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ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF AQUEOUS EXTRACT FROM LEAVES OF *SOLANUM TORVUM* (SOLANACEAE)

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## Abstract

*Solanum torvum* is used in Cameroonian traditional medicine for the management of pain and inflammation. The present work assesses the pain-killing and anti-inflammatory properties of the aqueous extracts of *Solanum torvum* leaves. Acetic acid- and pressure- induced pains were reduced by this extract while carrageenan-induced inflammation was inhibited at various doses of the extract. The extract therefore has both analgesic and anti-inflammatory properties.

**Key words:** Analgesic, inflammation, *Solanum torvum*, writhing, Analgesy Meter.

## Introduction

*Solanum torvum* (Solanaceae) also known as ‘top na aka’ in the Batoufam language, is a plant used in Cameroonian folk medicine for the treatment of fever, wounds, and tooth decay. This plant is also used for its haemostatic properties (Henty, 1973). Recent investigations demonstrated that *Solanum Torvum* (*S. torvum*) has antimicrobial activity (Chah et al., 2000). Importantly, isoflavonoids isolated from this plant showed significant antiviral properties (Arthan et al., 2002). Since no literature is currently available to substantiate the analgesic and anti-inflammatory properties of *S. torvum*, the present study was designed to provide scientific evidence of the claimed ethnopharmacological properties by investigating the analgesic and anti-inflammatory properties of this plant.

## Experimental

### Plant material

The fresh leaves of *S. torvum* were harvested in the month of September 2003, in the Yaoundé area, Cameroon and identified at the National Herbarium, Yaoundé, where a voucher specimen HNC N° 21103 was deposited. Harvested leaves were air-dried away from direct sunshine after which they were ground to powder in a food processor. 100g of ground leaves powder were macerated for 2h in 500 ml distilled water at 90°C. After filtration, the aqueous filtrate was concentrated by evaporation at 40°C and yielded 12.8 g of dry powder. Preliminary phytochemical screening of the aqueous extract of *S. torvum* revealed the presence of sterol, triterpenes, sugars, alkaloids and phenols.

### Animals

Wistar rats (weighing 160-220 g) and Swiss mice (weighing 20-30 g) of both sexes were bred in our animal house at room temperature and had access to food and water, *ad libitum*. Prior to treatment, the experimental animals were fasted overnight but allowed access to drinking water. Ethical approval for this study was obtained from the Research Board of the University of Yaoundé I.

## Drugs

Aspirin (Aspegic<sup>®</sup>, Sanofi-Synthelabo France), Indomethacine (Indocid<sup>®</sup>, Laboratoire Merck Sharp et Dohme France), Morphine (Sigma-Aldrich-Quinina S.A. Madrid-Spain), Tramadol (Laboratoire Pharmascience, France, Couveboie) and plant extract were dissolved in distilled water, Carrageenan (Sigma Chemical Co, St Louis, USA) in physiological saline.

## Noiceptive activities

The analgesic activity of *S. torvum* was measured against chemical and mechanical stimuli.

### Acetic acid-induced abdominal writhing test

30 swiss mice were divided into six groups of five animals each. Each group of animals was treated orally with one of the following: NaCl 9<sup>0</sup>/<sub>00</sub>, aspirin (100mg/kg), tramadol (25mg/kg), morphine (1.5mg/kg), *S. torvum* (300mg/kg) and (600mg/kg) respectively. One hour after administration of the test drugs, animals were injected intra-peritoneally with 1% acetic acid (1 ml/100 g body weight). The number of writhing responses such as contortions and stretching were recorded for 30 minutes. The results were evaluated by calculating the mean number of contortions per treated group and results compared to results obtained from control animals. Percentage pain inhibition was calculated as follows.

$$\text{Pain inhibition (\%)} = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nc}} \times 100$$

$\overline{Nc}$  : mean number of contortions of the control group

$\overline{Nt}$  : mean number of contortions per treated group

### Pressure test

In these experiments, the drugs cited above were tested at the same dose levels in rats using an Ugo Basile Analgesy Meter (N° 7200). A constantly increasing force was applied to the left hind paw of experimental animals by the Analgesy Meter plunger. Rats were restrained in the upright position while their left hind paws were placed between the plinth and the plunger. Pain was determined by the physical struggles of the animal to set itself free. The weight causing pain before treatment and then 1, 2 and 3 h after treatment of animals with the various test drugs was determined. Pain inhibition rates were calculated as follows.

$$\text{Pain inhibition (\%)} = \frac{\overline{Ft} - \overline{Fo}}{\overline{Fo}} \times 100$$

$\overline{Fo}$  : force at which the animal struggles to free its paw before administration of drugs.

$\overline{Ft}$  : force at which the animal struggles to free its paw after administration of drugs.

### Carrageenan-induced paw edema

Oedema was induced on the right hind paw of rats by a sub-plantar injection of 0.05 ml of a solution of 1% sterile carrageenan in saline (Lanhers *et al.*, 1991). The plant extract (300 and 600 mg/kg) and Indomethacine (10 mg/kg) were administered orally 30 minutes before carrageenan injection while control animals received the vehicle only. The volume of the injected paw was measured before and 1, 2, 3, 4 h after induction of inflammation using a Plethysmometer (Ugo Basile N° 7140). Inflammation was expressed as an increase in paw volume due to carrageenan injection. Oedema ( $\Delta V$ ) and inhibition rate (I %) were calculated as follows.

$$\Delta V = \overline{vt} - \overline{vo}$$

$$I\% = \frac{(\overline{vt} - \overline{vo})C - (\overline{vt} - \overline{vo})E}{(\overline{vt} - \overline{vo})C} \times 100$$

$\overline{vt}$  : right hind paw volume at time t. C: control group, E: experimental group

$\overline{vo}$  : right hind paw volume before sub-plantar injection of carrageenan

### Statistical analysis

One way ANOVA followed by Dunnett's multiple comparison tests were performed. The differences between groups were considered significant when  $P < 0.05$ . The statistical package used for these analyses was Instat. Results are expressed as mean  $\pm$  SEM

### Results

#### Antinociceptive effects

##### Acetic acid-induced abdominal writhing test

The results presented in Table 1 show that *S. torvum* extract at doses of 300 and 600 mg/kg *po* decreased the number of writhes by 23 % and 26 % ( $p < 0.05$ ) respectively. The reference drugs (Morphine, Tramadol and Aspirin) induced significant ( $P < 0.01$ ) reduction of the noted parameters by 61 %, 23 % and 48 % respectively. Pain inhibition by 300mg/kg of the extract was similar to the results obtained with tramadol. Although two doses were used, the higher dose (600mg/kg), did not elicit a greater protection from acetic acid induced abdominal writhing as would have been expected.

**Table 1:** Effect of the aqueous leave extracts of *S. torvum* on acetic acid-induced pain.

Group	Dose (mg/kg)	Contraction $\pm$ SEM	Pain inhibition (%)
Control	-	148 $\pm$ 14	-
Aspirin	100	77 $\pm$ 8**	48
Tramadol	25	113 $\pm$ 10*	23
Morphine	1.5	58 $\pm$ 8**	61
<i>S. torvum</i>	300	114 $\pm$ 9*	23
<i>S. torvum</i>	600	110 $\pm$ 13*	26

Test groups were compared with the control group and significant differences in response were noted. \*  $P < 0.05$ , \*\* $P < 0.01$ . n = 5

**Table 2:** Effect of the aqueous leave extracts of *S. torvum* on pressure-induced pain. Measurements (in gf (gram force)) were done before the administration of the various drugs and at various time intervals after drug administration. Every animal served as its own control.

Group	Dose (mg/kg)	Before drug administration	After drug administration (% analgesia)		
			1H	2H	3H
Control	-	86 $\pm$ 5	80 $\pm$ 9 (-7)	92 $\pm$ 5 (7)	86 $\pm$ 5 (0)
Aspirin	100	89 $\pm$ 4	105 $\pm$ 11 (18)	102 $\pm$ 6 (15)	95 $\pm$ 4 (7)
Tramadol	25	78 $\pm$ 5	92 $\pm$ 5 (18)*	130 $\pm$ 4 (67)**	104 $\pm$ 4 (33)**
Morphine	1.5	81 $\pm$ 5	107 $\pm$ 7 (32)*	136 $\pm$ 2 (68)**	106 $\pm$ 10 (31)*
<i>S. torvum</i>	300	80 $\pm$ 2	107 $\pm$ 8 (34)*	113 $\pm$ 7 (41)*	105 $\pm$ 6 (32)*
<i>S. torvum</i>	600	82 $\pm$ 4	104 $\pm$ 4 (27)*	117 $\pm$ 7 (43)*	132 $\pm$ 8 (61)**

Test groups were compared with the control group and significant differences in pain perception were noted.  $P < 0.05$ ; \*\* $P < 0.01$ . n = 5

**Table 3:** Anti-inflammatory activity of aqueous extract of *S. torvum* in carrageenan-induced hind paw edema: expressed as a percentage of volume variation ( $\Delta V$  in mL).

Group	Dose (mg/kg)	1H (% inhibition)	2H (% inhibition)	3H (% inhibition)	4H (% inhibition)
Control	-	0.35±0.06	0.52±0.08	0.61±0.07	0.55±0.05
Indomethacine	10	0.20±0.01 (43)	0.20±0.02 (62)**	0.11±0.02 (82)**	0.08±0.04 (85)**
<i>S. torvum</i>	300	0.24±0.03 (34)	0.36±0.06 (31)	0.37±0.07 (39)*	0.29±0.08 (47)*
<i>S. torvum</i>	600	0.33±0.08 (11)	0.51±0.08 (4)	0.37±0.08 (41)	0.29±0.07 (49)*

Test groups were compared with the control group and significant difference were noted.

\*  $P < 0.05$ , \*\* $P < 0.01$ . n = 5

### Pressure test

Aspirin (100 mg/kg) did not show significant antinociceptive effect on mechanical pain. The analgesic effects of the aqueous extract of *S. torvum* leaves (300 and 600 mg/kg *po*) were significant one hour after drug administration, and the results were similar to those obtained with both morphine (1.5 mg/kg) and tramadol (25 mg/kg). Whereas the maximal pain blocking activity of tramadol, morphine and 300mg/kg *S. torvum* extract were observed two hours after drug administration, 600mg/kg of *S. torvum* extract showed maximal activity ( $p < 0.01$ ) during the third hour after extract administration (Table 2). The analgesic effects of this extract dose was highest when the analgesic effects of all the other test drugs were already waning out.

### Carrageenan-induced paw edema

Control animals showed progressively increasing paw volume in response to carrageenan injection during the experiment. *S. torvum* leaves aqueous extract significantly attenuated paw swelling ( $P < 0.05$ ) 2 and 4 hours following oral administration (Table 3). The anti-inflammatory effect of the extracts were however not dose-dependent. Indomethacine had a greater inhibitory effect ( $P < 0.01$ ) on carrageenan-induced paw oedema compared to *S. torvum*.

### Discussion

The present study, made use of two models for the investigation of the analgesic effect of *S. torvum* extract. Acetic acid induced writhing and pressure tests were selected to investigate peripheral and central antinociceptive effects of the plant extract. Carrageenan induced paw oedema in rat was selected to represent a model of acute inflammation.

The present results showed that the aqueous extract of *S. torvum* induced dose dependant analgesic effect against the writhing syndrome indicating its peripheral effect (Atta and Afolabi, 1997). In peripheral tissues, prostaglandins and kinines would seem to play an important role in the pain process (Hajare *et al.*, 2000) and writhing induced by chemical substances injected intra-peritoneally is said to be the consequence of sensitisation of the chemosensitive nociceptors by prostaglandins (Maria *et al.*, 1997). These results suggest that the pain killing effect of this plant may be by the prostaglandins synthesis inhibition. This test also confirms the peripheral action of Aspirin (Rang *et al.*, 1995).

The pre-treatment of rats with *S. torvum* extract inhibited pain caused mechanically by a constantly increasing pressure on rat paw by the plunger and plinth of the Analgesy Meter. This system provides a model for the study of non inflammatory pain. The opioid-like analgesic drugs are more effective in inhibiting mechanically induced pain (Nkeh *et al.*, 2002). In this model of pain, the involvement of endogenous substances such as prostaglandins may be minimized (Dongmo *et al.*, 2001). The central protecting effect of *S. torvum* leaves extracts were comparable to tramadol and morphine test results. Tramadol is known to inhibit neuronal re-uptake of serotonin (Oliva *et al.*, 2002) while morphine activity is mediated by  $\mu$  opioid receptors (Raffa *et al.*, 1993). Aspirin did not show analgesic effect on this model of pain, thus confirming the previous

study in which Aspirin and Aspirin like drugs were ineffective against pain due to sensory nerve stimulation (Flower *et al.*, 1985).

In the last part of the study, the carrageenan experimental model of inflammation was used to evaluate the anti-inflammatory effect of *S. torvum*. Some authors (Hwang *et al.*, 1996; Lo *et al.*, 1987), think that the initial phase of carrageenan paw oedema is mediated by histamine and serotonin, while the later phase is suspected to be due to arachidonate metabolites (prostaglandins, leukotrienes) producing oedema dependent on mobilisation of neutrophils. Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflammatory process, inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors (Flower *et al.*, 1985).

In our experiments, rats pre-treated with *S. torvum* extract showed a significant oedema inhibitory response 2 h following carrageenan injection. This result suggests that *S. torvum* extract may act by suppressing the later phase of the inflammatory process by the inhibition cyclooxygenase, the enzyme involved in the formation of prostaglandins.

The presented data therefore, indicate that the oral administration of *Solanum torvum* extract showed analgesic and anti-inflammatory activities. These properties confirm its use in folk medicine.

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