Full Length Research Paper

Comparative studies on the anti-inflammatory and analgesic activity of the aqueous extracts from fresh, dried and boiled berries of *Solanum aculeastrum* Dunal.

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The berry of *Solanum aculeastrum* Dunal. is used for treating diseases such as rheumatism, gonorrhea, breast cancer and other inflammatory-related ailments in South Africa. The aqueous extracts of the fresh, dried and boiled berries at doses of 1 and 10 mg/kg body weight was evaluated for anti-inflammatory and analgesic effects in male Wistar rats using carrageenan-induced paw oedema as well as formalin, acetic acid induced writhing and tail immersion tests. Oral administration of the extract showed some inhibition of the paw oedema that was not dose dependent. The percentage reduction in inflammation diameter was more prominent in both concentrations of the boiled fresh berries than indomethacin. The extracts at 10 mg/kg prolonged the reaction time in the tail immersion-induced pain 60 min after administration. Although, only the extracts of the boiled dried berries (10 mg/kg) suppressed pain in the formalin test at the late phase, a more pronounced effect of all the extracts in a dose-dependent manner was observed in the late phase than the early phase. The results of the acetic acid induced writhing test showed that all the extracts possessed analgesic effect at the tested doses (1 and 10 mg/ml). In conclusion, the extracts of *S. aculeastrum* berry did not show considerable anti-inflammatory and analgesic effects in comparison to indomethacin. This observation in these models might in part be due to the low doses fed to the rats in this experiment.

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

INTRODUCTION

Inflammation is the way the body deals with infections and tissue damage (Simmons, 2006). Uncontrolled, inflammation can lead to a cascade of diseases such as rheumatoid arthritis, chronic asthma, psoriasis, cancer, multiple sclerosis and a host of other diseases (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Simmons, 2006). Various studies have shown that inflammation may be an initiator of carcinogenesis via genotoxic and cytotoxic effects (Platz and De Marzo, 2004). The longer inflammation persists, the higher the risk of cancer (Federico et al., 2007). Cancers of infectious etiology such as stomach, bladder, colon, liver and bile duct have been linked to inflammation (Coussens and Werb, 2002). Non-steroidal anti-inflammatory drugs (NSAIDs) are the main drugs used to reduce the consequences of inflammation (Vane and Botting, 1996). Indomethacin, an NSAID, for example has been found to block carcinogenesis in animals by reducing the production of inflammatory cytokines (Federico et al., 2007). A lower risk of cancer incidence has also been found in people regularly taking NSAIDs (Fosslien, 2000). Although, these drugs have been found to be effective, however, prolonged use has been reported to produce frequent adverse effects such as dyspepsia and severe gastrointestinal complications (Bures et al., 2002). This has therefore led to various researches to other effective drugs with less/no side effects.

Medicinal plants have been in use since ancient time in the treatment of various ailments. Plants such as *Catharanthus roseus* G. Don (Apocyanaceae), *Digitalis purpurea* Linn (Scrophulariaceae), *Rauwolfia serpentina* Plum ex Linn (Apocyanaceae), Salix species (Salicaceae), and Physostigma venenosum Balf. (Papilionaceae) have

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been used for centuries for the treatment of diseases. Since then, plants have served as sources of biologically active natural products which are either used as commercial drugs or as lead structures for the development of modified derivatives (Cordell et al., 1991). Extracts of *Solanum aculeastrum* Dunal. (Solanaceae) have been used to treat inflammation-related ailments in the local communities of South Africa. This plant also known as goat bitter apple is traditionally used by the Xhosa speaking people of South Africa in the treatment of breast cancer (Koduru et al., 2007a). *S. aculeastrum* is widely distributed in Southern Africa (Watt and Breyer-Brandwijk, 1962) and the bitter fruits are either used fresh, dried, ashed or boiled for treating jigger wound and gonorrhea (Agnew and Agnew, 1994), rheumatism as well as ringworm in cattle and horses (Watt and Breyer-Brandwijk, 1962). Pharmacological studies have revealed the antimicrobial and anticancer properties of the leaves and berries (Koduru et al., 2006a, b).

Previously, the authors studied the anti-inflammatory and analgesic effect of the fresh berries of *S. aculeastrum*. Although the extract was found to be active at the tested doses, toxicological studies indicated that those concentrations were not completely safe for use. Therefore, the main objective of the present study was to compare the anti-inflammatory and anti-nociceptive potentials of the fresh and boiled extracts of the berries in animal models in view of its use 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, was authenticated by Prof. D. S. Grierson at the Department of Botany, University of Fort Hare. A voucher specimen (SA/Med 01) deposited at the Giffens Herbarium of the University.

Extraction of plant materials

The fresh berries collected were divided into two portions. The first portion was oven dried at 40°C, a part of which (400 g) was boiled with distilled water (BDB) and the other (300 g) soaked with water (DB) by shaking for 24 h on an orbital shaker. The second portion of the fresh berries (1500 g) was soaked (FB) and boiled (2000 g) separately with distilled water (BFB). The extracts were filtered using a Buchner funnel and Whatman No 1 filter paper. This was thereafter freeze dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give yields of 2.03 g (BFB), 2.01 g (FB), 11.85 g (BDB), 13.16 g (DB) per 100g of plant material. The crude extracts were then reconstituted in distilled water to give the required doses for each experiment.

Animals

Male rats (*Rattus norvegicus*) of Wistar strains weighing between 120 and 180 g were obtained at the Experimental Animal House of the Agricultural and Rural Development Research Institute (ARDRI),

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.

Anti-inflammatory activity

Carrageenan-induced paw oedema

The animals were assigned into ten groups (A - J), each consisting of six rats. Group A (control) received only 0.5 ml of normal saline (the vehicle), group B received indomethacin (10 mg/kg) while groups C to J were treated with the same volume of the extract preparation corresponding to 1 and 10 mg/kg body weight of the extracts, respectively. The extracts were 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 1, 3 and 5 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:

Anti-inflammatory activity (%) = (1 - D/C) × 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:

% inhibition = $D0 - Dt/D0$ × 100.

where $D0$ was the average inflammation (hind paw oedema) of the control group at a given time; and Dt was the average inflammation of the drug treated (that is, extracts or reference indomethacin) rats at the same time (Gupta et al., 2005, Sawadogo et al., 2006).

Analgesic activity

Tail immersion test

Acute nociception was assessed using the tail immersion test. This method involves immersing extreme 3 cm of the rat tail in a waterbath (Buchi waterbath B480, Buchi Switzerland) containing water at a temperature of 55 ± 0.5°C. The time the animal's tail spent in the water before reacting to the pain is recorded with a stop watch. Each animal served as its own control. The average of the values was recorded as the initial reaction time (Tb). The test groups were given the extracts (1 and 10 mg/kg), indomethacin (10

Table 1. Effect of aqueous extract of *Solanum aculeastrum* berries and indomethacin on carrageenan-induced edema paws volume in male Wistar rats.

Treatment groups	Doses (mg/kg body weight)	Right hind paw volume (mm)		
		1 h	3 h	5 h
Control	0	1.26 ± 0.28^a	1.83 ± 0.56^a	1.85 ± 0.50^a
Indomethacin	10	0.53 ± 0.09^a (58.3)	0.65 ± 0.09^{ba} (62.6)	0.55 ± 0.02^{ba} (68.8)
Dried berries	1	0.22 ± 0.67^b (80.3)	1.03 ± 0.61^{bcd} (39.5)	0.19 ± 0.13^c (87.8)
	10	0.49 ± 0.23^b (56.4)	0.76 ± 0.25^c (52.2)	0.73 ± 0.04^c (54.6)
Boiled dried berries	1	0.59 ± 0.55^b (45.3)	1.17 ± 0.17^{bc} (25.7)	0.52 ± 0.18^c (64.8)
	10	0.55 ± 0.12^b (50.4)	0.78 ± 0.17^c (49.6)	0.47 ± 0.09^{dc} (68.8)
Fresh berries	1	0.49 ± 0^b (55.3)	0.84 ± 0.33^{cd} (46.2)	0.39 ± 0.21^c (74.0)
	10	0.73 ± 0.30^b (37.2)	1.00 ± 0.40^{bc} (39.1)	0.31 ± 0.20^{dc} (79.4)
Boiled fresh berries	1	0.44 ± 0.31^b (60.5)	0.65 ± 0.32^d (86.1)	0.73 ± 0.42^{cb} (95.2)
	10	0.58 ± 0.06^b (53.2)	0.56 ± 0.08^c (66.9)	0.07 ± 0.13^d (95.9)

Values are expressed in mean ± standard deviation (n = 6).
Values in brackets denote percentage inhibition of the edema paw volume.
[a-d] Test values carrying superscripts different from the control for each parameter are significantly different (P < 0.05).

mg/kg body weight) and normal saline. The reaction time (Ta) for the test groups were taken at 0, 1, 3 and 5 h after a latency period of 30 min, following administration of the extract and reference drug (Vogel and Vogel, 1997). The percentage analgesic activity was computed from the expression:

Percentage analgesic activity = (Ta - Tb)/ Tb × 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-plantar of the right hind paw. The number of times spent licking 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 were expressed as mean of six replicates and were subjected to one way analysis of variance (ANOVA). Means were separated by the Duncan multiple range test using SAS and complemented with student's t-test. Values were considered statistically significant at p < 0.05.

RESULTS

The sub-acute inflammation test with the carrageenan-induced inflammatory model showed that the inhibition of the paw oedema in all the extracts was not dose dependent (Table 1). The percentage reduction in inflammation diameter was highly significant at p < 0.05 and more prominent in both concentrations of the boiled fresh berries than indomethacin.

Regarding the inhibition of pain in the formalin nociception test, all the extracts at 1 mg/ml showed no significant difference with the control (Table 2). The boiled dried berry extract showed analgesic effect on both the early and late phase of the test, while the extracts of the dried, fresh and boiled fresh berries failed to demonstrate any significant change. Inhibition of pain resulting from inflammation was higher in all the extracts compared to the neurogenic induced pain.

The 30 min latency period following the oral administration of the aqueous extracts of the dried, fresh, boiled dried and fresh extracts of the berry showed that the extracts and indomethacin at 10 mg/kg significantly increased the reaction time. Indomethacin and the extract of the boiled dried berries at 10 mg/kg showed their maximal analgesic effect 1h post-treatment with percentage inhibitions of 28.04 and 25.11%, respectively. At 1 mg/kg, only the boiled extracts of the fresh berries demonstrated any significant effect on pain (24.08%) in the tail immersion test at the fifth hour (Table 3).

DISCUSSION

The results of the present study showed the effect of processing on the activity of the aqueous extracts of *S. aculeastrum* berries. The four extracts varied in their potential to act either centrally or peripherally in their effect on pain. The peripheral analgesic activity of this plant can be deduced from its inhibitory effect on chemically induced nociceptive stimuli. Only the boiled extracts of the dried and fresh berries exhibited both peripheral and centrally mediated protective effect on the animals.

Assessment of the analgesic response using the formalin test showed that at both concentrations tested, none of the extracts inhibited the phase of the formalin test which produces a biphasic response (Vasudevan et al., 2007). The study showed the extracts possessed

Table 2. Effect of oral administration of Solanum aculeastrum berries on formalin nociception response in rats.

Treatment	Dose (mgkg^{-1})	Number of times licked (mean ± SD)			
		Phase 1	% Inhibition	Phase 2	% Inhibition
Control	0	16.0 ± 4.00[a]	-	28.3 ± 7.64[a]	-
Dried berries	1	19.0 ± 5.57[a]	- 18.8	21.3 ± 3.21[ba]	24.7
	10	16.3 ± 2.52[ba]	- 2.08	20.3 ± 0.58[ba]	28.2
Boiled dried berries	1	14.3 ± 8.08[a]	10.4	14.0 ± 7.81[b]	50.6
	10	9.0 ± 1.73[bc]	43.8	12.7 ± 2.52[b]	55.3
Fresh berries	1	17.3 ± 1.53[a]	-8.3	19.0 ± 1.73[b]	32.9
	10	15.3 ± 3.21[ba]	4.2	17.3 ± 0.58[b]	38.8
Boiled fresh berries	1	20.0 ± 1.0[a]	- 25.0	22.3 ± 1.53[ba]	21.2
	10	20.3 ± 8.96[ba]	21.0 ± 9.64[ba]	- 27.1	25.9
Indomethacin	10	3.0 ± 1.0[b]	81.3	1.7 ± 0.58[c]	94.1

SD = Standard deviation; n = 6.
[a-c] Test values carrying superscripts different from the control for each parameter are significantly different (P < 0.05).

Table 3. Effect of oral administration of aqueous extracts of S. aculeastrum berries on tail immersion nociception response in rats.

Treatment groups	Doses (mg/kg body weight)	Time (s)			
		0 h	1 h	3 h	5 h
Control	0	2.83 ± 0.45[a]	3.77 ± 1.17[a]	4.08 ± 1.76[a]	2.88 ± 0.50[a]
Indomethacin	10	2.28 ± 0.49[ba]	2.92 ± 1.07[ba] (28.04)	2.50 ± 0.39[b] (9.96)	2.51 ± 0.31[ba] (10.32)
Dried berries	1	2.98 ± 1.01[a]	2.22 ± 0.42[bc] (- 25.42)	1.99 ± 0.70[b] (-33.03)	2.57 ± 0.72[ba] (- 13.55)
	10	2.47 ± 0.56[a]	2.50 ± 0.75[bc] (1.28)	1.92 ± 0.41[b] (-22.08)	2.71 ± 0.55[a] (9.86)
Boiled dried berries	1	2.44 ± 0.47[a]	2.30 ± 0.49[bc] (- 5.73)	1.60 ± 0.18[b] (-34.40)	1.98 ± 0.11[bc] (- 18.84)
	10	2.22 ± 0.38[a]	2.77 ± 0.19[b] (25.11)	2.60 ± 0.28[ba] (17.22)	2.40 ± 0.26[ba] (8.4)
Fresh berries	1	2.00 ± 0.17[a]	1.73 ± 0.24[bc] (- 13.73)	1.51 ± 0.12[b] (-24.46)	1.35 ± 0.11[c] (- 32.61)
	10	2.56 ± 0.22[a]	2.66 ± 0.56[b] (3.98)	2.53 ± 0.36[ba] (-1.17)	2.66 ± 0.89[ba] (3.85)
Boiled fresh berries	1	1.30 ± 0.08[b]	1.18 ± 0.04[c] (- 8.74)	1.38 ± 0.02[b] (6.78)	1.61 ± 0.13[c] (24.08)
	10	2.16 ± 0.04[a]	2.30 ± 0.03[b] (6.41)	2.33 ± 0.03[ba] (7.96)	2.44 ± 0.03[ba] (13.21)

Values are expressed in mean ± standard deviation (n = 6).
Percentages of protection against thermally induced pain by warm water are in parentheses.
[a-c] Test values carrying superscripts different from the control for each parameter are significantly different (P < 0.05).

significant effect in the second phase (resulting from an inflammatory pain) than in the first phase (caused by the stimulation of nociceptors) of the formalin test (Coderre and Melzack, 1992; Abbadie et al., 1997). Also, boiling had differing effects on the activity of the fresh and dried extracts. While inhibition increased when the dried extracts were boiled, the opposite trend was observed on the boiled, fresh extracts. Conversely, the tail immersion test showed that boiling had similar, positive effects on the inhibitory performance of both fresh and dry extracts.

The carrageenan induced oedema results from the ability of anti-inflammatory agents to inhibit mediators of acute inflammation. This study showed that the effect of the extracts on this model was not dose dependent. At 10 mg/kg, extracts of the dried, boiled dried and boiled fresh berries possessed higher inhibitory activity than the fresh extracts throughout the measurement intervals. This implies

that processing had a positive effect on the anti-inflammatory potential of the berries of S. aculeastrum.

In conclusion, the extracts of S. aculeastrum berry did show comparable anti-inflammatory and analgesic effects to indomethacin. Also, in all but the formalin test, processing improved anti-inflammatory and analgesic activity of the berry extracts.

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