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Research Article

Anti-inflammatory, analgesic and anti-lipid peroxidation studies on stem bark of *Ficus religiosa* Linn.

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Abstract

The methanol extract of the stem bark of ***Ficus religiosa* Linn.**, The Peepal tree, was screened for its anti-inflammatory activity in Wistar albino rats and analgesic effects in Swiss albino mice. A significant inhibition of carrageenan-induced rat paw oedema, comparable to that produced by indomethacin, the standard anti-inflammatory drug, was obtained with all the three doses of the extract, tested in the present study. A significant inhibition of acetic acid-induced writhing in mice was observed with two doses of the extract. The analgesic effect was comparable to that caused by the standard drug, aspirin. The methanol extract also showed significant anti-lipid peroxidant effects *in vitro*.

Keywords: *Ficus religiosa*, Peepal tree, Stem bark, Anti-inflammatory, Analgesic, Lipid peroxidation.

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Introduction

Plants, as illustrated throughout the history of civilization have served as the major source of medication for the treatment of human ailments. Herbal medicines are being accepted and used increasingly by general populations in both eastern and western countries not only as medicines but also as dietary supplements, along with modern chemotherapeutic agents. This is because of ethnic acceptability and compatibility having less side effects.

Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Although several modern drugs are used to treat these types of disorders but their prolonged use may cause severe adverse side effects[1]. Consequently, there is a need to develop new anti-inflammatory agents[2] with minimum side effects. Several plants are being used in traditional medicine for treating these disorders which are inflammatory in nature like rheumatism, arthritis, etc.

As most of the present day analgesic drugs exert a wide range of side effects[3], some times study on plant species that are traditionally used as pain killers should still be seen a logical and fruitful research strategy, in search of analgesic drugs[4].

***Ficus religiosa* Linn.**, The Peepal Tree (Family—Moraceae), is a large deciduous tree, cultivated throughout India and held sacred by the Hindus and Buddhists. It is locally known as *Arayal*. All parts of the tree are useful medicinally. The stem bark is used extensively in traditional Indian medicine to treat inflammation and glandular swelling of the neck. The bark is applied externally on ulcers and wounds and is also used as a mouthwash to relieve toothache. Although it is used extensively in traditional medicine as an anti-inflammatory[5] and analgesic[5] agent, these properties have not been scientifically evaluated. These facts prompted the present study on the anti-inflammatory and analgesic effects of this plant.

Materials and Methods

Plant material

The stem bark was collected from Tropical Botanic Garden and Research Institute during January 2006. It was authenticated by Dr Mathew Dan, plant taxonomist of the Institute and a voucher specimen was also preserved for future reference in Herbarium of the Institute (TBGT 57009).

Preparation of plant extract

The stem bark was shade-dried and powdered. The powder (50g) was then extracted with methanol (500ml)

The Peepal tree

Leaves

Stem bark

overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30ºC under reduced pressure in a rotatory evaporator. The yield (w/w) of the crude extract was found to be 13.5%. The crude extract was suspended in 0.5% Tween-80 to required concentrations and used for the experiments.

Experimental animals

Wistar albino male rats (170-190g) and Swiss albino male mice (27-35g), were grouped and housed in poly acrylic cages (two animals per cage) and maintained under standard laboratory conditions (temperature 24-28ºC, relative humidity 60-70% and 12 h light dark cycles). They were fed commercial rat feed (Lipton India Ltd, Mumbai) and boiled water, *ad libitum*. All experiments involving animals were done according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee.

Carrageenan-induced paw oedema in rats

Rats were divided into 5 groups (5 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% carrageenan solution in 0.9% saline into the sub plantar region of the left hind paw. Group I animals (carrageenan control) were injected with 0.1 ml of 0.9% saline solution into the sub plantar region of the left hind paw. Group II, the standard reference group was given p.o., an aqueous solution of indomethacin (5 mg/kg), 30 min prior to carrageenan injection. Groups III to V received p.o., 125, 250 and 500 mg/kg of the crude extract, 30 min prior to carrageenan injection. The paw volume was measured plethysmographically just before and 3h after carrageenan administration. The difference in the left and right paw volumes indicated the degree of inflammation[6].

Acetic acid-induced writhing test

Analgesia was assessed by the writhing test in mice[7]. Mice were divided into 5 groups (5 animals in each group). All the groups received i.p., 0.5% aqueous solution of acetic acid (10ml/kg). Group I, the acetic acid control group received a single dose of 0.5% Tween-80 (0.5 ml) p.o., 20 min prior to the administration of acetic acid. Group II, the standard control group received p.o., a single dose of aspirin (acetyl salicylic acid, 100 mg/kg), 20 min prior to the administration of acetic acid. Groups III to V received p.o., a single dose of 125, 250 and 500 mg/kg of the crude extract, respectively, 20 min prior to administration of acetic acid. The number of writhes (full extension of hind paw) per animal was recorded during the 20 min period, beginning 5 min after the injection of acetic acid. The experiment was repeated once.

***In vitro* anti-lipid peroxidation studies**

Anti-lipid peroxidant effect of ***F. religiosa*** was studied *in vitro* following the modified method of Yoshiyuki *et al*[8] and Masao *et al*[9]. The rat liver tissue (2.0g) was sliced and homogenized with 10 ml of 15 mM KCl-Tris-HCl buffer (*p*H 7.2). The reaction mixture (in triplicate) was composed of 0.25 ml of the liver homogenate, 0.1 ml of Tris HCl buffer (*p*H 7.2), 0.05 ml of 1 mM ascorbic acid, 0.05 ml of 4mM $FeCl_2$

Table 1: Effect of *Ficus religiosa* stem bark methanol extract (FR) on carrageenan-induced paw oedema in rats

Treatment	Dose (mg/kg)	Paw volume after 3 h (ml)	Per cent inhibition
Carrageenan Control	0	0.73±0.04	0
Indomethacin	5	0.42±0.02*	52.94
FR	125	0.44±0.01*	55.41
FR	250	0.42±0.01*	52.99
FR	500	0.45±0.03*	56.29

Values are the mean ±S.D. n=5, *ANOVA $P \leq 0.05$ vs Carrageenan control

Table 2: Effect of *Ficus religiosa* stem bark methanol extract (FR) on acetic acid-induced writhing response in mice

Treatment	Dose (mg/kg)	Mean number of writhes in 20 min	Per cent inhibition
Acetic acid Control	-	32.0±0.20	-
Aspirin	25	7.2±0.70*	77.51
FR	125	25.3±0.55	21.94
FR	250	9.1±0.35*	71.56
FR	500	10.9±0.41*	65.93

Values are the mean ±S.D. n=10, *ANOVA $P \leq 0.05$ vs Acetic acid control

Table 3: Inhibitory effect of *Ficus religiosa* stem bark methanol extract (FR) on $FeCl_2$-ascorbic acid -induced lipid peroxidation in rat liver homogenate *in vitro*

Groups	Plant extract concentration (µg/ml)	MDA (n mole/mg protein)	MDA inhibition (%)
Normal Control	-	1.35 ± 0.60	-
$FeCl_2$-AA control	-	2.31± 0.02	-
$FeCl_2$-AA + FR	25	1.20 ± 0.02*	48.1
$FeCl_2$-AA + FR	50	1.38 ± 0.01*	40.3
$FeCl_2$-AA + FR	100	1.20 ± 0.02*	48.1
$FeCl_2$-AA + FR	200	1.40 ± 0.01*	39.4

Values are the mean ± S.D. n = 3, *ANOVA $P \leq 0.05$ vs $FeCl_2$-AA control
MDA= Malondialdehyde

and 0.05 ml of various concentrations of extract (25, 50,100 and 200 µg/ml). The mixture was incubated at 37ºC for 1h in capped tubes. Then, 0.5ml of 0.1N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2 ml of 0.6% thiobarbituric acid (TBA) were added to each tube and the tubes were vigorously shaken. Following this, all the tubes were placed in a boiling water bath at 100ºC for 30 min. After cooling, the flocculent precipitate was removed by adding 5ml of n-butanol, mixed well and centrifuged at 1500 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm.

Behavioural and toxic effects

Mice were fasted overnight, but water was given *ad libitum*. Doses of 125, 250 and 500 mg/kg were administered p.o., to three groups of 10 animals. The controls were given the vehicle (0.5% Tween-80). They were observed continuously for 1h for any gross behavioural changes, symptoms of toxicity and mortality if any and intermittently for the next 6h and then again, 24h after dosing with the extract.

Statistical analysis

Statistical comparison between control and treated groups was made[10] using analysis of variance, followed by multiple comparisons.

Results

Anti-inflammatory activity

Injection of carrageenan into the rat paw induced an increasing oedema that could be quantitated after the second hour. The group treated with indomethacin

showed inhibition of oedema formation of 52.94% by the third hour, whereas the groups treated with the 3 doses of crude extract (125, 250 and 500 mg/kg) showed inhibition of oedema formation of 52.99, 55.41 and 56.29%, respectively by the third hour (Table 1). The study showed that this extract even at 125 mg/kg dose was almost as effective as indomethacin (5 mg/kg) in inhibiting oedema formation.

Analgesic property

Intraperitonial injection of acetic acid produced 32.0±0.20 writhes in the control group, 20 min after injection. The groups pretreated with the extract (250 and 500 mg/kg) and aspirin treated group (25 mg/kg) exhibited reduction in the number of writhing of 71.56, 65.93 and 77.51%. The lowest dose, used in the study (125mg/kg) did not cause significant inhibition of writhing. Increasing the dose beyond 250 mg/kg did not further inhibit the writhing response (Table 2).

***In vitro* anti-lipid peroxidant effect**

There was significant increase of malondialdehyde (MDA) in $FeCl_2$-AA treated rat liver homogenate, compared to normal control without $FeCl_2$-AA. Methanolic extract of ***F. religiosa*** stem bark (FR) significantly reduced the accumulation of lipid peroxides *in vitro* in a dose dependent manner up to 100µg/ml, beyond which there was lesser inhibition of lipid peroxidation (Table 3).

In the toxicity study, no mortality occurred within 24h with the three doses of FR tested. The LD_{50} was therefore, greater than 500 mg/kg p.o., in mice (data not shown).

Discussion

The mechanism involved in the genesis of the carrageenan-induced oedema can cause the release of prostaglandins and kinins, among other substances[10]. The time course of oedema development in carrageenan-induced paw oedema model in rats is generally represented by a biphasic curve[11]. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also due to the serotonin component[12]. Prostaglandins play a major role in the development of the second phase of reaction which is measured after an interval of 3 hours' time[13]. The presence of prostaglandin in the inflammatory exudates from the injected foot can be demonstrated after 3 hours and the period thereafter[14]. The carrageenan-induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis[15]. Based on these reports, it can be inferred that the inhibitory effect of ***F. religiosa*** stem bark extract on carrageenan-induced inflammation in rats observed in the present study could be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis.

Acetic acid-induced writhing response in mice is a simple and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs rapidly. It was found that bark extract significantly inhibited the acetic acid induced writhing response at the two doses used in the study (250mg/kg and 500mg/kg). The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. Therefore, it is possible that the stem bark exerts the analgesic effect probably by inhibiting synthesis or action of prostaglandins.

In the present study methanol extract of stem bark significantly reduced the accumulation of lipid peroxides *in vitro*. It has become evident that non-enzymatic or unspecified lipid peroxidation occurs during experimental inflammation in rats. Lipid peroxides may be pro-inflammatory and can damage the tissues directly[16]. Protection against free radical lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery[17]. There is abundant evidence that per oxidative decomposition of structural lipids in cellular and subcellular membranes is catastrophic in a living system. Lipid peroxidation results in mitochondrial swelling and disintegration[18]. Disintegration of lysosomes has been correlated with the peroxidative decomposition of lysosomal lipids[19].

The toxicity study indicates that the bark extract is fairly non-toxic. This is supported by traditional use of the plant as an anti-inflammatory and analgesic agent in Kerala.

The active compound responsible for the anti-inflammatory and analgesic activities of bark extract of ***F. religiosa*** remains to be identified, however, it is reported that the stem bark contains tannins[20]. It is significant to note in this context that the latex of ***Calotropis procera* (Ait.) R. Br.**, containing tannins showed potent anti-inflammatory effects. Volatile oils, resin, flavonoids, terpenoids

isolated from plant extracts are known to produce anti-inflammatory and analgesic effects[21]. Condensed tannin and polysaccharides are known for the anti-inflammatory effects of ***Rumex acetosa*** **Linn.** and ***R. patientia*** **Linn.**[22]. It is likely that tannin present in bark extract of ***F. religiosa*** is responsible for its anti-inflammatory and analgesic effects. Detailed studies are warranted in this direction to decipher the exact nature of the phytochemical compounds responsible for their anti-inflammatory and analgesic effects.

Conclusion

It can be concluded from the present study that the beneficial effect of ***F. religiosa*** stem bark extract may be from its role in the stabilization of lysosomes. The combination of anti-inflammatory and analgesic effects indicates the likelihood of intervention of prostaglandin synthesis as prostaglandins have been established as a common mediator in all these responses. However, this possibility remains to be investigated in detail.

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