Sesamum indicum leaf extract therapy for symptoms of acute malaria: Evaluation of anti-inflammatory, antipyretic and analgesic effects

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Abstract

As part of traditional medicine practice in some parts of Africa, an extract of Sesamum indicum leaves is drunk as a remedy for symptoms of malaria. This study was aimed at evaluating the effects of a standardized ethanol extract of S. indicum leaves on inflammation, fever and pain in mice and rats. The extract was prepared by maceration in 70% v/v ethanol; its total phenol content and HPLC profile were determined. Anti – inflammatory and antipyretic properties were evaluated using formalin – induced rat paw edema and Brewer’s yeast - induced pyrexia models in rats, while analgesic effect was assessed using acetic acid - induced abdominal writhing and tail flick tests in mice. The extract had a total phenolic content of 0.088 mg gallic acid equivalents per milligram, while its HPLC profile revealed gallic acid (7.75%), caffeic acid (6.08%), rutin (5.72%) and morin (0.98%) as some of its phenolic components. At 400 and 800 mg/kg doses, the extract reduced formalin-induced rat paw edema within 1 hour, by 38.89% (P < 0.001) and 33.33% (P < 0.01) respectively. It also significantly (P<0.05) reduced Brewer’s yeast-induced fever within 2 hours and significantly (P<0.05) inhibited acetic acid – induced abdominal writhes by 31.49 – 41.35 %, similar to a dose of 300 mg/kg aspirin. In the tail flick test for analgesic activity against thermal nociception, the extract increased response time by 13.87 - 47.82% (P>0.05), compared to morphine (10 mg/kg) which significantly (P < 0.01) increased response time by 72.75%. These findings show that S. indicum leaf extract possesses anti-inflammatory, antipyretic and analgesic effects, which may be due to the presence of bioactive phenolic compounds. These activities may likely be mediated by peripheral inhibition of prostaglandins. This study supports its use as remedy for malaria symptoms and indicates its potential as a candidate for further development.

Keywords: Fever; inflammation; pain; phenolics

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1.0 Introduction

Inflammatory processes underlie the pathophysiology of most communicable and non-communicable diseases. Most infectious diseases elicit inflammatory responses in the host, which are usually symptomized as weakness, aching muscles and fever. Malaria infection caused by *Plasmodium* species is characterized by the release of inflammatory toxins like hemozoin (Hz) and glycosylphosphatidylinositol (GPI) by the parasite. These trigger inflammations characterized by the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF) and interleukin-1 (IL-1) by the infected host [1]. These cytokines play a role in regulating homeostasis, but can also cause pathological changes when excessively released in some cases like malaria. Effective treatment to eliminate parasites abolishes deregulated action of these cytokines and restores normal homeostatic functions. However, the efficacy of conventional antimalarial therapy is gradually declining due to the emergence and spread of drug-resistant parasites in malaria-endemic regions [2]. Thus, there is a need to continue searching for effective alternatives to treat malaria and its symptoms. Medicinal plants have constituted an important part of human natural wealth for many centuries. Antimalarial drugs like artemisinin and quinine developed from plants were initially investigated on the basis of their observed use in treating fevers and related symptoms in ancient traditional medicine [3, 4]. *Sesamum indicum* L. (syn. *Sesamum orientale*) is an important plant with multiple purposes used for culinary, health and industrial purposes. It is commonly known internationally as sesame, and benniseed or simsim in Africa. *S. indicum* (family: Pedaliaceae) is an erect, herbaceous, annual plant considered to be one of the oldest oilseed crops. It has been cultivated for centuries particularly in Africa and Asia [5]. In Northern Nigeria, the leaves are popular as vegetable in soups [6]. A proximate analysis of the leaves revealed a high protein and crude fibre content of 18.59 and 27.58% respectively [7]. Among its ethnomedicinal uses, hot-water extract of the aerial parts of the plant is used in South Africa for sexual stimulation, while a decoction of the leaves is used for treating malaria [8]. However, to date, only few reports exist in the scientific literature to validate the traditional use of the leaves of this plant. Acute toxicity LD$_{50}$ testing revealed that the leaf extract of *Sesamum indicum* did not cause any mortalities in rats even at doses above 5000 mg/kg [9]. It has also been reported to possess antiulcer, antimicrobial and anti-leishmanial effects [9, 10, 11]. The various pharmacological activities of extracts and isolated compounds from leaves of sesame plant demonstrate its therapeutic potentials in treating metabolic, inflammatory, and infectious disease. Compounds isolated from *S. indicum* leaves include 3-epibartogenic acid, epigallocatechin and kaempferol 3-O-[2-O-(trans-pcoumaroyl)-3-O-α-1-rhamnopyranosyl]-β-d-glucopyranoside, all of which reportedly displayed alpha amylase inhibitory activity [12]. A literature search did not reveal any previous work done on anti-inflammatory or antimalarial activity of *S. indicum* leaf extract. The study is therefore designed to evaluate the effects of *S. indicum* leaf extract on fever, inflammation and pain.
2.0. Materials and Methods

2.1. Drugs and chemicals

Aspirin, Morphine, Brewer’s yeast (BDH, England), gallic acid (Santa Cruz Biotech, Germany), Folin Dennis reagent, formaldehyde, sodium carbonate, phenolic standards; apigenin, caffeic acid, ferulic acid, morin, quercetin and rutin (Sigma Aldrich, Germany).

2.2. Animals

Adult Swiss albino mice (6 weeks old, 20 – 26 g) and Wistar rats (8 weeks old, 100 – 120 g) of either sex were used. They were maintained in the Animal Facility Centre (AFC), National Institute for Pharmaceutical Research and Development (NIPRD) and acclimatized to laboratory conditions for two weeks before the study. They were fed with in-house rodent diet prepared at NIPRD and allowed unrestricted access to clean drinking water. All applicable institutional standard operating procedures and guidelines for the care and use of animals were adhered to [13], as contained in Institutional standard operating procedures on animal use (SOP no. 05:3:06).

2.3. Collection, identification and extraction of plant material

*S. indicum* leaves were collected in October 2017, from a farm in Suleja, Niger state, Nigeria. The plant material was identified by a botanist of the department of Medicinal Plant Research & Traditional Medicine, where a voucher specimen is maintained.

The leaves were air-dried under shade for a period of one week and mechanically pulverized to a coarse powder. An extract was prepared by maceration in 70% v/v ethanol for 24 h. After 24 h, the mixture was filtered and concentrated on a water bath maintained at 50°C to obtain the extract. The extract was then stored at 4°C in a refrigerator and freshly constituted before each test.

2.4. High Performance Liquid Chromatography Analysis

The bioactive constituents of the extracts were analysed by High Performance Liquid Chromatography (HPLC) equipped with a 4.6 × 150 mm VP-ODS C_{18} column (5 μm; Shimadzu Corporation, Kyoto Japan). A 10 μL volume of 100 mg/mL extract solution was injected into the column and isocratically eluted with a mobile phase comprising 0.2% v/v formic acid and acetonitrile (20:80) at a flow rate 0.6 mL/min. Components eluted were detected at a wavelength of 254 nm and total run time was 45 min. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, ferulic acid and morin were employed as standard phenolic constituents by comparing their retention time under similar experimental conditions [14].

2.5. Determination of total Phenolic content

The optimized method of George et al. [15] was adopted with slight modification for phenolic estimation. All tests were carried out in duplicates.
2.5.1. Preparation of gallic acid calibration plot

A 0.5 mg/mL stock solution of gallic acid was prepared and serially diluted (0.03125 – 0.5 mg/mL) with distilled water. To 3 mL of each dilution, 2.5 mL of Folin Denis reagent (diluted 1:9 in distilled water) was added and allowed to stand for two minutes at room temperature. Afterwards, 2 mL of sodium carbonate solution (75 g/L) was added to the mixture and maintained at 50°C for 15 min in a water bath. It was thereafter cooled in ice – cold water for 3 min then diluted with 15 mL of distilled water. Absorbance was read at 760 nm with a UV-Vis spectrophotometer (Cary 60, Agilent Technologies).

2.5.2. Phenolic content assay of extract

A solution of the extract (5 mg/mL) was prepared in distilled water and the test performed as described for gallic acid above. The concentration of phenolics was determined from the standard gallic acid plot.

2.6. Oral acute toxicity

A limit test was done according to the OECD guidelines for oral acute toxicity testing of chemicals [16]. Ten mice were fasted overnight but allowed access to drinking water prior to the test. Following the period of fasting, the body weight of each animal was determined and the dose was calculated according to the body weight. Five mice were given different volumes of 100 mg/mL extract prepared in distilled water, corresponding to a dose of 2000 mg/kg. Five mice served as control and received equivalent volumes of the vehicle. After the extract was administered, food was withheld for a further 1-2 hours. The mice were observed for signs of toxicity at 15 min, 30 min, 1, 2, 4 and 8 h, and then once daily for 14 days. Observations were recorded in the Malone and Robichaud Hippocratic screening table for drug materials [17].

2.7. Anti-inflammatory and antipyretic study

2.7.1. Formaldehyde – induced paw edema

The method of Brownlee [18] was used with slight modification. Acute inflammation was measured as increase of mouse hind paw volume induced by sub-plantar injection of 2.5%v/v formaldehyde solution. The initial volume of distilled water displaced by the right hind paw was measured using a digital plethysmometer (model 37140, Ugo Basile). Mice were randomized into five groups of five each, such that the mean paw volumes of the groups were near equal. Groups 1 and 2 served as negative control (distilled water, 10 mL/kg) and positive control (Aspirin, 300 mg/kg), while groups 3, 4 and 5 received 200, 400 and 800 mg/kg of the extract respectively. One hour after treatment, 2.5%v/v formaldehyde (0.02 mL) was injected into the sub-plantar region of the right hind paw to induce inflammation. The volume of distilled water displaced by the inflamed paw was recorded at 30, 60 and 120 min.

2.7.2. Brewer’s yeast induced pyrexia
Antipyretic activity was evaluated using Brewer’s yeast-induced pyrexia in rats as described by Kumar et al. [19]. Fever was induced by subcutaneously administering 10 mL/kg of 25% w/v aqueous suspension of Brewer’s yeast in normal saline. After 18 h, rats which exhibited a 0.8 – 1°C increase in rectal temperature were randomized into five groups of five rats each. Groups 1 and 2 served as negative control (distilled water, 10 mL/kg) and positive control (Aspirin, 300 mg/kg), while group 3, 4, and 4 received 200, 400 and 800 mg/kg of the extract respectively. Rectal temperature was taken with a digital thermometer before and at 1, 2, 3 and 4 h after treatment.

2.8. Analgesic studies

2.8.1. Acetic acid – induced abdominal writhing

Acetic acid-induced writhing method described by Koster et al. [20] was adopted for the evaluation of analgesic activity. Twenty-five mice were randomized based on body weight into five groups. Groups 1 and 2 served respectively as negative control (distilled water orally, 10 mL/kg) and positive control, the latter receiving an intraperitoneal (i.p.) injection of 10 mg/kg morphine. Groups 3, 4, and 5 received 200, 400, and 800 mg/kg of the extract, orally. Pain was induced with 0.6% v/v acetic acid (10 mL/kg, i.p.) at 30 min (for morphine-treated group) and 1 hour (distilled water and extract treated groups) after treatment. After 5 min, the mice were observed for writhing and the number of writhes by each mouse was counted for 5 min. Percentage inhibition was then calculated using:

\[
\text{Inhibition (\%)} = \frac{C_x - C_t}{C_x} \times 100
\]

Where \( C_x \) = mean writhes of control group, \( C_t \) = mean writhes of test group

2.8.2. Tail flick assay

Tail immersion test was conducted as previously described [21]. Twenty-five mice were randomized into five groups. Groups 1 and 2 served as negative (distilled water 10 mL/kg, orally) and positive (morphine, 10 mg/kg, i.p.) control groups respectively. Groups 3, 4, and 5 received 200, 400, and 800 mg/kg of the extract, respectively. After 30 min (morphine – treated group) and 1 hour (distilled water and extract treated groups), each mouse was held with minimal restraint to allow the distal tail portion to be immersed in a water bath preset at 50.5 ± 0.5°C. Latency to vigorous tail flick was recorded for each mouse. Cutoff time was 20 seconds, after which the mouse was removed regardless of response.

2.9. Data analysis

Results were expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) using Graph Pad Prism 6.0 software for Windows. The data obtained was further subjected to Dunnet’s post hoc test, differences between treated groups and the untreated control was accepted as significant at \( p < 0.05 \).

3. Results

3.1. Phenolic content and HPLC profile

The phenolic content of 5 mg/mL \( S. \) indicum leaf extract was equivalent to 0.088 mg/mL gallic acid, as determined from the gallic acid calibration plot \((y = 9.0468x + 0.0002;\)
R² = 0.9994). Four major peaks were detected as major components in the chromatogram, with respective retention times of 3.545, 3.644, 8.126 and 14.756 minutes (Figure 1). Compounds with retention times of 3.545, 4.763 and 6.739 and 19.23 minutes corresponded to gallic acid (7.75%), caffeic acid (6.08%), rutin (5.72%) and morin (0.98%) respectively.

3.2. Acute toxicity

The extract did not cause any observable sign of toxicity within 8 h after its administration. During the 14 – day inspection period, all the mice exhibited normal behaviour and no mortality was recorded.

3.3. Formaldehyde – induced paw edema

The effect of *S. indicum* on formaldehyde-induced paw edema in mice is presented in Table 1. At 400mg/kg and 800mg/kg doses, it produced a significant (p < 0.01) decrease in mean paw volume in treated mice at 60 min compared to the control group. A similar effect was also produced in the aspirin-treated group, which exhibited a significant (p < 0.001) decrease in mean paw volume of the treated mice at 60 min (Table 1).

3.4. Brewer’s yeast induced pyrexia test

Fever produced by Brewer’s yeast was observed to be significantly (p < 0.05) reduced compared to the control group, following treatment with *S. indicum* (Table 5). This effect was evident at extract doses of 400 and 800 mg/kg as well as in the aspirin-treated groups at 1 – 4 h post-treatment, but significant (p < 0.05) effects were produced from 3 h and upwards. A dose of 200 mg/kg also ameliorated fever, but this effect was statistically insignificant (p > 0.05).

3.5. Acetic acid – induced abdominal writhing

Abdominal writhing response produced by acetic acid injection was reduced in all the *S. indicum*-treated groups, but was observed to be significant (p < 0.05) only in the 200 and 400 mg/kg groups (Table 3). The anti-inflammatory effect produced was comparable with the effect of aspirin, but not with morphine (10mg/kg), which significantly (p < 0.001) inhibited writhing response by 92.31%.

3.6. Tail flick test

The analgesic effect of *S. indicum*-treated groups in response to thermal pain (tail flick) is presented in Figure 2. It caused a non-dose dependent increase in response time, but this effect was not statistically significant (p > 0.05). In contrast however, a dose of 10 mg/kg morphine significantly (p < 0.01) increased response time by 72.75%, compared to the control group.

4.0. Discussion

*Sesamum indicum* leaf extract used as malaria remedy showed no signs of acute toxicity in mice and was able to produce anti-inflammatory, antipyretic and analgesic effects in this study. Inflammatory pain induced by acetic acid injection is caused by
endogenous release of cytokines such as TNFα (tumor necrosis factor α), IL (Interleukin)-1β and chemokines, which act synergistically with other cyclooxygenase products and cysteinyl leukotrienes to induce a writhing response [22, 23, 24]. The characteristic writhing response in this model of pain is reduced in response to pre-treatment with opioids, cyclooxygenase inhibitors, and some natural products [25, 26, 27]. Because of the lack of specificity in this model, other models are often warranted to confirm positive results obtained in the writhing test. The aspirin-like effect of *S. indicum* on acetic acid-induced writhing suggests that its action was likely elicited by inhibition of prostaglandins peripherally. This was supported by the tail flick test which distinguishes compounds with central or peripheral analgesic effects. In this test, thermal pain is mediated mainly by nerve stimulation of nociceptors, in response to an actual or potential noxious stimulus or tissue damaging event [28]. Findings in this study indicate that the analgesic effect produced by *S. indicum* was not comparable with that of the centrally-acting drug morphine, which elicits its analgesic effects by inhibiting the stimulation of nociceptive receptors.

The anti-inflammatory effect of *S. indicum* was also evident against formaldehyde-induced paw edema. The first phase (0 to 5 min) of this test is reported to be caused by the neurogenic action of an inflammatory neuropeptide, substance P, while the second or late phase (10 to 60 min) is thought to be induced by inflammatory cytokines including substance P, prostanoids, 5-hydroxytryptamine and histamine [29]. Both of these phases show an increase in vascular permeability, but only the late phase is inhibited by cyclooxygenase inhibitors such as indomethacin, aspirin and ketoprofen [30]. The observed late phase (60 min) onset of anti-inflammatory effect of *S. indicum* may be due to the inhibition of the inflammatory mediators produced in the second phase of inflammation, similar to the effect of aspirin.

Paroxysmal malaria fever is a symptom of an unchecked host immune response to the rupture and release of mature schizonts from infected erythrocytes [31]. High fever, often reaching 41°C, can lead to convulsions with resulting fatality especially in infants. It has been shown that febrile temperature can increase *P. falciparum* adherence to endothelial cells and its virulence, through an increased expression of phosphatidylyserine on the surface of infected red cells [32]. Reducing fever is therefore important for eliminating malaria progression and development of disease complications. Brewer’s yeast mimics malaria fever induced by schizonts, by stimulating endogenous pyrogenic cytokines that internally signal thermoregulatory regions of the brain to elevate body temperature through a series of cascading events [33]. *S. indicum* was able to inhibit pyrexia induced by Brewer’s yeast similar to aspirin, likely via inhibition of prostaglandin E₂ in the brain especially in the hypothalamus, by an inhibitory effect on action on cyclooxygenase (COX)-3 [34].

The demonstrated anti-inflammatory, antipyretic and analgesic properties can be attributed to the presence of some bioactive components. The chromatographic profile revealed some of these as morin, rutin, gallic acid and caffeic acid. Gallic acid, caffeic acid and rutin reportedly display good anti-inflammatory effects, attributed in part to the down regulation of interleukin-6, prostaglandin E₂ and inhibition of neutrophil...
migration in inflammation [35, 36, 37]. These phenolics also display antioxidant properties which may contribute to the observed properties of *S. indicum*.

**5.0. Conclusion**

This study supports the use of *S. indicum* leaves as remedy for malaria symptoms and indicates its potential as a candidate for further development.

**Acknowledgement**

The authors acknowledge Mr. Muazzam for assistance in plant identification and collection. We also acknowledge the assistance provided by staff of the Animal Facility Center, NIPRD.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**References**


Table 1 - Effect of *S. indicum* on formaldehyde-induced paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water 10ml/kg</td>
<td>0.10±0.02</td>
<td>0.13±0.01</td>
<td>0.18±0.01</td>
<td>0.16±0.01</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Extract 200mg/kg</td>
<td>0.09±0.01</td>
<td>0.22±0.01</td>
<td>0.19±0.01</td>
<td>0.20±0.01</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Extract 400mg/kg</td>
<td>0.09±0.01</td>
<td>0.17±0.02</td>
<td>0.11±0.02</td>
<td>0.15±0.01</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Extract 800mg/kg</td>
<td>0.08±0.01</td>
<td>0.19±0.01</td>
<td>0.12±0.01</td>
<td>0.17±0.02</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>Aspirin 300mg/kg</td>
<td>0.09±0.01</td>
<td>0.14±0.01</td>
<td>0.11±0.01</td>
<td>0.16±0.01</td>
<td>0.19±0.01</td>
</tr>
</tbody>
</table>

***p < 0.001, compared to control

Table 2 - Effect of *S. indicum* extract on Brewer’s yeast - induced pyrexia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
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<tr>
<td>Distilled Water</td>
<td>37.70±0.26</td>
<td>38.41±0.32</td>
<td>38.92±0.51</td>
<td>38.73±0.30</td>
<td>38.52±0.36</td>
<td>38.13±0.40</td>
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<tr>
<td>200mg/kg Extract</td>
<td>37.56±0.20</td>
<td>38.41±0.25</td>
<td>38.58±0.11</td>
<td>38.11±0.39</td>
<td>38.00±0.37</td>
<td>37.99±0.34</td>
</tr>
<tr>
<td>400mg/kg Extract</td>
<td>37.13±0.41</td>
<td>38.37±0.34</td>
<td>38.03±19</td>
<td>37.71±0.24</td>
<td>37.13±0.45*</td>
<td>37.28±0.44</td>
</tr>
<tr>
<td>800mg/kg Extract</td>
<td>36.74±0.36</td>
<td>38.34±0.35</td>
<td>38.26±0.31</td>
<td>37.75±0.27</td>
<td>37.10±0.49*</td>
<td>36.74±0.45*</td>
</tr>
<tr>
<td>300mg/kg Aspirin</td>
<td>36.68±0.33</td>
<td>38.44±0.29</td>
<td>38.53±0.26</td>
<td>37.60±0.30</td>
<td>36.64±0.49**</td>
<td>36.71±0.26*</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, compared to control
Table 3- Effect of *S. indicum* extract on acetic acid – induced abdominal writhing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Writhes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (10 mL/kg)</td>
<td>20.80±2.40</td>
<td>-</td>
</tr>
<tr>
<td>Extract 200 mg/kg</td>
<td>13.60±1.57*</td>
<td>34.62</td>
</tr>
<tr>
<td>Extract 400 mg/kg</td>
<td>12.20±2.31*</td>
<td>41.35</td>
</tr>
<tr>
<td>Extract 800 mg/kg</td>
<td>14.25±0.48</td>
<td>31.49</td>
</tr>
<tr>
<td>Aspirin 300 mg/kg</td>
<td>14.25±0.48</td>
<td>38.46</td>
</tr>
<tr>
<td>Morphine 10 mg/kg</td>
<td>1.60±1.03***</td>
<td>92.31</td>
</tr>
</tbody>
</table>

*p < 0.05, ***p < 0.001, compared to control

Figure 1: HPLC chromatogram of *S. indicum* leaf extract
Figure 2: Effect of *S. indicum* extract on tail flick response to thermal pain

**p < 0.01, compared to control**