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Effect of aqueous leaf extract of *Ficus benghalensis* on nociception and sedation in Rats

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Abstract

The aim of this study was to investigate the effects of aqueous leaf extract (WE), of *Ficus benghalensis* Linn. (Family: Moraceae) on nociception and sedation using male rats. Different doses of the WE (0,125, 250, 500 or 1000 mg/kg) were orally administered and one hour later the effects on nociception and sedation were determined using hot plate and tail flick test and rat hole board technique . The WE had no analgesic or sedative effect but exhibited marked hyperalgesic action determined in the hot plate test indicating that this effect was mediated supraspinally. The hyperalgesic activity was dose-dependent with an ED_{50} value of 585.7 mg/kg. The WE extract was well tolerated even after subacute treatment; with no sums of overt toxicity, hepatotoxicity, haematoxicity, stress or motor impairments. Further, WE did not induce any aversive behaviour characteristic of pain-like syndrome.

Key words: ***Ficus benghalensis,*** **nociception, hyperalgesia, analgesia, sedation**

1. Introduction

Sri Lanka with its great diversity of flora possesses many plants species of medicinal value. Many of the medicines used today are derived straight from plants and quite a few of the prescription drugs are from tropical forest species. The plant kingdom represents a virtually untapped reservoir of new and exciting chemical compounds, many of them extraordinarily biodynamic.

The ethno pharmacological uses of F*icus* species indicate that the genus *Ficus* (Family - Moraceae) could be a rich source of phytomedicine. In Sri Lanka there is an abundant population of *Ficus benghalensis* Linn. and *Ficus religiosa* Linn.

Ficus benghalensis (E. Banyan) is a large tree, remarkable with numerous aerial roots. Growing down from the branches, these take root in the soil and form prop roots or secondary trunks. In this manner the tree spreads over a large area. As the tree ages, the original trunk decays and the tree breaks up into several sectors, the props becoming separate trunks for various sections (Jayaweera, 1982).

The chloroform extract of the fruits of F. *benghalensis* has showed anti-tumor and antibacterial activities (Mousa, et al., 1994) while the ethanol extract of the hanging roots showed anti-diarrhoeal activity (Mukherjee et at., 1998) in various experimental models. 3,5-dimethyl ether of leucocyanadin-3-*O*-β-**D**-galactosylcellobioside, 5,7-dimethyl ether of leucopelargonidin-3-*O*-α-**L** rhamnoside and 3',5',7-trimethy ether of leucodelphinidin-3 –*O*-α- **L** rhamnoside isolated from the stem bark (Subramanian and Misra, 1977) of *F. bengahalensis* exhibited various activities such as anti-diabetic activity, serum insulin raising effect (Kumar. et al., 989, Cherian et al., 1992, Geetha, et al.,1994, Augusti. et al., 1994), insulin sparing effect (Kumar and Augusti, 1994, Cherian and Augusti, 1995), hypolipidemic effects (Cherian and Augusti, 1993, Kumar and Augusti, 1989) and antioxidant effect (Daniel et al., 1998) as well as effects on various enzyme activities (Kumar and Augusti, 1989). The chloroform extract of the dried fruits of *F.religios* showed anti-tumour and anti-bacterial activities (Mousa, et. al, 1994). β-sitosteryl-**D**-glucoside isolated from the hot ethanol extract of the bark showed hyperglyceamic activity, central nervous system stimulation and reversal of reserpine induced depression (Ambike and Rao, 1967). Bergapten and bergaptol isolated from *F.religiosa* showed anti-bacterial activity against certain microorganisms (Swanii and Bisht, 1996). The water extract of the trunk bark of *F.religiosa* showed anti-fertility (Ratnasooriya and Dharmasiri, 1999) and anxiolytic (suppression of anxiety) activity (Ratnasooriya et al., 1998). There are no previous records that attempt to identifying any biological activity present in the leaves of *F. benghalensis* or *F.religiosa.* As an initial step to investigate this aspect the leaves of *F. benghalensis* were studied to determine the nociceptive (reception of pain) and sedative effects of the water extract of the leaves.

2. Materials and Methods

Mature leaves of *Ficus benghalensis* were collected form the tree located in the Science Faculty of the University of Colombo in December 2000-March 2001 and was identified by Dr G. I. Seneveratne of the Botany Department

of the University of Colombo and a voucher specimen was deposited The leaves were washed under running water and cut into small pieces. The pieces (520 g) were macerated with water and were then refluxed with 31 water for two days in a round bottom flask fitted to a Leibig condenser. The brownish red solution was filtered and freeze-dried (32 g) and stored air tight at room temperature (30-32^0 C). The freeze dried powder was dissolved in distilled water to obtain the required dosage concentration in 1 ml. solution (125, 250 or 500 mg/kg)

Experimental Animals

Healthy adult cross breed albino male rats (weight: 200-250 g) were used in the study. The animals were kept in plastic cages (six per cage) under standardized animal house conditions (temperature: 28-31^{0}C, photo period: approximately twelve hours natural light per day, relative humidity. 50-55%, with free access to pelleted food (Master Peed Ltd., Colombo, Sri Lanka) and tap water. Except at the time of experimental procedures the animals were handled only during cage cleaning.

Drug Treatment

Two groups of (one for the hot plat and the other for the tail flick test) sixty four male rats were selected and randomly divided into six groups. Food was withheld from these rats for 16 hours and was orally administered with the water extract or vehicle (control) in the following manner.

Group 1 (n = 24) with 1 ml of water group 2 (n = 8) with 1 ml 125mg/kg of extract, group 3 (n = 8) with 1 ml 250 mg/kg of extract, group 4 (n = 8) with 1 ml 500 mg/kg of extract. Group 5 (n = 8) with 2 ml 500 mg/kg of extract and group 6 (n = 8) with 2 ml of water.

Evaluation of nociception

One hour after administration of the extract or vehicle, the rats were subjected to hot plate and tail flick lest (Langerman et.al, 1995). A cut off time of 20 sec was allowed to avoid tissue damage. In the hot plate test, the time taken to lick the hind paw (reaction time) when placed on a hot plate (model MK 35A Muroma Co. Ltd. Tokyo, Japan) maintained at 55^{0}C was recorded. In the tail flick test, the time taken to flick the tail (the reaction time) when the tail was immersed (5-6 cm from the tip) in a water bath at 55 ^{0}C was noted.

Evaluation of sedative activity

Each of the above rats were placed on a rat hole-board apparatus and was given 7.5 minute trial (File and Wardill, 1975). During this period, the number of head dips, rears and locomotory activity were scored.

Evaluation of effects on muscle coordination and strength

Eighteen rats were treated either with 500 mg/kg extract (n = 9) or vehicle (n = 9) thrice a day for seven consecutive days. On the day I post treatment, the rats were subjected to the Bridge test, bar holding test (Plaznik et al, 1993) and righting reflex test (Mortin et al., 1993) and the respective latencies were recorded.

Observation of overt signs of toxicity, stress and aversive behaviours

These rats used in the above investigation were observed each day of treatment (6-8 hours) and on day 1 post treatment for overt signs of toxicity (such as salivation, diarrheoa, yellowing of hair, loss of hair, postural abnormalities, behavioural changes, marked impairments of food and water intake and body weight), stress (fur erection, exopthalmia) and aversive behaviours (biting and scratching behaviour, licking at tail, paw and penis, intense grooming behaviour or vocalization).

Evaluation of effect on Haematological Parameters and Enzyme Levels

Eighteen rats were treated either with 500 mg/kg extract (n=9) or vehicle (n=9) thrice a day for seven consecutive days. On day 1 post treatment, blood (1.5-2.0 ml) was collected from the tail of these rats using aseptic precautions and the WBC, RBC, DC counts and haemoglobin content of fresh blood was determined as described previously (Ghai, 1993). Another aliquot of blood was centrifuged at 3200 rpm for 5 mins and the serum was collected and the SGOT (EC 2.6.1.1) and SGPT (EC 2.6.1.2) levels determined using Randox kits (Randox Laboratories Ltd., Co., Antrium, UK) and a spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan).

Statistical Analysis

In all experiments, the responses of drug treated animals were always assessed in parallel to those of vehicle treated rats to minimize interference of possible fluctuation in responsiveness. The data are expressed as the mean ± SEM of the difference between the responses elicited by drug treated and those elicited by the vehicle treated. Statistical analysis was performed using Mann-Whitney U test after an analysis of variance (ANOVA). Significant values were set at $P \leq 0.05$. Linear regression analysis was performed to assess dose dependencies.

3. Results

Results of the control treatments were pooled as there was no difference ($P > 0.5$) between 1 ml and 2 ml vehicle treatments.

As shown in Table 1, 250, 500 and 1000 mg/kg of extract caused a significant ($P<0.05$) shortening of the reaction time in the hot plate test, both compared to the control and respective pre-treatment values. This effect was dose-dependent ($r = 0.95$; $P< 0.05$). The EC_{50} value for this hyperalgesic (increased perception of pain) effect was 585.7 mg/kg. (a tailing off effect was observed around 1000mg/kg and hence this value was not included in calculation of the ED_{50} value). As shown in Table 2 there was no significant alteration ($P > 0.05$) in the tail flick reaction time in any of the extract treated rats as compared to control rats. In the rat hole-board test all four doses of the extract failed ($P>0.05$) to significantly alter any of the parameters monitored. (See Table 3)

Table 1. The effect of oral administration of different doses of aqueous leaf extract of Ficus benghalensis on the hot plate reaction time of rats. (mean ±SEM; ranges in brackets)

Treatment	Sample size n	Hot plate reaction time (in sec.) (mean± SEM)	
		Pre treatment	Post treatment
Pooled control (water)	32	14.4±0.6 (8.0 -20.1)	15.6 ±0.6 (8.4 -19.8)
Extract			
125 mg/kg	8	13.7 ±1.2 (7.1-16.7)	13.0±1.0 (9.7-17.0)
250 mg/kg extract	8	14.3±1.0 (9.0-18.6)	11.8±0.7* (9.3-14.6)
500 mg/kg dose	8	14.2 ±0.8 (10.5-16.8)	10.1 ±1.3* (5.2-16.7)
1000mg/kg dose	8	13.5 ±0.7 (11.0 - 16.0)	8.2± 0.7* (4.4-10.3)

Values are significant at $P<0.05$

Table 2. The effect of oral adminitration of different doses of the aqueous leaf extract of Ficus benghalensis on tail flick reaction time of rats. (mean± SEM; ranges in brackets)

Treatment	Sample size n	Tail flick Reaction time (in sec.) (mean± SEM)
Pooled control (water)	32	3.6±0.3 (1.5-7.3)
Extract		
125 mg/kg	8	3.5 ±0.5 (2.3-6.5)
250 mg/kg extract	8	3.6±0.5 (1.5-5.6)
500 mg/kg dose	8	4.2 ±0.3 (3.3-5.6)
1000mg/kg dose	8	3.0 ±0.6 (1.0 - 6.7)

Table 3. The effect of oral administration of different doses of aqueous leaf extract of *Ficus benghalensis* on different parameters of rat hole board test. (mean ±SEM; ranges in brackets)

Treatment	Sample size n	Number of crossings	Number of head dips	Number of rears
Pooled control (control)	32	8.0 ± 1.2 (0-25)	4.3± 0.7 (0-20)	9.6± 1.4 (0-25)
Extract				
125mg/kg	8	7.5± 1.7 (1-18)	4.4 ± 1.2 (0-14)	7.0± 1.7 (3-13)
250 mg/kg	8	8.25 ± 1.4 (4-14)	4.8 ± 1.3 (1-11)	14.6 ± 3.0 (5-28)
500mg/kg	8	10.0± 1.9 (1-16)	3.3± 0.6 (0-5)	7.0 ± 1.1 (3-12)
1000mg/kg	8	7.9± 1.4 (4-13)	4.0 ± 1.6 (0-14)	8.2 ± 1.6 (3-13)

Muscle Coordination and Muscle Strength

As shown in Table 4, none of the latencies investigated was significantly (P>0.05) altered.

Table 4: The effect of oral administration of 500mg/kg (thrice a day for seven consecutive days) of aqueous leaf extract of the leaves of Ficus benghalensis on Bridge test, bar holding test and righting reflex test (mean± SEM)

Treatment		Pre treatment time (in sec.)			Pos treatment time (in sec.)		
	n	bar holding test	bridge test	righting test	bar holding test	bridge test	righting test
Control (water)	9	25.9± 5.4	18.1 ±3.4	0.30±.02	31.3 ±.2.4	12.3±. 2.9	0.3±. 0.04
500mg/kg of extract	9	33.7±. 4.2	24.8±. 5.9	0.2 ±.0.03	40.1±. 6.8	16.1±. 4.2	0.3 ±.0.05

Toxicity, Stress and Aversive Behaviour

Sub-acute treatment of the extract did not elicit overt signs of toxicity, stress or aversive behaviour.

Effect on Haematological Parameters and Enzyme Levels

None of the haematological parameters (results not shown) or enzyme levels (SGOT: control vs treatment; 46 ± 9.2 U/*l* vs 55.1 ± 8.8 U/*l* and SGPT: control vs treatment, 24.3±3.9 U/*l* vs 21.5 ± 3.8 U/*l*) were altered significantly (P>0.05) by the extract.

4. Discussion

The results show that the aqueous leaf extract of the of *F.benghalensis* had no analgesic or sedative effects but marked and significant hyperalgesic effect when tested with male rats. This is important and novel finding because reports of such activity are rare with natural products; evident only with capsaicin isolated from chillies (Piovezan *et al.*, 1998). The hyperalgesic effect had a fairly rapid onset and was dose-dependant with a ED_{50} value of 585.7 mg/kg. This effect was mediated supraspinally: tail flick test measures spinal reflexes whereas the hot plate test measures supraspinally organized responses to heat (Wong et. al, 1994).

This hyperalgesic effect was not associated with overt signs of acute or sub-chronic toxicity (in terms of salivation, diarrhoea, yellowing of hair, loss of hair, postural abnormalities, behavioural changes, marked

impairments of food and water intake and body weight), stress (exopthalmic and erection of fur), hepatatoxicity (in terms of SGOT and SGPT), haemotoxicity (in terms of Hb content arid RBC, WBC and DC counts) or motor impairments (muscle co-ordination or muscle relaxation). Further, the extract did not induce any aversive behaviours (biting and scratching behaviour, licking at tail, paw and penis, intense grooming behaviour) characteristic of pain-like syndrome evident with other reported hyperalgesics (Narita et al, 1996). This suggests that *F benghalensis*-induced hyperalgesic is unlikely to be due to release of pain mediators such as histamine, serotonin, bradykinin or prostaglandins (Obata et.al 2000). On the other hand this hyperalgesic effect could have resulted from alteration in the membrane excitability of brain nociceptors (Siddall and Cousings, 1995).

Leucoanthocyanidin derivative such as 3, 5-dimethyl ether of leucocyanadin-3-*O*-β-**D**-galactosylcellobioside, 5, 7-dimethyi ether of leucopelargonidin-3-*O*- α -**L** rhamnoside and 3′, 5′, 7- trimethyl ether of leucodelphinidin-3-O-α **L**-rhamnoside isolated from the stem bark were implicated with many of the biological activities *(vide infra)* reported with *F. benghalensis.* However, the water extract of the stem bark of *F. benghalensis* failed to induce any analgesic or hyperalgesic effect when tested on rats (our unpublished observations). This suggests that the hyperalgesic effect observed in this study is unlikely to be mediated by leucoanthocyanidins, if they were present, in the water extract of *F. benghalensis* leaves.

Isolation of the active compound/s from plant would serve as yet another step. This could be used in model studies of acute and chronic pain. Such compounds may be important in discovery/synthesis of inhibitors of enzymes which bring about analgesic and hyperalgesic effects.

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