Research Article

Anti-inflammatory and analgesic effects of methanol extract of *Stellaria media* (L.) Vill leaf

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ABSTRACT: The anti-inflammatory and analgesic effect of the methanolic extract of *Stellaria media* (L.) Vill leaf was studied using albumen-induced paw oedema and formalin-induced paw lick in rats as the anti-inflammatory test models; acetic acid-induced writhing, hot plate and tail flick tests in mice as the analgesic models. Three groups of five rats or mice each were administered orally with the leaf extract of *S. media* at 100mg/kg, 300mg/kg or 500mg/kg body weight respectively. A fourth group was administered with Indomethacin (5mg/kg b.w) and distilled water (10mls/kg b.w) for the control group. The extract of *S. media* dose-dependently, significantly (p<0.05) inhibited egg albumen-induced paw oedema as effectively as Indomethacin. The late phase of the formalin response was also inhibited. The extract at 300mg/kg body weight produced a significant (p<0.05) inhibition of the acetic acid-induced abdominal constrictions in mice compared to the control group and mice administered with indomethacin. The analgesic property of the extract was also exhibited in the tail flick test as the extract significantly (p<0.05) increased the tolerance of the mice to pain relative to indomethacin-treated mice. The methanolic extract of *S. media* showed potent peripherally and centrally mediated anti-inflammatory and analgesic properties. The analgesic effect appears mediated through inhibition of release of histamine, serotonin and kinins, prostaglandin, cyclooxygenase and slow reacting substance.

Keywords: Medicinal plant, anti-nociceptive, rat, mice

INTRODUCTION

*Stellaria media* (L.) Vill of the plant family *Carophyllaceae* (Britton and Brown, 1913), commonly known as chickweed, is a cosmopolitan plant found in most regions of the World including Britain and it grows as a common garden weed (Chiej, 1984). In both Europe and North America, the plant is common in gardens, fields, and disturbed grounds. It is very competitive with small grains, and produces up to 80% yield losses among barley (Davis *et al.*, 2005). *S. media* is edible and nutritious, and is used as a leaf vegetable, often raw in salads. There are several closely related plants referred to as chickweed which lack the culinary and medicinal properties of plants in the genus *Stellaria*. *S. media* can easily be distinguished from other members of its family. *S. media* has fine hairs on only one side of the stem in a single band while other members of the family have hairs uniformly covering the entire stem.

*S. media* is used medicinally as a tonic, diuretic, demulcent, expectorant, and mild laxative (Haragan, 1991). It is traditionally recommended for treatment of asthma, bronchitis, or congestion and aids in the control of obesity. *S. media* relieves itching and inflammation and has soothing and moisturizing effects. It is used for minor skin infections or irritations, and is an ingredient in a number of commercial skin care products but most of these traditional uses are not supported by scientific data (Howard, 1987). This study was therefore designed to investigate the anti-inflammatory and analgesic activities of *S. media* leaves using laboratory animal models.
MATERIALS AND METHODS

Experimental animals
Male Swiss mice (18 -20g) and Wistar rats (150 -180g) of both sexes were used for the study. The experimental animals were housed in a 12 hour light: dark condition and maintained on standard rat and mouse diets and water ad libitum. Twenty five animals were randomly and equally divided into five groups for each protocol. Three groups were administered with extract of 100, 300 or 500mg/kg body weight. A group was administered with 5mg/kg b.w of Indomethacin as the reference drug and another was administered with 10ml/kg of distilled water. The Experimental Animal Use and Ethical Committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria approved the research protocols.

Plant materials
Fresh leaves of S. media were collected from Mokuro, Ile-Ife, Nigeria in September, 2008 by Mr. Ademoriyo of Herbarium section, Department of Botany, Obafemi Awolowo University. Samples of the plant were deposited at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife with voucher specimen number UHI 16380. The leaves were air-dried for 72 hours after which they were pulivered into powder form. A 500g of the powder was soaked in methanol for 72 hours and thereafter filtered. The filtrate was concentrated in a rotary evaporator set at 40°C and freeze dried at -20°C. The extract obtained weighed 17.5 g (3.5% of the plant material) and this was stored in a dessicator.

Acute toxicity
The acute toxicity and lethality (LD50) of the plant extracts in rat were estimated using an Up-and-Down Method for Acute Toxicity testing described by Bruce (1985). 6 rats were administered with 3,000 mg/Kg body weight of the extract. The rats were observed for mortality for 2 days. No mortality was observed, after which 4 more rats were administered with 5,000 mg/Kg b.w and observed for mortality for another 5 days. There was no death recorded for the first and second groups within 7 days of observation.

Anti-inflammatory study

Albumen-induced paw oedema in rat: Pedal oedema was induced according to the method described by Okoli et al., (2008). Following an overnight fast, doses of methanolic extract of S. media were orally administered to rats in three groups Indomethacin was administered to animals in the reference group (5mg/kg p.o) and control animals received the vehicle (distilled water at 10mls/kg b.w). One hour after treatment, an injection of 0.2ml (2% v/v) egg albumen was injected into the right hind paw of each rat under the sub plantar region. The paw sizes were measured before and at intervals of 30, 60, 90 and 120 minutes after egg albumen injection by using the cotton thread method (Hess and Milonig, 1972, Olajide et al., 2000). Cotton thread was wrapped around the paw and the circumference was measured with a meter rule. The inhibitory activity was calculated according to the following formula;

\[
\text{Percentage inhibition} = \frac{(C_0 - C_t) \text{ control} - (C_t - C_0) \text{ treated}}{(C_t - C_0) \text{ control}} \times 100
\]

Where C0 is paw size after albumen injection, C1 is paw size before albumen injection.

Formalin paw lick: The method of Dubuisson & Dennis (1977) modified by Tjolsen et al., (1992) was adopted in this experiment. Formalin test is biphasic, and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0 – 5 minutes) being a result of direct stimulation of nociceptors measures centrally mediated effects and is insensitive to anti-inflammatory agents. The second phase (20-30 minutes) is qualitatively different from the first phase and is dependent on peripheral inflammation and changes in central procession due to chemical mediators released from damaged cells that stimulate nociception and thus induced pain. Following an overnight fast, doses of the plant extract, distilled water and indomethacin was orally administered to rats in the same pattern described above. Thirty minutes after treatment, formalin was injected sub-cutaneously into the sub-plantar surface of rat left hind paw at the rate 50 μl of 2.5% solution. Responses were measured 0-5 minutes after formalin injection, for the first phase and the second phase were taken 20-30 minutes after the injection. The licking of the injected paw and the duration was indicative of pain.

Analgesic study

Acetic acid writhing response in mice: Mice were divided into five groups and administered with extract, Indomethacin or distilled water as earlier mentioned. One hour after, 1mg/kg b.w of 3% acetic acid was injected intraperitoneally to each mouse. 5 minutes following acetic acid administration, the number of
abdominal contractions that occurred within the next 20 minutes were counted and recorded for each mouse.

**Hot plate test:** Twenty five rats were randomly and equally divided into five groups and treated as mentioned above. The animals were dropped gently on the hot-plate (Ugo Basile, Socrel DS-37) set at 55±1°C at 0, 30 and 60 minutes after. The reaction time was recorded as the interval between placement of the animals on the hot plate and the time it either licks its fore-paws or jumps off the plate.

**Tail flick test:** This experiment was conducted according to the modified method adopted by Sanchez-Mateo et al. (2006) using hot water bath. Groups of five mice each were administered with the extract at 100, 300, 500mg/kg b.w. Indomethacin (5mg/kg b.w) and distilled water respectively. Thereafter, the terminal 2 cm of the mice tail were immersed in hot water contained in a 500 ml beaker maintained at 55±1°C. A thermometer was placed inside the water to monitor the temperature. Their responses to thermal pain were taken at 30, 60 and 90 minutes after administration of extract, Indomethacin or distilled water.

**Statistical analysis**
Data were analysed using one way analysis of variance (ANOVA) on GraphPad Prism 4.0 version. The result obtained were expressed as mean values ± standard error of mean (SEM). The statistically significant difference between the mean values were determined at p<0.05.

**RESULTS**

**Acute toxicity test**
There was no mortality recorded for up to 5,000 mg/kg body weight of the extract within 7 days. The acute toxicity test conducted therefore showed that the lethal dose (LD₅₀) of methanolic extract of *S. media* was higher than 5000 mg/kg body weight.

**Albumen induced paw edema**
Rats administered with the extract recorded varying degrees of inhibition of inflammation, with 500mg/kg dose having the highest percentage inhibitions of 54% at 90 and 120 minutes post administration. These were comparable to rats administered with Indomethacin which had 30% and 55% percentages of inhibition 90 or 120 minutes post administration. Rats in the control group had significantly (p<0.05) larger paw volumes (0.8±0.05cm, 1.0±0.07cm, 1.1±0.07cm and 1.1±0.08cm) compared to other rats in the study (Table 1).

**Formalin paw lick in rats**
The early (60.00±3.90 seconds) and late phase (18.25±8.00 seconds) reaction time of rats administered with 300mg/kg b.w and those administered with 500mg/kg b.w (58.00±9.30 seconds and 18.25±8.00 seconds) of extract were comparable but significantly (p<0.05) longer than that of Indomethacin-treated rats (42.00±3.08 seconds and 7.00±4.60 seconds). Rats that served as control animals recorded significantly longer reaction time to both the early and late phases of inflammatory pain in this experiment (Table 2).

**Acetic acid writhing response in mice**
The mean number of writhes recorded in the control mice was higher than in mice administered with 300 (p<0.05), 500 mg/kg b.w (p<0.05) of the extract or 5mg/kg b.w of Indomethacin (p<0.05). Indomethacin treated mice had lower number of writhing movement with mice administered with 100 mg/kg (p<0.05), 300 mg/kg (p<0.05) or 500 mg/kg b.w (p>0.05) of the extract (Table 3).

**Table 1:**
Changes in paw volume (cm) by methanolic extract of *S. media* or indomethacin in albumen induced paw oedema test in rats.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>90 minutes</th>
<th>120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (100mg/kg)</td>
<td>0.88±0.07ᵃ(0)</td>
<td>0.74±0.15ᵇ(20)</td>
<td>0.64±0.18ᵇ(27)</td>
<td>0.7±0.03ᵇ(27)</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>0.86±0.15ᵃ(0)</td>
<td>0.86±0.09ᵇ(10)</td>
<td>0.88±0.11ᵇ(18)</td>
<td>0.76±0.08ᵇ(27)</td>
</tr>
<tr>
<td>Extract (500mg/kg)</td>
<td>0.90±0.13ᵃ(0)</td>
<td>0.62±0.14ᵇ(30)</td>
<td>0.6±0.09ᵇ(54)</td>
<td>0.6±0.13ᵇ(54)</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>0.98±0.02ᵃ(0)</td>
<td>0.76±0.07ᵇ(30)</td>
<td>0.86±0.1ᵇ(30)</td>
<td>0.56±0.1ᵇ(55)</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>0.8±0.05ᵃ</td>
<td>1.0±0.07ᵃ</td>
<td>1.1±0.07ᵃ</td>
<td>1.1±0.08ᵃ</td>
</tr>
</tbody>
</table>

*Values with different superscripts in a column are statistically significant (p<0.05); Values in bracket are percentage inhibitions.*
An antinociceptive effect of Stellaria media

Table 2: The effect of methanolic extract of S. media or Indomethacin on reaction time (in seconds) to pain in Formalin paw lick test in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Early phase (sec)</th>
<th>Late phase (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (100mg/kg)</td>
<td>68.75 ± 13.10a</td>
<td>16.00 ± 9.68ab</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>60.00 ± 3.90ab</td>
<td>18.25 ± 8.00b</td>
</tr>
<tr>
<td>Extract (500mg/kg)</td>
<td>58.00 ± 9.30ab</td>
<td>17.00 ± 7.80b</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>42.00 ± 3.08a</td>
<td>7.00 ± 4.60b</td>
</tr>
<tr>
<td>Control</td>
<td>64.35 ± 6.67ab</td>
<td>65.00 ± 2.08a</td>
</tr>
</tbody>
</table>

Values with different superscripts in a column are statistically significant (p<0.05)

Table 3: The effect of methanolic extract of S. media or Indomethacin on abdominal writhing in mice injected with acetic acid intraperitonealy.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Average number of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (100mg/kg)</td>
<td>36.3±9.9a</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>19.6±4.3b</td>
</tr>
<tr>
<td>Extract (500mg/kg)</td>
<td>26.0±3.3ab</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>16.4±2.7b</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>35.2±1.2a</td>
</tr>
</tbody>
</table>

Values with different superscripts in a column are statistically significant (p<0.05)

Table 4: The effect of methanolic extract of S. media or Indomethacin on reaction (in seconds) to thermal pain induced in Hot plate method in mice.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>0</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (100mg/kg)</td>
<td>3.55 ± 0.20ab</td>
<td>3.33 ± 0.51ab</td>
<td>2.99 ± 0.42b</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>3.41 ± 0.16b</td>
<td>4.76 ± 0.37a</td>
<td>4.46 ± 0.50a</td>
</tr>
<tr>
<td>Extract (500mg/kg)</td>
<td>3.40 ± 0.17ab</td>
<td>4.18 ± 0.64a</td>
<td>3.19 ± 0.26ab</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>5.33 ± 0.90a</td>
<td>4.06 ± 1.10a</td>
<td>3.27 ± 0.46ab</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>3.96 ± 0.52ab</td>
<td>2.89 ± 0.16b</td>
<td>1.92 ± 0.30b</td>
</tr>
</tbody>
</table>

Values with different superscripts in a column are statistically significant (p<0.05)

Table 5: The effect of methanolic extract of S. media or Indomethacin on response (in seconds) to thermal pain induced by tail flick method in mice.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (100mg/kg)</td>
<td>3.50±0.33ab</td>
<td>5.53±1.43b</td>
<td>6.11±0.90a</td>
</tr>
<tr>
<td>Extract (300mg/kg)</td>
<td>2.89±0.92a</td>
<td>4.69±0.54b</td>
<td>6.37±1.08a</td>
</tr>
<tr>
<td>Extract (500mg/kg)</td>
<td>3.60±0.24ab</td>
<td>3.66±0.44b</td>
<td>4.55±0.72b</td>
</tr>
<tr>
<td>Indomethacin (5mg/kg)</td>
<td>5.33±0.93a</td>
<td>4.10±1.09b</td>
<td>4.00±0.56b</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>2.00±0.20a</td>
<td>2.00±0.23a</td>
<td>1.80±0.30a</td>
</tr>
</tbody>
</table>

Values with different superscripts in a column are statistically significant (p<0.05)
DISCUSSION

The animal models of inflammation and analgesia used in this study showed that methanolic extract of Stellaria media possesses potent anti-inflammatory and analgesic property, especially at 300mg/kg body weight of the extract.

The extract of S. media dose-dependently inhibited paw oedema induced by egg albumen as effectively as Indomethacin. Egg albumen induces inflammation in body tissue with the release of serotonin, histamine and prostaglandin from the tissue, resulting in oedema, which has been shown to be inhibited by antihistaminic agents (Mossa et al., 1995 Olaleye et al., 2004). Ilavarasan (2005) corroborated these submissions with the study of Cassia fistula bark which was concluded to have an anti-inflammatory effect in acute inflammatory conditions. These strongly point to the fact that the anti-inflammatory effect of S. media is mediated through inhibition of modulation of nociception by serotonin, histamine or prostaglandins.

The anti-inflammatory effect of S. media obtained from the egg albumen paw oedema test was corroborated by the formalin paw lick test. This test is not only useful for assessing antinociceptive property, but also in understanding the mechanism of anti-inflammatory action. The early (neurogenic) phase is as a result of direct stimulation of nociceptors in the paw which culminates in centrally mediated pain with release of substance P, while the late phase is due to the release of histamine, serotonin, bradykinin and prostaglandins (Zeashana et al., 2009). Drugs that act primarily on the central nervous system, such as narcotics, inhibit both phases equally while peripherally acting drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory only inhibit the late phase (García et al., 2004; Zeashana et al., 2009). S. media effectively inhibited the late phase of the formalin response similarly in this study thus reinforcing inhibition of prostaglandin synthesis as its possible mechanism of action.

The acetic acid-induced writhing is a visceral pain model and widely used for the evaluation of peripheral antinociceptive activity (Du et al., 2007). The intraperitoneal administration of an agent that irritates the serous membranes cause a stereotypical behaviour in mice which is characterized by abdominal contractions, movements of the body as a whole, twisting of the dorsal abdominal muscles, and a reduction in the motor activity and coordination (Zeashana, et al., 2009). Findings from the present study showed that the methanolic extract of S. media at 300mg/kg body weight produced a significant (p<0.05) inhibition of the acetic acid-induced abdominal contractions in mice compared with mice in the control group. The inhibition produced by the extract was also comparable to that produced by Indomethacin. This indicates a marked evidence of analgesic property. Acetic acid causes an increase in the peritoneal fluid level of prostaglandins (PGE2 and PGF2α) as well as lipoxygenase production, which partially involves peritoneal receptors and inflammatory pain by inducing capillary permeability (Collier et al., 1968; Choi, 2007). S. media therefore probably disrupt prostaglandins synthesis through inhibition of lipoxygenase and cyclooxygenase.

In this study S. media significantly (p<0.05) prolonged the reaction time to thermal pain. The reaction time to pain in mice administrated with 300mg/kg b.w of the extract was longer than in mice administered with indomethacin. The hot-plate test of analgesia is considered selective for opioid-like receptors. Although the central and peripheral analgesics act by inhibiting the number of contractions provoked by chemical pain stimuli, only the central analgesics increase the time of response in the hot plate test (García, et al, 2004). This test therefore suggests an involvement of centrally mediated mechanism of analgesic action of S. media, as well as peripheral mechanism as initially mentioned in the formalin induced paw lick test.

The analgesic property of the extract was also exhibited in the tail flick test with the extract significantly (p<0.05) increasing the tolerance of the mice to pain, more than was observed for Indomethacin. Tail flick test is a standard method for investigating nociception and analgesia, with the measurement of the response to a brief, noxious stimulus which appears to be a spinal reflex, modulated by supraspinal inhibitory mechanism. The test is selective for centrally acting analgesics (Ramabadran et al., 1989) indicative of morphine like effect (Domer, 1990) and NSAIDs which inhibit cyclooxygenase in peripheral tissues, thereby interfering with the mechanism of transduction in primary afferent nociceptors (Fields, 1987). This test also confirms that the mechanism of analgesia is both centrally and peripherally mediated.

In conclusion, the methanolic extract of Stellaria media has potent peripherally and centrally mediated anti-inflammatory and analgesic properties. The antinociceptive property appeared mediated through inhibition of release of histamine, serotonin and kinins, prostaglandin, cyclooxygenase and slow reacting substances. Further study is aimed at isolating and elucidating the chemical structure of the bioactive principles responsible for the anti-inflammatory and analgesic properties.
REFERENCES