ANTI-INFLAMMATORY PROPERTIES OF METHANOLIC BARK EXTRACTS OF TERMINALIA BROWNII IN WISTAR ALBINO RATS

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ABSTRACT

Objective: This study investigated the anti-inflammatory properties of methanolic bark extract of Terminalia brownii in Wistar albino rats (Rattus norvegicus).

Methods: The experimental animals were divided into six groups of five rats each; normal control, negative control, positive control and three experimental groups. Carrageenan was used to induce inflammation. Diclofenac was used as the reference drug, and the three experimental groups were treated with the extract at the dose levels of 50, 100 and 150 mg/kg bw. The extract was screened for the presence or absence of selected phytochemical secondary metabolites using standard procedures.

Results: The methanolic bark extracts of *T. brownii* significantly [p < 0.05] reduced the carrageenan-induced paw edema by between 1.57%-20.41% while diclofenac reduced it by between 11.12%-25.33%. Phytochemical screening of the extract indicated the presence of alkaloids, flavonoids, phenols, saponins, steroids and terpenoids.

Conclusion: The present study revealed that *T. brownii* bark extract is a potential candidate for the development of a novel anti-inflammatory formulation.

Keywords: *Terminalia brownii*, Methanolic bark extracts, Anti-inflammatory activity

INTRODUCTION

The use of traditional medicine has been in existence before the development and spread of modern medicine and it is still in use today [1]. About 75-80% of the world populations rely on herbal medicine for primary health care [2]. The use of herbal medicine exceeds that of conventional drugs by 2-3 times [1]. However, there is little information regarding the literature on medicinal plants use in Kenya [3].

Inflammation is the basic way in which the body reacts to irritation, infection, or other tissue injuries, the key features being a pain, swelling, redness and warmth [4]. The inflammatory response is important because it disposes of pathogens and cell debris, prevents damaging agents from spreading to the nearby tissues, and sets the stage for the repair process [5]. Although inflammation acts as a warning for microbial infection, allergens, burns and noxious stimuli to the body it causes much discomfort to the victims and hence lowering their productivity [6].

The most commonly prescribed anti-inflammatory drugs are Non-Steroidal Anti-inflammatory Drugs [NSAIDs] like diclofenac, indomethacin, meloxicam, ibuprofen and sulindac [7]. These drugs inhibit the activity of cyclooxygenase [COX-2] enzyme that converts arachidonic acid to the key inflammatory mediator, prostaglandins [8]. However, NSAIDs are expensive, have low efficacy [9] and have been reported to have various severe side effects like adverse effects on the kidney, liver, heart, spleen, the gastrointestinal tract, and the coagulation system [10].

These drawbacks of the conventional drugs have therefore triggered continuous research, especially on medicinal plants to discover new compounds as therapeutic alternatives [11]. Naturally occurring medicinal plants are a rich source of anti-inflammatory formulations and are the best alternatives because of their lesser side effects, affordability, and ready availability [12]. One of the important medicinal plants is *Terminalia brownii* belonging to the family Combretaceae. It is widely distributed in Africa, Kenya, Ethiopia, Tanzania and the Democratic Republic of Congo [13]. *T. brownii* has a wide range of medicinal uses in African traditional medicine. It is used to treat body swellings, malaria, epilepsy, urogenital problems, stomach ache, jaundice, liver cirrhosis and colic [13].

Although *T. brownii* is extensively used traditionally for the treatment of inflammation, there is no scientific validation of this ethnomedical claim. This study, therefore, evaluated the anti-inflammatory properties of the methanolic bark extracts of *T. brownii*.

MATERIALS AND METHODS

Collection and preparation of plant materials

The bark samples of *T. brownii* were collected from regional areas of Kitui County, Kenya with the help of a local herbalist and authenticated by taxonomist from the National Museums of Kenya. The samples were then packed in polythene bags and transported to the Biochemistry and Biotechnology laboratories at Kenyatta University [KU] where they were shade dried at room temperature before grinding them into fine homogeneous powder.

Solvent extraction

300 g of the sample’s powder was soaked in 2 L of methanol and stirred for 6 h. The fraction was separated using a muslin cloth and filtered through Whatman filter paper No 1. The filtrate was concentrated using the Buchi rotary evaporator [R-200, Switzerland] and the concentrate was stored at 4 °C in airtight containers before use in bioassay studies.

Experimental animals

The present study used 2-3 months old adult male Wister albino rats weighing 140-180 g [15]. These experimental animals were obtained from the Department of Biochemistry and Biotechnology breeding unit. The experimental rats were put in standard cages and allowed to acclimatize for two days under standard laboratory conditions before the experiments begun. The experimental rats...
were fed on rodent pellets and were provided with water ad libitum [16]. The experimental procedures were in compliance with the recommendations of the internationally accepted principles for using animal models and also with the ethics committee of the institution on research on animal models.

**Sensory motor test**

The sensory motor test was conducted on every experimental rat after the administration of the respective treatments as the exclusion criteria for the rats that may have been paralyzed as described by [17].

**Anti-inflammatory assay**

Carrageenan [Sigma type, Batch No. C1013-2259] was used to induce paw edema [18]. Diclofenac [Bhumi Pharmaceuticals, India] was used as the reference drug. Thirty rats were randomly divided into six groups of five rats each and the treatments were carried out as shown in table 1.

**Table 1: Treatment protocol used for the evaluation of anti-inflammatory activities of methanolic bark extracts of *T. brownii* in *R. norvegicus***

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>Carrageenan+DMSO</td>
</tr>
<tr>
<td>III</td>
<td>Positive control</td>
<td>Carrageenan+DMSO+15 mg/kg diclofenac</td>
</tr>
<tr>
<td>IV</td>
<td>Experimental group A</td>
<td>Carrageenan+DMSO+50 mg/kg extract</td>
</tr>
<tr>
<td>V</td>
<td>Experimental group B</td>
<td>Carrageenan+DMSO+100 mg/kg extract</td>
</tr>
<tr>
<td>VI</td>
<td>Experimental group C</td>
<td>Carrageenan+DMSO+150 mg/kg extract</td>
</tr>
</tbody>
</table>

Before inflammation was induced, an SDC041 digital vernier calipers [Xuzhou Smile Trading Company Ltd., Jiangsu, China] was used to measure the initial paw diameter [mm]. 0.1 ml of 1% carrageenan was then injected subcutaneously into the sub-plantar tissue of the left hind paw 1 h after administration of the various treatments.

The normal control group received no treatment; the negative control group received 10% dimethyl sulfoxide [DMSO] in normal saline; the positive control group received 15 mg/kg Diclofenac and the three experimental groups received the extract at the dose levels of 50, 100 and 150 mg/kg bw. Paw diameter was then measured 1 h after induction of inflammation up to the 4th h [19]. Paw diameter measured prior to the carrageenan injection was then compared with the diameter of the same paw after carrageenan injection by calculating the percentage inhibition and percentage change using the formulae below:

\[
\text{% inflammation inhibition} = \frac{\text{Ct} - \text{Tt}}{\text{Ct}} \times 100
\]

Where,

\(\text{Ct} = \text{Paw diameter at 1 hour after Carrageenan administration}\)

\(\text{Tt} = \text{Paw diameter after Treatment}\)

**Qualitative phytochemical screening**

Methanolic bark extract of *T. brownii* was subjected to qualitative phytochemical screening as described by [20, 21] to test for the presence or absence of selected phytochemical secondary metabolites. The secondary metabolites that were tested for include; saponins, flavonoids, terpenoids, phenolics, alkaloids, and sterols.

**Statistical analysis**

The results were expressed as mean±standard error of the mean. Statistical variances were assessed using ANOVA. Significant differences (p<0.05) between the means were identified by Turkey’s test using the Minitab statistical software version 17.1.0 [Penn State University, 1972].

**RESULTS**

The anti-inflammatory activity of methanolic bark extracts of *T. brownii* on carrageenan-induced edema in *R. norvegicus*

The methanolic bark extract of *T. brownii* demonstrated anti-inflammatory activity on the carrageenan-induced paw edema in the experimental rats, and this was indicated by the reduction of the paw diameter after the administration of the treatments [fig. 1, table 2]. In the first hour after administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug, diclofenac, did not demonstrate anti-inflammatory activity; -4.11%, -5.39%, -3.08% and -0.28% respectively [fig. 1, table 2]. The extract at the three dose levels showed no significant difference [p>0.05, table 2] and at the dose levels of 50 and 150 mg/kg bw, the extract was comparable to the reference drug [p>0.05, table 2]. In this hour, the extract at the three dose levels did not exhibit a significant difference when compared to the negative control group [p>0.05, fig. 1 and table 2].

**Table 2: Anti-inflammatory activity of methanolic bark extracts of *T. brownii* in *Rattus norvegicus***

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>% Change in paw diameter after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Normal Control</td>
<td>DMSO</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Carrageenan+DMSO</td>
<td>[0.00±0.00]</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Carrageenan+Diclofenac+DMSO</td>
<td>[0.00±0.00]</td>
</tr>
<tr>
<td>Experimental Group A</td>
<td>Carrageenan+50 mg/kg+DMSO</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>Experimental Group B</td>
<td>Carrageenan+100</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>Experimental Group C</td>
<td>Carrageenan+150</td>
<td>100.00±0.00</td>
</tr>
</tbody>
</table>

The values were expressed as mean±SEM for five rats per group. Statistical comparisons were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey’s post hoc test [p > 0.05]. Percentage reduction in paw diameter is given in the brackets. Carrageenan = 1%; DMSO = 10%; Diclofenac = 15 mg/kg.
The present study evaluated the anti-inflammatory activity of methanolic bark extracts of *T. brownii* on carrageenan-induced paw edema which is one of the most feasible methods used to screen anti-inflammatory agents [22]. Freund's adjuvant [23], dextran [24], cotton pellet granuloma [6] and formalin [25] are other inflammatory models. Carrageenan-induced paw edema is a simple and routine model for the evaluation of inflammation [26] and produces an inflammatory response in two phases [27, 28] and hence, the phlogistic agent of choice in this study.

Carrageenan is a natural carbohydrate derived from a number of seaweeds of the class Rhodophyceae [26]. Subplantar injection of carrageenan in the rat hind paw induced a biphasic edema; the early and late phases [29, 30]. The key inflammatory mediators detectable during the early phase [1 hour] of the carrageenan-induced edema include; serotonin, histamine and kinins [22]. The late phase occurs after the first one hour of the carrageenan-induced edema and the key mediators detectable in this phase include prostaglandins and inducible Cox-2 [26].

The methanolic bark extracts of *T. brownii* demonstrated a significant anti-inflammatory activity on the carrageenan-induced hind paw edema in *Rattus norvegicus*. These findings were consistent with the findings of previous similar studies [8, 31, 32, 33]. Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as indomethacin, aspirin and diclofenac are the conventional drugs used to manage inflammation [11]. The anti-inflammatory effect of NSAIDs is attributed to their inhibitory effect on the activity of a COX-2 enzyme that converts arachidonic acid to the inflammatory mediator prostaglandins [8]. Two types of COX enzymes exists; COX-1 enzyme, produces prostaglandins responsible for supporting platelets and protecting the stomach, and COX-2 enzyme, produces prostaglandins responsible for inflammation [34]. Therefore, NSAIDs inhibit only the late phase of carrageenan-induced inflammation where prostaglandins and COX-2 enzymes are the detectable mediators [26]. It can, therefore, be suggested that the methanolic bark extracts of *T. brownii* reduced the carrageenan-induced paw edema by inhibiting the activity of the COX-2 enzyme.

The dose levels used in this study for the evaluation the anti-inflammatory activities of the methanolic bark extracts of *T. brownii* were 50, 100 and 150 mg/kg bw. These dose levels were similar to dose ranges used in other similar previous studies [35, 36, 37]. The methanolic bark extracts of *T. brownii* demonstrated a dose-dependent response on the carrageenan-induced paw edema. A similar trend was also observed by [11] in their study on the evaluation of the anti-inflammatory properties of DCM: methanolic extracts of *Coelosiplana volteni* and *M. obscura* in mice. The study on the evaluation of the anti-

In the second hour after the administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema in the left hind paw of the experimental rats by 2.88%, 5.82%, 14.65% and 20.69% respectively [Fig. 1, table 2]. The three dose levels of the extract exhibited a dose-dependent response [Fig. 1, table 2]. The anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was significantly different from the anti-inflammatory activity of the extract at the dose levels of 50 and 100 mg/kg bw [p < 0.05, table 2]. Compared to the reference drug, the extract at the dose level of 150 mg/kg bw did not show a significant difference [p>0.05, table 2]. A significant difference was noted when the extract at the three dose levels and the reference drug were compared to the negative control group [p < 0.05, Fig. 1 and table 2].

In the third hour after the administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema by 5.56%, 11.21%, 20.14% and 25.33% respectively [Fig. 1, table 2]. The extract at the three dose levels demonstrated a dose-dependent response [Fig. 1, table 2]. The anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was significantly different from the anti-inflammatory activity of the extract at the dose levels of 50 and 100 mg/kg bw [p < 0.05, table 2]. However, compared to the reference drug, the extract at the dose level of 150 mg/kg bw did not show a significant difference [p>0.05, table 2]. In this hour, the reference drug and the extract at the three dose levels demonstrated a significant difference when compared to the negative control group [p < 0.05, Fig. 1 and table 2].

In the fourth hour after the administration of the treatments, the methanolic bark extracts of *T. brownii* at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema by 11.12% respectively and routine model for the evaluation of inflammation [26] and produces an inflammatory response in two phases [27, 28] and hence, the phlogistic agent of choice in this study. Carrageenan is a natural carbohydrate derived from a number of seaweeds of the class Rhodophyceae [26]. Subplantar injection of carrageenan in the rat hind paw induced a biphasic edema; the early and late phases [29, 30]. The key inflammatory mediators detectable during the early phase [1 hour] of the carrageenan-induced edema include; serotonin, histamine and kinins [22]. The late phase occurs after the first one hour of the carrageenan-induced edema and the key mediators detectable in this phase include prostaglandins and inducible Cox-2 [26].

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**Phytochemical screening**

Qualitative phytochemical screening of the methanolic bark extracts of *T. brownii* showed the presence of steroids, flavonoids, alkaloids, phenols, saponins and terpenoids.

**Fig. 1: Anti-inflammatory activity of methanolic bark extracts of *T. brownii* in *Rattus norvegicus***
inflammatory activity of the methanolic extract of *Rhodiola rosea* L. Rhizomes also demonstrated a dose-dependent response on the carrageenan-induced paw edema [22]. Evaluation of the *in vivo* anti-inflammatory activities of *Caesalpinia bonducella* F. also demonstrated a dose-dependent response [8].

The methanolic bark extract of *T. brownii* demonstrated a minimal anti-inflammatory activity at the lower dose levels of 50 and 100 mg/kg body weight as compared to the highest dose level of 150 mg/kg body weight and the reference drug in all the four hours after treatment. These findings indicate that the extract at the dose level of 150 mg/kg was able to inhibit the activity of COX-2 better than the lower dose levels probably due to the presence of a sufficient concentration of the active principle. However, the anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was comparable to the anti-inflammatory activity of the standard drug indicating that the two treatments inhibited the activity of COX-2 with a similar magnitude.

The extract at the three dose levels achieved maximum anti-inflammatory activity in the fourth hour indicating a slow but steady passive diffusion of the bioactive chemical constituents across the cell membrane into the peritoneal cavity [38]. However, the reference drug and the extract at the three dose levels did not inhibit the carrageenan-induced edema during the first hour. This can be explained by the absence of prostaglandins in this early phase of inflammation since the treatments were working by inhibiting the biosynthesis of prostaglandins just like the NSAIDs [26].

The phytochemical screening of the methanolic bark extracts of *T. brownii* revealed the presence of various phytochemicals in the extract some of which could have been responsible for its anti-inflammatory activity. Flavonoids inhibit the activity of the prostaglandin enzyme synthetase and hence, their anti-inflammatory activity [22]. Flavonoids have also been reported as potent anti-inflammatory agents in another study [39]. Steroids reduce inflammation by inhibiting phospholipase A2 which is responsible for the hydrolyzation of arachidonic acid from the membrane phospholipids leading to the formation of prostanoids and leukotrienes [40]. Triterpenoids inhibit the production of prostaglandins and also suppresses the function of macrophages and neutrophils, hence their anti-inflammatory activity [41]. This study, therefore, suggested that the flavonoids, steroids, and terpenoids observed in the extract, acting either individually or synergistically, could have been responsible for its anti-inflammatory activity.

**CONCLUSION**

In summary, the results obtained in our study revealed significant anti-inflammatory properties of methanolic bark extracts of *T. brownii*. We showed that the carrageenan-induced inflammation in rats was considerably ameliorated, in a dose-dependent trend, by the use of *T. brownii* methanolic extract. The anti-inflammatory activity of the extract was comparable to the anti-inflammatory activity of the reference drug. The methanolic bark extracts of *T. brownii* could, therefore, be an alternative bio-resource for the development of anti-inflammatory drugs in animals [22]. Flavonoids have also been reported to be effective individually or synergistically, could have been responsible for its anti-inflammatory activity.

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**CONFLICT OF INTERESTS**

Authors declare that no conflicting interests exist.

**REFERENCES**


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