Anti-inflammatory and Analgesic Properties of Ethanolic Stem Bark Extract of *Ficus trichopoda* in Rats.

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ABSTRACT

Introduction: *Ficus trichopoda* is a ficus species growing in wet places – swamp forest, river banks and swamp grassland. It is well used in the management of inflammation - related conditions locally. This present work was undertaken to investigate anti-inflammatory and analgesic properties of aqueous ethanolic extract of *Ficus trichopoda* bark in rats. **Methods:** The anti-inflammatory effect was investigated by using the acute inflammatory model of carrageenan - induced paw oedema and its analgesic activity using formalin test and tail flick test in rats. **Results:** The study for preliminary phytochemical secondary metabolites revealed the presence of saponins, tannins, alkaloids and free amines/amino acids. Oral administration of the aqueous ethanolic extract of *F. trichopoda* in doses up to 5000 mg/kg body weight did not produce any mortality and any visible signs of toxicity. *Ficus trichopoda* extract at the doses (125 - 500 mg/kg) showed significant ($p < 0.05$) dose dependent inhibition of oedema formation in the carregeenan induced model. The extract showed significant dose dependent inhibition of the inflammatory (late) phase but not the neurogenic (early) phase of the formalin test in rats. The analgesic activity in tail flick method showed significant ($p < 0.05$) elevation in pain latency threshold from 30, 60, 90 and 120 minutes after pretreatment. **Conclusion:** The results obtained suggest marked analgesic and anti-inflammatory activity of ethanolic extract (125- 500 mg/kg). This finding supports that the stem bark is useful in inflammatory and painful conditions.

Key words: Bark, *Ficus trichopoda*, Inflammation, Pain, Phytochemicals

INTRODUCTION

Medicinal plants have long been used in traditional medicine for therapeutic purposes and their healing effects have well been recognized since ancient times. *Ficus trichopoda* Baker is a medicinal plant belonging to the Moraceae family (commonly called fig trees) used popularly as a 'multi-purpose' medicinal plant in Uganda. Forty-four species are known from Uganda.[1-2]

Ficus trichopoda is a ficus species growing in wet places – swamp forest, river banks and swamp grassland. The vernacular name is 'kaboga' in Luganda language of Uganda. It is widely used for live fence, building poles, fibre, firewood and ground water.[3] The majority of the medicinal uses of figs in humans are based on historical reports or anecdotal evidence with only a few reports coming from modern clinical trials. The reported medicinal properties of different *Ficus* species includes: anti-inflammatory, antineoplastic, antioxidant, antiulcer and antidiarrheal.[4] Species of the genus *Ficus* (Moraceae) are used in many parts of East Africa for the treatment and management of many clinical and gynaecological problems.[5-6]

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Despite the popular use of these species in management of inflammation – related conditions locally, there are no literatures on biological studies addressing the anti-inflammatory and analgesic effects of *F. trichopoda*. This study reported the investigation into the anti-inflammatory effects of the plant extract using carrageenan-induced oedema in rats, and its analgesic activities using formalin (chemical) and thermal (tail immersion) pain induction methods.

MATERIALS AND METHODS

Plant collection and extract preparation

The bark of *F. trichopoda* was collected in November 2009 in Ishaka, Western Uganda. The plant was identified by its local name and was later authenticated at the Botany Department of Makerere University Kampala. A voucher specimen was prepared and deposited at the Kampala International University, School of Pharmacy herbarium.

The plant bark was air dried at room temperature and later ground to powder. The air- dried powdered bark of *F. trichopoda* was extracted with 70% ethanol by cold maceration for 48 hours with occasional shaking. The extract was concentrated by evaporation and dried in an oven at 40^0C to obtain a brown solid powder. A freshly prepared solution in distilled water was used for pharmacological studies.

Animals

Male Wistar rats weighing 120 to 200g and male Swiss albino mice weighing 25 to 40g were obtained from the Pharmacology department laboratory animal facility. Animals were maintained in ordinary animal cages under constant 12 h/12 h light/dark cycle. They were acclimatized in the laboratory environment for at least two weeks, and were fed with standard pellet diet (Nuvita animal feed Ltd, Uganda) and water *ad libitum*. All experiments were carried out with strict compliance to The "Principle of Laboratory Animal Care" (NIH Publication No. 85-23)[7] and ethical guidelines for investigation of experimental pain in conscious animals.[8]

Drugs and Reagents

Indomethacin (MSD, Canada), Carrageenan (Sigma Chemical Co., USA) and Formaldehyde (M & B, UK) were used.

Phytochemical Screening

Conventional standard protocols (Trease and Evans, 1983)[9] for detecting the presence of different chemical constituents in the plant extract were employed. Secondary metabolites tested include alkaloids, tannins, saponins, glycosides, flavonoids, digitalis, and phenols.

Acute toxicity study

Acute toxicity study was carried out using the method of Lorke[10] with slight modification using the oral route. In the first phase, nine mice randomly divided into three groups of three mice per group were given 500, 1000 and 5000 mg/kg body weight of ethanol extract of *F trichopoda* orally (via a cannula), respectively. The mice were observed for signs of adverse effects and death for 24 h and then weighed daily for 14 days. The geometric mean of the least dose that killed mice and the highest dose that did not kill mice was taken as the median lethal dose.

Carrageenan-induced paw oedema in rats

Pedal inflammation in rats was produced according to the method described by Winter et al.[11]. Animals in treatment groups were orally administered with 125, 250, and 500mg/kg of ethanol extract of *F. trichopoda*, and indomethacin (10 mg/kg). At the same time, control animals received 10 ml/kg saline. One hour later, all animals were injected with 0.1ml of 1% carrageenan in the right hind foot under the subplantar aponeurosis. Measurement of paw size was carried out by gently wrapping a piece of white cotton thread round the paw, and measuring the circumference on a metre rule. This method has been successfully used in previous studies.[12, 13, 14] Measurements of paw (oedema) sizes were carried out just before, and at hourly intervals for five hours after carrageenan injection. Readings were taken at least twice on each occasion and the mean value for each reading was recorded.

Many studies showed that oedema in the right hind paw of rats induced with carrageenan peaks at third hour following the inhibitory activity of test agents and indomethacin against carrageenan –induced rat paw oedema. The inhibitory activity was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{(Ct - Co)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - Co)\ \text{control}} \times 100$$

where Ct – paw (oedema) sizes at time 3h after carrageenan injection and Co – paw (oedema) sizes before carrageenan injection.

Formalin test

This test was carried out in five groups of rats (n = 5) pretreated with either saline; the extracts (125, 250 and 500 mg/kg p.o.) and indomethacin. Formaldehyde (0.05 ml,

2.5%) was injected into the sub-plantar surface of the rat left hind paw 30 min after treatment. Severity of pain was rated using Dubuisson and Dennis,[15] pain scoring measurements in the following manner: (0), normal weight bearing on the injected paw; (1), light resting of the paw on the floor; (2), elevation of the injected paw; and (3) for licking, biting and grooming of paw. These responses were observed and recorded for a total of 60 min. The first 10 min were considered as the early phase and represents aphasic pain while the period between 15 and 60 min was recorded as the late phase (representing tonic pain).

Tail-Flick test

The study as described by Yaro et al[16] was adopted with minor modification for our local laboratory settings. The rats were initially screened for the test by immersing about 2cm of their tails into hot water maintained at 50 ± 1°C. The animals that lifted their tail within 5 seconds were selected for the study.

Twenty five rats that shows response (withdrawing the tail within 5 s) were selected and grouped into five (n = 5). The rats were treated with extracts (125, 250 and 500 mg/kg), indomethacin (10 mg/kg), or saline (10 ml/kg). The tail withdrawal reflex period (latency/pain threshold) was taken at 30, 60, 90 and 120 minutes after the administration for each rat.

Data analysis

Results were expressed as mean ± standard error of mean (SEM). Student t-test was used to analyze level of statistical significance between groups and Analysis of Variance (ANOVA) among groups. All level of significance were set at $p < 0.05$.

RESULTS

Phytochemical Screening

Phytochemical analysis of the crude extract gave positive reactions for the following secondary metabolites: saponins, tannins and alkaloids, free amines/amino acids. Glycosides, flavonoids, digitalis, phenols, resins and volatile oils were absent.

Acute toxicity studies

Oral administration of the aqueous ethanolic extract of *F. trichopoda* in doses up to 5000 mg/kg body weight did not produce any mortality and any visible signs of toxicity when observed up to 72 hrs after administration. It was observed that there was no weight loss, no loss of appetite, and no mortality up to 14 days after treatment.

Carrageenan-induced paw oedema in rats

The result obtained from this experiment is shown in Table 1. Pre-treatment with Extract (125 – 500 mg/kg) and indomethacin (10 mg/kg) produced statistically significant ($p<0.05$) dose dependent inhibition of the oedematous response. At the third hour post-carrageenan, oedema was inhibited by 19.23, 23.08, and 38.46% by 125, 250 and 500 mg/kg of extract respectively. Indomethacin administered at 10mg/kg inhibited oedema by 61.53%.

Formalin test

The extract exhibited a dose dependent howbeit insignificant inhibition of the early phase of the formalin test showing a pain inhibition of 26.96, 37.39 and 46.09%. In the late phase (15 – 60 mins), pain inhibition was significant ($P < 0.05$) showing % inhibition of 41.13, 61.47, and 60.60%

Table 1: Effect of ethanolic extract of *Ficus trichopoda* bark (FTB) on carrageenan-induced paw oedema in rats.

Treatment	Dose (mg/kg)	Paw Sizes (cm) at					
		0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
Control Distilled water	10 ml/kg	2.22 ± 0.04	2.60 ± 0.03	2.60 ± 0.03	2.74 ± 0.02	2.72 ± 0.06	2.70 ± 0.04
FTB Extract	125	2.24 ± 0.05	2.56 ± 0.02	2.64 ± 0.04	2.66 ± 0.02* (19.23)a	2.66 ± 0.05	2.70 ± 0.07
FTB Extract	250	2.22 ± 0.07	2.54 ± 0.05	2.66 ± 0.06	2.62 ± 0.02* (23.08)a	2.50 ± 0.06	2.54 ± 0.04
FTB Extract	500	2.26 ± 0.04	2.42 ± 0.06	2.56 ± 0.05	2.58 ± 0.04* (38.46)a	2.64 ± 0.06	2.50 ± 0.05
Indomethacin	10	2.24 ± 0.02	2.52 ± 0.04	2.48 ± 0.09	2.44 ± 0.05* (61.53)a	2.46 ± 0.05	2.44 ± 0.05

Each value is the mean ± SEM of five rats (n = 5)
*Each value is significant at $p < 0.05$, compared with control using the Student's t – test.
[a]Inhibition (%) of oedema in treated group vs control

for 125, 250 and 500 mg/kg of the extract respectively (Table 2). Higher pain inhibition was, however, observed in the late phase.

Tail immersion test

The results of this test are shown in Table 3. There was significant (P< 0.05) dose dependent increase in pain response threshold against heat induced stimulus. This effect started 30 mins after treatment and persisted throughout the 120 min duration of the experiment.

DISCUSSION

The absence of death in the groups of mice treated with *Ficus trichopoda* bark extract at doses up to 5000 mg/kg body weight is suggestive that the extract is practically non –toxic acutely and is relatively safe with low risk of acute intoxication. This study evaluated the scientific basis for the traditional use of *Ficus trichopoda* against inflammation and pain. The anti-inflammatory and analgesic effects were analysed using different stimuli such as chemical agents (carrageenan, formalin) and heat (tail-flick). The results demonstrated a modest but significant anti-inflammtory activity of the ethanolic extract of *Ficus trichopoda* bark (19.23, 23.08, and 38.46 %) as compared to indomethacin (61.53 %). The carrageenan-induced rat paw oedema model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation, the result of this study is an indication that *F. trichopoda* can be effective in acute inflammatory disorders. Formalin as a potent oedematous agent produced inflammation through the release of several inflammatory mediators including prostaglandins.[17] Sub-cutaneous injection of formalin produces distinct biphasic pain, termed early and late phases. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase.[18] The formalin test for ethanolic extract of *Ficus trichopoda* bark demonstrated a significantly (P < 0.005) higher percentage of inhibition in the late phase (inflammatory phase). The tail immersion test is a thermal painful stimuli model and is selective for the evaluation of centrally, but not peripherally acting analgesic drugs.[19] Our findings showed significant dose-dependent increase in latency period of response to heat. The data concerning the analgesic effects of the ethanolic extract of *Ficus trichopoda* bark is indicative of morphine -like[20] and also NSAIDs-like effects by inhibiting cyclooxygenase in peripheral tissues thereby interfering with the mechanism of transduction in primary afferent nociceptors.[21]

The ability of the extract to reduce the size of oedema produced by carrageenan and the paw licking in formalin test, suggests that it contained chemical component(s) that may be active against inflammatory conditions.

Table 2: Effect of the ethanolic extract of Ficus trichopoda bark (FTB) on formalin –induced nociception in rats.

Treatment	Dose (mg/kg)	Score of pain[a]			
		0 -10 mins	% inhibition	15- 60 mins	% inhibition
Control Distilled water	10 ml/kg	2.30 ± 0.31	-	2.31 ± 0.18	-
FTB Extract	125	1.68 ± 0.19	26.96	1.36 ± 0.09*	41.13
FTB Extract	250	1.44 ± 0.27	37.39	0.89 ± 0.12*	61.47
FTB Extract	500	1.24 ± 0.20	46.09	0.91 ± 0.94*	60.60
Indomethacin	10	1.44 ± 0.29	37.39	1.06 ± 0.12*	54.11

[a]Each value is the mean ± SEM of five rats (n = 5)
*Each value is significant at p < 0.05, compared with control using the Student's t – test.

Table 3: Effect of ethanolic extract of Ficus trichopoda bark (FTB) on tail immersion test in rats

Treatment	Dose (mg/kg)	Mean Reaction Latency (s)[a]			
		30 mins	60 mins	90 mins	120 mins
Control	Saline	4.64 ± 0.14	4.01 ± 0.19	3.62 ± 0.17	3.70 ± 0.20
FTB Extract	125	4.85 ± 0.15	5.78 ± 0.10*	5.47 ± 0.12*	5.21 ± 0.10*
FTB Extract	250	6.43 ± 0.17*	6.65 ± 0.10*	6.53 ± 0.08*	6.81 ± 0.06*
FTB Extract	500	6.70 ± 0.08*	6.97 ± 0.14*	6.95 ± 0.07*	6.87 ± 0.12*
Indomethacin	10	6.91 ± 0.13*	7.29 ± 0.07*	7.24 ± 0.11*	7.23 ± 0.07*

[a]Each value is the mean ± SEM of five rats (n = 5)
*Each value is significant at p < 0.05, compared with control using the ANOVA.

The demonstrated analgesic and anti-inflammatory activities may be due to the presence of saponins, tannins and alkaloids either singly or in combination in the ethanolic extract of *F trichopoda* bark as demonstrated also by Zaku et al.,[22] in the bark extract of *F. racemosa*.

In conclusion, the ethanolic extract of *Ficus trichopoda* bark presented with an anti-inflammatory and analgesic effects and this supports the use of this plant in folk medicine in treatment of inflammatory pain.

ACKNOWLEDGEMENTS

We acknowledge the contributions and support of Kampala International University Complementary and Alternative Medicine Research Group (CAMRES), Uganda.

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