

Anti-Pyretic Properties of Methanolic Bark Extracts of *Terminalia brownii* in Wistar Rats (*Rattus novogicus*)

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Abstract

The conventional drugs used to manage fever are usually not affordable, not easily available and have adverse side effects. Alternative therapeutic agents, like medicinal plant derivatives, should therefore be developed because they have been reported to be more affordable, more readily available and have lesser side effects. *Terminalia brownii* is traditionally used to manage fever but this ethno-medicinal claim lacks scientific validation. The present study therefore evaluated the anti-pyretic activity of *T. brownii* in Wistar rats. Fresh bark samples of *T. brownii* were collected from Kitui County, Kenya. This study used 30 adult male Wistar rats that were 2-3 months old and weighing 140-180 g was used for the experiments. Steam-distilled turpentine was the pyrogen used to induce pyrexia and Aspirin was used as the reference drug. The extract reduced the elevated rectal temperatures by between 1.15-4.38% while aspirin reduced the elevated rectal temperatures by between 0.00-4.85%. The present study showed a significant dose-dependent anti-pyretic activity of methanolic bark extracts of *T. brownii* hence validating its folklore use as a fever remedy.

Keywords: Anti-pyretic activity; Aspirin; Methanolic bark extracts; *Terminalia brownii*

Introduction

Microbial infections, body tissue damage, chronic diseases and graft rejection elevates the body temperatures beyond the normal range of between 36.5-37.5°C, leading to pyrexia or fever [1,2]. Cytokines such as tumor necrosis factor α and β (TNF α and TNF β) and interleukins (IL-6, IL- α and IL- β) produced when the body is invaded by micro-organisms or body tissues are damaged stimulates the conversion of arachidonic acid to prostaglandins, the major fever mediator, in the hypothalamus [3]. Prostaglandins in turn trigger the hypothalamus to produce responses that elevate the body temperatures [2]. Although pyrexia causes unnecessary suffering and discomfort, it is considered as a natural defense mechanism that creates an environment where infectious agent or damaged tissue cannot survive [4].

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are used in the management of pyrexia [5]. However, their use is declining due to their adverse gastrointestinal effects like bleeding, perforations and peptic ulcers [6]. These conventional drugs have also been reported to be unaffordable and less effective [7]. Continuous search for alternative therapeutic agents with much lesser adverse effects is therefore necessary. Naturally occurring plant-based derivatives are considered as better alternatives due to their lesser side effects, ready availability and inexpensive nature [8]. Herbal medicine is increasingly gaining popularity. Currently, approximately 25% of available synthetic drugs are formulated either directly or indirectly from medicinal plants [9].

Terminalia brownii [Combretaceae] is native in Kenya, Democratic Republic of Congo, Tanzania and Ethiopia [10]. The tree grows near rivers and wadies in dry areas and in moist savannas of semi-arid regions, thriving best on sandy loam soils [11]. *T. brownii* is used as a folklore remedy for jaundice, stomach ache, urogenital infections, malaria, gastric ulcers, epilepsy, cough, hepatitis and liver cirrhosis in different parts of Eastern and Central Africa [10]. Although *T. brownii* is used as a traditional remedy for fever, no studies have been conducted

to validate this ethno-medicinal claim. The present study was therefore aimed at evaluating the anti-pyretic properties of methanolic bark extracts of *T. brownii*.

Materials and Methods

Collection and preparation of plant materials

With the help of a local herbalist, fresh bark materials were collected from Kitui County, Kenya. The samples were sorted, cleaned and packed in polythene bags and transported to the Biochemistry and Biotechnology laboratories at Kenyatta University (KU) for further processing. A voucher specimen of the tree species was collected and botanically authenticated at the East African Herbarium. The samples were cut into small pieces and dried at room temperature for two weeks before being ground into a fine homogenous powder using an electric mill.

Extraction

To obtain the extract, 2 L of methanol was used to soak 400 g of the sample's powder. The mixture was stirred for 6 h and left to stand for 24 h. The extract was then soaked through a Whatman's filter paper (No. 1) and the percolate concentrated under reduced pressure and vacuum using R-200 Buchi rotary evaporator (Sigma Aldrich, Switzerland). The concentrate was packed in airtight containers and stored at 4°C.

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Received July 25 2016; **Accepted** August 19, 2016; **Published** August 24, 2016

Citation: Mbiri JW, Kasili S, Mbinda W, Kisangau PD, Piero NM (2016) Anti-Pyretic Properties of Methanolic Bark Extracts of *Terminalia brownii* in Wistar Rats (*Rattus novogicus*). J Pharmacogn Nat Prod 2: 121. doi:10.4172/2472-0992.1000121

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Experimental animals

The rules for caring and using experimental animals in laboratories [12] and the standards set by the Kenyatta University's ethics committee were keenly followed. 30 adult male Wistar rats weighing 140-180 g and 2-3 months old [13] were used to evaluate the anti-pyretic properties of *T. brownii*. The rats were placed in standard cages in the KU's animal house where they were left to acclimate for 1 wk under standard laboratory conditions before being used for the experiments. The rats were fed on standard commercial food for rodents and provided with water spontaneously [14].

Anti-pyretic assay

The antipyretic activity of *T. brownii*'s methanolic bark extracts was evaluated on steam-distilled turpentine-induced pyrexia in *Rattus norvegicus* [2]. Aspirin (Zhongshan Yuanhang E-Commercial Co., Ltd., Guangdong, China), dissolved in 10% DMSO, was used as the standard drug. The rats were divided into six groups with each group having 5 rats. Table 1 displays a summary of the treatments.

In this assay, the weights and initial rectal temperatures of the rats were taken before fever was induced. A properly lubricated thermistor probe of a YB-009 digital thermometer (Shenzhen Osykyoo Technology Co., Ltd., Guangdong, China) was imbedded about 3 cm in to the rectum [15] to take the rectal temperatures. After obtaining the initial rectal temperatures, fever was induced by intraperitoneal administration of 25 ml/kg bw steam-distilled turpentine.

The rats that experienced a temperature rise by 0.8°C after an hour were considered pyretic and therefore given the respective treatments. Fever was not induced in the normal control group but it was induced in the rest of the groups. The normal control group therefore received no treatment, negative control group received 0.5 ml of 10% DMSO, positive control group received 100 mg/kg bw Aspirin, experimental group A received 50 mg/kg bw extract, experimental group B received 100 mg/kg bw extract and experimental group C received 150 mg/kg bw extract.

Rectal temperatures were recorded at hourly intervals up to the fourth hour after administration of the treatments. Rectal temperatures before and after treatments were compared by calculating percentage inhibition using the following formula [16];

$$\frac{B - C_n}{B} \times 100$$

Where,

B: Rectal temperature, 1 h, after turpentine administration

C_n: Rectal temperature after drug administration

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

Table 1: Treatment procedure for the evaluation of antipyretic activities of methanolic bark extracts of *T. brownii* in *R. norvegicus*.

Statistical analysis

The data obtained was recorded and tabulated on Excel spreadsheet and the Minitab statistical software version 17.1.0 was used to analyze the data. The results were presented as mean ± standard error of mean (SEM). The statistical significance difference among the various groups was evaluated using ANOVA (One Way) and this was followed by Turkey's tests to separate the means and get the precise significant differences among the different groups. Values of $p \leq 0.05$ were regarded as significant.

Results

The methanolic bark extracts of *T. brownii* showed antipyretic activity on the turpentine-induced pyrexia in rats and this was indicated by reduction in the rectal temperatures after extract administration (Figure 1, Table 2). In the 1st h, the methanolic bark extracts of *T. brownii* at the dose levels of 50, 100 and 150 mg/kg bw and aspirin, the reference drug, lowered the elevated rectal temperatures by 1.15%, 2.76%, 4.28% and 4.85% respectively (Table 2). The extract at the three dose levels showed a dose dependent response (Figure 1, Table 2). The antipyretic activity of the extract among the three dose levels was significantly different ($p < 0.05$, Table 2). The antipyretic activity of the plant extract at 150 mg/kg bw dose level was commensurable to that of the reference drug ($p > 0.05$, Table 2). The extract at the three dose levels was significantly different from the negative control group ($p < 0.05$, Table 2).

In the 2nd h after administration of the treatments, the *T. brownii* extract at the dose levels of 50, 100 and 150 mg/kg bw and Aspirin, reference drug, decreased the elevated rectal temperatures by 2.19%, 2.66%, 4.38% and 4.22% respectively (Figure 1, Table 2). The extract at the three dose levels demonstrated a dose dependent response (Figure 1, Table 2). The antipyretic activity of the extract at the dose of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at 50 and 100 mg/kg bw dose levels ($p < 0.05$, Table 2). In this hour, the antipyretic activity of the extract at 100 and 150 mg/kg bw dose levels was as good as that of Aspirin ($p > 0.05$, 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 the 3rd h, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the turpentine-induced pyrexia by 1.98%, 2.55%, 4.02% and 4.01% respectively (Figure 1, Table 2). The extract at the three dose levels showed a dose dependent response (Figure 1). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at the dose levels of 50 and 100 mg/kg bw ($p < 0.05$, Table 2). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was comparable to that of the standard drug ($p > 0.05$). The extract at the three dose levels was significantly different from the negative and normal control groups ($p < 0.05$, Table 2).

In the 4th h 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 elevated rectal temperatures by 2.55%, 2.65%, 3.76% and 4.01% respectively (Figure 1 and Table 2). The extracts of *T. brownii* at the three dose levels demonstrated a dose dependent response on the turpentine oil-induced pyrexia (Figure 1). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at the dose level of 50 mg/kg bw ($p < 0.05$). Compared to aspirin and the extract at 100 mg/kg bw dose level, the extract at the dose level of 150 mg/kg bw did not show a significant difference ($p > 0.05$, Table 2). The extract at the three dose levels was significantly different from the negative and normal control

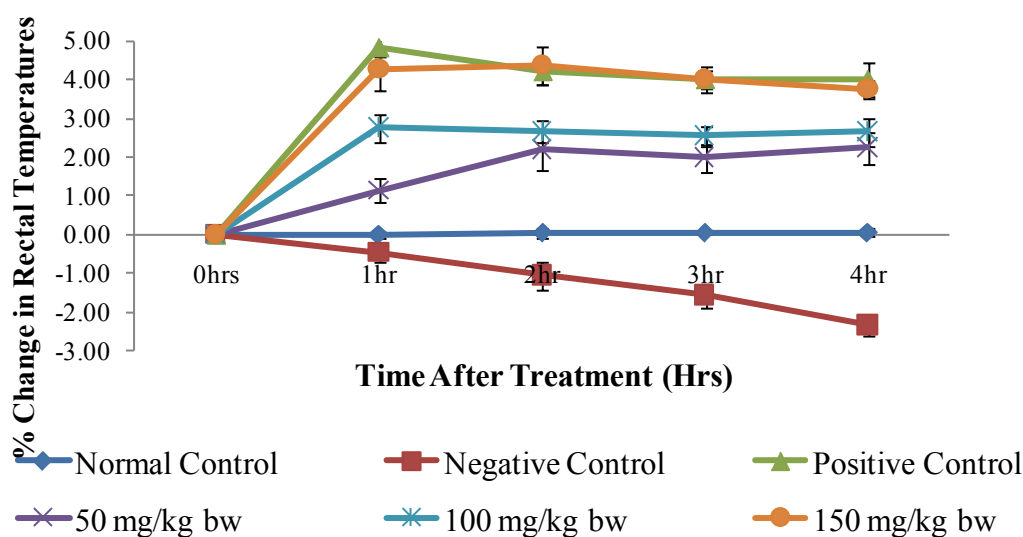


Figure 1: Antipyretic activity of the methanolic bark extracts of *T. brownii* in *R. novogicus*.

Group	Treatment	% Change in Rectal Temperatures after Treatment				
		0 h	1 h	2 h	3 h	4 h
Normal Control	DMSO	100.00 ± 0.00 (0.00%)	100.00 ± 0.09 ^{ab} (0.00%)	99.95 ± 0.13 ^a (0.05%)	99.95 ± 0.05 ^b (0.05%)	99.95 ± 0.10 ^b (0.05%)
Negative Control	Turpentine + DMSO	100.00 ± 0.00 (0.00%)	100.47 ± 0.23 ^a (-0.47%)	101.04 ± 0.36 ^a (-1.05%)	101.57 ± 0.30 ^a (-1.57%)	102.35 ± 0.24 ^a (-2.35%)
Positive Control	Turpentine + Aspirin + DMSO	100.00 ± 0.00 (0.00%)	95.42 ± 0.23 ^d (4.85%)	95.78 ± 0.32 ^{cd} (4.22%)	95.99 ± 0.34 ^d (4.01%)	95.99 ± 0.46 ^d (4.01%)
Experimental Group A	Turpentine + 50 mg/kg + DMSO	100.00 ± 0.00 (0.00%)	98.90 ± 0.29 ^b (1.15%)	97.81 ± 0.50 ^b (2.19%)	98.02 ± 0.37 ^c (1.98%)	97.76 ± 0.40 ^c (2.25%)
Experimental Group B	Turpentine + 100 mg/kg + DMSO	100.00 ± 0.00 (0.00%)	97.24 ± 0.35 ^c (2.76%)	97.34 ± 0.29 ^{bc} (2.66%)	97.45 ± 0.27 ^c (2.55%)	97.35 ± 0.35 ^{cd} (2.65%)
Experimental Group C	Turpentine + 150 mg/kg + DMSO	100.00 ± 0.00 (0.00%)	95.72 ± 0.55 ^d (4.28%)	95.62 ± 0.48 ^d (4.38%)	95.99 ± 0.23 ^d (4.02%)	96.24 ± 0.26 ^d (3.76%)

The values were expressed as Mean ± SEM for five rats per group. Statistical comparisons were done within a column and values with a similar superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Percentage reduction in rectal temperature is given within the brackets. Turpentine oil=25 ml/kg BW; DMSO=10%; Aspirin=15 mg/kg

Table 2: Antipyretic activity of methanolic bark extracts of *T. brownii* in *R. novogicus*.

groups ($p < 0.05$, Table 2). The extract at the dose level of 150 mg/kg bw exhibited the highest antipyretic activity in all the four hours.

Discussion

Turpentine oil is an exogenous pyrogen that is widely used to induce fever in experimental rats and mice [17]. Other exogenous pyrogens that have been used to induce fever include; brewer's yeast [18], Lipopolysaccharides [19] and polyinosinic: Polycytidylic acid [20]. Steam-distilled turpentine induces pyrexia faster [within an hour or two], experimental animals acquire a higher tolerance to it than to other exogenous pyrogens and produces a steady pyrexia trend [2]. It is against this background that turpentine oil was chosen as a model for screening for the antipyretic activity of the methanolic bark extracts of *T. brownii* in this study. In the present study, crude turpentine was distilled to eliminate impurities and obtain pure turpentine oil. These impurities could have interfered with the tests or even killed the experimental rats as it was observed in the pilot study.

The most commonly prescribed drugs for the management of pyrexia are the NSAIDs such as diclofenac, aspirin and ibuprofen [17]. NSAIDs inhibit the activity of the enzyme Cyclooxygenase (COX-2)

whose function is to convert arachidonic acid to PGE₂ [21]. PGE₂ are the main fever mediators [2]. The findings of this study therefore suggest that the methanolic bark extract of *T. brownii* was able to inhibit the activity of COX-2 enzyme hence its antipyretic activity.

The methanolic bark extracts of *T. brownii* demonstrated a significant antipyretic activity in all the four hours after the administration of the treatments. These results relate to the findings of [22] who demonstrated a significant antipyretic activity of some medicinal plants from Cholistan desert Pakistan, [23] who affirmed significant antipyretic properties of virgin coconut oil and [24] who showed that *Azima tetracantha's* friedelin possess significant antipyretic effect. The dose levels of the extract used in the present study to evaluate the antipyretic activity of *T. brownii* were 50, 100 and 150 mg/kg bw and were similar to those used [17,25,26]. The methanolic bark extracts of *T. brownii* exhibited a dose-dependent response on the turpentine oil-induced pyrexia in the experimental rats. These findings were consistent with the findings of other studies on medicinal plants from Cholistan desert Pakistan [22], *Piper cubeba* L. essential oil [5] and ethyl acetate roots extracts of *Ocimum sanctum* [27].

The extract at the dose level of 150 mg/kg bw demonstrated the highest antipyretic activity compared to the dose levels of 50 and 100 mg/kg bw in all the four hours after the administration of the treatments. This can be explained by the possible existence of an adequate concentration of the active principle(s) in the dose level of 150 mg/kg bw in comparison to the lower dose levels of the extract. It can also be explained by the quick metabolism and expulsion of the effective principle(s) present in inadequate concentrations in the lower dose levels of the extract. The reference drug, Aspirin, demonstrated the highest antipyretic activity compared to the three dose levels of the extract. This can be suggested to be as a result of the reference drug exhibiting a better blockage of the biosynthesis of prostaglandins than the extract [17].

The extract at the dose levels of 100 and 150 mg/kg bw and the standard drug achieved their maximum antipyretic activity in the 1st, 2nd and 3rd h respectively and then their activity decreased subsequently. This could probably be due to the metabolism and excretion of the respective treatments. However, the extract 50 mg/kg bw dose level achieved its maximum activity at the fourth hour. This could be due to the gradual diffusion of the functional principle(s), present in very low concentrations in the extract, across the cell membrane into the peritoneal cavity [28].

Several studies have revealed a number of phytochemical secondary metabolites present in *T. brownii*. The phytochemical screening of methanolic bark extracts of *T. brownii* conducted by Wanja et al. [29] affirmed the presence of terpenoids, phenols, steroids, saponins and flavonoids in that extract. Methanolic leaf extracts of *T. brownii* have been reported to possess phytosterols, coumarins, flavonoids, tannins, polyphenols, and saponins [30]. Fourier Transform Infrared (FTIR) spectroscopy of crude dry powder of *T. brownii* revealed the presence of saponins [31]. Phytochemical screening of ethyl acetate stem bark extract of *T. brownii* revealed the presence of 3-O- β -D-glucopyranosyl- β -sitosterol, an oleanane-type triterpenoid, seven ellagic acid derivatives and seven known triterpenoids [32]. Ethyl acetate and n-hexane extracts of *T. brownii* revealed the presence of five compounds; arjungenin, β -sitosterol, betulinic acid, monogynol A and stigmasterol [33].

The anti-pyretic activity of the methanolic bark extract of *T. brownii* could therefore be attributed to the activity of one or more of the phytochemical secondary metabolites that have been reported to be present in this tree. Steroids and terpenoids have been reported to inhibit the activity of prostaglandin synthetase, the enzyme that stimulates the production and release of prostaglandins, the primary mediator in fever induction [34]. Flavonoids can inhibit fever in two ways; by decreasing the release of arachidonic acid or by interfering with the eicosanoids biosynthesis pathways involved in fever production [6]. These two actions in turn suppress mediators like prostaglandins responsible for fever [6]. Saponins also have antipyretic activity [35]. The present study therefore suggests that the methanolic bark extracts of *T. brownii* demonstrated an anti-pyretic activity due to the presence of steroids, terpenoids, flavonoids and saponins that have been revealed to possess anti-pyretic activity.

Conclusion

Significant anti-pyretic activity of the methanolic bark extract of *T. brownii* was demonstrated in the present study. This study concludes that the anti-pyretic activity of the extract could be due to the presence of bioactive principles with a pharmacological potential. The extract demonstrated a dose-dependent response to the turpentine

oil-induced fever with its highest anti-pyretic activity at 150 mg/kg bw dose. The anti-pyretic activity of the extract at 150 mg/kg bw dose level was similar to the anti-pyretic activity of Aspirin. The barks of *T. brownii* can therefore be considered as a probable candidate that can be used to develop a neoteric anti-pyretic formulation that is more affordable, readily available and has less adverse effects. The present study therefore supports and provides a scientific validation of the local use of *T. brownii* as a remedy for fever.

Acknowledgement

The authors are thankful to the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya, for availing their facilities to carry out this research.

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Citation: Mbiri JW, Kasili S, Mbinda W, Kisangau PD, Piero NM (2016) Anti-Pyretic Properties of Methanolic Bark Extracts of *Terminalia brownii* in Wistar Rats (*Rattus novogicus*). J Pharmacogn Nat Prod 2: 121. doi:10.4172/2472-0992.1000121

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