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Evaluation of anti-oxidant and anti-inflammatory activity of stem bark of *Bridelia airy - shawii* in rats

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Absract

Objective: To investigate antioxidant and anti-inflammatory activity of the petroleum ether, chloroform, methanol and aqueous extracts of Stem Bark of *Bridelia airy - shawii* (Li). Materials and Methods: The anti-inflammatory activity of the petroleum ether, chloroform, methanol and aqueous extracts of the stem bark of *Bridelia airy - shawii*. (Euphorbiaceae) were evaluated by using carrageenan induced rat paw edema and cotton pellet granuloma method in rat. The anti oxidant activity of all the extracts was determined by using Nitric oxide scavenging assay. Results: It was observed that, all the extracts significantly inhibited the carrageenan induced rat paw edema at the dose of 200 mg/kg b.w. orally, when compared with control and standard drug Ibuprofen. Extracts also suppressed the granulomatous tissue formation of chronic inflammation, when compared with standard drug Diclofenac sodium. The stem bark of *B. airy - shawii* extracts showed significant free radical scavenging activity against nitric oxide ($NO^{\bullet}$) induced release of free radicals. Conclusion: Therefore, present study suggests, potential of stem bark of *B. airy shawii* in inhibition of both acute and chronic phases of an inflammatory process.

Key words: *Bridelia airy - shawii*; Carrageenan induced edema; Cotton pellet granuloma, Nitric Oxide inhibition.

1. Introduction

Inflammation is a complex pathophysiological process. Inflammatory diseases including different types of rheumatic disorders are very common through out the world [1]. Prolong use of presently available synthetic agents has been associated with many side effects like gastrointestinal irritation. Several natural medicinal plants are available for the management of inflammatory diseases.

Bridelia retusa Spreng.,Syn: *Bridelia airy - shawii* (Euphorbiaceae) is a small to moderate sized deciduous tree, found through out the India up to an altitude of 1000 m, except in very dry regions. The bark contains 16-40 % of tannin. In pharmacological trials, the bark exhibited antiviral, hypoglycemic and hypotensive properties [2]. According to Ayurveda, the bark is good for removal of urinary concretions.

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The bark is useful in lumbago and hemiplegia [3]. It is also documented to be used ethanobotanically to promote antifertility activity [4, 5, 6]. The bark is used as a liniment with gingelly oil in rheumatism [7]. Among the various species of *Bridelia*, the stem bark of *Bridelia ferruginea* has been reported to have anti-inflammatory activity [8]. Based on these ethanobotanically clues, this study was first time undertaken for the evaluation of various extracts of stem bark of *B. retusa* on acute and chronic models of inflammation along with anti oxidant activity.

2. Materials and Methods

2.1 Animals

Male Wistar rats (125-150 g) were used for the study. Animals were maintained under standard enviornmental conditions and had free access to feed (Amrut feeds, Pune, India) and water ad libitum. The animal study was approved by institutional animal ethical committee 651/02/BC/ CPCSEA of R.C.Patel College of Pharmacy, Shirpur, Maharashtra, India..

2.2 Plant materials

The fresh stem bark of *B. retusa* Syn: *Bridelia airy - shawii* was collected from Toranmal area of Nandurbar District, India in the month of August 2005 and authenticated by Botanical Survey of India (BSI), Pune, India. Voucher herbarium specimen number of the plant is RAN-472 and kept in the institute.

2.3 Preparation of plant extract

The bark was shade dried and pulverized to make the powder. Bark powder was then successively extracted with petroleum ether (60-80°C), chloroform and methanol in soxhlet extractor for 72 hours. All the extracts were concentrated under reduced pressure at 50°C using Rota vacuum evaporator(Roteva, Equitron, Mumbai) and dried in a vacuum dryer. The fresh powder was also macerated with chloroform water by cold maceration for three days with constant shaking. Thus, various dried extracts obtained were used directly for the assessments of anti-inflammatory & antioxidant activity.

2.4 Carrageenan-induced rat paw edema

Inflammation in rats was produced by carrageenan according to the method described by [9]. The rats were divided into six groups, each group consist of six animals. Tween 80 was used as a vehicle for suspending the extracts as well as standard antiinflammatory drug Ibuprofen. The group I served as control and received only vehicle, the group II received standard drug Ibuprofen (100 mg/kg p.o.). Group III to VI were treated with pet. ether, chloroform, methanol and aqueous extracts of bark of *B. retusa* respectively at the dose of 200 mg/kg p.o. After 30 minutes of sample treatment, acute inflammation was produced by sub-planter injection of 0.1 ml of 1 % carrageenan in normal saline in the right hind paw of rat. Mean increase in paw volume were measured plethysmometrically (UGO Basile, Italy) at 0 hr, 1 hr, 2 hr and 3 hr after carrageenan injection to each group. 0 hr readings are considered as initial paw volume of the animals. Percentage inhibition of paw volume was calculated at each hour by using the formula:

% of Inhibition = 1- (Vt/Vc) X 100

Where, Vt is the paw volume of test group and

Vc is the paw volume of control group.

2.5 Cotton pellet granuloma

The method was described by [10]. In this method, some giant cells and connective tissue can be observed besides the fluid infiltration after several days of subcutaneous implantation of pellets of cotton in rats [11]. Rats were divided into six groups of six animals each. Sterile cotton pellets (10±0.7mg) were implanted

subcutaneously in axilla under ether anesthesia. The I group served as control, received vehicle only, II group received standard drug Diclofenac sodium (13.5 mg/kg p.o.) and animals of III to VI group were treated with pet. ether, chloroform, methanol and aqueous extracts of the bark at the dose of 200 mg/kg p.o. for seven consecutive days from the day of cotton pellet implantation. On the eighth day, animals were sacrified and granulation tissue with cotton pellet was removed and dried to constant weight at 60°C and dry weight was taken.

2.6 Phytochemical screening

A phytochemical analysis of various extracts of stem bark of *B. airy - shawii* was conducted according to the method of Trease & Evans [12].

2.7 Nitric oxide scavenging assay

Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of pet. ether, chloroform, methanol and aqueous extracts of *Bridelia retusa* was dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 h, 0.5 ml of incubation solution containing nitrite was pipetted and mixed with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water) and allowed to stand for 5 min completing diazotization. The absorbance of chromophore formed was measured at 546 nm against the corresponding blank solutions using micro titer plate reader (BIO-Tek Powerwave TM XS, Model-96well micro plate). The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula 100-[100/blank absorbance × sample absorbance [13, 14]. The activity was compared with ascorbic acid, which was used as a standard antioxidant. IC_{50} for test extracts was calculated using log-dose inhibition curve.

2.8 Statistical analysis

All the data are expressed as mean ± S.E.M. Statistical significance was determined by one way ANOVA (Analysis of variance) followed by Dunnett's test.

Table 1. Effect of various extracts of *Bridelia airy - shawii* stem bark on carrageenan induced rat paw edema

Group (n=6)	Dose(mg/kg p.o.)	Mean increases in paw volume (ml) (mean ± SEM)[a]			
		0 hr.	1 hr.	2 hr.	3 hr.
Control	-	0.834±0.012	1.508±0.085	1.982±0.087	2.170±0.011
Pet. ether extract	200	0.848±0.066	1.220±0.086 [c] (19.09)	1.348±0.081 [b] (31.98)	1.224±0.012 [b] (43.59)
Chloroform extract	200	0.910±0.037	1.246±0.084 [c] (17.37)	1.369±0.003 [b] (30.92)	1.30±0.050 [b] (40.09)
Methanol extract	200	0.964±0.049	1.286±0.013 [c] (14.72)	1.480±0.013 [b] (25.32)	1.410±0.097 [b] (35.02)
Aqueous extract	200	0.964±0.066	1.328±0.033 [c] (11.93)	1.658±0.059 [b] (16.34)	1.519±0.003 [b] (29.95)
Ibuprofen	100	0.926±0.065	1.090±0.059 [c] (27.71)	1.302±0.093 [b] (34.30)	1.184±0.049 [b] (45.30)

Each value is presented as Mean ± S.E.M. ([b]$p<0.01$, [c]$p<0.05$) one way ANOVA followed by Dunnett's test.
Figures in parentheses indicate the % of anti-inflammatory activity.

Table 2. Effects of various extracts of stem bark of *Bridelia airy - shawii* on cotton pellet induced granuloma in rats.

Group (n =6)	Dose (mg/kg, p.o.)	Granuloma dry wt. (mg)	Inhibition (%)
Control	-	30.16 ± 1.73	-
Pet. ether extract	200	17.16 ± 0.58 [b]	43.90
Chloroform extract	200	20.83 ± 0.76 [b]	30.94
Methanol Extract	200	21.83 ± 0.94 [b]	27.62
Aqueous extract	200	25.43 ± 1.16 [b]	15.68
Diclofenac sodium	13.5	16.83 ± 0.72 [b]	44.20

Each value is presented as Mean ± S.E.M. (bp<0.01) one way ANOVA followed by Dunnett's test.

Table 3. IC_{50} Value of different extracts of stem bark of *Bridelia airy - shawii in* Nitric Oxide scavenging assay

Sr. No.	Test Material	IC_{50}
1	Ascorbic Acid	19.86
2	Pet. Ether	79.16
3	Chloroform Extract	77.52
4	Methanolic Extract	54.15
5	Aqueous Extract	51.06

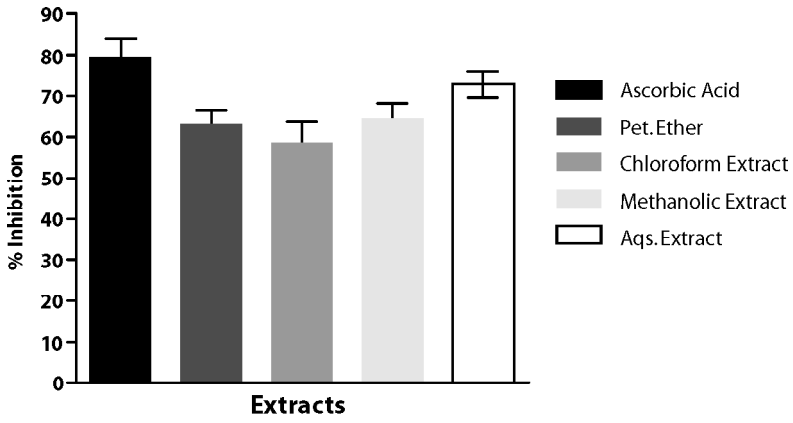

Fig. 1
Nitric Oxide Scavenging effect on different extracts of stem bark of Bridelia airy shawii at 125 µg/ml Conc.

3. Results

3.1 Carrageenan induced rat paw edema

In the acute inflammation model, i.e. carrageenan induced rat paw edema, all the four extracts exhibited significant inhibition of paw volume when compared to control and standard group. Pet. ether extract shows potent antiinflammatory activity among the four extracts. (Table-1).

3.2 Cotton pellet granuloma

All the extracts of stem bark of *B. airy - shawii* at the dose of 200 mg/kg, produced significant reduction in granuloma tissue formation when compared to control group. The pet. ether extract exhibited pronounced inhibition of increase in granuloma weight and the effects were comparable to that of the standard drug Diclofenac sodium and control group (Table-2).

3.3 Nitric Oxide scavenging activity

The stem bark of *B. retusa* extracts showed significant free radical scavenging activity against nitric oxide (NO) induced release of free radicals. IC_{50} for pet. ether, chloroform, methanol and aqueous extracts of *Bridelia retusa* was found to be 79.16, 77.52, 54.15, and 51.06 µg / ml respectively in comparison with standard ascorbic acid 19.86 µg / ml. (Table-3).

4. Discussion and conclusions

Carrageenan induced inflammation is biphasic event. The initial phase which last for one hour is attributed to the release of histamine and serotonin. The second phase (3rd hr) is related to release of bradykinin and prostaglandins [15, 16]. The second phase is sensitive to most chemically effective anti-inflammatory drugs [17, 18]. Pet ether, chloroform, methanol and aqueous extracts showed reduction in paw volume from 1st hr to 3rd hr. But maximum reduction in paw volume was observed at 3rd hr after administration of carrageenan. From the data it is evident that, the pet ether extract showed a greater percentage of decrease in paw volume at second phase.

Similarly in cotton pellet granuloma model of inflammation, all the extracts inhibited the granuloma tissue formation significantly ($P<0.01$) indicating that, the extracts can also inhibit chronic inflammatory process. The pet ether extract of bark of *B. retusa* was found to be more significant among the four extracts for reduction in weight of cotton pellet when compared with control and standard Diclofenac sodium. The cotton pellet granuloma method has been widely employed to evaluate the transudative, exudative and proliferative components of chronic inflammation.

Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases. Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process [19]. Studies have suggested that free radicals liberation causes inflammation and tissue damages [20]. Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for $NO^{\bullet}$ scavenging activity. Nitric oxide (NO) is recognized as a mediator and regulator of inflammatory responses [21]. NO can also interact with molecular oxygen and super oxide anion to produce reactive nitrogen species that can modify various cellular functions.

Some sterols and triterpenoids are responsible for anti-inflammatory activity of both acute and chronic model [22]. Tannins and other polyphenols are also reported to exhibit inhibitory effects on proinflammatory mediators

of cytokines As qualitative chemical analysis of stem bark of *B. airy - shawii* revealed the presence of sterols and triterpenoids in pet ether & chloroform extracts and presence of tannins & polar components in methanolic and aqueous extract, they might suppress the formation of prostaglandins and bradykinins.

In conclusion, this study has shown that the bark of *B. retus* does possess significant anti-inflammatory and anti-oxidant effects. Thus the results support the traditional use of this plant in rheumatic conditions. Further detailed studies for anti-inflammatory and anti-oxidant are currently under way to isolate and characterized the active principles of the plant extract.

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