Research Article

In Vitro and *In Vivo* Determination of Anti-Inflammatory Activities of *Garuga pinnata* Roxb

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ABSTRACT

The present investigation on anti-inflammatory activities revealed that various parts of *Garuga pinnata* tested at all concentrations proved to possess significant *in vitro* and *in vivo* anti-inflammatory activities. Initially, the *in vitro* anti-inflammatory activities are determined as the ability of plant extract for the inhibition of egg albumin denaturation and *in vivo* anti-inflammatory activities are determined by carrageenan induced paw edema test. Among the various parts of *Garuga pinnata*, stem bark exhibited highest *in vitro* and *in vivo* anti-inflammatory activities. Methanol and aqueous extracts of stem bark noticed significant inhibition of protein denaturation and reduction of edema at various concentrations tested. The percentages noticed with inhibition of protein denaturation by methanol and aqueous extracts of stem bark at 50, 100 and 150 mg/ml concentrations are 35, 65, 95 and 30, 56, 78 respectively. The IC_{50} values calculated for methanol and aqueous extracts were found 65 and 56 at 100 mg/ml concentration respectively. The inhibition percentage of edema noticed by methanol and aqueous extracts at 50, 100 and 150 mg/ml concentrations are 49, 52 83, and 31, 42, 59 respectively The IC_{50} values calculated for methanol and aqueous extracts were found 52 and 59 at 100 and 150 mg/ml concentration respectively. Stem bark methanol extract showed highest reduction of paw volume at 150 mg/kg^{-1} is 1.35±0.01 after 4 h of carrageenean induction which is considered as 50% of the volume decrease comparing to the negative control 2.07±0.02, and 80% with reference drug diclofenac sodium 1.09±0.02.

Keywords: Anti-inflammatory, Albumin, Carrageenean, *In vitro, In vivo*.

INTRODUCTION

Term inflammation has been derived from the *Inflammare* a Latin word, which give meaning as burning. All types of human body injuries results in chemical changes in the injured area. Inflammation is considered as spontaneous response shown as a consequence of many injuries caused by physical injury or human pathogenic organisms. Generally, the process of inflammation is associated with the activation and involvement of secretion of cytokines such as IL-1β, IL-6, and TNF-α, by activated cells which play major role in host defense mechanisms. The use of steroidal and non-steroidal anti-inflammatory drugs have been used for rapid and effective for only temporary relaxation and are inadequate and are inadequate.[1-4] Therefore, it is crucial to identify a new and safe drug for preventing or treating IBD.[5]

To minimize hazardous effects generated by these synthetic drugs, there is an, urgent search is required for alternative approach for development of drugs with minimum side effects. The natural compounds symbolize to the drugs synthesized chemically in reference to action and create a safer environment.[6] Owing to bio-integrity and ease in isolation of natural drugs, from medicinal plants, modern research has been focused on the development ethanomedicine for treatment of inflammatory diseases.[7] *Garuga pinnata* (Burseraceae) commonly called as kondavepa in Andhra Pradesh, and seen to bloom throughout Indian forests. This plant is commonly used as hub for the isolation of natural drugs that attribute to curative properties and share good contribution for the discovery and development of alternative medicine. [8-10] It has been proved that stem bark aqueous extract of this plant inhibit lipid peroxidation mechanism.[11] It has been also evidently proved that methanol extract of this plant is also much potent to scavenge free radicals generated *in vitro*.[12] Pheophorbide-α and-β methyl esters which are isolated from methanol crude extracts of this plant are reported paramount cytotoxic activity against KB and its drug-resistant human cancer cell lines.[13] Methanol extract of stem bark also reported prominent anticancer activities on human breast cancer cell lines such as MCF-7 and MDA-MB 231.[14] Garuganins I and II compounds isolated stem bark hot petrol and methanol extracts exhibits analogous mechanisms of antibacterial action.[14-16] Methanol and aqueous extracts of stem bark observed with high *in vitro* α- amylases and α- glucosidase inhibition and also notably reduced serum glucose levels *in vivo* in streptozotocin induced diabetic rats proving its anti-diabetic activities.[17] With the reference to contemporary above mentioned pharmacological properties of *Garuga pinnata*, the present investigation is carried out for the evaluation of anti-inflammatory activities *in vitro* and *in vivo.*

MATERIAL AND METHODS

Plant Material

Various parts of *Garuga pinnata* such as, Leaves, Fruits, Stem and Stem bark collected from Rampet village, Warangal district, Andhra Pradesh, India. Plant has been authenticated by Professor V.S Raju, Department of Botany, Kakatiya University.

Extraction Procedure

All the parts were chopped in to smaller fragments, shade dried and grinded in homogenizer in to coarse powder. The 100 grams of powdered material is extracted with methanol, ethyl acetate, chloroform, acetone, petroleum ether and aqueous which were concentrated under rotavapour at their boiling points.

Chemicals

Carrageenan was purchased from SRL chemical Laboratories, Mumbai, India. Diclofenac sodium is purchased from local market. Whereas, all other chemicals purchased were off research grade.

Animals

Albino rats of Wistar strain, of male sex, weighing 150 – 250 g were purchased from National Institute of Nutrition, Hyderabad, India and housed under standard environmental conditions (temperature: 24 ± 1^{o} C, light / dark cycle: 10/14 h). The rats were fed with standard pellet diet (Amrut laboratory animal feed, Maharashtra, India) and water *ad libitum*. Animals were acclimatized to laboratory conditions at least 1 week before conducting the experiments according to the guide lines of CPCSEA – New Delhi, (Registration No. - 915/ac/05/CPCSEA).

Preliminary Studies on Anti-Inflammatory Activities Of Extracts Of *Garuga Pinnata*

Preliminary studies on anti-inflammatory activities of extracts of *Garuga pinnata* were determined by the described method.[18] About of 5 mL reaction mixture includes of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) add 2 mL of *Garuga pinnata* (Leaf, Fruit, Stem and Stem bark) various extracts at 50, 100 and 150 µg/mL. Double-distilled water with same volume used as control. The mixtures were incubated at (37±2) ^{0}C in BOD incubator for about 15 min and followed by heating at 70 ^{0}C for 5 min. After attaining room temperature, absorbance was measured at 660 nm using vehicle as blank and viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (10.15, 20 µg/mL) was used as reference drug. The inhibition percentage of protein denaturation was calculated using the following formula:

% inhibition = 100 × (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Carrageenan-Induced Rat Paw Edema Assay

In vivo anti-inflammatory activity of *Garuga pinnata* methanol extract was conducted using carrageenan-induced rat hind paw edema according to Winter et al., 1962.[19] Briefly, acute inflammation was induced 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 1h after the oral administration of test materials. The paw volume was measured by plethysmometer at 1, 2, 3, and 4 h after the carrageenan injection. The extract was administered at 50,100 and 150 mg/kg body weight. Diclofenac sodium 10, 15, 20 mg/kg body weight was used as standard anti-inflammatory agent.

Statistical Analysis

Values of paw volume reduction by plant extract and standard were expressed as the mean ± SEM. Differences between mean values are calculated by using SAS 9.1 and student "*t*" test. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Preliminary Studies on Anti-Inflammatory Activities Of Extracts Of *Garuga Pinnata*

Among various parts of *Garuga pinnata* stem bark is noticed to associate with high *in vitro* anti-inflammatory activity by significant inhibition of protein denaturation. Among the extracts of stem bark, methanol and aqueous extracts were evidently showed highest inhibition percentage when compared with other extracts. Methanol extract showed prominent inhibition percentage of protein denaturation at 150 mg/ml 95 % which is very much near and more comparable with reference drug Diclofenac sodium 99 % at 20 µg/ml concentration (Table 1). On the other hand, aqueous extract also exhibited good activity against protein denaturation. The inhibition percentages noticed by methanol and aqueous extracts of stem bark at 50, 100 and 150 mg/ml concentrations are 35, 65, 95 and 30, 56, 78 respectively. The IC_{50} values calculated for stem bark methanol and aqueous extracts were found 65 and 56 at 100 mg/ml concentration respectively. Next to stem bark leaf methanol and aqueous extracts are also showed good anti-inflammatory activities (Table 1).

Carrageenan-Induced Rat Paw Edema Assay

In vivo anti-inflammatory activities of stem bark methanol and aqueous extracts revealed, significant inhibition of edema in a concentration dependent manner (Table 2-3). Methanol extract exhibited highest inhibition percentage of swelling 83 % at 150 mg/kg^{-1} after 4 h of carrageenan induction which is comparable with 93 % noticed with reference drug Diclofenac sodium at 20 µg/ml (Table 1

and 4). On the other hand, aqueous extract also showed good inhibition percentage 59 % at 150 mg/kg^{-1} (Table 3).

The inhibition percentages of edema noticed at 50, 100 and 150 mg/ml concentrations by methanol and aqueous extracts are 49, 52 83, and 31, 42, 59 respectively. The IC_{50} values calculated for methanol and aqueous extracts were found 52 and 59 at 100 and 150 mg/ml concentration respectively. The reduction of paw volume by both extracts was found significant. Methanol extract recorded highest decrease of paw volume at 150 mg/kg^{-1} is 1.35±0.01 after 4 h of carrageenan induction, which were comparable with reference drug Diclofenac sodium 1.09±0.02. Whereas, aqueous extract was also showed reduction of paw volume. The highest reduction was recorded with aqueous extract at 150 mg/kg^{-1} is 1.55±0.01.

The present studies were carried out for the assessment of *in vitro* and *in vivo* anti-inflammatory activities of *Garuga pinnata* extracts. The preliminary study of anti-inflammatory activities *in vitro* is conducted for the inhibition of protein denaturation by the various extracts. According to the current data obtained, clearly it has been understood that methanol and aqueous extracts of stem bark noticed significant inhibition of protein denaturation in concentration dependent manner comparing with that from other extracts. Denaturation of tissue proteins is due to the generation of auto antigens which leads to different types of inflammatory diseases especially, rheumatoid arthritis. Decrease in absorbance can establish a direct correlation with the ability of plant extract in inhibition of the protein denaturation mechanism. This method is a well executed method and postulated for the evaluation of *in vitro* anti-inflammatory activities of plant extracts.[20] Based on the *in vitro* anti-inflammatory reports methanol and aqueous extracts were further confirmed for their *in vivo* anti-inflammatory activities using animal models.

Table 1: Percentage inhibition of protein denaturation by hydrolic extracts of *Garuga pinnata* various extracts

mg/ml	Petroleum Ether			Chloroform			Ethyl acetate			Acetone			Methanol			Aqueous			Diclofenac sodium		
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	10	15	20
Leaf	22	29	35	28	39	50	26	37	48	20	31	46	30	48	71	26	42	59	45	78	99
Fruit	20	24	27	25	30	35	23	28	31	22	26	31	29	36	35	24	32	38	45	78	99
Stem	18	24	28	22	28	33	20	26	30	22	28	32	28	37	48	26	37	46	45	78	99
Stem bark	24	35	44	30	49	57`	32	48	54	35	44	54	35	65	95	30	56	78	45	78	99

Table 2: *In vivo* anti-inflammatory activities of *Garuga pinnata* stem bark methanol extract on carrageenan induced paw edema in rats

carrageenean induced								
Treatment	1 Hour		2 Hour		3 Hour		4 Hour	
	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition
N. control	2.07±0.02	---		---		---		---
#50 mg/kg^{-1}	2.04±0.01*	5	1.80±0.08*	9	1.72±0.02*	14	1.60±0.02*	49
#100 mg/kg^{-1}	1.95±0.01*	7	1.74±0.02*	13	1.60±0.01*	23	1.45±0.01*	52
#150 mg/kg^{-1}	1.92±0.02*	9	1.61±1.4*	21	1.50±0.01*	44	1.35±0.01*	83

Garuga pinnata stem bark methanol extract; *P- value is considered less than 0.05 (n=3 and P<0.05)

Table 3: *In vivo* anti-inflammatory activities of *Garuga pinnata* stem bark aqueous extract on carrageen induced paw edema in rats

Carrageenan induced								
Treatment	1 Hour		2 Hour		3 Hour		4 Hour	
	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition
N. control	2.07±0.02	---		---		---		---
#50 mg/kg^{-1}	2.03±0.5*	3	1.98±0.03*	8	1.86±0.3*	19	1.74±0.02*	31
#100 mg/kg^{-1}	2.00±0.005*	5	1.94±0.02*	18	1.80±0.02*	36	1.61±0.01*	42
#150 mg/kg^{-1}	196±0.01*	8	1.86±0.01*	29	1.72±0.03*	42	1.55±0.01*	59

Garuga pinnata stem bark aqueous extract; *P- value is considered less than 0.05 (n=3 and P<0.05)

Reduction of paw volume by both of extracts were found significant, however, methanol extract recorded more significant than that of aqueous extract. The reduction of paw volume was gradually decreased every hour after treatment with both extracts. The extracts exhibited concentration dependent anti-inflammatory activities.

It is reasonable to assume the speculated reasons for the anti-inflammatory activity of *Garuga pinnata* extracts is due to the synergistic effects exhibited by the compounds.

The chemical constituents of *Garuga pinnata* is remarkably contain high amounts of phytochemicals such as flavonoids, phenolic compounds.[12] Many research reports elucidate the cellular mechanism of flavonoids in anti-inflammatory activity in various inflammation processes.[21] It has been reported that flavonoids regulate the activities of arachidonic acid (AA) metabolizing enzymes viz., COX-2, LOX iNOS and phospholipase A2(PLA2).[22- 28] Flavone and its amino-substituted flavones were also reported to possess inhibitory effects of NO production.[29-30] IL-1, IL-6, and TNF-α, production was also inhibited by flavonoids.[31,-32] The high anti-inflammatory activities of methanol extract of stem bark is might because of its high amounts of flavanoids.[12] Our data correlates with other research reports especially, the role of flavonoids as anti-inflammatory agents. The presence of high amounts of flavonoids in the stem bark of *Garuga pinnata* can establish its significant *in vitro* and *in vivo* anti-inflammatory activities.

Table 4: *In vivo* anti-inflammatory activities of Diclofenac sodium on *carrageenan induced paw edema in rats*

Carrageenean induced								
Treatment	1 Hour		2 Hour		3 Hour		4 Hour	
	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition	Paw volume(ml)	% inhibition
N. control	2.07±0.02	---		---		---		---
#10 µg/ml	1.90±0.02*	10	1.82±0.01*	18	1.68±0.01*	32	1.48±0.02*	66
#15 µg/ml	1.76±0.02*	15	1.69±0.01*	28	1.46±0.01*	39	1.24±0.02*	78
#20 µg/ml	1.63±0.02*	21	1.52±0.01*	43	1.29±0.02*	68	1.09±0.02*	94

Various concentrations of Diclofenac sodium; *P- value is considered less than 0.05 (n=3 and P<0.05)

CONCLUSION

In conclusion, we demonstrate that *Garuga pinnata* stem bark is a potent inhibitor of various inflammation processes. Further studies are underway for the identification and isolation of the active components from *Garuga pinnata* for actual their mechanism of action.

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