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Anti-Inflammatory and Analgesic Potential of Methanolic Extract of *Emilia Sonchifolia* (Compositae) Leaves in Rodents

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ABSTRACT: *Emilia sonchifolia* (Lin)DC (compositae) has found various medicinal uses in folkloric medicine, as a cure for various ailment such as sore-throat, tonsillitis, styptic, vulnery, wounds healing, stomach ache, conjunctivitis, depurative, infantile tympanitis, anticonvulsants, bowel complaints and sores; aqueous extract is used to treat internal heat among pregnant women. The need to pharmacologically establish these claims stimulated this investigation of the plant. The LD_{50}, preliminary phytochemical screening, anti-inflammatory and analgesic potentials of the methanolic extracts of *Emilia sonchifolia* (ES) were investigated in mice using carragenin, egg albumin, capsaicin-induced paw oedema, formalin-induced paw licking, acetic acid induced writhing and hot plate nociception in mice. The LD_{50} i.p. was calculated to be 2874.02mg/kg; phytochemical screening revealed the presence of terpines, flavonoids, tannins, saponins and alkaloids. The extract (287.4, 574.8, 862.2, ASA (100mg/kg) and 574.8 + ASA (mg/kg) (i.p.) produced a dose dependent (p<0.05-0.001) inhibition carragenin, egg-albumin, capsaicin, formalin-induced paw licking, acetic acid-induced writhing and hot plate nociception in mice. Preliminary phytochemical screening revealed the presence of flavonoids, tannins and alkaloids. Results from this study show that ES may be useful as anti-inflammatory and analgesic agent.

Key Words: *Emilia sonchifolia*, anti-inflammatory, analgesic, Acute toxicity, Phytochemical screening.

INTRODUCTION

*Emilia sonchifolia* (Compositae) is an annual herbaceous plant growing up to 0.6m heigh. It flowers from July to October and the seed ripen from August to October. The flowers are hermaphrodite and insect pollinated. The plant is pantropic and probably originates in South Asia (Huxley, 1992). The plant is used in folklore medicine for the treatmhas a folkloric uses in tumour, inflammation, cough, rheumatism, cuts, and wounds. In China, the leaves of this plant are used against fever and dysentery (Autor, 1969). The arial parts of the plant have been reported to contain alkaloids, flavoniods and terpenes (Cheng et al., 1984). Active anti-cancer fraction isolated from the plant is reported to have cytotoxic as well as Ehrlich ascitic carcinoma (EAC) and anti-Dalton’s lymphoma ascites (DLA) activities in mice (Shylesh et al., 2000). The anticonvulsant activity of *E. Sonchifolia* leaf extracts has been reported in chicks and mice (Asije et al., 2006). The tea made from the leaves of *E. Sonchifolia* is used in African folk medicine for treatment of dysentery (Duke and Ayensu 1985). The juice of the leaf is used in the treatment of eye inflammations, night blindness, cuts and wounds and sore ears (Chopra et al., 1986). The plant is astringent, depurative, diuretic, expectorant, febrifuge and sudorific (Manandhar, 2002). It is used in the treatment of infantile tympanitis and bowel complaints (Chopra et al., 1986). The juice of the root is used in the treatment of diarrhoea. The flower heads are chewed and kept in the mouth for about 10 minutes to prevent tooth decay (Manandhar, 2002).
In Akwa Ibom State, Nigeria, the leaf is used to treat inflammation, convulsion, fever, muscular aches and pains. It is also used as cure for sore-throat, tonsillitis and styptic (stop bleeding), vulnery (accelerates healing of cuts, wounds and sores); aqueous extract of the leaf (“mmon ndobbo udo or mmonunnu” (Efik, Nigeria) is used to treat internal heat among pregnant women. It is used as emetic, stomach ache, conjunctivitis, depurative diaphoretic, diuretic, dysentery, infantile tympanitis and bowel complaints (Inyang, 2003; Etukudo and Inyang, 2001). Information investigating the pharmacological activity, as anti-inflammatory and analgesic, is scanty in literature. This study is executed to evaluate these activities so as to justify its safety and the folkloric basis of its uses.

MATERIALS AND METHODS

Plant material: Fresh roots of *E. sonchifolia* were collected in June 2007 from Ibiono Local Government area, Akwa Ibom State, Nigeria and authenticated by Dr. (Mrs.) Margaret Bassey, a taxonomist in the Department of Botany, where a voucher specimen is maintained. The fresh leaves weighing 4.5kg was cleaned cut into smaller pieces, dried under the sun and reduced to a coarse powder using mortar and pestle. The powdered plant material (120 g) was extracted by macerating in methanol (350ML) for 72 hours. The liquid extract was strained with a muslin cloth, allowed to cool and filtered. The filtrate was concentrated in vacuo at 40 °C. The extract was stored in refrigerator at 4 °C until used for the experiment. The methanolic extract was subsequently subjected to phytochemical analysis using conventional methods (Harbone, 1984).

Pharmacological tests

Animals:
Adult Swiss albino Swiss mice (18–30 g) of both sexes were obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy and Toxicology, University of Uyo, Nigeria. Animals were housed in steel cages within the facility under standard conditions and allowed free access to standard pellets and water. Prior to their use, they were allowed two weeks for acclimatization within the work area environment. All animal experiments were in conformity with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

Preliminary Phytochemical screening: Preliminary phytochemical analysis of the extract was carried out as described by the procedure of Odebiyi and Sofowora, (1978).

Acute toxicity: The acute toxicity (LD$_{50}$) of ES in mice (n = 12) was estimated using the method described by Lorke, 1983. In stage one of the test, animals received oral administration of 10, 100, or 1000 mg/kg (n = 3) of ES methanolic extracts and observed for 24 hours for a number of deaths. Since no death occurred in any of the groups in the first stage of the test, 1600, 2900 and 5000 mg/kg doses of the extract were administered to a fresh batch of animals (n = 3) and death was recorded within 24 h. From the number of deaths, the i.p LD$_{50}$ in mice was calculated to be 2874.02 mg/kg.

Effect of ES Extracts on Carrageenan–Induced Inflammation in Mice:
Increase in the mice hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Winter et. al., 1992; Arya and Kumar; 2005). The phlogistic agent employed in this study was carrageenin. Adult albino mice of either sex (18-30g) were used after 24hours fast and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1ml of a 1% (w/v) solution of carageenin in 0.9% saline solution into the sub-plantar surface of the right hind paw. The linear circumference of the injected paw was measured before injection and thereafter 30mins interval for the next 5 hours (Adeyemi et al., 2005; Mbagwu et al., 2007; Besra et. al., 1996). Oedema (inflammation) was assessed as the difference between the control and 0.5 to 5.0 hrs interval after administration of the phlogistic agent (Hess and Melonig, 1972; Asongalem et al., 2004., Tijani et al., 2008; Gupta et al., 2008). The drugs were administered intraperitonealy (IP) 1h before inducing inflammation. The rats were divided into six groups with six animals per group. Group 1 received only Carrageenan which serves as control. Group 2-4 receives a dose of extracts 284.4, 574.8 and 862.2mg/kg i.p respectively. Group 5 received only ASA (100mg/kg dissolved in 5% sodium carbonate. Group 6 received ASA and extract (575.8mg/kg). Average oedema was calculated (Asuju et al., 1999; Ajay-Abe et al., 1999). The degree of inflammation was measured using vernier callipers (Table 1).

Effect of ES Extracts on Egg Albumin–induced Inflammation in Mice:
Increase in the mice hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Winter et. al., 1992; Arya and Kumar; 2005). The phlogistic
agent employed in this study was egg-albumin (Akah, and Nwambie, 1994; Ekpendu, et. al., 1995). Adult albino mice of either sex (18-36g) were used after 24hours fast and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1ml of egg-albumin on the subplantar surface of the right hind paw. The linear circumference of the injected paw was measured before and 30mins to 5h (at every 30mins interval) after administration of the phlogistic agent (Adeyemi et al., 2005; Mbagwu et al., 2007; Besra, 1996). Oedema (inflammation) was assessed as the difference between the control and 0.5 to 5.0h interval after administration of the phlogistic agent (Hess and Milonig, 1972., Asongalem et al., 2007; Besra, 1996). Oedema (inflammation) was assessed as the difference between the control and 0.5 to 5.0h interval after administration of the phlogistic agent (Adeyemi et al., 2005; Mbagwu et al., 2007; Besra, 1996). Oedema (inflammation) was assessed as the difference between the control and 0.5 to 5.0h interval after administration of the phlogistic agent (Hess and Milonig, 1972., Asongalem et al., 2004., Tijani et al., 2008; Gupta et al., 2008). The drugs were administered i.p 1h before inducing inflammation. The mice were divided into six groups with six animals per group. Group 1 received only egg-albumin which served as control. Group 2-4 received a dose of extracts 284.4, 574.8 and 862.2mg/kg i.p respectively. Group 5 received only ASA (100mg/kg dissolved in 5% sodium carbonate) dissolved in 5% sodium carbonate. Group 6 received ASA and extract (575.8mg/kg). Average oedema was calculated (Okoli et al., 2007a; Iwueke et al., 2006; Ahmed et al., 1993) (Table II).

**The Effect of ES Extract on Capsaicin–induced Inflammation in Mice:**

Increase in the mice hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Winter et al. 1992; Arya and Kumar; 2005). The phlogistic agent employed in this study was capsaicin (Akah and Nwambie, 1994; Ekpendu, et. al., 1995). Adult albino mice of either sex (20-36g) were used after 24hours fast and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1ml of 5µg/kg capsaicin dissolved in 20% Tween 80 into the subplantar surface of the right hind paw. The linear circumference of the injected paw was measured before and 30mins to 5h (at every 30mins interval) after administration of the phlogistic agent (Akah, and Nwambie, 1994; Ekpendu, et. al., 1995; Besra, 1996). Oedema (inflammation) was assessed as the difference between the control and 0.5 to 5.0h interval after administration of the phlogistic agent (Hess and Milonig, 1972; Ajay-Abe et al., 1999). The drugs were administered i.p 1h before inducing inflammation. The mice were divided into six groups with six animals per group. Group 1 receives only capsaicin which served as control. Group 2-4 received a dose of extracts 284.4, 574.8 and 862.2mg/kg i.p respectively. Group 5 receive only ASA (100mg/kg dissolved in 5% sodium carbonate. Group 6 received ASA and extract (575.8mg/kg). Average oedema was calculated (Okoli et al., 2007a; Iwueke et al., 2006; Ahmed et al., 1993). The degree of inflammation was measured using vernier callipers. Group 1 served as standard. Group 2- 4 were pre-treated with extract (287.4, 574.8 and 862.2mg/kg i.p) respectively 30mins before been challenged with buffered formalin. Group 5 receives only ASA (100mg/kg dissolved in 5% sodium carbonate which serve as standard. Group 6 received ASA and extract 575.8mg /kg 30mins before been challenged with buffered formalin. The amount of time spent licking the paw was timed, and was considered as indicative of pain.

**Effects of ES Extracts on Formalin–induced Hind Paw Licking In Mice:**

Adult albino mice of either sex (20 – 36g) were used. The procedure was essentially similar to that described previously by (Mbagwu et al., 2007; Iwueke et al 2006; Adeyemi et al., 2004; Hunskaa and Hole, 1989., Cornea and calixto, 1993; Gorski., 1993). These mice were used to analyze the first phase of formalin – induced licking. 20µl of 2.5% formalin solution (0.9%) of formaldehyde, made up in phosphate buffer solution (PBS; concentration, NaCl 137mM; KCl 2.7mM and phosphate buffer 10mM) was injected subcutaneously under the surface of the right hind paw. The first of the nociceptive response normally peaked 5mins after formalin injection and the second phase 15 - 30mins after formalin injection representing the neurogenic and inflammatory pain responses respectively (Mbagwu et al., 2007; Gorski. et al.1993). The mice were divided into six groups. Group 1 received subcutaneous injection of formalin in saline which serve as control. Group 2- 4 were pre-treated with extract (287.4, 574.8 and 862.2mg/kg i.p.) respectively 30mins before been challenged with buffered formalin. Group 5 receives only ASA (100mg/kg dissolved in 5% sodium carbonate which serve as standard. Group 6 received ASA and extract 575.8mg /kg 30mins before been challenged with buffered formalin. The amount of time spent licking the paw was timed, and was considered as indicative of pain.

**Effects of ES Extracts on Acetic Acid-Induced Writhing Reflex on Mice:**

The abdominal constrictions resulting from intraperitoneal (i.p.) injection of acetic acid (3%) consisting of the contraction of abdominal muscle together with a stretching of hind limbs, were carried out according to the procedure described by Mbagwu et al., 2007; Santos et al., 1994; Correa et al., 1996; Nwafor et al., 2002. The mice were grouped into six groups with six mice per cage. Group 1 served as control and received only Acetic Acid (3%). Group 2- 4 was treated with extract (287.4, 574.8 and 862.2mg/kg i.p.) respectively. Group 5 received only ASA (100mg/kg dissolved in 5% sodium carbonate which serve as standard. Group 6 received ASA and extract 575.8mg /kg 30mins before been challenged with acetic acid. After 30 minutes, acetic acid was administered (i.p). The numbers of writhing movement were counted.
Effect of ES Extracts on Thermal Nociception In Mice:
The method of Vaz et al., 1996 was used to establish the analgesic effects of ES. Mice of both sexes were used. Mice were placed in glass beaker of 50 cm diameter kept on Eddy’s hot plate surface having a constant temperature 55±0.1°C. The time in second between placement and shaking or licking of the paws or jumping out of the beaker was recorded before and after administration of extract as index of response latency (Mbagwu et al., 2007). The animals were divided into six groups of 6 mice per group. Group 1 served as the control and received 5ml/kg of 20% Tween 80. Group 2-4 was treated with extract (287.4, 574.8 and 862.2mg/kg i.p.) respectively. Group 5 received only ASA (100mg/kg) dissolved in 5% sodium carbonate which serves as standard. Group 6 received ASA and extract (575.8mg/kg) 30mins before pain was induced. The reaction time was taken as interval extending from the instant the mouse reached the hot plate until the instant the animal licked its feet or jumped out of the cylinder.

Statistical analysis:
Results are expressed using multiple comparisons of Mean ± SEM by one way analysis of variance(ANOVA), followed by Turkey- Krammar multiple comparisons test. Results were considered statistically significant at a probability level of less than 5%.

Table 1:
Effect of Extract of E. Sonchifolia leaf on Caragenin-induced inflammation in mice.

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>0.5 HR</th>
<th>1.0HR</th>
<th>2.0HRS</th>
<th>3.0HRS</th>
<th>4.0HRS</th>
<th>5.0HRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.43±0.01</td>
<td>34.2±0.01</td>
<td>35.9±0.01</td>
<td>35.9±0.00</td>
<td>35.9±0.01</td>
<td>34.2±0.01</td>
</tr>
<tr>
<td>287.4</td>
<td>27.8±0.00</td>
<td>29.7±0.00</td>
<td>27.8±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.8±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.7±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.5±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>574.8</td>
<td>28.6±0.01</td>
<td>30.6±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.6±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.5±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.2±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.4±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>862.2</td>
<td>20.6±0.01</td>
<td>18.2±0.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.6±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.9±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.0±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>29.4±0.01</td>
<td>31.4±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.4±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.3±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.0±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.0±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>574+ASA</td>
<td>21.2±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.2±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.1±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.3±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.3±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent : Mean ± SEM significance relative to control: <sup>a</sup>p<0.05; <sup>b</sup>p<0.01; <sup>c</sup>P<0.001; ASA= acetyl salicylic acid; n=6; Percentage inhibition at 3 hours.
Table 2:
Effect of Extracts of E. Sonchifolia Leaf on Egg-Albumin Induced Inflammation in Mice

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>Increase in paw oedema (%)</th>
<th>Inhibition* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 HR</td>
<td>1.0HR</td>
</tr>
<tr>
<td>Control</td>
<td>28.9±0.01</td>
<td>28.9±0.01</td>
</tr>
<tr>
<td>287.4</td>
<td>30.6±0.02</td>
<td>28.6±0.01c</td>
</tr>
<tr>
<td>574.8</td>
<td>35.1±0.00</td>
<td>31.4±0.00c</td>
</tr>
<tr>
<td>862.2</td>
<td>27.0±0.01</td>
<td>22.9±0.01c</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>34.4±0.01c</td>
<td>30.0±0.01c</td>
</tr>
<tr>
<td>574+ASA</td>
<td>31.3±0.01c</td>
<td>29.0±0.01c</td>
</tr>
</tbody>
</table>

Values represent: Mean ±SEM; Significance relative to control: *p<0.05; **p<0.01; ***P<0.001; ASA= acetyl salicylic acid; *Percentage inhibition at 4 hours; n=6

Table 3:
Effect of Extracts of E. Sonchifolia Leaf on Capsaicin- Induced Inflammation in Mice

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>Increase in paw oedema (%)</th>
<th>Inhibition* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 HR</td>
<td>1.0HR</td>
</tr>
<tr>
<td>Control</td>
<td>18.8±0.01</td>
<td>23.5±0.01</td>
</tr>
<tr>
<td>287.4</td>
<td>30.3±0.00</td>
<td>32.4±0.01</td>
</tr>
<tr>
<td>574.8</td>
<td>25.0±0.01</td>
<td>25.0±0.01</td>
</tr>
<tr>
<td>862.2</td>
<td>25.7±0.01c</td>
<td>27.8±0.01c</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>17.9±0.01c</td>
<td>14.8±0.01c</td>
</tr>
<tr>
<td>574.8 ASA</td>
<td>18.5±0.00e</td>
<td>18.5±0.01c</td>
</tr>
</tbody>
</table>

Values represent: Mean ±SEM; significance relative to control: *p<0.05; **p<0.01; ***P<0.001; ASA= acetyl salicylic acid; *percentage inhibition at 2 hours.

Capsaicin induced inflammation: The effects of the extract on capsaicin induced oedema in mice is summarised in Table 3. The extract at the dose of 287.4mg/kg produce significant (p<0.01) reduction of pore oedema at 2 hours; the median dose 574.8mg/kg showed significant (P<0.05-0.01) from 1-5 hours and the maximal dose 862.2mg/kg showed more significant (p<0.01-0.001) reduction of pore oedema at 0.5 and 4-5hours comparable to Acetyl salicylic acid and Acetyl salicylic acid plus the median dose of the extract(574.8mg/kg p.o.).

Analgesic Studies:

Acetic Acid induced writhing assay: The extract pre-treated mice also exhibited dose dependent significant (p<0.05-0.001) reduction of abdominal constrictions and hind limb stretching induced by intraperitoneal administration of acetic acid. The effects are shown in table 4.

Formalin induced rat paw licking in mice: The extract pre-treated mice showed a dose dependent significant (p<0.05-0.001) reduction of the hind paw licking caused by formalin in mice. The effects of reduction in hind pore licking was less in early than the latter phase of formalin induced pain (Table 5).

Thermal nociception: The extract (287.4, 574.8 and 862.2mg/kg; i.p.) pre-treated mice produce significant (P<0.05-0.001) dose dependent increased in the reaction time. The standard drug Acetyl salicylic acid produced reaction time twice that of the maximal dose of the extract. Acetyl salicylic acid plus the median dose of the extract (574.8mg/kg p.o.) gave a marginally increased in reaction time from 3.0±0.26 to 3.6±0.19 minutes.
**Table 4:**
Effect of Extract of *E. sonchifolia* leaf on Acetic Acid-Induced Writhing In Mice.

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>5MINS</th>
<th>10MINS</th>
<th>15MINS</th>
<th>20MINS</th>
<th>25MINS</th>
<th>30MINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00±0.00</td>
<td>8.67±1.17</td>
<td>1.95±2.4</td>
<td>1.76±0.92</td>
<td>19.50±1.33</td>
<td>15.00±1.2</td>
</tr>
<tr>
<td>287.4</td>
<td>0.00±0.00a</td>
<td>2.67±0.55a</td>
<td>7.00±1.31a</td>
<td>7.50±0.62a</td>
<td>7.83±1.25a</td>
<td>5.05±0.83a</td>
</tr>
<tr>
<td>574.8</td>
<td>0.00±0.00a</td>
<td>0.67±0.23a</td>
<td>2.50±0.34a</td>
<td>1.30±1.03a</td>
<td>3.67±0.80a</td>
<td>1.33±0.33a</td>
</tr>
<tr>
<td>862.2</td>
<td>0.00±0.00a</td>
<td>0.33±0.33a</td>
<td>1.00±0.26a</td>
<td>1.67±0.56a</td>
<td>1.50±0.62a</td>
<td>0.67±0.21a</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>0.00±0.00a</td>
<td>8.00±0.00a</td>
<td>5.17±1.22a</td>
<td>8.00±0.96a</td>
<td>5.83±1.14a</td>
<td>5.50±1.10a</td>
</tr>
<tr>
<td>574.8 +ASA</td>
<td>0.00±0.00a</td>
<td>0.00±0.00a</td>
<td>0.00±0.00a</td>
<td>0.00±0.00a</td>
<td>0.52±0.22a</td>
<td>1.00±0.26a</td>
</tr>
</tbody>
</table>

Values represent Mean± SEM significance relative to control: *p<0.001.  ASA= acetyl salicylic acid N=6

**Table 5:**
Effect of Extract of *E. Sonchifolia* Leaf on Formalin-Induced Hind Paws Linking in Mice

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>5mins</th>
<th>10 Mins</th>
<th>15mins</th>
<th>20mins</th>
<th>25mins</th>
<th>30mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.33±0.98</td>
<td>0.50±0.34</td>
<td>4.83±0.20</td>
<td>2.00±0.85</td>
<td>2.50±0.56</td>
<td>3.50±0.33</td>
</tr>
<tr>
<td>287.4</td>
<td>9.00±0.85</td>
<td>0.00±0.00a</td>
<td>1.33±0.20</td>
<td>0.83±0.41</td>
<td>1.33±0.10</td>
<td>0.50±0.20</td>
</tr>
<tr>
<td>574.8</td>
<td>7.17±0.14</td>
<td>0.00±0.00a</td>
<td>1.00±0.30</td>
<td>0.50±0.20</td>
<td>0.20±0.20</td>
<td>0.20±0.20</td>
</tr>
<tr>
<td>862.2</td>
<td>7.33±1.40</td>
<td>0.00±0.00a</td>
<td>0.50±0.33</td>
<td>0.50±0.22</td>
<td>0.50±0.34</td>
<td>0.17±0.30</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>8.67±0.99</td>
<td>0.33±0.33a</td>
<td>0.50±0.34</td>
<td>0.50±0.22</td>
<td>0.17±0.17</td>
<td>0.17±0.17</td>
</tr>
<tr>
<td>574.8 +ASA</td>
<td>4.00±0.85</td>
<td>0.00±0.00a</td>
<td>0.17±0.17</td>
<td>0.33±0.22</td>
<td>0.17±0.17</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Values represent: Mean ± SEM significance relative to control: *p<0.05; bP<0.01; cP<0.001  ; ASA= acetyl salicylic acid  N=6

**TABLE 6:**
Effect of Extract of *E. Sonchilia* Leaf on Hot Plate Reactions Time In Mice.

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>TIME (Mins.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>287.4</td>
<td>1.20±0.13a</td>
</tr>
<tr>
<td>574.8</td>
<td>1.20±0.12a</td>
</tr>
<tr>
<td>862.2</td>
<td>1.60±0.12c</td>
</tr>
<tr>
<td>ASA(100)</td>
<td>3.00±0.26c</td>
</tr>
<tr>
<td>574.8 +ASA</td>
<td>3.60±0.19c</td>
</tr>
</tbody>
</table>

Values represent: Mean ± SEM significance relative to control: *p<0.05; cP<0.001  ASA= acetyl salicylic acid

**DISCUSSION**

Inflammation can be induced by variety of chemical agents which have no coherent and consistent correlation between obtained pharmacological activity and chemical structure (Nwafor et al., 2007b; Adeyemi et al., 2005; Atta and Alkohafi, 1998; Sertie et al., 1990). Besides, as a result of the complexity involved in the reparative and proliferative phenomenon in tissues injury (Whaley and Burt, 1996; Okoli et al., 2007b) and the absence of one generally acceptable pharmacological assay to predict efficacy of tested extracts in human necessitated the utilization of multivariable experimental models in conduction of pharmacological trials for putative anti-inflammatory agent (Sertie et al., 1990). Therefore, carragenin, egg albumin and capsacin were utilised in this study to elucidate the anti-inflammatory properties and formalin-induced paw licking; acetic acid-induced writhing, and thermal nociception were also used to investigate the analgesic potentials of ES methanolic leaf extract. Carragenin-induced paw edema is always taken as a prototype of early exudative phase of inflammation which is an important feature of inflammatory pathway (Gupta et al., 2008; Ajay-Abe et al., 1997) and a fastest screening procedure of involvement of cyclooxygenase catalobic or anabolic...
products of arachidonic acids. The results show that the extract exhibited a dose dependent anti-inflammatory activity on the various models used. The extract pre-treated mice produced dose dependent anti-inflammatory effect on carragenin-induced paw oedema. The extracts produced maximal effects after 1 hour of induction of oedema at all the doses investigated, but the dose of 862.2mg/kg produce greater effect at 1-hour than that produced by the standard drug ASA 100mg/kg.

Carragenin model is an acute model of investigation of inflammation and it is utilised in the study of non-steroidal anti-inflammatory drugs (Adeyemi et al., 2005; Atta and Alkohafi, 1998). Carragenin induction of inflammation involves three distinct phases of mediators releases: histamine and 5-HT in the first phase, bradykinin in the second phase and prostaglandin in the third phase. Bradykinins and prostaglandins mediate the prolonged delayed phase of onset responses in inflammation (Di-Rosa et al., 1971; Singh et al., 1996). Prostaglandin is known to cause or enhance all the cardinal signs of inflammation (Singh et al., 1996; Atta and Alkohafi, 1998). In the beginning of the carragenin injection, there were a sudden elevation of paw volume under 1 hour (and this is attributed to acute release of histaminic and serotoninergic mediators of inflammation) which continues to rise thereafter until 3 hours before a decrease was observed (and this is attributed to prostaglandins and kinins). Similarly, the extract pre-treated mice significantly produced dose dependent reduction of paw oedema induced by egg albumin. The extract at all the tested doses significantly inhibited mice paw oedema than the standard drug, ASA 3-4 hours after injection of egg albumin. The effects of the extract (287.4, 574.8 and 862.2mg/kg; i.p.) on overall oedema induced was dose dependent, but the highest anti-inflammatory response was exerted at a dose of 574.8mg/kg. Egg albumin is known to induce two mediators of inflammation which are basically histamine and 5HT (Pearce, 1986) which the extract dose dependently inhibited to reduce inflammation. Also the extract 574.8 and 862.2mg/kg was only very effective 4-5 hours after injection of capsaicin and its effect is comparable to standard drug, ASA 100mg/kg. Capsaicin induces neurogenic inflammation mediated by neupeptides such as substance P, neuropepin A, vasoactive intestinal peptides and calcitonin gene related peptides released from capsaicin stimulated neurons. That the extract exhibits significant effects in carragenin and egg-albumin induced oedema suggests that the extract may exhibit in part the inhibition of cyclooxygenase synthesis, histamine and serotonin releases. The inhibition of capsaicin suggests that its anti-inflammatory effects may be related to its anti-inflammatory neurogenic and non-neurogenic properties (Nwafor et al., 2007a).

Oedema is associated with increase in vascular permeability and is elicited by numerous primary stimuli mediated by two mechanisms. One is autacoids mediated by non-neurogenic stimulation and the second by bradykinins induced neurogenic stimulation of primary sensory neurons leading to release of substance P from peripheral nerve endings (Gamse et al., 1980; Amico-Roxas et al., 1984). The neurogenic provoked substance P release from nerve endings mediate the stimulation of histamine release from mast cells which promote the non-neurogenic cascade of plasma extravasations and sustained oedema. The neuronal blockade of the stimulation of substance P by the extract, in part may be the pharmacological basis of countering the increased vascular permeability and fluid extravasations induced by nociceptive stimuli.

The data obtained from the analgesic studies revealed that the extract significantly inhibited the first phase of formalin test and increased the reaction time of the hot plate test suggesting that the analgesic property of the extract is in part centrally mediated like morphine. Besides, the inhibition of acetic acid-induced writing in mice and the late phase formalin test suggest that the analgesic effects of the extract are peripherally mediated. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Rxas et al., 1984) while formalin induces neurogenic and inflammatory pains (Vas et al., 1996) and hot plate induce algesia indicates narcotic involvement (Besra et al., 1996). The preliminary phytochemical screening revealed the presence of terpines, alkaloids, flavoniods, tannins and saponins in the extract. Flavoniods (Sharma et al., 1996; Trease and Evans, 1989; Nwafor et al., 2003), tannins and alkaloids (Tijani et al., 2008) have been shown to have inhibitory effects on prostaglandin synthesis and as compounds with anti-inflammatory properties. The various in vivo induced inflammatory models utilised in this studies revealed that the ES methanolic extract have anti-inflammatory and analgesic potential; though the exact mechanism of anti-nociceptive properties may not be fully understood but it may not be unrelated to the present findings: suppression of early phase capillary permeability and exudation through neurogenic and non-neurogenic pathways. Work is in progress in our laboratory to pharmacologically characterize the active ingredient.

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REFERENCES


Anti-Inflammatory and analgesic effects of Emilia sonchifolia


