Anti-nociceptive and anti-inflammatory activities of the aqueous extract of fresh Solanum aculeastrum Dunal. berries in male Wistar rats

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The berry of Solanum aculeastrum is used among other remedies, for treating inflammatory-related ailments in South Africa. The aqueous extract of the fresh berries at 25, 50 and 75 mg/kg body weight was evaluated for anti-inflammatory and analgesic effects in rats using histamine and carrageenan-induced paw oedema, as well as formalin and tail immersion tests. The result of the phytochemical screening indicated that the berries possess alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides. Oral administration of the extract significantly reduced the formation of oedema induced by carrageenan and histamine after 3 h. The extracts also prolonged the reaction time in the tail immersion-induced pain 60 min after administration. In addition, the extract significantly suppressed the nociceptive response in the early and late phases of the formalin-induced pain in a dose-dependent manner, with more pronounced effect on the late phase. These results also compared well with those of indomethacin, the reference drug used in this study. This study therefore gives credence to the traditional uses of S. aculeastrum in the treatment of certain conditions associated with inflammatory pain.

Key words: Solanum aculeastrum, anti-inflammatory, analgesic.

INTRODUCTION

Inflammation is a process that involves the response of cellular tissues to foreign bodies such as pathogenic micro-organisms, toxic chemicals, parasites and injury (van Kempen et al., 2006). Several studies have established a role for inflammation in the initiation of diseases such as diabetes, rheumatoid arthritis and cancer (Balkwill and Mantovani, 2001; Coussens and Werb, 2002). Inflammatory-related ailments are treated mainly with non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are used to reduce the consequences of inflammation (Vane and Botting, 1996). For example, indomethacin has been found to block carcinogenesis in animals by reducing the production of inflammatory cytokines (Federico et al., 2007). However, prolonged use of these drugs has been reported to produce frequent adverse effects such as dyspepsia and severe gastrointestinal complications (Bures et al., 2002). Therefore, there is a need for potent drugs with fewer side effects. As a result, the search for other alternatives seems necessary and beneficial.

Plants possess biologically active compounds of medicinal value (Talhouk et al., 2007). Extracts of these plants have been used to treat inflammation-related ailments in the local communities of developing countries. Solanum aculeastrum Dunal. (Solanaceae) commonly known as Umthuma by the Xhosa speaking people in South Africa is one of such plants. This plant, also known as goat bitter apple, is widely distributed in Southern Africa (Watt and Breyer-Brand wijk, 1962). It is a thorny perennial plant that grows up to 2 to 3 m in height with white flowers and lemon shaped berries that become yellow-green when ripe (Wanyonyi et al., 2002). The bitter fruits of S. aculeastrum is used fresh, dried,ashed or boiled for treating jigger wound and gonorrhoea (Agnew and Agnew, 1994), rheumatism as well as ringworm in cattle and horses (Watt and Breyer-Brand wijk, 1962). Pharmacological studies have revealed the antimicrobial

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and anticaner properties of the leaves and berries (Koduru et al., 2006a, b). Steroidal alkaloids, the major components of the berries of S. aculeastrum produced mollusccidal and anticaner effects in vitro (Koduru et al., 2007; Wanyonyi et al., 2002; Drewes and Van Staden, 1995). Toxicological studies of the aqueous extracts of the fresh, dried and boiled berries of this plant indicated that these extracts may have consequential effect on the normal functioning of the organs of male Wistar rats (Aboyade et al., 2009).

The main objective of the present study was to evaluate the anti-inflammatory and anti-nociceptive potentials of S. aculeastrum berries in animal models in view of the use of the berry in the local treatment of some painful inflammatory conditions.

MATERIALS AND METHODS
Collection of plant materials and authentication
Fresh berries collected from Kayalethu village in the Eastern Cape province of South Africa, were authenticated by Prof. D.S Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (SA/Med 01) is deposited at the Giffen’s herbarium of the University.

Extraction of plant materials
The fresh berries (500 g) was washed, cut into small pieces and then extracted in 1000 ml of distilled water for 48 h on a mechanical shaker (Stuart Scientific Orbital Shaker SO1, United Kingdom). The extract was filtered using a Buchner funnel and Whatman No 1 filter paper. The resulting filtrate was freeze-dried using Vir Tis benchtop K freeze dryer (Vir Tis Company, Gardner, NY) to give a yield of 7.50 g. This was reconstituted in distilled water to give the required doses for each experiment.

Experimental animals
Male rats (Rattus norvegicus) of Wistar strains (120) weighing between 120 and 180 g were obtained at the Experimental Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were housed in clean metabolic cages placed in well ventilated house with optimum condition (temperature 28 ± 1°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). They were also allowed free access to food (Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and water. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals, as well as internationally accepted principles for laboratory animal use and care.

Chemicals
Carrageenan, Tween-80, indomethacin and histamine were obtained from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. All other chemicals used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Phytochemical screening
The screening of some chemical constituents of the extract was carried out as described for tannins and triterpenes (Odebiiy and Sofowora, 1978), alkaloids (Harbourne, 1973), phenolics and flavonoids (Awe and Sadipo, 2001), cardiac glycosides, saponins and steroids (Edoega et al., 2005), cardenolides, dienolides and anthraquinones (Trease and Evans, 1989).

Anti-inflammatory activity
Carrageenan-induced paw oedema
Thirty animals were grouped into five of six animals each. While the first group served as the negative control, the second, third, fourth and fifth groups received indomethacin (10 mg/kg body weight) and extract (25, 50 and 75 mg/kg body weight), respectively. The extract was dissolved in normal saline, while indomethacin was suspended in 3% Tween 80 in normal saline. Carrageenan solution (0.1 ml of 1%) was injected into the sub plantar region of right hind paw of the rats. 1 h after intraperitoneal administration of normal saline, indomethacin and the extract (Moody et al., 2006). The paw volume was measured at 0.5, 1, 2, 4 and 6 h after administration of drug and extract using a micrometer screw gauge (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India). The anti-inflammatory effect of the extract was calculated using the expression:

\[
\text{Anti-inflammatory activity} \% = \left(1 - \frac{D}{C}\right) \times 100
\]

Where \(D\) represents the average paw volume after extract was administered to the rats and \(C\) was the average paw volume of the negative control animals. The percentage inhibition of inflammation was calculated from the expression:

\[
\% \text{ Inhibition} = \frac{D_0 - D_t}{D_0} \times 100
\]

Where \(D_0\) was the average inflammation (hind paw oedema) of the control group at a given time and \(D_t\) was the average inflammation of the drug treated (extracts or reference indomethacin) rats at the same time (Gupta et al., 2005; Sawadogo et al., 2006).

Histamine-induced paw oedema
Using the method described by Perianayagam et al. (2006), paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw volume was recorded before the histamine injection. Rats (six per group) were orally administered with 0.5 ml of the extract corresponding to 25, 50 and 75 mg/kg body weight 30 min prior to the administration of histamine. The controls were administered with 10 mg/kg body weight of indomethacin (positive control) and 0.5 ml of normal saline (negative control). Histamine was administered intraperitoneally 1 h after the administration of the extract and indomethacin. The right hind paw volume was measured at 1, 2, 3 and 6 h using a micrometer screw gauge. The anti-inflammatory activity was calculated as described earlier for carrageenan-induced oedema.

Analgesic activity
Tail immersion test
Acute nociception was assessed using the tail immersion test described by Vogel and Vogel (1997). Briefly, this method entails immersing the extreme 3 cm of the rat’s tail in a water bath (Buchi water bath B-480, Buchi, Switzerland) maintained at a temperature of 55.00 ± 0.5°C. The time spent by the animal before reacting to
the pain was measured with a stop watch as the initial reaction time (Tb). The various groups of the animals were orally administered with the extracts (25, 50 and 75 mg/kg body weight), indomethacin (10 mg/kg body weight) and distilled water. The response latency between the onset of immersion and the withdrawal of the tail (Ta) following the administration of the extract and the reference drug was recorded at 30, 60 120, 240 and 360 min after a latency period of 30 min. The percentage analgesic activity was computed from the expression:

\[
\text{Percentage analgesic activity} = \left(\frac{\text{Ta} - \text{Tb}}{\text{Tb}}\right) \times 100
\]

**Formalin-induced pain test**

The formalin-induced pain test was carried out according to the procedure as described by Correa and Calixto (1993). Briefly, 0.05 ml of 2.5% formalin solution was injected into the sub-planter of the right hind paw. The number of times the rat licked the injected paw was recorded and was considered as indicative of pain. The animals were pretreated with normal saline, indomethacin and extracts, 30 min before the administration of formalin, and the responses were observed for 30 min.

**Statistical analysis**

Data was expressed as mean of six replicates. Statistical difference between the control and the treated groups were tested by Student’s t-test. Values were considered statistically significant at \( p < 0.05 \).

**RESULTS**

Phytochemical screening of the aqueous extract of *S. aculeastrum* berries showed the presence of alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides. In the carrageenan-induced inflammatory model, oedema was reduced by the aqueous extract of *S. aculeastrum* at concentrations of 25, 50 and 75 mg/kg body weight in a dose-dependent manner. The maximum inhibition of paw oedema was observed at the end of 6 h (Table 1). The percentage reduction in inflammation diameter was highly significant (\( p < 0.05 \)) in the group treated with 75 mg/kg of the extract after 3 h compared to the vehicle control. Furthermore, the sub-acute inflammation test with histamine showed an inhibition of the paw oedema with indomethacin and the extract in a dose-dependent manner, while an increase in paw volume of the control group was observed (Table 2). The extract at 75 mg/kg body weight and indomethacin exhibited comparable activity at 6 h (\( p < 0.05 \)).

All the doses of the extract (25, 50 and 75 mg/kg body weight), had analgesic effect on both early and late phases of the formalin test as shown on Table 3. These phases correspond to neurogenic and inflammatory pains respectively. Inhibition of pain resulting from inflammation was higher than the neurogenic-induced pain at all concentrations tested. The highest dose (75 mg/kg b.wt) exhibited more prominent response to the pain at both phases compared to indomethacin. Following 30 min latency period, 50 and 75 mg/kg of orally administered extract caused reduction of the painful sensation due to tail immersion in warm water (Figure 1). The antinociceptive property of the extract at 75 mg/kg was more pronounced than that of indomethacin at 60 min.

**DISCUSSION**

The study indicated that the aqueous extract of fresh *S. aculeastrum* berry possess both peripheral and central analgesic properties as well as anti-inflammatory potential. The ability of anti-inflammatory agents to inhibit mediators of acute inflammation is the basis of carrageenan-induced inflammation test. These mediators include histamine, serotonin and bradykinin, which are responsible for the induction of inflammation in the first phase (Kasahara et al., 2002). The second, late phase of inflammation is induced mainly by prostaglandins that are released from the third hour (Di-Rosa et al., 1971). The pronounced anti-inflammatory effect of *S. aculeastrum* berry observed in both the carrageenan and histamine-induced oedema test from 4 h is an indication of the potential of the extract to inhibit the release of prostaglandins (Di-Rosa et al., 1971). These results indicate the effectiveness of *S. aculeastrum* on acute inflammatory conditions.

Phytochemical screening of the extract shows that *S. aculeastrum* berries possess alkaloids, saponins, phenolics, flavonoids, cardenolides and dienolides. Of these, flavonoids are the main compounds possessing anti-inflammatory activities (Talhouk et al., 2007). Many studies have also demonstrated the anti-inflammatory and analgesic properties of alkaloids, phenols (Ahmadiani et al., 2001) and saponins (Peana et al., 1997). The study of Felderman and Kovacs (1969) reported that the oral administration of tomatoine, one of the steroidal alkaloids found in *S. aculeastrum*, exerted significant dose-dependent inhibitory activity on oedema formation. Therefore, it could be suggested that the anti-inflammatory potential of the berries of *S. aculeastrum* may be due to the presence of these active constituents.

The formalin test represents a more valid model for the assessment of clinical pain (Tjolsen et al., 1992) and it produces distinct biphasic responses (Vasudevan et al., 2007). Various analogs differ in their response to the formalin test. Drugs such as opioids inhibit both phases of the formalin test because they act on the central nervous system (Shibata et al., 1989), while aspirin, hydrocortisone and dexamethasone act peripherally, thereby inhibiting only the second phase (Vasudevan et al., 2007). The first (neurologic) phase results from the stimulation of nociceptors, while the second phase is of an inflammatory pain origin. Assessment of the antinociceptive response using the formalin test showed that all the doses (25, 50 and 75 mg/kg b.wt), inhibited both phases of the formalin test. The highest dose (75 mg/kg b.wt) exhibited more prominent response to the pain at
both phases compared to indomethacin (Table 3). Therefore, the results of this study suggest that the S. aculeastrum extract acts centrally and peripherally in the formalin test. The study also showed that the extract of S. aculeastrum possess a centrally acting protective effect in the tail immersion test. The result indicated that after 30 min latency period, 50 and 75 mgkg\(^{-1}\) of orally administered extract caused reduction of the painful was sensation by the warm water. The protective effect at 75 mgkg\(^{-1}\) b.wt more pronounced than indomethacin, which was inactive.

In conclusion, the anti-inflammatory and analgesic properties of S. aculeastrum berry observed in these models might in part be due to the presence of compounds such as alkaloids, saponins, phenolics and flavonoids. The results obtained in this study suggest a rational for the traditional use of this plant in Southern Africa for some painful inflammatory conditions. However, the medicinal use of the plant may pose considerable

### Table 1. Effect of intraperitoneal administration of S. aculeastrum berries on carrageenan-induced rat paw oedema in rats (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Differences in right hind paw (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.72 ± 0.08(^{a})</td>
</tr>
<tr>
<td>SAB</td>
<td>25</td>
<td>0.58 ± 0.09(^{a}) (11.9)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.62 ± 0.06(^{a}) (7.3)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.36 ± 0.04(^{a}) (46.5)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.45 ± 0.09(^{a}) (40.4)</td>
</tr>
</tbody>
</table>

** Test values are significantly different from the control down the group for each hour (P < 0.05). Percentage inhibitions are indicated in brackets.

### Table 2. Effect of oral administration of S. aculeastrum berries on histamine-induced rat paw oedema in rats (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Differences in right hind paw (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.78 ± 0.06(^{a})</td>
</tr>
<tr>
<td>SAB</td>
<td>25</td>
<td>0.71 ± 0.06(^{a}) (5.1)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.69 ± 0.17(^{a}) (9.9)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.55 ± 0.07(^{a}) (26.9)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.54 ± 0.09(^{a}) (19.2)</td>
</tr>
</tbody>
</table>

\(^{a}\) Test values are significantly different from the control down the group for each hour (P < 0.05). Percentage inhibitions are indicated in brackets.

### Table 3. Effect of oral administration of S. aculeastrum berries on formalin nociception response in rats (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mgkg(^{-1}))</th>
<th>Number of times licked (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase 1</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>14.00 ± 1.41(^{a})</td>
</tr>
<tr>
<td>Extract</td>
<td>25</td>
<td>10.67 ± 3.21(^{a})</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.00 ± 1.63(^{b})</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>5.00 ± 1.41(^{c})</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>5.25 ± 1.71(^{b})</td>
</tr>
</tbody>
</table>

\(^{a}\) Test values are significantly different from the control down the group for each hour (P < 0.05).
Figure 1. Effect of oral administration of S. aculeastrum berries (SAB) on tail immersion nociception response in rats (n = 6).

health risks, since investigation conducted into the plant's safety revealed that the berry extracts may not be completely safe as an oral remedy (Aboyade et al., 2009).

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