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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 which resemble the plant 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.

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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 pulverised into powdery 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 40^0 C and freeze dried at -20^0 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 (LD_{50}) 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;

Percentage inhibition

$$= \frac{(C_1 - C_0)\ \text{control} - (C_1 - C_0)\ \text{treated}}{(C_t - C_0)\ \text{control}} \times 100$$

Where C_t is paw size after albumen injection, C_0 is paw size before albumen injection

Formalin paw lick: The method of Dubuission & 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 (LD_{50}) 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.

Treatment Groups	30 minutes	60 minutes	90 minutes	120 minutes
Extract (100mg/kg)	0.88 ± 0.07^{a} (0)	0.74 ± 0.15^{ab} (20)	0.64 ± 0.18^{b}(27)	0.7 ± 0.03^{b} (27)
Extract (300mg/kg)	0.86 ± 0.15^{a} (0)	0.86 ± 0.0^{ab} (10)	0.88 ± 0.11^{ab} (18)	0.76 ± 0.08^{b}(27)
Extract (500mg/kg)	0.90 ± 0.13^{a} (0)	0.62 ± 0.14^{b} (30)	0.6 ± 0.09^{b} (54)	0.6 ± 0.13^{b}(54)
Indomethacin (5mg/kg)	0.98 ± 0.02^{a} (0)	0.76 ± 0.07^{ab} (30)	0.86 ± 0.1^{ab}(30)	0.56 ± 0.1^{b} (55)
Control (Distilled water)	0.8 ± 0.05^{a}	1.0 ± 0.07^{a}	1.1 ± 0.07^{a}	1.1 ± 0.08^{a}

Values with different superscripts in a column are statistically significant ($p<0.05$); Values in bracket are percentage inhibitions.

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

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

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.

Treatment Groups	Average number of writhing
Extract (100mg/kg)	36.3±9.9[a]
Extract (300mg/kg)	19.6±4.3[b]
Extract (500mg/kg)	26.0±3.3[ab]
Indomethacin (5mg/kg)	16.4±2.7[b]
Control (Distilled water)	35.2±1.2[a]

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.

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

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

Hot plate test

The time of 4.76±0.37 or 4.46±0.50 seconds taken to react to thermal pain in mice group administered with 300mg/kg b.w at 30 or 60 minutes post administration was non-significantly (p>0.05) longer than for those administered with Indomethacin (4.06±1.10 or 3.27±0.46 seconds) for the same period. The reaction time in mice administered with 500mg/kg b.w of extract was non-significantly (p>0.05) different from values recorded for those mice in 300mg/kg dose. However, the mice in 300mg/kg dose recorded the longest time taken to react to thermal pain (4.76±0.37) at 30 minutes post administration (Table 4).

Tail flick test

The onset of reaction to thermal induced pain was significantly (p<0.05) shorter in the control rats than in those administered with extract or Indomethacin for any period of evaluation of pain. Indomethacin administered mice exhibited longer reaction time to pain than for mice administered with 100 mg/kg (p>0.05), 300 mg/kg (p<0.05) or 500 mg/kg (p>0.05) body weight of extract at zero (0) minute post administration. At 30 minutes post administration, the time taken to react to pain was non-significantly (p>0.05) shorter in indomethacin treated mice than in mice pre-treated with 100 or 300 but longer for 500 mg/kg b.w. At 60 minutes post administration, the value was significantly (p<0.05) shorter in indomethacin treated mice than in mice pre-treated with 100 mg/kg or 300 mg/kg but non-significant (p>0.05) with 500mg/kg b.w. (Table 5).

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

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

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 constrictions 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 (PGE_2 and PGF_{2a}) as well as lipooxygenase 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 lipoxoygenase 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 administered 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 cycloxygenase 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 anti-nociceptive 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.

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