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PHARMACOLOGICAL EVALUATION OF FICUS RELIGIOSA

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Abstract

Ficus religiosa is known to be a native Indian tree. *F. religiosa* is a large evergreen tree found throughout India, wild as well as cultivated. It is a familiar sight in Hindu temples, Buddhist monasteries and shrines, villages and at roadsides. It is known to be a sacred plant in India and since ancient times it is widely being used to treat various ailments like skin diseases, heart diseases, diabetes, vomiting, burns, nervous disorder, constipation, dysentery*,* snakebite and important constituent of various traditional herbal preparations like shankha vati, chandraprabha vati and kaminivindravan rasa. In recent studies, *F. religiosa* has been investigated for presence of a wide range of phytoconstituents in Leaves yield campestrol, stigmasterol, isofucosterol, - amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, nonacosane, nhentricontanen, hexa-cosanol and noctacosan. Extract of the plant leaves posses Anti inflammatory activity, wound healing activities and antidiabetic activity, Analgesic activity.

Keywords: *Ficus religiosa*, Anti inflammatory activity, wound healing activities, Antidiabetic activity, Analgesic activity.

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INTRODUCTION

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care (Kamboj, 2000) [1]. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998) [2]. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994) [3]. *Ficus religiosa* Linn (Moraceae) commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart shaped, long tipped leaves on long slender petioles and purple fruits growing in pairs. *F. religiosa* is known to be a native Indian tree, and thought to be originating mainly in Northern and Eastern India, where it widely found in uplands and plane areas and grows up to about 1650 meters or 5000 ft in the mountainous areas. It is also found growing elsewhere in India and throughout the subcontinent and Southern Asia, especially in Buddhist countries, wild or cultivated. Preliminary phytochemical screening of *F. religiosa* , showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Babu et al., 2010; Jiwala et al., 2008). The *F. religiosa* showed the presence of bergapten, bergaptol, lanosterol, -sitosterol, stigmasterol, lupen-3-one, -sitosterol-d glucoside (phytosterolin), vitamin K1 (Swami et al., 1989; Joseph and Justin, 2010; Margareth and Miranda, 2009). *Ficus religiosa posses* Hypolipidemic activity, Antimicrobial and antiviral activities, Antioxidant activity, Antiasthmatic activity Immunomodulatory activity, Anti-acetylcholinestrase activity. Wound healing, Anti inflammatory, Anti diabetic activity, Analgesic activity

EXPERIMENTAL

COLLECTION OF PLANT MATERIALS

The leaves of *Ficus religiosa* was collected from local area of Rimmanaguda, Medak,Telengana state ,India. The plant was identified and authenticated in the Department Of Botany, Osmania University,Telengana, India.

PREPARATION OF PLANT EXTRACTS

The leaves of the plant were shade dried on a laboratory table for 14 days and reduced to powder by using dry grinder. This powder (100gm) was then packed into soxhlet apparatus and extracted using 95% ethanol at 40-50 °C. The extraction was carried out for 40hrs.The extract obtained was dried at 45°C in hot air oven till green colour semisolid mass was obtained. The yield obtained was 4.5% and the semisolid extract was stored in a refrigerator at 4°C for further use.

EXPERIMENTAL DESIGN

EXCISION WOUND MODEL [4]

Under light ether anaesthesia an impression of 500 sq mm was made on the shaved back of the rat as described in Morton and Malone 4. The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made consider as day '0' (Zero). Animals divided into four groups of each with 5 animals. Group A consider as control and treated with simple ointment (eg. Bees wax, Cetosteryl alcohol etc.), group B consider as standard and treated with 5% w/w Povidine iodine ointment, group C and group D are *Ficus religiosa* treated group and applied ointment 5% and 10% respectively (Table-1).

WOUND HEALING ACTIVITY [5]

The wound healing activity was investigate by excision and incision wound models using *F. religiosa* leaf extracts, prepared as ointment (5 and 10%) were applied on Wistar albino strain rats. Povidine iodine 5% was used as Standard drug. High rate of wound contraction, decrease in the period for epithelialisation, high skin breaking strength were observed in animals treated with 10% leaf extract ointment when compared to the control group of animals. It has been reported that tannins possess ability to increase the collagen content, which is one of the factor for promotion of wound healing.

STATISTICAL ANALYSIS

The results were subjected to statistical analysis by using ANOVA followed by Turkey Krammer Multiple Comparison Test.

ANTI-INFLAMMATORY ACTIVITY [6]

Carrageenan-induced hind Paw edema in rats in present study anti-inflammatory activity was determined in albino rats of either sex according to the method. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw volume was measured plethysmometrically (Ugo Basile) at '0' and '3' hours after the carrageenan injection (Table 1). Aspirin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug.

STATISTICAL ANALYSIS

Results are expressed as mean ± S.E.M. statistical evaluations were made using ANOVA followed by t-test (Prism 3.0) and P values less than 0.05 were considered significant. Data are represented as mean ± S.E.M.

ANALGESIC ACTIVITY [7-10]

HOT PLATE METHOD

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56 °C . This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Swiss albino rats weighing between 100-150g were used for evaluation of analgesic activity; in each group six albino rats were kept. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water.

Test - 1 : A solution of *Ficus religiosa* (10mg/kg/10ml),

Test - 2 : A solution *Ficus religiosa* extract (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared.

Wistar albino rats of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 minutes, the animals are placed on the hot plate and the observations were recorded and at the time interval of 90, 120 and 180 minutes.

STATISTICAL ANALYSIS

The results of the study were subjected to one-way analysis of variance followed by Dunnett.s t-test for multiple comparisons. Values with $P < 0.05$ were considered Significant.

ANTI-DIABETIC ACTIVITY

ALLOXAN INDUCED DIABETIC MODEL [11]

Alloxan monohydrate was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg b.w. intraperitonially. After one hour of alloxan administration the animals were given feed ad libitm and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic Phase. The animals were kept under observation and after 48 hours blood glucose was measured by a gluco-meter. The diabetic rats (glucose level > 300 mg/dl) were separated and divided into six different groups for experimental study; each group contained six animals.

EXPERIMENTAL DESIGN

The animals were divided in to five groups and each group consisted of 6 rats.

1. Normal control (vehicle only)

2. Diabetic control (untreated rats)

3. Diabetic rats treated with Glibenclamide 5mg/kg

4. Diabetic rats treated with *Ficus religiosa* ethanolic extract 100mg/kg b.w (FREE)

5. Diabetic rats treated with *Ficus religiosa* ethanolic extract 200mg/kgb.w (FREE)

This experimental design was followed in both the alloxan induced model and OGGT model but in the latter model, normal rats loaded with glucose were used instead of diabetic rats.

BODY WEIGHT MEASUREMENT

Body weight was measured four times during the course of the study period (i.e., on, before alloxan induction (initial values), days 1, 4, 7 of the treatment period), using a digital weighing scale obtained from KERN (EMB), Germany.

STATISTICAL ANALYSIS

The results of the study were subjected to one-way analysis of variance followed by Dunnett.s t-test for multiple comparisons. Values with $P < 0.05$ were considered Significant.

RESULTS

Table 1: **Anti-inflammatory effect of *Ficus religiosa* extracts on carrageenan-induced paw edema in rats**

Groups	Dose orally (mg/kg, p.o.)	Change In Mean Paw Volume (ml)		Inhibition (%)	
		3h	4h	3h	4h
Control	-------	0.86±0.20	0.92±0.24	-----	-----
PPEE	200	0.54±0.03	0.46±0.02*	38.8%	53.6%
	500	0.46±0.12**	0.33±0.03**	52.3%	59.8%
	1000	0.34±0.02**	0.36±0.03**	61%	64.6%
ASA	300	0.23±0.01***	0.26±0.01**	76%	74.5%

n=6 in each group; Values are mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ significant;FREE- Ficus religiosa ethanolic extract.

Table 2: Analgesic Activity by Hot Plate Method in Rats:

Group	Treatment	Dose (mg/kg)	Reaction Time in seconds at time(minutes)(mean ±sem)				
			0	60	90	120	180
Control	Normal Saline	--------	3.17 ± 0.045	3.24 ± 0.038	3.96 ± 0.146	3.55 ± 0.145	3.98 ± 0.256
Test – 1	FREE	50	3.42 ± 0.041	7.78 ± 0.186 c	8.16 ± 0.296 c	8.57 ± 0.274 c	8.10 ± 0.293 c
Test – 2	FREE	100	3.25 ± 0.055	7.41 ± 0.296 c	8.28 ± 0.328 c	8.64 ± 0.314 c	8.19 ± 0.293 c
Standard	Ibuprofen	100	3.33 ± 0.081	6.635 ± 0.064 c	7.86 ± 0.2498c	8.24 ± 0.268 c	7.945 ± 0.297 c

Each value is the mean ± SEM for 6 rats, a $P < 0.05$; b $P < 0.01$; c $P < 0.001$ compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

Table 3: Effect of Ficus religiosa extracts on blood glucose level of alloxan induced diabetic albino rats after sub-acute treatment

Gr.no.	Treatment	Blood glucose level mg/dl						
		Basal volume	1hr	3hr	5hr	3rd day	5th day	7th day
I	Normal control (vehicle only)	80.00 ±1.693	80.83 ±1.722	80.83± 1.425	79.83±1. 376	81.33±0. 987	79.83±0. 834	81.16±0. 7924
II	Diabetic control	322.33 ±7.775	327.50 ±7.945	329.50 ±7.386	336.67± 6.516	369.00±6 .110	388.33±1 6.592	413.50± 4.752
III	*Ficus religiosa* extract 100mg	307.66 ±5.747	267.66 ±3.201 **	260.5± 4.128* *	256.66± 4.758**	203±12.9 46**	182.5±10 .686**	169.33± 9.736**
IV	*Ficus religiosa* extract200mg	309.66 ±8.511	282.33 ±7.932 *	273.5± 8.160* *	268±5.2 85**	196.33±3 .547**	163±9.58 5**	113.33± 6.663**
V	Glibenclamide 5mg/kg	277.33 ±7.933	206.65 ±6.280 **	174±7. 095**	154.84± 5.043**	125.33±6 .960**	114.33±5 .251**	105.66± 5.098**

amg/kg/day for 7 days. Values are means±SEM; N=6. Values are statistically signal can't at *$P<0.05$ and more signiÞcant at **$P<0.01$. ns= not significant, **$P<0.01$vs diabetic control. (ANOVA).

Table 4: Effect of various extracts on body weight after treatment in diabetic rats

Groups	Treatment (n=6)	Average body weight (g) ±SEM	
		Initial value	**Day 7**
1	Normal control (NC) (vehicle only)	166.33 ±2.974	182.3 ±2.525
2	Glibenclamide 5mg/kg (GLB)	183.5 ±2.078	205.33 ±1.65
3	Diabetic control(DC)	147.5 ±2.952	110.3 ±2.37
4	Ethanolic extract 100mg (FRAE)	144.3 ±2.124	128.0 ±2.98
5	Ethanolic extract 200mg (FREE)	147.8 ±2.915	163.3 ±3.48**

One-way ANOVA followed by Dunnett.s test. Values are expressed as mean±SEM. **$P<0.01$ as compared to normal control group; n= number of animals

Table 5: Effect of hydro alcoholic leaf extracts ointment of *Ficus religiosa* on Excision wound parameters

Group	% Wound contraction on				Epithelization time (Days)
	4th day	**8th day**	**12th day**	**16th day**	
A Control	15.77 ± 3.00	30.93 ± 2.50	44.87 ± 3.20	61.68 ± 4.21	23.5±0.65
B Standard Povidine iodine5%(w/w)	33.51 ±2.63***	59.93 ± 3.37***	88.19 ± 4.11***	96.61 ± 3.74***	18 ±0.54***
C *Ficus religisa* 5% (w/w)	26.38 ± 2.14*	46.57 ±3.56**	59.46 ± 4.49***	77.76 ± 4.80***	20.2±0.58*
D *Ficus religiosa* 10% (w/w)	31.78 ±3.86***	57.46 ±2.90***	86.47 ±3.00***	93.25 ± 3.72***	18 ±0.60***

• The values are expressed as Mean ± SEM, n=5 in each group. If * $P<0.05$, **$P<0.01$ and ***$P<0.001$ vs control.

DISCUSSION

ACUTE TOXICITY STUDY

In acute toxicity study ethanolic extract of *Ficus religiosa* leaves at the tested dose level of 5000mg/kg body weight did not show significant toxicity signs when observed for the parameters

during the first four hours and followed by daily observations for 14 days. No mortality was observed and the drug was found to be safe.

WOUND HEALING ACTIVITY

The results indicates the topical application of *Ficus religiosa* extract in different concentration (5% and 10%) have demonstrated significant reduction in the wound area.(Table 5). The 10% ointment of *Ficus religiosa* treated animals showed faster epithelialisation of wound (18 ±0.60) than the animals treated with 5% extract ointment (20.2±0.58) . The period of epithelialisation was (18 ±0.54) in the case of standard drug 5% w/w standard Povidine Iodine Ointment treated animals. In incision wound model, *Ficus religiosa* (10% and 5%) extract ointment treated animals showed increase in breaking strength (562.2±6.93), (476.9±7.25), respectively when compared to the control (430.4±6.46). The mean breaking strength was also significant in animals treated with standard drug (571±8.84) Results was tabulated in Table-5

ANTI –INFLAMMATORY

The results of the present study suggest that the *Ficus religiosa* at doses of 100 and 200mg/kg significantly suppressed carrageenan-induced paw edema in rats (Table 1). The results were found to be highly significant ($P<0.001$) in comparison to the control. The anti-inflammatory effects of the extract on acute inflammatory process such as carrageenan-induced edema in rats paw was dose dependent. At 100 mg/kg, the extract showed at least 50% inhibitory activity throughout the measurement intervals was comparable to 500 mg/kg of the extract. It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process. Phytochemical screening of the ethanolic extract showed the presence of flavonoids and saponins. Flavonoids act as an anti-inflammatory response in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins..

ANALGESIC ACTIVITY

Hot plate method

All the test and standard drugs significantly ($p<0.001$) reduce the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. (Table-2) As all the drugs are standard analgesics, by applying Student Newman-Keuls test, it was shown that no significant difference between the tests and standard. The standard drug exhibited awrithing inhibition percentage of 62.5% , test-1 (65.06) and test-2 (68.74) as

comparision to control group. The Hot plate and Tail immersion test useful in the elucidating centrally mediated anti nociceptive responses, which focuses mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.001$) reduce the pain as compare to the control group. (Table-2)

ANTI –DIABETIC ACTIVITY

In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and alloxan-induced model. As expected, in the diabetic control, there was severe hyperglycemia as compared to the normal animals. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly bringing it nearly back to normal. The single dose of ethanolic extract (100 mg/ kg b.w.) of *Ficus religiosa* has more significantly ($P<0.01$) reduced the blood glucose level as compare to diabetic control at 7th day of the study. Ethanolic extract (200 mg/kg b.w shown significant reduction of blood glucose after one hour whereas 100mg/kg showed significant reduction at three hours [Table 3]. The effects of different extracts on glucose tolerance test in normal rats were evaluated. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. Glibenclamide treated group (5mg/kg) prevented glucose induced hyperglycemia significantly. at 30 min and 90min (171.83 ± 4.214 and 106.16 ± 4.316) as compare to normal control (167.83 ± 2.301 and 146.83 ± 2.960) respectively. Maximum glucose tolerance in *Ficus religiosa* extracts was observed in 200mg/kg (121.00 ± 2.966) and the glucose tolerance was observed in aqueous extract (155.33 ± 3.018) was observed in 90 minutes compared with the normal and control [Table 4]. In the present study(100mg/kg), diabetic rats had lower body weights, high blood glucose level as compared to normal rats. This may be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic - cells in diabetic rats. The exact biologically active constitutents responsible for the said effect have not been reported nor was the exact mode of action of the antidiabetic activity reported earlier, with the lone observation that it is used in folklore diabetic treatments.

CONCLUSION

The present study was carried out to determine the wound healing, anti inflammatory, anti diabetic, Sanalgesic of ethanolic leaf extract of *Ficus religiosa.* Ethanolic extract of leaves of *Ficus religiosa* shows potent wound healing activity by topical application of leaves extract at

different concentrations, significantly suppressed the oedema of the paws and decreased the paw volume in carragenan induced hind paw oedema, anti diabetic activity in alloxan induced diabetic rats, potent analgesic activity in hot plate method. Hence this plant material has really shown great interest to develop a novel phytomedicine in these area.

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