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Evaluation on anti-inflammatory effects of *ficus religiosa* (linn.) in carrageen an induced acute inflammation in golden Syrian hamsters

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ABSTRACT

The present investigation was carried out to find the effect of aqueous and ethanolic extracts of bark of *Ficus religiosa* for its anti-inflammatory activity in Hamsters. Anti-inflammatory activity was evaluated using acute inflammatory models like carrageenan induced paw edema models. The biochemical parameters like thiobarbituric acid reactive substances (TBARS), enzymatic anti-oxidants and non enzymatic anti-oxidants were carried out in blood and tissues of control and experimental animals in each group. Oral administration of the ethanolic extract (*Ficus religiosa* bark) at the dose 100mg/kg body weight (b.w) showed significant effect than aqueous extract and was also much comparable to that of standard drug, Ibuprofen. The mechanism of anti-inflammatory effect of *Ficus religiosa* is probably due to their inhibitory action on the release of mediators of inflammation.

Introduction

Inflammation is a complex serotypical reaction of body expressing the response to damage of its cells and vascularized tissues. It covers a series of reparative and protective responses in tissue injury, whether caused by infection, autoimmune stimuli or mechanical injury. However, a plenty of effecter mechanisms capable of defending the body against such antigens and agents have developed and these can be mediated by soluble molecules or by cells. Carrageenan induced acute inflammation is widely used inflammatory model to test the efficacy of anti-inflammatory agents. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan induced inflammation (Di and Willoughby 1971) here as prostaglandins are detectable in the late phase inflammation (Salvemini et al., 1996).

Free radicals and other oxygen derivatives are constantly generated *in-vivo* both by accident of chemistry and for specific metabolic purposes. The reactivity of oxygen species vary with many agents causing inflammation or even damage to structure and function of bio-membrane and biological molecules. Anti-oxidants form the first line of defense against free radical mediated oxidative damage. Disturbances in oxidant and anti-oxidant status have been well documented in inflammatory conditions.

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "Reactive Oxygen Species" (ROS). Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hydrochloride radical and various lipid peroxides. The most important characteristics of free radicals *in-vivo* or *in-vitro* are peroxidation of lipids in biomembrane. Lipid peroxidation is a radical mediated chain reaction that includes three major steps namely, initiation, propagation and termination. Free radical induced lipid peroxidation has been implicated in ageing and various pathological conditions (Pratico *et al.*, 2004, Vasudevan *et al.*, 2006 and Semb *et al.*, 1990).

Medicinal plants and herbs play a vital role in the management of various inflammatory diseases including rheumatoid arthritis. Ayurvedha and Siddha systems of Indian medicine recommend a number of herbal remedies for the treatment of inflammatory diseases. *Ficus religiosa* is one of the medicinal plants, recommended for the treatment of several disorders. Bark of *Ficus religiosa* is considered beneficial for inflammatory disorders. However, there has been no scientific basis for claims that *Ficus religiosa* has anti-inflammatory properties.

Ficus religiosa is a medium sized deciduous tree with spreading branches. It is commonly known as peepal tree with spreading branches, it is commonly known as peepal tree in English and Arasamaram in Tamil. Different parts of *Ficus religiosa* such as bark, fruits, seeds, leaf buds and latex are used in traditional medicine for the treatment of several diseases. It is considerably beneficial for mouth sores, atrophy emaciation, rheumatism, smallpox, carbunde, rinder peast, mucus in urine, spermatorrhoea, gravel, cholera etc. Leaves and young shoots are purgative. A bark is astringent and is found efficacious in gonorrhea (Joshi., 2000).

Hence, the present study was designed to study the anti-inflammatory effect of *Ficus religiosa* in acute inflammation in Golden Syrian Hamsters.

Materials and methods

Animals

Male Golden Syrian Hamsters (*Mesocricetus auratus)* 8-10 weeks old weighing 80-120g was purchased from National

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Institute of Nutrition, Hyderabad, India. The animals were housed in a polypropylene cage and provided standard pellet diet and water *ad libitum.* The animals were maintained under controlled conditions of temperature and humidity with a 12hrs light/dark cycle.

Chemicals

The carrageenan was obtained from Sigma Aldrich Chemical Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Plant Materials

Ficus religiosa (Linn.) bark was collected in and around Namakkal, Tamil Nadu, India. The Botanist, Arignar Anna Goverment Arts College, Namakkal verified the identification of plant species.

Preparation Of Plant Extracts

The aqueous and ethanolic extracts of *Ficus religiosa* bark were prepared according to the method of Hossain *et al*., (1992).

Ethanolic Extract Of *Ficus Religiosa*

500g of *Ficus religiosa* bark were dried, powdered and then soaked separately in 1500ml of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48hrs and filtered again. The above two filtrates were mixed and the solvent were evaporated in a rotavapour at 40-50°C under reduced pressure. A 14% semisolid light brown material of *Ficus religiosa* bark obtained was stored at 0–4°C until used.

Aqueous Extract of *Ficus Religiosa*

100g of dried fine powder of *Ficus religiosa* bark were suspended separately in 250ml of water for 2hrs and then heated at 60-65^{0}C for 30 minutes. The extracts were collected separately and the processes were repeated three times with the residual powder, each time collecting the extract. The collected extracts were pooled and passed through fine cotton cloth. The filtrates were evaporated at 40-50^{0}C in a rotavapour under reduced pressure. A 21% semisolid light brown material of *Ficus religiosa* bark obtained was stored at 0–4°C until used. A known amount of the residual extracts were suspended in distilled water and was orally administered to the animals by gastric intubation using force-feeding needle during the experimental period.

Experimental Protocol

The Institutional Animal Ethics Committee, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu, has approved the experimental design. A total number of 24 animals were randomized into four groups. Group I animals were served carrageenan with 2% gum acacia solution. Group IV animals were served as positive control and received Ibuprofen 100 mg/kg body weight. Group II and III animals were received aqueous and ethanolic extract of bark of *Ficus religiosa (*100 mg/kg b.w) respectively. Food was withdrawn overnight, however, adequate water was given to the Hamster before the experiments.

All the drugs (plant extracts and Ibuprofen) were orally administered to the animals with the help of oral catheter. After 1hr subplanter injection of 0.1ml of 1% solution of carrageenan was administered in the left hind paw to all the groups. The paw volume was measured in all the animals by using plethysmograph, immediately after carrageenan injection. The paw volume was again measured after 3hrs in all the experimental animals. The percentage of anti-inflammatory effect of *Ficus religiosa* bark was compared with Ibuprofen treated animals.

The percent inhibition of edema volume between drug treated and carrageenan alone treated groups were calculated as follows ().

$$\text{Percent Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc-Vt and Vc represented the mean increase in paw edema volume in control and drug treated groups.

Biochemical Analysis

Biochemical estimations were carried out in blood and tissues of experimental animals in each group. Plasma was separated from heparinized blood by centrifugation at 3000rpm. After plasma separation, the erythrocyte membrane was prepared by the method of Dodge et al., (1962) modified by Quist (1980).

Thiobarbituric Acid Reactive Substances (TBARS) in erythrocytes and erythrocyte membranes were estimated for method described by Donnan (1950) and TBARS in plasma were assayed by the method of Yagi (1987). The reduced glutathione level was determined by the method of Beutler and Kelley (1963). Lipid peroxidation (TBARS) in tissue was estimated by the following method of Ohkawa *et al.,* (1979). Superoxide dismutase activity was assayed by the method of Kakkar *et al.,* (1984). The activity of catalase was assayed by the method of Sinha (1972). The activity of glutathione peroxidase was determined method described by Rotruck *et al.,* (1973). Vitamin E was estimated in plasma and erythrocyte membrane by the method of Desai (1984) based on the classical Emmerie Engle reaction. The concentration of total vitamin E in tissues was estimated by the method of Palan *et al.,* (1973).The level of plasma vitamin C was determined by the method of Omaye *et al.,* (1979).

Statistical Analysis

The data are expressed as mean followed by Standard Deviation (SD), statistical comparisons were performed by One-way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). The null hypothesis was rejected for $p<0.0$.

Results

The levels of paw volume of experimental animals in each group were presented in Table 1. The levels of paw volume were significantly increased in carrageenan alone treated Hamster as compared to drug treated animals. Aqueous and alcoholic extracts of bark of *Ficus religiosa* significantly reduced the levels of paw volume in carrageenan treated animals. Hamsters treated with Ibuprofen showed no significant differences in paw volume levels as compared to *Ficus religiosa* treated Hamsters.

The levels of TBARS were significantly increased in plasma and the erythrocyte of carrageenan treated Hamsters as compared to drug treated animals. Aqueous and alcoholic extracts of bark of *Ficus religiosa* significantly reduced the levels of TBARS in plasma of carrageenan treated animals. Hamster treated with Ibuprofen showed no significant difference in TBARS levels as compared to *Ficus religiosa* treated Hamsters (Table 2 and 3).

The levels of non-enzymatic antioxidants were significantly decreased in plasma and the erythrocyte of carrageenan treated hamster as compared to drug treated animals. Aqueous and alcoholic extracts of bark of *Ficus religiosa* significantly increased the levels of non-enzymatic antioxidants in plasma and erythrocytes in carrageenan treated animals. Hamsters

treated with Ibuprofen showed no significant differences in non-enzymatic antioxidants level as compared to *Ficus religiosa* treated Hamsters (Table 4 and 5).

The activities of enzymatic antioxidant erythrocyte were significantly increased in erythrocytes of carrageenan treated Hamster as compared to drug treated animals aqueous and alcoholic extracts of bark of *Ficus religiosa* significantly reduced the activities of enzymatic antioxidants in erythrocytes in carrageenan treated animals. Hamster treated with Ibuprofen showed no significant difference in enzymatic antioxidant activities as compared to *Ficus religiosa* treated hamsters (Table 6 and 7).

Discussion

In the present study, we have observed an increased oedema volume in hamsters hind paw after three hours of carrageenan injection. Oral administration of *Ficus religiosa* bark of ethanolic extract was more potent than aqueous extract and was also much comparable to that of standard drug and Ibuprofen. The anti-inflammatory activity is probably due to the presence of one or more bioactive principles and their synergistic effect. Carrageenan induced inflammation, a biphasic response is mediated by release of histamine and serotonin followed by kinin release (I phase) and then prostaglandin from the tissue arachidonic acid (II phase). The anti-inflammatory effect of *Ficus religiosa* is probably due to their inhibitory action on the release of inflammatory mediators.

Reactive oxygen species (ROS) play an important role in immunological host defense, providing anti-microbial, anti-viral and anti-tumor activity as well as being involved in apoptosis and cell survival (Nardi *et al.,* 2005; Cuzzorea *et al.,* 2001). Over production of ROS are cytotoxic and may cause tissue damage through lipid peroxidation, oxidation of amino acid side chains, protein cross linking and fragmentation and DNA damage (Comporti *et al.,* 1989). Free radical induced lipid peroxidation has been implicated in the pathogenesis of several disorders including inflammatory diseases (Conner *et al.,* 1996). In the present study, an elevation in plasma, erythrocytes and inflammatory tissues TBARS were observed in carrageenan induced Hamsters. It has been reported that reactive oxygen species were generated during acute and chronic inflammatory diseases. In the present study, the reactive oxygen species were increase by the treatment with ethanolic extract of *Ficus religiosa* in inflammatory hamsters.

Erythrocytes are more susceptible to lipid peroxidation due to their high content of iron and poly unsaturated fatty acids. Increased plasma TBARS is an indicator of tissue damage (Donnan 1950). We therefore feel that the elevated plasma lipid peroxidation as evidenced by TBARS formation in carrageenan treated Hamsters is due to overproduction and diffusion of lipid peroxides from inflammatory tissues and damaged erythrocytes with subsequent leakage in to plasma.

Antioxidant defense systems scavenge and minimize free radical formation. In the present study the non-enzymatic anti-oxidants were decreased in plasma and erythrocytes. This is probably due to their utilization by inflammatory tissues to scavenge the excess radicals and to combat the deleterious effects of Oxidative damage. Increased activities of enzymatic anti-oxidants have been well documented in inflammatory conditions (Cotran *et al.,* 1997). Our results were also supported these observations.

Oral administration of *Ficus religiosa* significantly reduced the levels of TBARS and improved the status of anti-oxidants in carrageenan induced acute inflammation in Hamsters. Our results thus indicated that the plant extracts have potent free radical scavenging activity and anti-oxidant functions in acute inflammatory diseases.

Thus, we have demonstrated and validated the anti-inflammatory effects of *Ficus religiosa* bark extracts in carrageenan induced acute inflammation. Further studies will be focused on isolation and characterization of bioactive anti-inflammatory principles from the barks of *Ficus religiosa.*

Conclusion

This study inferred that aqueous and ethanolic extract of bark *Ficus religiosa* possess significant anti-inflammatory property. The anti-inflammatory effect is probably due to their inhibitory effect on the release of mediators of inflammation. The effect is also due to the presence of one or more anti-inflammatory principles and their synergistic effects. Further studies are necessary to isolate and characterize the active principles from the bark of *Ficus religiosa.*

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Table: 1: Paw Oedema volume of experimental animals in each group.

Groups	Paw Oedema Volume*
Group I (Carrageenan treated animal)	0.73±0.06[a]
Group II (carrageenan+ aquous *Ficus religiosa* bark extract)	0.51±0.07[b]
Group III (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	0.43±0.05[c]
Group IV (Carrageenan+ Ibuprofen)	0.46±0.03[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table 2: Plasma TBARS levels in experimental animals of each group.

Groups	Plasma TBARS (n mol/ ml)
Group I (Carrageenan treated animal)	3.59±0.41[a]
Group II (carrageenan+ aquous *Ficus religiosa* bark extract)	2.78±0.36[b]
Group III (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	2.31±0.32[c]
Group IV (Carrageenan+ Ibuprofen)	2.17±.019[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table: 3: Erythrocyte membrane TBARS in experimental animals of each group.

Groups	Erythrocyte membrane TBARS (n mol/mg)
Group I (Carrageenan treated animal)	0.93±0.11[a]
Group II (carrageenan+ aquous *Ficus religiosa* bark extract)	0.64±0.07[b]
Group III (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	0.42±0.05[c]
Group IV (Carrageenan+ Ibuprofen)	0.36±0.03[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table 4: The levels of non-enzymatic antioxidants in plasma of experimental animals in each group.

Groups	Vitamin E (mg/dl)	Vitamin C (mg/dl)	*GSH (mg/dl)*
Group I (Carrageenan treated animal)	0.82±0.07[a]	0.75±0.09[a]	20.1±1.7[a]
GroupII carrageenan+ aquous *Ficus religiosa* bark extract)	1.17±0.11[b]	0.98±0.07[b]	23.8±2.1[b]
GroupIII (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	1.32±0.13[c]	1.27±0.14[c]	29.5±2.2[c]
GroupIV (Carrageenan+ Ibuprofen)	1.23±0.15[c]	1.07±0.08[c]	28.7±1.3[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table: 5: The levels of erythrocyte non-enzymatic antioxidants in experimental animals of each group.

Groups	Vitamin E (mg/dl)	GSH (mg/dl)
Group I (Carrageenan treated animal)	1.37±0.09[a]	30.6±3.4[a]
Group II (carrageenan+ aquous *Ficus religiosa* bark extract)	1.86±0.12[b]	37.3±2.9[b]
Group III (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	2.11±0.23[c]	45.1±3.6[c]
GroupIV (Carrageenan+ Ibuprofen)	1.93±0.11[c]	42.7±2.8[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table: 6: The activity of plasma glutathione peroxidase in experimental animals of each group. Values are mean ± SD (n=6).

Groups	GPx
Group I (Carrageenan treated animal)	148.7±14.2[a]
GroupII (Carrageenan+ aquous *Ficus religiosa* bark extract)	125.5±9.8[b]
GroupIII (Carrageenan+ Ethanolic *Ficus religiosa* bark extract)	118.7±12.5[c]
Group IV (Carrageenan+ Ibuprofen)	119.9±8.9[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table: 7: The activities of enzymatic antioxidants in inflammatory tissues of experimental animals in each group. Values are mean ± SD (n=6).

Groups	GPx (mg/dl)	SOD (mg/dl)	CAT (mg/dl)
Group I (Carrageenan treated animal)	13.3±1.6[a]	16.5±2.1[a]	8.1±0.73[a]
Group II (carrageenan+ aqueous *Ficus religiosa* bark extract)	10.5±0.92[b]	12.4±1.7[b]	5.8±0.69[b]
GroupIII (Carrageenan+Ethanolic *Ficus religiosa* bark extract)	8.7±0.26[c]	9.7±0.7[c]	4.7±0.58[c]
GroupIV (Carrageenan+ Ibuprofen)	9.1±0.54[c]	10.9±0.6[c]	5.1±0.37[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**

Table: 8: The levels of TBARS in inflammatory tissues of experimental animals in each group. Values are mean ± SD (n=6).

Groups	Inflammatory tissues TBARS (n moles / 100 mg protein)
Group I (Carrageenan treated animals)	116.7±12.8[a]
Group II (Carrageenan + aquous *Ficus religiosa* bark extract)	95.9±6.2[b]
Group III (Carrageenan + ethanolic *Ficus religiosa* bark extract)	80.7±9.2[c]
Group IV (Carrageenan + ibuprofen)	83.9±7.6[c]

*** Values are not sharing a common superscript significantly differ at *p<0.05* (DMRT).**