

Hot plate test results

Table 1 represents the hot plate results. The 50 mg / kg with a latency of 4.4 ± 0.3 seconds and 100 mg / kg with a latency of 4.8 ± 0.3 seconds showed no significant effects compared to the vehicle treated animals (4.1 ± 0.3). However the 200 mg / kg dose (6.3 ± 0.5 sec) showed a very highly significant effect ($p < 0.001$) compared to the controls (4.1 ± 0.3). The control drugs morphine 5 mg / kg with a latency of 7.9 ± 0.4 and acetyl salicylic acid (ASA) 100 mg/kg with a latency of 7.5 ± 0.4 also showed a very highly significant effects ($p < 0.001$) compared to the controls (4.1 ± 0.3) (table 1).

Table 1. Antinociceptive effects of the *T. asiatica* root bark extract in the hot plate test in mice.

Group	Dose	Latency (s)
Vehicle	0 mg /kg	4.1 ± 0.3
	50 mg /kg	4.4 ± 0.3
<i>T. asiatica</i> root bark extract	100 mg /kg	4.8 ± 0.3
	200 mg /kg	6.3 ± 0.5***
Morphine	5mg /kg	7.9 ± 0.4***
Acetyl salicylic acid	100mg /kg	7.5 ± 0.4***

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

Table 2. Antinociceptive effects of the *T. asiatica* root bark extract in the tail flick test

Group	Dose	Latency (s)
Vehicle	0mg /kg	4.9 ± 0.2
	50	5.9 ± 0.2
<i>T. asiatica</i> root bark extract	100	6.8 ± 0.5*
	200	7.8 ± 0.5***
Morphine	5mg /kg	7.9 ± 0.6***
Acetyl salicylic acid	100mg /kg	5.6 ± 0.3

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

Tail flick test results

Table 2 shows the tail flick results. Administration of the root bark extract (50, 100 and 200 mg / Kg i. p.) given 1 hour prior to the test, elicited a dose related increase in the tail flick response latency (Table 2). The 50 mg/kg dose with a latency of 5.9±0.2 seconds showed no significant effect while the 100 mg/kg with a latency of 6.8±0.5 showed a significant effect (p < 0.5) compared to the control (4.9±0.2 seconds). The 200 mg / kg dose (7.8±0.5) showed a very highly significant effect (p<0.001) compared to the control (4.9±0.2). Morphine 5mg/kg dose with a latency of 7.9±0.6 seconds also showed a very highly significant effect (p < 0.001) compared to the control (4.9±0.2), but ASA 100 mg / kg did not have any significant effect compared to the control (4.9±0.2) (Table 2).

Discussion

The acetic acid-induced writhing test is widely used methods for evaluating peripheral analgesic effects (Gene et al., 1998) and in this study the acetic acid induced writhing test was used as the screening test. Three doses of the root bark and the leaf extracts were compared to the vehicle. The results indicate that the root bark extract showed a significant (p < 0.01) antinociceptive effect at dose 100 mg/kg dose while the leaf extract showed no significant effect. At 200mg/kg dose both the leaf and the root bark showed significant (p < 0.01) antinociceptive effect. The root bark extract showed significant effect at a lower dose indicating it has higher analgesic properties than the leaf extract.

The hot plate test is used in evaluating analgesics effects of pharmacological agents in rodents and especially thermal nociception (Bannon and Malmberg, 2001; Le Bars et al. 2001).

The hot plate response is centrally integrated (Le Bars et al., 2001). In this study, the root bark extract showed a highly significant ($p < 0.001$) antinociceptive effect at the 200mg/kg dose compared to the vehicle in the hot plate test. The antinociceptive effects were comparable to those of morphine (5 mg/ kg) and of ASA (100 mg / kg). This indicates that the extract may be acting supraspinally.

The Tail flick test is a widely used convenient method for evaluating 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). The tail flick response is a spinally integrated reflex, although the response latencies have been shown to be sensitive to pharmacological manipulation with analgesics acting at supraspinal levels (Bannon and Malmberg, 2001; Le Bars et al., 2001). The root bark extract (100mg/kg) of *T. asiatica* showed no significant antinociceptive activity, whereas the 200mg/kg dose of the extract produced a highly significant antinociceptive effect which are comparable to the effects of the reference drug morphine (5 mg/kg). These results from the tail flick test suggest that higher doses of *T. asiatica* extract may be acting at the spinal level as well as supra spinal.

The root extract of *T asiatica* does possess significant antinociceptive activities in the writhing test as well as the hot plate and the tail flick tests in mice. The data supports the traditional/folkloric use of *T asiatica* as a plant used by the communities for the management of pain and validates their use. (Kanui, 2006; Kariuki, *et. al.*, 2012; Njoroge and Bussman, 2007). The root bark extracts of *T. asiatica* possess significant antinociceptive activities in the animal models of nociception. This supports the anecdotal use of *T. asiatica* in the management of pain and related disorders.

Acknowledgements

The research was supported by a grant from University of Nairobi, Deans Committee and carried out in the Department of Medical Physiology, School of Medicine, College of Health Sciences, University of Nairobi. The authors appreciate the technical assistance of Mr. Mutiso of the School of Biological Sciences herbarium, University of Nairobi and the careful animal handling of the staff of Department of Medical Physiology animal house.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Balasubramaniam LA, Manivannan R, Paramaguru R, Vijayakumar M, (2011). Evaluation of Anti-inflammatory and Antioxidant Activities of Stem Bark of *Todalia asiatica* (L.) Lam. Using Different Experimental Models. *Global Journal of Pharmacology* 5 (2), 67-72.
- Bannon A, Malmberg A, (2001). Models of Nociception: Hot-Plate, Tail-Flick, and Formalin Tests in Rodents. *Current Protocols in Neuroscience* 41, 8.9.1-16.
- Blyth F.M, March L.M, Brnabic A.J.M, Jorm L.R, Williamson M, Cousins M.J, (2001). Chronic pain in Australia: a prevalence study. *Pain* 89:127-34.

- Corrêa C.R, Kyle D.J, Chakravarty S, Calixto J.B, (1996). Antinociceptive profile of the pseudopeptide B2 bradykinin receptor antagonist NPC 18688 in mice. *British Journal of Pharmacology* 117, 552–558.
- De Sousa D.P, (2011). Analgesic-like Activity of Essential Oils Constituents *Molecules*, 16, 2233-225.
- Gene R.M, Segura L, Adzet T, Marin E, Iglesias J, (1998). Heterothecal inuloides: anti-inflammatory and analgesic effect. *Journal of Ethnopharmacology* 60, 157–160.
- Guide for the use of laboratory animals DHHS, PHS, NIH Publication No. 85-23 (1985 Revised).
- Hao X.Y, Peng L, Ye L, Huang N.H, Shen Y.M, (2004). A study on anti-inflammatory and analgesic effects of alkaloids of *Toddalia asiatica*. *Journal of Chinese integrative medicine* 2 (6), 450-452.
- Hunskar S, Berge O.G, Hole K. (1986b). A modified hot plate test sensitive to mild analgesics. *Behavioral Brain Research*, 21:101-108.
- Kanui T.I, (2006). Multipurpose shrubs used in the management of arthritis. *Proceedings of the Annual DAAD Scholars Conference Kenya*. 26-32.
- Kariuki H.N, Kanui T.I, Yenesew A, Mbugua P.M, Patel N.B, (2012). Antinociceptive activity of the root extracts of *Rhus natalensis* Kraus and *Senna singueana*. *Phytopharmacology* 2, 312-317.
- King T.E, Joynes R.L, Grau J.W, (1997). Tail-flick test: II. The role of supraspinal systems and avoidance learning. *Behavioral Neuroscience* 111, 754–767.
- Koster R, Anderson M, De-Beer E.J, (1959). Acetic acid for analgesic screening. *Federation proceedings* 18: 412–418.
- Le Bars D, Gozariu M, Cadden S.W, (2001). Animal Models of Nociception. *Pharmacology Reviews*, 53, (4), 597-652.
- McCurdy C.R, Scully S.S, (2005). Analgesic substances derived from natural products. *Natureceuticals. Life Science*; 78 (5): 476-484.
- Njoroge G.N, Bussmann R.W, (2006). Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). *Journal of Ethnobiology and Ethnomedicine* 2, 8.
- Orwa J.A, Jondiko I.J.O, Minja R.J.A, Bekunda M, (2008). The Use of *Toddalia asiatica* (L) Lam. (Rutaceae) in traditional Medicine Practice in East Africa. *Journal of Ethnopharmacology*, 115, (2): 257-262
- Schlage C, Mabula C, Mahunnah R.L.A, Heinrich M, (2000). Medicinal plants of the Washambaa (Tanzania): Documentation and Ethnopharmacological Evaluation. *Plant Biology* 2: 83-92.