Anti-Nociceptive, Anti-Inflammatory and Anti-Oxidant Effects of the Methanol Leaf Extract of Sterculia tragacantha Lindl

1R.I. Udegbanum, 2U.I. Asuzu, 1R.O.C. Kene, 1S.O. Udegbanum and 2Chinaka Nwaehujor
1Department of Veterinary Surgery, University of Nigeria, Nsukka, Nigeria
2Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria

Corresponding Author: R.I. Udegbanum, Department of Veterinary Surgery, University of Nigeria, Nsukka, Nigeria Tel: +234 08035647718

ABSTRACT

The anti-nociceptive, anti-inflammatory and anti-oxidant activities of Sterculia tragacantha were evaluated in this study. The extract was also subjected to acute toxicity and phytochemical tests. It was demonstrated that pretreatment of mice with the extract significantly inhibited acetic acid induced pain and carrageenan-induced paw edema. Daily dosing of 300 and 600 mg kg^{-1} of the extract significantly suppressed granuloma formation in mice. The extract showed significant anti-oxidant activity. Phytochemical screening showed that the extract contained alkaloids, flavonoids, tannins, glycosides and saponins. It was concluded that Sterculia tragacantha used in the present study, possesses anti-inflammatory, anti-nociceptive and anti-oxidant activities.

Key words: Sterculia tragacantha, anti-inflammatory, anti-nociceptive, anti-oxidant, phytochemistry

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli (Ferrero-Miliani et al., 2007). It is protective and enables the removal of the injurious stimuli and initiates the healing process. However, severe inflammation may lead to oxidative stress induced by deleterious substances such as reactive oxygen species, neutrophil-derived free radicals and reactive nitrogen species produced by neutrophils and macrophages during inflammation (Valko et al., 2006).

In African traditional medicine, ethno medicines prepared from plants materials are used to treat a wide range of disease conditions including pain and inflammation. These ethno medicines are relied on by local West African dwellers for their primary health care since the plant materials used in their preparation are cheap and readily available (Jodi et al., 2008).

Clinical experiments have validated the efficacy and safety of some plants used traditionally to manage pain and inflammation (Musa et al., 2007; Narendhirakannan et al., 2007; Woode et al., 2009a). Some of these studies have shown that plant constituents with anti-oxidant activity protect tissues from damage caused by products of oxidative stress (Choi and Hwang, 2004; Okoli et al., 2008; Tanas et al., 2010).

Medicinal plant discovery continues and there is need to screen more plants for biologic activity. Among such plants is Sterculia tragacantha Lindl locally known as Uhobo by Nsukka dwellers.
It is a medium sized tree seen in the edges of lowland rain forests of Eastern Nigeria (Keay, 1989). It has been reported that the leaves, bark, shoots and seeds are used to prepare ethno medicines for the treatment of diarrhea, dysentery, arthritis, rheumatism, edema, gout and whitlow (Iwu, 1993). In Nsukka area, its leaves are popularly used by traditional bone setters to prepare ethno medicines used for pain relief after fracture reduction. Its methanol and aqueous extracts have been reported to show significant anti-ulcer, anti-cholinergic, antispasmodic and smooth muscle relaxant properties (Aguwa and Ukwe, 1997).

There is yet no scientific report validating the ethno medicinal uses of the plant in the treatment of inflammatory pain. This lead us to investigate the anti-nociceptive and anti-inflammatory effects of the methanol leave extract of Sterculia tragacantha in mice. Acute toxicity, phytochemical and anti-oxidant tests were also performed with the extract.

MATERIALS AND METHODS

Plant material: Fresh leaves of Sterculia tragacantha were collected in September, 2009 from Okpata in Nsukka area. They were authenticated by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethno medicine and Drug Development, Nsukka. A voucher specimen (INTERCEED/819) was deposited in their herbarium.

Preparation of extract: The fresh leaves were air dried and later pulverized. The plant materials (1.0 kg) were cold macerated in 80% v/v of methanol for 48 h with intermittent shaking. Thereafter, filtration was done using filter papers and glass funnel into an already weighed beaker. The solvent was allowed to evaporate in a rotary evaporator at 40°C to obtain a greenish sticky extract (yield: 11.1%). The extract was stored at 4°C.

Animals: Adult male albino mice weighing 28-38 g were used for the experiments. They were housed at 25±5°C under a 12-h light/12-h night conditions with free access to standard pelleted feed and clean drinking water. All experiments carried out in this study were approved by the Animal Ethics Committee, University of Nigeria, Nsukka. Each experimental group consisted of eight mice housed together. This study lasted from October to November, 2009.

Acute toxicity: Acute toxicity of the extract was investigated as described by Lorke (1983). Five groups of mice were injected i.p. with the extract (200, 400, 800, 1600 and 3200 mg kg⁻¹). The control group received distilled water (1 mL kg⁻¹). They were observed for 48 h for mortality and adverse reactions.

Phytochemical analysis: The extract was screened for the presence of tannins, saponins, alkaloids, flavonoids, glycosides, reducing sugars, terpenes, polyuronides and arthroquinones using standard methods (Harborne, 1984).

Acetic acid-induced writhing: Writhing was induced in mice as described by Koster et al. (1959). Thirty minutes after administration of extract (150, 300 and 600 mg kg⁻¹, i.p.) to mice in three experimental groups, 0.7% acetic acid (10 mL kg⁻¹, i.p.) was injected to induce pain. Mice in the control groups were injected with indomethacin (10 mg kg⁻¹, i.p.) and distilled water (1 mL kg⁻¹, i.p.) before acetic acid administration. The number of contortions by each mouse was counted for 30 min after acetic acid injection. Inhibition (%) was calculated as described by Okoli et al. (2006).
Anti-inflammatory studies: In the anti-inflammatory studies, mice were randomized into three extract (150, 300 and 600 mg kg\(^{-1}\), i.p.) treated groups as well as two control groups treated with indomethacin (10 mg kg\(^{-1}\), i.p.) and saline (1 mL kg\(^{-1}\), i.p.), respectively.

Carrageenan induced paw edema: Acute inflammation of the paw was induced as described by Winter et al. (1962). The paw thickness of each mouse was measured using a venire caliper before edema induction. Acute inflammation was induced 30 min post treatments by injecting 0.02 mL of 1% carrageenan s.c into the left hind paw of mice. The paw thickness of each mouse was re-measured at 1, 2, 3, 4 and 5 h after carrageenan injection. Paw edema was obtained by subtracting the original paw thickness from the paw thickness obtained post edema induction.

Cotton pellet-induced granuloma: Cotton pellet induced granuloma test was performed as described by Niemegeers et al. (1975). Formation of granuloma was induced by subcutaneous implantation of 20 mg of sterile cotton pellets into the left and right axillae of each mouse under pentobarbitone (35 mg kg\(^{-1}\)) anesthesia. Post cotton pellet implantation, the mice were treated once daily with the extract, indomethacin and distilled water i.p. for a period of 7 days. On day 8, the mice were euthanized using chloroform and the pellets covered by granulomatous tissues were carefully dissected out. The moist pellets were weighed and dried at 60°C for 24 h. The dry pellets were then weighed. The weights of the granuloma formed were calculated as the difference between the wet and dry weights.

Free radical scavenging activity: The free radical scavenging activity of the extract (10, 50, 100, 200 and 400 μg mL\(^{-1}\)) was evaluated by the diphenyl picryl hydrayl (DPPH) radical scavenging method (Mensor et al., 2001). Ascorbic acid was used as control. Absorbance at 517 nm was taken after 30 min of incubation of the mixture of extract and DPPH in the dark at room temperature. The percentage antioxidant activity was calculated as follows:

\[
\% \text{ antioxidant activity} = 100 \times \frac{(\text{Absorbance of sample} - \text{Absorbance of blank})}{\text{Absorbance of control}}
\]

Ferric reducing antioxidant activity: The ferric reducing power (FRAP) of the extract (10, 50, 100, 200 and 400 μg mL\(^{-1}\)) was evaluated as described by Benzie and Strain (1999).

Statistical analysis: The mean number of contortions, paw edema and granuloma weights of the groups were compared using one-way ANOVA in the SPSS 12.0 software followed by Duncan multiple range tests. p<0.05 was accepted as statistically significant.

RESULTS
Acute toxicity: No mortality or adverse reaction was detected in mice during the 48 h observation period following i.p injection of the extract up to a dose of 3200 mg kg\(^{-1}\).

Phytochemical analysis: The result of the phytochemical analysis of the extract showed the presence of carbohydrate, starch, glycosides, alkaloids, flavonoids, terpenes, tannins and saponins.
Acetic acid-induced writhing: The result of the analgesic study presented in Table 1 showed that the extract (150, 300 and 600 mg kg⁻¹) significantly inhibited acetic acid induced pain. Their effects were comparable to that of indomethacin.

Carrageenan-induced paw edema: Pretreatment of mice with of 300 and 600 mg kg⁻¹ of the extract significantly inhibited edema formation from one to 4 h (Table 2). At 3, 4 and 5 h post edema induction, 300 and 600 mg kg⁻¹ of the extract showed better anti inflammatory effect compared to indomethacin.

Cotton pellet-induced granuloma: The result of the cotton pellet granuloma test (Table 3) showed that daily injection of 300 and 600 mg kg⁻¹ of the extract significantly inhibited granuloma formation.

Free radical scavenging activity: The result of the DPPH assay is presented in Fig. 1. The result of the assay showed that the extract at 200 and 400 µg mL⁻¹ significantly scavenged DPPH radicals with 63 and 78% activity.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg kg⁻¹)</th>
<th>No. of writhings</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>-</td>
<td>183.5±14.3*</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>31.8±9.6*</td>
<td>82.7</td>
</tr>
<tr>
<td>*Extract</td>
<td>150</td>
<td>43.9±6.5*</td>
<td>76.6</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>25.1±4.4*</td>
<td>86.3</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>22.5±6.4*</td>
<td>87.7</td>
</tr>
</tbody>
</table>

Different superscripts in a column indicate significant difference at p<0.05. * Extract: 150, 300 and 600 mg kg⁻¹ S. tragacantha

<table>
<thead>
<tr>
<th>Treat</th>
<th>Dose (mg kg⁻¹)</th>
<th>Paw edema (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>NS</td>
<td>-</td>
<td>0.06±0.01*</td>
</tr>
<tr>
<td>Indo.</td>
<td>10</td>
<td>0.04±0.01*</td>
</tr>
<tr>
<td>*Extract</td>
<td>150</td>
<td>0.02±0.01*</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.04±0.00*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.03±0.00*</td>
</tr>
</tbody>
</table>

Different superscripts in a column indicate significant difference at p<0.05. NS: Normal saline; Indo: Indomethacin; *Extract: 150, 300 and 600 mg kg⁻¹ S. tragacantha.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg kg⁻¹)</th>
<th>Granuloma wt (g)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>-</td>
<td>0.25±0.02*</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.22±0.02*</td>
<td>12</td>
</tr>
<tr>
<td>Extract</td>
<td>150</td>
<td>0.29±0.02*</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.12±0.02*</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.07±0.01*</td>
<td>72</td>
</tr>
</tbody>
</table>

Different superscripts in a column indicate significant difference at p<0.05
Fig. 1: Percentage anti-oxidant activity of the extract and ascorbic acid

Fig. 2: FRAP values of the extract and ascorbic acid

**Ferric reducing anti-oxidant power:** The result of the FRAP assay presented in Fig. 2 showed that the extract (200 and 400 μg mL⁻¹) significantly reduced ferric oxide with FRAP values of 1.34 and 1.63, respectively.

**DISCUSSION**

In this study we investigated the anti-nociceptive, anti-inflammatory and anti-oxidant activities of the methanol extract of *S. tragacantha*. Preliminary acute toxicity and phytochemical tests of the extract were also performed.

Abdominal injection of acetic acid is used to evaluate drugs for peripheral analgesic activity (Chakraborty *et al*., 2004). It has been reported that acetic acid irritates the peritoneal cavity leading to stimulation of local nociceptors located at the surface of the peritoneal cavity (Bentley *et al*., 1983). This leads to the release of prostaglandins and other algogens with subsequent stimulation of pain nerve endings (Choi *et al*., 2006). The inhibition of acetic acid-induced pain by the extract suggests that it probably reduced the pain response to acetic acid injection by suppressing the release of inflammatory mediators like prostaglandin, bradykinin and histamine (Mehmet, 2002). No scientific report exist on the analgesic effect of *S. tragacantha*, however plant extracts have been shown to effectively inhibit acetic acid induced pain (Okoli *et al*., 2006; Zakaria *et al*., 2006; Santos *et al*., 2007).

Acute inflammation often leads to exudation and release of chemical mediators which increase tissue permeability and cell migration (Jones and Hamm, 1977). The first phase of inflammation is histamine mediated while the second phase is induced by algogens like prostaglandin, bradykinin and serotonin (Ialenti *et al*., 1992; Sumen *et al*., 2001). To experimentally induce acute inflammation, carrageenan was injected subcutaneously into the plantar surface of the mice paw.
The injection of this irritant reproduced the classical phases of acute inflammation in the paw of the mice in the negative control group. The extract significantly inhibited the edema formation in the paw of mice in both phases of inflammation. This is an indication that it was able to inhibit various chemical mediators involved in the early and late phases of acute inflammation (Woode et al., 2007, 2009b). The extents of inhibition of edema by 300 and 600 mg kg\(^{-1}\) of the extract were more than the anti-inflammatory drug indomethacin. A similar report has been documented by Zakaria et al. (2008) following the investigation of the anti-inflammatory effect of Melastoma malabathricum chloroform leaf extract. These authors found out that the effect of the anti-inflammatory drug aspirin (100 mg kg\(^{-1}\)) was lower than that of the extract throughout the experimental period. The reason for this finding was not postulated. However, it has been shown that plants contain several phytochemical constituents which may exhibit complex interactions producing synergistic responses (Savelev et al., 2003).

The high dose of the extract was able to inhibit the proliferative phase of chronic inflammation (Zhang et al., 2008). This is an indication that it was able to inhibit fibroblast activity and collagen synthesis which play important roles in granuloma formation (Swingle and Shideman, 1972). Similar clinical studies have shown that medicinal plant extracts inhibit granuloma formation in experimental animals (Sulaiman et al., 2010; Shahavi and Desai, 2008).

The anti-inflammatory effects of the plant were found to be similar to those of indomethacin, a known non-steroidal anti-inflammatory drug. The plant extract was however more effective than indomethacin in the suppression of chronic inflammation. Thus, since the repeated use of the NSAIDs such as indomethacin for pain relief in chronic and recurrent joint pain leads to unacceptable side effects (Insel, 1990), the use of this plant extract in the management of inflammatory conditions may be preferred with the hope that its use may not only suppress the inflammatory pain but also produce eventual elimination of the cause of inflammation and pain.

The presence of alkaloids and flavonoids in the extract may account for its anti-inflammatory and analgesic activities. A diterpene alkaloid, 3 acetylaconitine (ACC) isolated from the root of Aconitum flavum was reported to possess analgesic activity (Tang et al., 1986). Experiments have also shown that flavonoids isolated from medicinal plants possess anti-inflammatory and antioxidant properties (Musa et al., 2007). This effect has been attributed to their potent inhibition of prostaglandins and oxidative enzymes (Middleton, 1998; Narayana et al., 2001).

The results of the antioxidant assays showed that the methanol leaf extract of Sterculia tragacantha has anti-oxidant properties. It was able to scavenge the free radicals produced by DPPH. The oxidative stress in inflammation is associated with shifts in redox balance, increased generation of Reactive Oxygen Species (ROS), lipid oxidation and decreased antioxidant defenses. These ROS and free radicals generated in the body are known to induce cellular and tissue damage by reacting with proteins, lipids, polysaccharides and nucleic acids (Satoh et al., 1979; Pakoja et al., 1998). Various studies have shown that plant constituents with anti-oxidant activity protect tissues from damage caused by products of oxidative stress (Okoli et al., 2008). Thus it is likely that the anti-oxidant activity of this plant may contribute to the effectiveness of its ethno medicines in the management of inflammatory diseases.

Substances which exhibit high reducing power donate electrons which react with free radicals converting them to more stable products (Duh, 1998). In the FRAP assay, electron donating anti-oxidants donate electrons to Fe\(^{III}\) -tripyridyltriazine (a colorless compound) leading to the formation of a blue colored Fe\(^{II}\) -tripyridyltriazine (Benzie and Strain, 1989). The extract at 200 and 400 \(\mu\)g mL\(^{-1}\) reduced ferric oxide appreciably leading to a change in absorbance of the solutions.
This implied increased production of Fe\textsuperscript{III}-triarylurea from Fe\textsuperscript{III} by the action of the extract. This agrees with the report from similar works done using plant extracts (Ogunlana and Ogunlana, 2008; Ogunlana et al., 2008).

CONCLUSION

The present study showed that the extract of Sterculia tragacantha possesses anti-nociceptive, anti-inflammatory and anti-oxidant activities. These results authenticate the use of the leaves of this plant in the preparation of ethno medicines used in the treatment of ailments associated with pain and inflammation. The inhibition of acetic acid induced writhing as well as the late phase of carrageenan-induced edema by the extract suggests that its anti-nociceptive and anti-inflammatory activities might involve inhibition of prostaglandin synthesis.

REFERENCES


