Full Length Research Paper

Analgesic, anti-inflammatory and antiulcer properties of the extract of *Uapaca guineensis* (Euphorbiaceae)

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Uapaca guineensis is an euphorbiaceae used traditionally for the management of pain and inflammation. The present study was aimed to investigate the analgesic, anti-inflammatory and anti-ulcer properties of the methanol extract of stilt root barks of *U. guineensis* in rats and mice. The analgesic properties of *U. guineensis* were investigated using the acetic acid writhing, hot plate as well as pressure-induced pain models. This was followed by a study of the anti-inflammatory properties of this plant using the carrageenan-induced acute inflammation method. The anti-ulcer test was done using high doses of both the extract and indomethacin. The extract showed significant (p < 0.05) reduction of pain induced by all three models of nociception. It also had significant (p < 0.05) anti-inflammatory properties and showed a non-ulcerative effect on gastric mucosa. Our results show that the methanolic extract of *U. guineensis* has both analgesic and anti-inflammatory properties and importantly does not corrode gastric mucosa.

Key words: Pain, inflammation, nociceptive, writhing, ulcer.

INTRODUCTION

Inflammation is the response of living tissues to infection and injury. The hall marks of inflammation include pain and swelling (Tracy, 2006). Most human diseases have a pain and inflammation component which leads to individuals seeking medical attention (Merskey, 1986). As such, analgesic and anti-inflammatory drugs are among the most prescribed drugs in clinical practice (Lim and Yap, 1999). Despite the progress in the discovery of anti-inflammatory and analgesic drugs, the chronic use of these drugs is hampered by their adverse effects such as gastric lesions or tolerance as seen with NSAIDs and opiate analgesics respectively. Therefore, it is important to search for potent analgesic and anti-inflammatory drugs with less adverse effects from plant sources. One such potential plant is *Uapaca guineensis*

(Euphorbiaceae). *U. guineensis* is a plant used in Cameroonian folk medicine for treating fever, inflammation, pain, skin diseases and sexual dysfunction. Locally known as '*Assam*' in the Beti language (Vivien and Faure, 1996), this plant is found in the humid forests in the central region of Cameroon (Betti, 2004). The objective of the present study was therefore, to investigate the analgesic, anti-inflammatory and the effect of UG on gastric mucous integrity.

MATERIAL AND METHODS

Methanol extract of the stilt root bark extract of *U. guineensis* is abbreviated as UG in the text

Plant material

The whole stilt roots of UG were collected in Yaoundé, Cameroon, in September, 2006. Identification and authentication of the plant material was done at the National Herbarium, Yaoundé, Cameroon, where a voucher specimen has been registered (No. 41501/HNC).

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Preparation of the methanol extract of the stilt root bark of UG

Methanol extract was prepared by soaking the air-dried powdered stilt root bark of UG in the ratio 1:20 (w/v) for 72 h at room temperature. The suspension was filtered using Whatman filter paper No. 1. After evaporation of methanol, the extract separated into two phases. The methanol soluble phase was aspirated and air dried for this study. Preliminary phytochemical screening of the extract revealed the presence of flavanoids, terpenoids, saponins, alkaloids and sterols. The dried methanol extract was dissolved to prepare stock solutions of doses 62 mg/kg and 124 mg/kg in a 5% DMSO (vehicle) solution for use in all experiments (Schiller et al., 1979).

Animals

Swiss albino mice of either sex weighing 20 - 25 g and Wistar rats weighing 180 - 200 g were obtained from the University animal holding facility. Animals were housed in a standard environmental conditions and 12:12 h light/dark cycle. All animals had free access to water and standard rat chow. In all experiments each group was made up of five animals. This study was approved by the Ethical Committee of the Walter Sisulu University. Reference No: Ethics 0009-07.

Drugs

Indomethacin (Indocid®, Merck Sharp Laboratories, France), Morphine (Sigma-Aldrich-Quiinina S.A. Madrid-Spain), Celecoxib (Pfizer, South Africa) and plant extract were dissolved in distilled water while Carrageenan (Sigma Chemical Co, St Louis, USA) was dissolved in physiological saline.

Acetic acid-induced writhing test

This test was performed as described by Fontenele et al., (1996). Mice were randomly divided into five groups and each group was orally pretreated with: 0.9% NaCl, indomethacin (10 mg/kg), morphine (10 mg/kg) or UG (62 and 124 mg/kg). Pain was induced by the intraperitoneal injection of 0.1 ml/10 ml acetic acid (1% v/v) 1 hour after pretreatment which is within the efficient therapeutic duration of morphine. Animals showed characteristic stretching behaviour (writhing). The number of abdominal contortions was recorded for 30 min and results were evaluated by calculating the mean number of abdominal contortions per group.

Hot plate test

This test was carried out as described by Wagh et al. (2006). In this experiment, drugs were tested in mice using Eddy's hot plate (DS37-Socrel). The temperature was maintained at $55 \pm 0.2°C$; - a temperature hots enough to cause discomfort without tissue damage. Animals licked their forelegs and/or jumped as an indication of pain. Similar doses of extract and test drugs as those used in the writhing test were used in this test. The reaction time for each animal was recorded before treatment and every hour for 4 h after treatment. Pain inhibition rates were calculated as:

Pain inhibition = $R_t - R_0$.

Where R_t = reaction time at any given time.

R_0 – reaction time before treatment with drugs.

Pressure-induced pain

In these experiments, the drugs were tested at the same doses as mentioned before. Nociceptive thresholds (in gf) were estimated using an analgesimeter (Ugo Basile, Milan, Italy). A constantly increasing pressure was applied to the right hind paw until the rats vocalized or withdrew their hind paws. This indicated the level at which the rats felt pain (Ito et al., 2001). The weight causing pain before treatment and 1 h, 2 h and 4 h after treatment with the drugs was determined. Pain inhibition was calculated thus:

Pain inhibition = $F_t - F_0$

Where F_t – is force causing pain at a given time

F_0 – is force causing pain before treatment with drugs

Anti-inflammatory test: carrageenan induced paw edema

The carrageenan-induced paw edema test was used to determine the anti-inflammatory activity of UG. The right hind paw just below the tibiotarsal junction was plunged into the plethysmograph for volume determination. Standard anti-inflammatory drugs and UG were administered orally to rats 60 min prior to the injection of carrageenan into the right paw (0.1 ml, 1% w/v solution in sterile saline). Paw volumes were measured before treatment and 30 min 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Anti-inflammatory activity was determined by subtracting the paw volume before pretreatment from paw volume at given times after pretreatment.

Ulcerogenic properties

Male and female Wistar rats were starved for 48 h with access to water *ad libitum*. Four groups of animals each received 0.9% NaCl solution, 30 mg/kg indomethacin, 124 or 240 mg/kg UG. Rats were decapitated 6 hrs post treatment followed by laparotomy. With stomach *in situ*, two ligatures were made, at the pyloric sphincter and at the end of the oesophagus. The stomach was then sectioned out of the animal and filled with 10 ml of 4 % formalin for fixation of ulcers. Ten minutes later, the stomach was opened along the greater curvature, rinsed with tap water and the ulcers evaluated in mm^2 using scores as described by Martin et al. (1993). The percentage of the ulcerated surface (US) was calculated as follows:

$$\%US = \frac{TUS}{675\,mm^2} \times 100$$

Where TUS = Total Ulcerated Surface.

$675mm^2$ = Calculated total surface area of the rat stomach.

Cotton was used to dry the gastric membrane and a spatula used to scrape out the mucus in the stomach which was subsequently weighed.

Statistical analysis

All data are presented as mean ± SEM. One-way ANOVA followed by Dunnett's multiple comparison tests were used to analyze and compare data. Level of significance was set at $P < 0.05$. All statistical calculations were carried out using Graphpad Instat® Prism 3.0 (USA) statistical software.

RESULTS

Analgesic effects of UG extract

Acetic acid-induced writhing: The UG extract showed a significant ($p < 0.01$) dose-dependent inhibition of acetic acid induced writhes in mice at both the 62 and 124 mg/kg, compared to controls. However, the effect of UG was greater than that observed for the non-steroidal anti-inflammatory drug indomethacin (10 mg/kg) and the opioid morphine (10 mg/kg). Of the two analgesic standard drugs used, indomethacin showed more effective inhibition of acetic acid-induced writhes (Figure 1). The effect of 124mg/kg UG was significantly ($p < 0.05$) greater than that observed with morphine.

Hot plate method: The UG extract induced significant ($p < 0.05$) dose related analgesia in mice as shown by an increase in the reaction time. Specifically, 124 mg/kg induced significant analgesic effects compared to controls by the 2nd h, similar to morphine but the analgesic effect had dissipated by the 4th hour. The morphine analgesic effect continued to be significantly different from controls in the 4th h. The lower dose of 62 mg/kg showed a delayed onset of the analgesic effects, which became evident at 3 hours and similar to the higher dose, had dissipated by the 4th hour. However, morphine was significantly ($p < 0.05$) more effective than both doses of UG (Figure 2).

Pressure-induced pain test: At 124 mg/kg body weight, UG extract showed early onset of analgesic effect to rat paw pressure from 1 h post treatment to 5 h post treatment, similar to morphine. At the lower dose of 62 mg/kg there was late onset of analgesic effects which became evident at 4 h post treatment but was still significantly different from controls after 5 h. Indomethacin did not show significant protective effect against this pain model (Figure 3).

Anti-inflammatory test: Carrageenan-induced progressive paw inflammation which peaked two hours post carrageenan injection followed by slow and progressive resolution of the inflammation as shown by the results obtained with the control animals. This pattern of inflammation development was inhibited by treatment with UG and the standard anti-inflammatory drugs indomethacin and celecoxib. The anti-inflammatory properties of UG were dose dependent. The 124 mg/kg dose of UG showed sustained significant anti-inflammatory properties from 1 h post treatment to beyond 6 h similar to celecoxib. The 62 mg/kg dose had a delayed onset of activity (2 h post treatment) but sustained activity to 6 h post treatment. Celecoxib was more potent and its effect lasted beyond 6 h. Indomethacin, on the other hand had significant anti-inflammatory effects from 2 - 5 h post treatment only (Figure 4).

Ulcerogenic test: The UG extract at 124 and 248 mg/kg body weight did not cause gastric mucosal ulceration in rats. Furthermore amount of gastric mucus was similar to controls. However, indomethacin (30 mg/kg) induced ulcerations of stomach wall as well as significantly ($p < 0.05$) reduced the amount of gastric mucus produced compared to controls (Table 1).

DISCUSSION

In the present study, the analgesic, anti-inflammatory and ulcerogenic effects of UG were evaluated. This extract was found to have remarkable antinococeptive, anti-inflammatory and non-ulcerative properties, thus confirming its use for the treatment of inflammation and pain in Cameroonian folk medicine.

Analgesic effects of UG were evaluated using chemical, thermal and mechanical models of nociception. In the chemical model using acetic acid-induced writhing test, UG at both lower and higher dose showed inhibition of the writhing syndrome in a manner similar to indomethacin, a peripheral analgesic. Acetic acid induces pain by the release of endogenous mediators of pain such as prostacyclines through the activity of the enzyme cyclooxygenase (COX) (Satyanarayana et al., 2004; Ballou et al., 2000) As such, this model of nociception is one of peripherally mediated pain and therefore should be effectively inhibited by peripheral analgesics through COX inhibition. Our results therefore show that UG has peripheral analgesic properties similar to indomethacin, probably due to inhibition of COX activity and further inhibition of the release of other endogenous pain mediators. Indeed, UG at 124 mg/kg body weight was more effective at inhibiting acetic acid-induced pain than morphine, an opioid which is more effective in managing central pain. However, acetic acid-induced writhing is a non-specific test responding to analgesics as well as other classes of drugs such as anticonvulsants (Meymandi and Sepehri, 2008). In order to confirm these analgesic properties, the hot plate method which involves spinal reflexes and is regarded as one of the more suitable methods for studying the involvement of central acting analgesics (Lavich et al., 2005) was carried out.

Two models for testing the effects of UG on central pain were used. The hot plate method is a thermal model of inducing central pain with possible narcotic involvement. The UG extracts showed analgesic effects at both doses. The higher dose of UG showed early onset of analgesic effects similar to morphine but by the 4th h the UG analgesic effects waned off. At the lower dose of 62 mg/kg body weight, UG extract had a later onset of analgesia which waned off by the 4th h, similar to the higher dose. At all time points the analgesic activity of morphine was consistently better than UG. However, because in the hot plate test four paws are involved and the reaction may be modified by the presence of other

Writing test
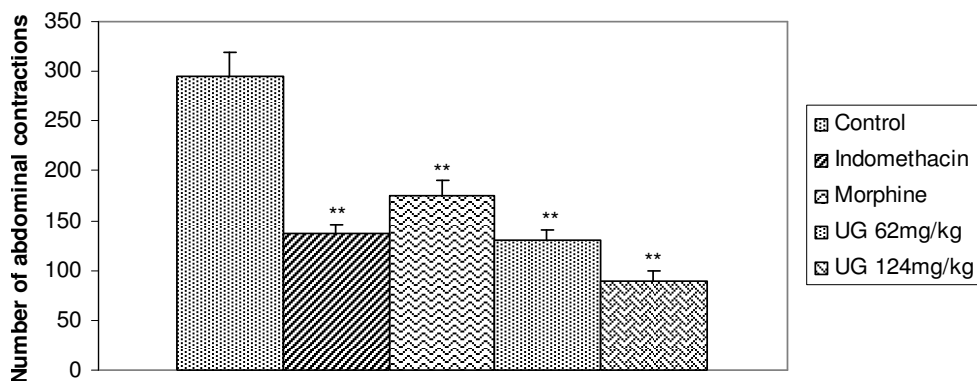
Figure 1. Effects of UG on acetic acid-induced abdominal contractions. Each column represents the mean of 5 animals ± S.E.M. **p < 0.01, significantly different from the control group.

Hot plate test
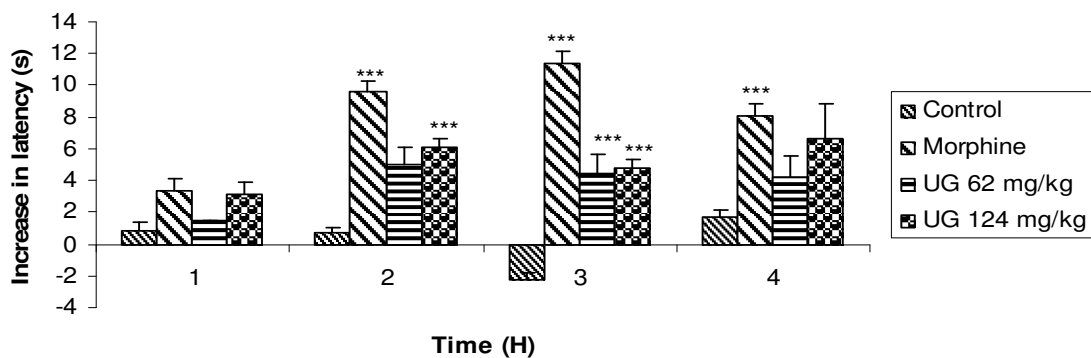

Figure 2. Effect of UG on reaction time when animals were placed on the hotplate. Each value represents the mean of 5 animals ± S.E.M. *p < 0.05, ***p < 0.001, significantly different from the control group

Response to mechanically induced pain
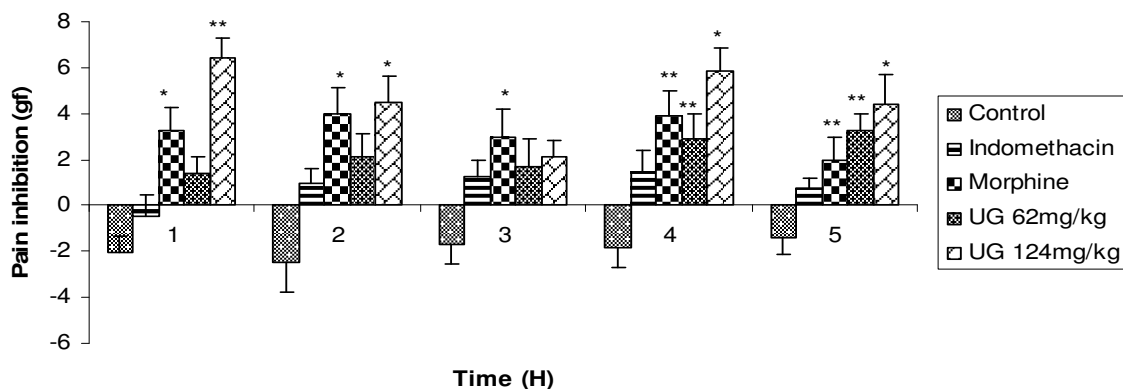
Figure 3. Effects of UG and reference drugs on mechanically-induced pain. Values represent the mean of 5 animals ± S.E.M. *p < 0.05, **p < 0.01, significantly different from the control group.
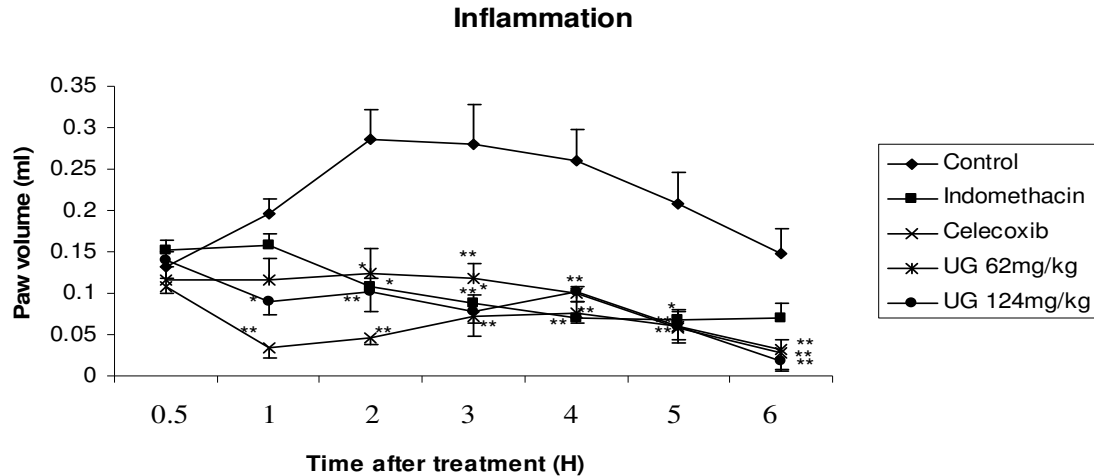
Inflammation

Figure 4. Time dependent effects of UG, indomethacin and celecoxib on carrageenan-induce inflammation. Values in this graph represent the mean of 5 animals ± S.E.M. *p < 0.05, **p < 0.01, significantly different from the control group.

Table 1. Ulcerogenic effects of UG compared to effects of indomethacin.

	Average mass of mucus (mg)	Ulcerated surface (mm^2)	Ulceration index	% Ulceration	% of rats with ulcers
NaCl (0.09%)	27.1 ± 4.7	0.0	0.0	0.0	0
Indomethacin (30 mg/kg)	12.6 ± 1.1*	13.3 ± 2.0**	2.5 ± 0.0	9.8 ± 00	100
UG 124 mg/kg	25.5 ± 4.0	0.0	0.0	0.0	0.0
UG 248 mg/kg	23.7 ± 3.0	0.0	0.0	0.0	0.0

Results are expressed as mean of 5 animals ± SEM. *p < 0.05 and **p < 0.01.

more painful stimuli, we decided to confirm our findings by using the paw pressure test which is another model of testing for central analgesia.

In the mechanical pain model of centrally mediated pain, UG showed similar patterns of early onset of analgesic effects for the higher dose as in the hot plate test but with sustained analgesic effects similar to morphine. The paw pressure pain test is a non-inflammatory pain model which is sensitive to centrally acting antinociceptive agents (Vivancos et al., 2004). The pre-treatment of rats with UG inhibited mechanically-induced pain, an indication of the central acting properties of the plant. Cervero et al. (1988) showed that mechanically-induced pain may result in stimulation of either Aÿ and C fibres or both. Thus the central analgesic properties of UG may therefore, be due to the inhibition of either Aγ or C fibres or both. Indomethacin did not produce significant analgesic effect on this model since it is more sensitive to peripheral and inflammatory pain (Hwang et al., 2008).

The carrageenan-induced paw edema test was used to study the anti-inflammatory effects of UG. The higher dose of UG (124 mg/kg) prevented inflammation from the first to the sixth hour post carrageenan injection. This

anti-inflammatory drug. At 62 mg/kg, UG showed significant anti-inflammatory effects although its effect was manifest only from the second hour post treatment. Both doses of UG produced anti-inflammatory effects which were more like those of celecoxib after the 4th h post treatment. Indomethacin prevented inflammation between 2 and 5 h post treatment after which its effect became non-significant. Carrageenan-induced paw inflammation in rats is considered to be one of the best methods for screening for anti-inflammatory properties of a drug (Morris, 2003). This model causes an inflammatory response which occurs in two phases: the first phase occurs within the first hour post carrageenan-injection followed by the second phase which lasts beyond three hours post injection (Garcia-Pastor et al., 1999). The first phase involves the release of histamine and 5HT while the second phase is dependent on the release of pro-inflammatory mediators such as prostaglandin E2 (Kulkarni et al., 1986; Suleyman et al., 2003). Like celecoxib, the extract of UG was effective against both phases of carrageenan-induced inflammation. Indome-thacin on the other hand, was effective only on the second phase of this model of inflammation confirming its effect as a peripheral cyclooxygenase inhibitor. The

ability of UG to inhibit paw inflammation indicates that it has effects on both the early and late phases of carrageenan-induced inflammation.

The prolonged use of NSAIDs for the management of chronic pain is often associated with gastric ulcer formation. Therefore the ulcerogenic effects of UG were investigated. High doses of (124 and 248 mg/kg) had no ulcerative effects on gastric mucosa nor was mucous secretion decreased. However, high doses of indomethacin produced visible gastric ulcers in animals as well as significantly ($p < 0.05$) reduced mucous secretion. Gastric ulcers are reportedly the result of the inhibition of prostaglandin on gastric mucosa (Radi and Khan, 2006). Indomethacin like other NSAIDs is a potent inhibitor of prostaglandin biosynthesis (Filaretova et al., 2007). Prostaglandins are known to play an important role in maintaining the integrity of gastric mucosa (Wallace, 2008) as well as stimulation of mucous and bicarbonate secretion. UG had no effect on gastric mucosa suggesting that its mode of action may not be by the inhibition of COX-1. These results suggest that the anti-inflammatory properties of UG may not be using mechanisms similar to the non-steroidal anti-inflammatory drugs. The present study confirms the folklore use of UG for pain relief and as an anti-inflammatory decoction.

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