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Antinociceptive Properties of Methanolic Bark Extracts of *Terminalia brownii* in Wistar Rats

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Abstract

Background: The barks of the medicinal plant, *Terminalia brownii,* are widely used in African folk medicine for the management of pain. However, this ethno-medicinal allegation has not been scientifically validated. This study was therefore designed to verify the antinociceptive potential of the methanolic bark extract of *T. brownii* in Wistar rats (Rattus novegicus).

Methods: Fresh barks of *T. brownii* were obtained from Kitui County, Kenya with the guidance of a residential herbalist. The study used thirty 2-3 months old male Wistar rats, weighing 140-150 g. The rats were randomly divided into 6 groups; three control groups (normal, negative and positive) and three experimental groups (50, 100 and 150 mg/kg extract treatment). Each group had five rats. The analgesic properties of the extract were evaluated on formalin-induced pain and diclofenac was used as the standard drug.

Results: The methanolic bark extracts of *T. brownii* demonstrated significant antinociceptive activity ($p<0.05$) by reducing the paw licking time by between 4.62%-44.96% in the early phase and 35.77%-58.89% in the late phase. Diclofenac reduced the paw licking time by 44.79% in the early phase and 55.33% in the late phase.

Conclusion: Our study results strongly support the antinociceptive activity of the barks of *T. brownii* and rationalize the traditional use of the barks in management of pain.

Keywords: Antinociceptive; Methanolic bark extract; *Terminalia brownii*; Wistar rats

Introduction

Pain is an unpleasant sensory affliction and emotional experience usually associated with actual or potential tissue damage, or described in terms of such damage [1]. Pain ranges from mild irritation, through sensations of pricking and itching to unbearable sensations like throbbing and stabbing [2]. In response to external noxious stimuli such as chemical, mechanical, electrical and thermal stimuli, the nervous system triggers behavioral responses that help in protection or avoidance of tissue damage [3]. Pain therefore represents the principal symptom for the diagnosis of numerous disease conditions and is extensively accepted as one of the significant determinants of quality of life [4]. Non-steroidal anti-inflammatory drugs like piroxicam, diclofenac, meloxicam, ibuprofen, ketoprofen, indomethacin, among others are the conventional drugs commonly prescribed for the management of pain [5]. However, long term use of these drugs is unsatisfactory in terms of efficacy, tolerability, toxicity and affordability [4,6]. Opoids like morphine, fentanyl, nalorphine, and hydromorphone are also used for the management of pain [7]. Opoids bind to and modulate the descending and ascending pathways related to pain [8].

However, Opoids have been reported to cause adverse side effects like respiratory depression, constipation, sexual dysfunction, nausea and muscle rigidity [7]. Search for alternative remedies for pain is therefore inevitable due to the limitations associated with the conventional analgesics. Medicinal plants derivatives have been suggested to be better alternatives since they are more effective, more affordable, associated with fewer side effects and readily available [9,10]. *Terminalia brownii* (Combretaceae) is an important medicinal plant that is widely used in African traditional medicine [11]. It is found in Eastern and Central Africa; Kenya, Tanzania, Sudan, Ethiopia and Democratic Republic of Congo [12]. *Terminalia brownii* is used as a folklore remedy for back and rheumatic pains, tooth-ache, tonsillitis, cough, typhoid and snake bites [13-16]. As part of our interest in search for pharmacological effect of natural products for the management of pain and because *T. brownii* is widely used a pain remedy, this work focused to confirm the popular use of this plant by assessing the antinociceptive effects of its methanolic bark extract in animal models.

Materials and Methods

Collection and preparation of plant materials

Fresh barks of *T. brownii* were obtained from Kitui County, Kenya with the guidance of a residential herbalist. The bark samples were identified and authenticated at the East African Herbarium. The study was conducted for one month at the Kenyatta University's Biochemistry and Biotechnology laboratory. The samples were cleaned using running tap water, cleaved into small pieces and shade dried until completely dry at room temperatures. An electric mill was used to grind the dry samples into fine consistent powder. The powdered materials were kept at room temperature away from direct sunlight in closed dry paper bags until use.

Extraction

The powder was macerated with 100% methanol for 2 days at room temperature. After filtration, the extract was concentrated using a rotary evaporator (Buchi rotary evaporator, Sigma Aldrich, Switzerland), before storing the crude extract in airtight containers at 4°C.

Experimental animals

Wistar rats (2-3 months old) weighing 140-180 g were used in this study [17]. The animals were housed under a controlled temperature (25 ± 2°C) on a 12 h light/12 h dark photoperiod cycle and allowed to acclimate for 7 days. The rats were fed on standard rodent pellets and provided with water ad libitum. We conducted the present study in accordance with the international rules accepted for the care and use of experimental animals in laboratories [18] and the guidelines provided by our Institution's Ethics Committee. A research permit (No. NACOSTI/P/16/48885/11446) was also obtained from the National Commission for Science, Technology and Innovation.

Determination of the antinociceptive activity

To investigate the possible antinociceptive properties of methanolic bark extracts of *T. brownii*, formalin-induced pain was used according to the method described by [19] with some modifications. The antinociceptive activity of the extract was compared to diclofenac (Bhumi Pharmaceuticals, Gujarat, India), the reference drug. The experimental rats were partitioned into 6 groups of 5 rats each and the treatments' summary is as presented in (Table 1).

Group	Status	Treatment
I	Normal control	None
II	Negative control	Formalin+DMSO
III	Positive control	Formalin+15 mg/kg diclofenac+DMSO
IV	Experimental group A	Formalin+50 mg/kg extract+DMSO
V	Experimental group B	Formalin+100 mg/kg extract+DMSO
VI	Experimental group C	Formalin+150 mg/kg extract+DMSO

DMSO=10%; Formalin=2.5%

Table 1: Treatment procedure used for evaluation of the anti-nociceptive activities of methanolic bark extracts of *T. brownii* in Wistar rats.

To induce nociceptive effect, 0.1 ml of 2.5% formalin was administered subcutaneously into the sub plantar region of the left hind paw leading to nociceptive behaviors of biting, licking and lifting [20].

Formalin (Shijiazhuang Xinlongwei Chemical Co., Ltd., Hebei, China) was administered thirty minutes after the various treatments were given. One rat at a time was placed in a transparent glass cage to allow proper observation of the nociceptive behaviors. The time that the rats spent licking and biting the injected paw was scored in two distinct phases. The early phase was scored for the first five minutes after formalin injection and the late phase was scored 15-30 minutes after formalin injection.

The following formula was then used to calculate percentage inhibition of paw licking;

$[(C-T)/C] *100$

Where;

C- The vehicle treated control group value for each phase

T - The treated group value for each phase

Data analysis

All data obtained was analyzed using ANOVA with Minitab statistical computer software v.17 (Minitab Inc., Pennsylvania, U.S.A). Means were separated using Tukey's Honest Significant Difference test at a confidence level of 95% ($p \leq 0.05$).

Results

The administration of the methanolic bark extract of *T. brownii* reduced the formalin-induced pain in both early and late phases and this was indicated by the reduction in paw licking time (Table 2). In the early phase, treatment with the extract at the dose levels; 50, 100 and 150 mg/kg bw exhibited a dose dependent trend and reduced paw licking time by 4.62%, 23.59% and 44.96% respectively (Table 2).

In addition, the reference drug (diclofenac) reduced the paw licking time by 44.79%. The antinociceptive effects extract at the dose levels of 50, 100 and 150mg/kg bw were significantly different amongst each other ($p<0.05$, Table 2). At the dose level of 150 mg/kg bw, the extract was comparable to the standard control drug ($p>0.05$, Table 2).

The extract at the dose levels of 100 and 150 mg/kg bw was significantly different from the negative and normal control groups ($p<0.05$, Table 2). In this phase, the extract at the dose level of 150mg/kg bw manifested the maximum antinociceptive activity.

In the late phase, the methanolic bark extracts of *T. brownii* exhibited a dose dependent response on the formalin-induced pain. The standard drug and the extract at the dose levels of 50, 100 and 150 mg/kg bw reduced the paw licking time by 55.33%, 35.77%, 49.79% and 58.89 respectively (Table 2).

The antinociceptive effect of the extract at the dose levels of 100 and 150mg/kg was not significantly different ($p>0.05$, Table 2). However,

the antinociceptive activity of the extract at the dose levels of 100 and 150 mg/kg bw was significantly different from the antinociceptive activity of the extract at the dose level of 50 mg/kg ($p<0.05$, Table 2).

The antinociceptive activity of the extract at dose levels 100 and 150 mg/kg bw was comparable to that of the reference drug, diclofenac ($p>0.05$, Table 2). The extract at the three dose levels was significantly different from the negative and normal control groups ($p<0.05$, Table 2). In this phase, the extract at the dose level of 150 mg/kg bw manifested maximal antinociceptive activity.

Group	Treatment	Paw Licking Time After Treatment (Sec)	
		Early Phase	Late Phase
Normal control	None	0.00 ± 0.00^{d} (100.00%)	0.00 ± 0.00^{d} (100.00%)
Negative control	Formalin+DMSO	117.00 ± 4.11^{a} (00.00%)	191.20 ± 4.80^{a} (00.00%)
Positive control	Formalin+diclofenac+DMSO	64.60 ± 2.29^{c} (44.79%)	85.40 ± 3.78^{c} (55.33%)
Experimental Group A	Formalin+50 mg/kg bw+DMSO	111.60 ± 4.34^{a} (4.62%)	122.80 ± 4.89^{b} (35.77%)
Experimental Group B	Formalin+100 mg/kg bw+DMSO	89.40 ± 4.43^{b} (23.59%)	96.00 ± 5.44^{c} (49.79%)
Experimental Group C	Formalin+150 mg/kg bw+DMSO	64.40 ± 5.95^{c} (44.96%)	78.60 ± 7.11^{c} (58.89%)

Values were expressed as Mean ± SEM for the five rats per group. Statistical comparisons were made within a column and values with the same superscript were not significantly different by ANOVA followed by Tukey's post hoc test ($p>0.05$). Values in brackets indicate percentage paw licking inhibition.

Table 2: Anti-nociceptive activity of the methanolic bark extracts of *T. brownii* in Wistar rats.

Discussion

The antinociceptive activity of the methanolic bark extract of *T. brownii* was evaluated on formalin-induced pain in the left hind paw of male Wistar rats. Acute thermal assays like the hot plate, tail flick and Hargreave's tests are other models that have been used to screen for the antinociceptive activities of various agents [21]. Acetic acid can also be used to induce pain [22]. However, the formalin assay was chosen over other models because it has the potential to mimic human clinical pain conditions [23] with the freely moving unrestrained animals allowing for the observation of spontaneous pain-related responses [24]. Formalin produces a response in two distinct phases and this allows researchers to model both acute and chronic pain using a single noxious chemical.

Formalin evoked a biphasic pain response; phase I known as neurogenic pain and phase II also known as inflammatory pain [22]. The first phase starts from 0-10 minutes after formalin injection [25] and mediators such as amino acids and kinins [22] are released. This phase results when the primary afferent sensory neurons are directly activated [24] and therefore, drugs that act mainly on the central nervous system, can inhibit neurogenic pain [25]. The second phase starts from 20-30 minutes after formalin injection leading to the release of inflammatory mediators like bradykinin, histamine, prostaglandins, ILs and TNF-α [25]. This phase reflects both the peripheral input and sensitization of the spinal cord sensitization [22] and it's sensitive to peripherally-acting drugs like NSAIDs and corticosteroids [25].

In the present study, 2.5% formalin was injected into the left hind paw of the experimental animals. This concentration of formalin was chosen because it evokes a maximum response and according to [26], higher concentrations of formalin may lead to other behavioral responses that may interfere with the primary antinociceptive behavior. Under normal physiological conditions, rats tend to lick their forepaws [27] and so as to show that the paw licking was entirely due to formalin, the hind paw was chosen.

The methanolic bark extracts of *T. brownii* demonstrated a significant antinociceptive activity by reducing the paw licking time in both phases. These results showed that the extract was able to inhibit the activation of the primary afferent sensory neurons and the release of inflammatory pain mediators. It can therefore be suggested that the methanolic bark extracts of *T. brownii* contain centrally and peripherally acting analgesic phytochemicals. These results are similar with the results of previous studies that have evaluated and revealed the antinociceptive activities of other medicinal plants like the studies by [28-30].

The dose levels of the extract used in this study were 50, 100 and 150 mg/kg bw and were in a similar dose range used by [27,31,32]. The three dose levels of the methanolic bark extracts of *T. brownii* produced a dose-dependent response to the formalin-induced pain and this kind of response was also obtained by [33]. In the present study it was observed that the extract at the lower dose levels of 50 and 100 mg/kg bw was not as effective as the higher dose level of the extract, 150 mg/kg bw. This could be explained by the fast metabolism and clearance of the effective principle(s) that was/were in a low concentration in these lower dose levels of the extract [34]. It can also

be suggested that at the dose level of 150 mg/kg bw, the extract demonstrated the highest antinociceptive activity due to the presence of a sufficient concentration of the effective principle(s) in the extract.

The phytochemical composition of *T. brownii* has been widely studied and various phytochemical constituents have been affirmed to exist in this medicinal plant. They include saponins, polyphenols, flavanoids, phytosterols, tannins, coumarins, alkaloids, terpenoids, phenols, and steroids [35-37]. Several compounds have also been identified in various extracts of *T. brownii* including; betulinic acid, β-sitosterol, arjungenin, stigmasterol, monogynol A [38], seven ellagic acid derivatives, 3-O-β-D-glucopyranosyl-β-sitosterol and an oleanane-type triterpenoid [9].

The antinociceptive properties of some of these secondary metabolites have been revealed. Flavonoids exhibit antinociceptive activity [39]. Flavonoids inhibit the activity of the enzyme endo-peroxidase (prostaglandin synthetase) leading to a reduction of the synthesis and release of prostaglandins, a pain mediator [33]. Prostaglandins are involved in pain perception; therefore, agents that inhibit their synthesis are possible remedies for pain. Several studies have also revealed that alkaloids, saponins and terpenoids do possess antinociceptive properties [34,40-42]

The present study therefore suggests that the antinociceptive effect of the methanolic bark extracts of *T. brownii* could be due to the activity flavonoids, alkaloids, terpenoids and saponins that have been confirmed to be present in this medicinal plant. One limitation of this study is that biochemical tests were not conducted to determine the effects of the methanolic bark extract of *T. brownii* on internal organs like the liver and the kidney. However, we recommend this as an area of further research.

Conclusion

Our study showed a significant dose-dependent antinociceptive activity of the methanolic bark extracts of *T. brownii* in both early and late phases. The antinociceptive activity of this extract is probably associated with the presence of phytochemical secondary metabolites that were able to inhibit the activation of the primary afferent sensory neurons and the release of inflammatory pain mediators. The extract's antinociceptive effect was comparable to that of diclofenac, reference drug. In addition, the antinociceptive effect of the extract was maximum at the dose level of 150 mg/kg bw in both the early and late phases. The methanolic bark extracts of *T. brownii* can therefore be used to develop new formulations against pain as alternative medication to the conventional drugs used to manage pain. Our study therefore provides a scientific confirmation on the ethno medicinal claim of the use of *T. brownii* barks as a pain remedy.

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