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RESEARCH ARTICLE

ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *BARRINGTONIA ACUTANGULA* LINN. BARK EXTRACTS ON RATS

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ABSTRACT

Barringtonia acutangula Linn (*B.actangula)* commonly known as Indian oak is an indigenous medicinal plant which is found throughout India. The objectives of present study are to evaluate the effects of ethanolic and aqueous extracts of *B.actangula* Linn bark on anti-oxidant and anti-inflammatory properties. The *B.actangula* bark extracts were prepared by using soxhlet with ethanol and successive aqueous extract was prepared using same marc. The extracts were subjected for phytochemical analysis to identify different phytoconstituents. Acute toxicity study of extracts was carried according to OECD guidelines 423. With the extracts (500mg/kg) anti-oxidant activity property and anti-inflammatory activity was carried out. Antioxidant models such as, ethanol induced oxidative stress in rats and histamine induced oxidative stress in guinea pigs for the estimation of SOD, MDA. Acute and chronic studies of anti-inflammation models like Carrageenan induced paw edema and cotton wool granuloma models were carried out by using extracts of *B.acutangula*. A significant pharmacological activities of *B .acutangula* Linn extracts was observed in the present investigation may be due to synergistic potentiative action of its phytoconstituents since, they contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of anti-inflammation activities.

INTRODUCTION

Oxygen is required for all living organisms for their survival. But at the same time it is potentially toxic. Oxygen has been described as a double edged sword. It is vital to life but at the same time because of its highly reactive nature, it is capable of becoming a part of potentially damaging molecules called free radicals (Pendyala *et al*., 2008). Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack our cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins, and enzymes present in the body (Aher *et al*., 2011). The living organisms have adapted themselves to an existence under a continuous efflux of free radicals. Among the different adaptive mechanisms the antioxidant defense mechanism is of major importance (Pendyala *et al*., 2008). Free radicals tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals (O2•-, and •OH) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and ozone (O3).

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About 100 disorders have been well attributed to ROS (Govindarajan *et al*., 2005). Minor free radical concentrations are considered harmless for the cells because relatively large amounts of protective enzymes and free radical scavengers are present in the cells. Mitochondria as well as cytosol contain considerable amounts of the enzyme superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase neutralises superoxide to hydrogen peroxide whereas, hydrogen per oxide is neutralized by catalase and glutathione peroxidase 2'7"4"5. Reduced glutathione is an essential co-factor for the enzymatic action of glutathione peroxidase. Cellular protection against free radical attack is also conferred by substances such as alpha- tocopherol (Vitamin E) and beta-carotenes because they scavenge free radicals (Singh, 1922). The most commonly used synthetic antioxidants like BHA, BHT, propylgalate and *tert*-butylhydroquinone have suspected of being responsible for liver damage and carcinogenicity (Sumanont *et al*., 2004). Therefore there is a need of antioxidants which are more effective and have lesser side effects (Govindarajan *et al*., 2005). Natural antioxidants include flavonoids, vitamins, carotenoids and hydroxycinnamates. Among these, flavonoids form the largest group of antioxidants which are ubiquitous in nature in most of the fruits, vegetables and plants (Adeneye and Benebo, 2008).

Inflammation is the reaction of tissue and its micro circulation to a pathogenic tissue (Rubin, 2005) . Among the conditions that may produce inflammation are pathogens, abrasions, chemical irritations, distortion or disturbances of cells and extreme temperature (Gerad, 2003). Inhibitors of NOS activate reduce the severity of inflammation and support the role of NO in the pathophysiology associated with various models of inflammation. There is a large amount of evidence to show that the production of ROS such as $O2^{-}$, H2o2 and OH- occurs at the site of inflammation and contributes to tissue damage. The early phases of inflammation process is related to the production of histamine, lekotriens, PAF and cycloxygenase products where as delayed phase of inflammation and the production of neutrophil derived free radicals and oxidants such as H2O2, O2and OH as well as the release of other neutrophil derived mediators (Cuzzocrea *et al*., 2001). Though the advances in modern medicines are significant, there remains an ever increasing demand for herbal medicines. Effective and potent herbal medicines require evaluation by standard scientific methods so as to be validated for the treatment of diseases. Based on these facts, the present work aimed to evaluate the anti-oxidant property and anti-inflammatory activity of *B.actangula* by using ethanolic and aqueous extracts.

MATERIALS AND METHODS

Wistar albinos both sex weighing between (200-250g) were purchased from Shri Venkateshwara traders, Bangalore, Karnataka. The animals were maintained under standardized environmental conditions (22-28°C, 60-70% relative humidity, 12 h dark/light cycle). They were allowed to access to food and water *ad libitum*. All experimental protocols described in this study were in accordance with the rules and regulation of the Animal Ethics rules obtained from Institutional Animal Ethical Committee (IAEC), The Oxford College of Pharmacy, Bangalore (60/CP/2011-12, Dt 21/5/2011).

Plant Materials

*Barringtonia acutangula Linn*Bark was collected from Chennai, Tamil Nadu and authenticated by Dr P Santhan, Plant taxonomist, Natural Remedies private limited, Bangalore.

Extraction

The powdered plant material (627g) was extracted using ethanol (2.5l) in a Soxhlet extractor (hot extraction). The extract was evaporated by distillation method and then on water bath.The aqueous extract was prepared using the same marc by the process of maceration. The percentage yield of ethanolic ext (40gm@6.4%) and Aqueous Ext (18gm@2.8%), colour and consistency of the extract were recorded.

Phytochemical Analysis

The phytochemical tests were performed and the chemical constituents that are present in both the extracts are carbohydrates, saponins, amino acids, flavanoids, phenols, tannins steroids, triterpenoids (Trease, 2001).

Acute Toxicity Studies

The acute oral toxicity study was performed according to the OECD guidelines 423 by following UP and DOWN method[11]. *Barringtonia acutangula* extracts 50 mg/kg, 500mg/kg, 1000mg/kg, and 2000mg/kg orally. All doses were safe up to 2000 mg/kg, so $1/4^{th}$ (500mg/kg) was taken as safe dose from ethanolic and aqueous extract of *B. acutangula* Linn.

Anti Oxidant Activity

Ethanol induced oxidative stress in rats (Kath and Gupta, 2006)

Estimation of superoxide dismutase (SOD)

Wistar rats of either sex was taken and divided in 5 groups (n=6) in which

Group I: Control group received vehicle
Group II: Received Control- (1ml ethanol)
Group III: Received 500 mg /kg of ethanolic ext of *B.acutangula*
Group IV: Received 500 mg /kg of aqueous extract of *B.acutangula*
Group V: Standard group received Ranitidine (20mg/kg) orally to all rats.

Treatment was followed by 7 days excluding the 2^{nd} group rest of the groups Absolute ethanol was administered on the 7^{th} day After 30 minutes of drug treatment blood samples will be collected from animals in all the five groups for estimation of serum malondialdehyde and serum Superoxide dismutase after 4 hours of ethanol administration.

Sample Collection

The serum was homogenized with a polytronhomogenizerinice-cold Tris- buffer to produce 10% homogenate. The homogenate was centrifuged at 10,000rpm for 15min at 4°C. Aliquot of supernatant 0.1ml was added to 1.2ml of 0.052M sodium pyrophosphate buffer (pH8.3) followed by addition of 0.1ml of 186µM phenazoniummethosulphate, 0.3ml of 300µM nitrobluetetrazolium, 0.2ml of 780µM NADH. Reaction mixture was incubated for 90 sec at 30°C, and the reaction was stopped by the addition of 1.0ml of glacial acetic acid. Reaction mixture was stirred vigorously and shaken with 4.0ml of n-butanol and centrifuged at 4000rpm for 10min the absorbance of organic layer was measured at 560nm. A control was prepared using 0.1ml of distilled water devoid of 0.1ml of homogenate. One unit of the enzyme activity is defined, as enzyme concentration required inhibiting the absorbance of chromogen production by 50% in control sample under the assay conditions.

Formula for estimation of SOD

Histamine induced oxidative stress in guinea pigs (Shay, 1945)

Guinea pigs of either sex was taken and divided in 5(n=6) groups in which

Group I: Control group received vehicle
Group II: Received Control- (1ml Histamine Acid Phosphate 1mg/ml)
Group III: Received 500 mg /kg of ethanolic ext of *B.acutangula*
Group IV: Received 500 mg /kg of aqueous extract of *B.acutangula*
Group V: Standard group received Ranitidine (20mg/kg) orally to all rats.

7 days of treatment was followed, on the 7th day excluding the 2nd group animals will be administered histamine acid phosphate. After 30 minutes of drug treatment blood samples will be collected from animals in all the five groups for estimation of serum malondialdehyde and serum Superoxide dismutase after 4 hours of histamine administration.

Sample Collection

MDA levels in the serum were measured by the method developed by Yagieta l.0.1ml serum was taken in to the centrifuge tube containing 4ml of 0.083N sulphuric acid. Then 0.5ml of 10% phosphotungsticacid was added to the tube and mixed then kept at room temperature for 5min. The mixture was centrifuged at 4000rpm for 10min. The sediment was suspended in 2ml of 0.083N sulphuric acid and 0.3 ml of 10% phosphotungstic acid and the mixture was centrifuged at 4000rpm for 10min. The sediment was suspended in 4ml of distilled water and 1ml of TBA reagent was added. The reaction mixture was heated for 60 min at 95°C. After the mixture was cooled to room temperature, then 5ml of n-butanol was added and the mixture was centrifuged at 4000rpm for 10 min and n-butanol layer was separated and used for colorimetric measurement at 532 nm.

Anti-Inflammatory Activity

Acute Model - Carrageenan-induced Paw Edema in Rats (Winter *et al*., 1962)

Animals are divided into four groups (n=6) starved overnight with water ad libitum prior to the day of experiment. The animals were treated as,

Group I: Control group received vehicle
Group II: Received 500 mg /kg of ethanolic ext of *B.acutangula*
Group III: Received 500 mg /kg of aqueous extract of *B.acutangula*
Group IV: Standard group received 10mg/ kg of Indomethacin orally to all rats.

One hour after dosing, the rats are challenged by a subcutaneous injection of 0.1ml of 1% solution of carrageenan into the sub-plantar side of the left hind paw. The paw volume is measured again at 1, 2, 3, 4 and 5 hours after challenge. The percent Inhibition is calculated using the formula as follows.

% edema inhibition = [1- (Vt / Vc)] X 100

Vt and Vc are edema volume in the drug treated and control groups respectively (Crunkhorn and Meacock, 1971, Vinegar *et al*., 1969).

Chronic Model - Cotton Pellet-Induced Granuloma in Rats (Goldstein SA, 1976)

Rats of either sex were taken and divided into four groups (n=6), fasted overnight and allowed free access to water. The animals were treated as,

Group I: Control group received vehicle
Group II: Received 500 mg /kg of ethanolic ext of *B.acutangula*
Group III: Received 500 mg /kg of aqueous ext of *B.acutangula*
Group IV: Standard group received 10mg/ kg of Indomethacin orally to all rats.

One hour after the first dosing, the animals are anesthetized with anesthetic ether and 20 mg of the sterile cotton pellet is inserted one in each axilla and groin of rats by making small subcutaneous incision. The incisions are sutured by sterile catgut. On the 8th day animals were sacrificed by using excess anaesthesia and cotton pellets are removed surgically. Pellets are separated from extraneous tissue and dried at 60°C until weight become constant. The net dry weight, i.e. after subtracting the initial weight of the cotton pellet will be determined. The average weight of the pellet of the control group as well as of the test groups is calculated. The percent change of the granuloma weight relatively with vehicle control is determined and statistically evaluated. The percentage inhibition increase in the weight of the cotton pellet is calculated.

% Inhibition = Wc - Wd / Wc X 100

Wd = difference in pellet weight of the drug treated group.
Wc = difference in pellet weight of the control group.

RESULTS

Anti-Oxidant results

Ethanol induced oxidative stress in rats

The bark extracts of *B.acutangula* Linn showed a potent antioxidant activity. The extracts of *B.acutangula* Linn (500mg/kg,*p.o*.) showed significant increase SOD and MDA, when compared with control Table 1and Figure1. But the ethanolic extract of *B. acutangula* Linn showed a significant effect when compared with aqueous extract of *B. acutangula* Linn.

Table 1. Effect of various extracts of *B. acutangula* on ethanol induced oxidative stress on rats

Treatment	SOD Units / Mg	Malondialdehyde
Normal control	160.11 ± 0.23	7.34 ± 2.44
Control- (1ml ethanol)	56.20 ± 3.56	5.71 ± 2.03
Ethanol extract of *B. acutangula* Linn. (500mg/kg)	134.23±3.85***$^{+++}$	13.75 ± 3.15***$^{+++}$
Aqueous extract of *B. acutangula* Linn. (500mg/kg)	104.34 ± 2.11**	9.91 ± 2.73**
Ranitidine (20mg/kg)	156.34 ± 1.34***	6.19 ± 2.01*

All the values are mean ± SEM, n=6. *p<0.05, **p<0.01, ***p<0.001 Vs control, $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 Vs aqueous extract .Data was analyzed using 1 way ANOVA & TUKEY test.

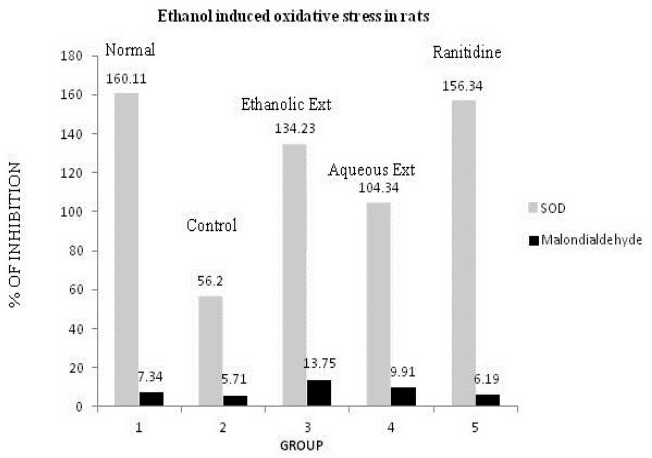

Figure 1. Effect of various extracts of *B. acutangula* on ethanol induced oxidative stress on rats

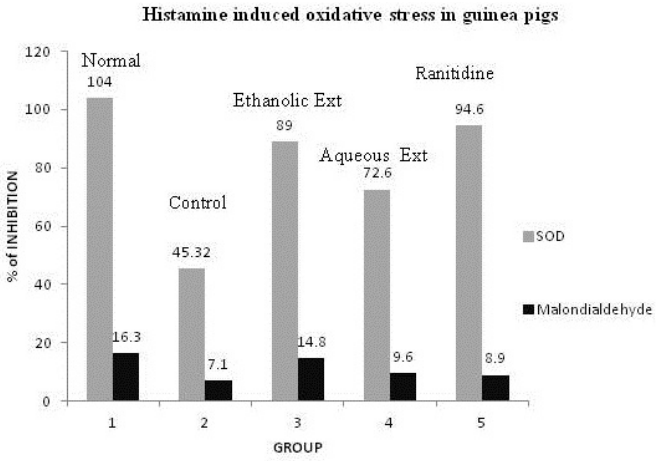

Figure 2. Effect of various extracts of *B. acutangula* on histamine induced oxidative stress in guinea pigs

Histamine induced oxidative stress in guinea pigs

B.acutangula Linn extracts of 500mg/kg body wt. was administered orally, caused a dose dependent increase in SOD and MDA in histamine induced oxidative stress in guinea pigs model. Ethanol extract at a dose of 500mg/kg showed maximum SOD and MDA activity Table 2 and figure 2, whereas the aqueous extract at a dose of 500mg/kg showed minimum SOD and MDA activity when compare with control after treated with various drugs for 7 days.

Table 2. Effect of various extracts of *B. acutangula* on histamine induced oxidative stress in guinea pigs

Treatment	SOD, Units / Mg	Malondialdehyde, mmoles/mg
Normal control	104 ± 3.61	16.3 ± 1.6
Control- (1ml ethanol)	45.32 ± 1.67	7.1 ± 2.03
Ethanol extract of *B. acutangula*. (500mg/kg)	89 ± 2.47***+++	14.8 ± 1.5***+++
Aqueous extract of *B. acutangula* Linn. (500mg/kg)	72.6 ± 1.03**	9.6 ± 0.68***
Ranitidine (20mg/kg)	94.6 ± 1.93***	8.9 ± 0.68*

All the values are mean ± SEM, n=6. *p<0.05, **p<0.01, ***p<0.001 Vs control, $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 Vs aqueous extract. Data was analyzed using 1 way ANOVA & TUKEY test.

Anti-inflammatory activity

Carrageenan induced paw edema

Carrageenan induced paw edema is an acute model for evaluating anti-inflammatory drugs. The paw volume was measured using plethysmograph. The extracts of *B. acutangula Linn* were found to reduce the edema induced by Carrageenan. The percentage reduction in paw volume was calculated. The percentage inhibition of edema by a dose (500 mg/kg) of aqueous and ethanolic bark extracts of *B. acutangula* Linn were compared with standard indomethacin. The effect of standard indomethacin at dose of 10 mg/kg b/w and extracts of *B. acutangula* Linn at dose of 500 mg/kg b/w showed significant effect on 3rd and 4th hr respectively, whereas aqueous ext was significant in 5th hr on Carrageenan induced acute inflammation is tabulated in Table 3 and also shown in Figure 3.

Cotton wool granuloma

Cotton pellet granuloma model is a chronic model for evaluating anti-inflammatory drugs. Reduction in the growth of granuloma tissue by the extracts indicates that they possess anti-inflammatory activity. The weight of granuloma tissue developed in extracts and standard treated animals is compared

Table 3. Effect of various extracts of *B. acutangula* on carrageen induced acute inflammation

S.No	Treatment	Dose	1st	2nd	3rd	4th	5th
1	Indomethacin	10 mg/kg	58.2±0.39	70.31±0.69	76.42±0.42	81.42±0.47	79.2±0.49
2	Ethanolic Ext	500 mg/kg	45.77±0.38***	58.80±0.52***	67.13±0.52***	77.99±0.33***	71.0±0.44***
3	Aqueous Ext	500mg/kg	42.45±0.89***$^{++}$	45.36±0.51***$^{+++}$	54.54±0.48***$^{+++}$	65.53±0.24***$^{+++}$	74.0±0.55***$^{++}$

All the values are mean ±SEm N=4 ***P<0.001 compare Vs Standard , $^{+++}$P<0.00 compare vs ethanolic extract $^{++}$P<0.01 compare vs ethanolic Extract . Data was analysed using 1 way ANOVA & TUKEY test.

Table 4. Effect of various extracts of *B. acutangula* on cotton pellet induced granuloma method in rats

S.No	Treatment	Dose mg/kg weight	Weight of cotton pellet	%of inhibition of Granuloma tissue
1	Control		53.75±0.47	
2	Indomethacin	10	25±0.064***	52.5±1.21%
3	*B.acutangula* Ethanolic	500	31±0.40***	42.1±1.15%
4	*B.acutangula* Aqueous	500	36±0.408***	34.15±%1.46

All the values are mean ±SEm N=4 ***P<0.001 compare Vs Control, Data was analyzed using 1 way ANOVA & TUKEY test

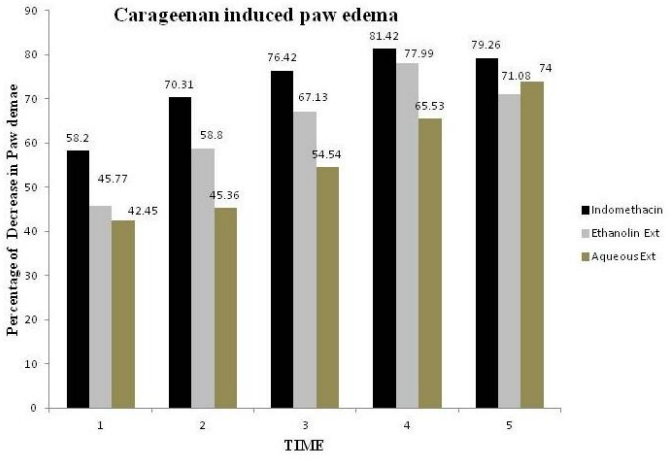

Figure 3. Effect of various extracts of *B. acutangula* on carrageen induced acute inflammation

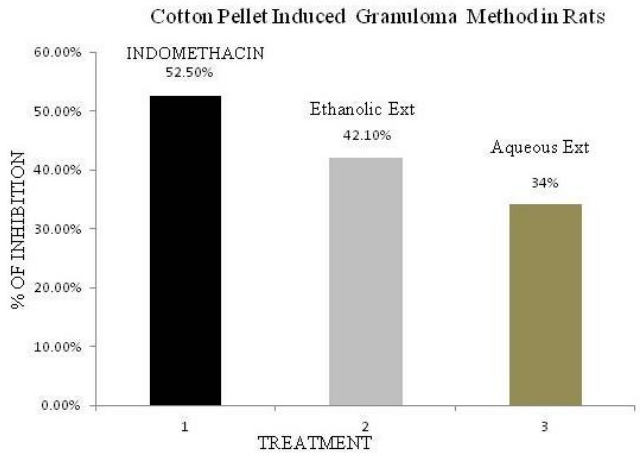

Figure 4. Effect of various extracts of *B. acutangula* on cotton pellet induced granuloma method in rats

with that of the control animals. Decrease in granuloma tissue development in extract treated group as compared with the control group signifies the anti-inflammatory activity of the extracts. Weight of granuloma tissue and percentage decrease in granuloma tissue developed was evaluated and tabulated in Table 4 and Figure 4.

DISCUSSION

Majority of the diseases are reported on the shift in balance of pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress, or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidant (Govindarajan *et al*., 2005). A strategy of preventive oxidation could therefore be formulated as prevention by diversion i.e. by channelling an attacking species into a less harmful product, hence lowering the risk of further damage. Phenolic compounds, the biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups[18] (Kathirvel and Sujatha, 2012). *B. acutangula* Linn has been reported to be a good source of flavonoids, Saponins, tannins, Phenols, etc (Saumya and Basha, 2011). Results from anti-oxidant activity showed a significant effect of increase in SOD and MDA levels in both models. From the results, it can be noted that the flavonoid content of the extracts had an immense potency which reasoned the significant antioxidant activity of *B. acutangula*. In addition, the phenolic content of the extracts had been a greater support to this activity. Results from anti-inflammatory activity indicate that ethanolic and aqueous extracts of *B Acutangula* were able to produce anti-inflammatory activity in both carrageen and cotton pellet granuloma models. In the acute model of inflammation, carrageen was used as a phlogistic agent and it produced paw edema when administered into the sub plantar region. Various mediators are responsible for the development of carrageen induced inflammation at the different time intervals namely, serotonin and histamine in the first phase (0 to <2h), kinins in the second phase (3 h) and prostaglandin in the third phase (>4h) (Deshpande, 2003) . Ethanolic and aqueous extracts of *B Acutangula* at both the doses were capable of reducing edema induced by carrageen at all the time intervals it was evaluated for. This indicates that anti-inflammatory activity of *B Acutangula* was by inhibiting more than one chemical mediator responsible for inflammation. The ethanolic and aqueous extracts of *B Acutangula* were able to reduce the growth of granuloma tissue in cotton pellet granuloma model which is a chronic model of inflammation. This indicates that it is active in proliferative phase of inflammation. Significant (*P<0.001*) reduction in granuloma tissue was produced in animals treated with ethanolic and aqueous extracts of *B Acutangula* at both the doses. The activity was found to be dose dependent.

Flavonoids are reported to produce enzyme inhibitory activity. It inhibits both COX and LOX enzymes thereby preventing the mechanism of inflammation like PG synthesis. PG also indirectly activates other mediators of inflammation through inhibition of enzymes which also aids anti-inflammatory activity of flavonoids (Kim *et al*., 2004). Preliminary phytochemical studies conducted have indicated the presence of flavonoids, which is also supported by the reported chemical constituents in the *B.acutangula.* The flavonoids present in *B.acutangula* may be partly responsible for anti-inflammatory activity against Carrageenan induced paw edema model. The various chemical constituents present in *B.acutangula* would have acted synergistically to produce anti-inflammatory activity.

Conclusion

The results obtained in this study are noteworthy, *In-vitro* antioxidant studies indicate that *B.acutangula* possess antioxidant activity, by the virtue of which, anti-inflammatory activity may be produced. Based on the promising pharmacological property of the *B.acutangula* bark extracts, it can be used as a safe nutritional supplement without any adverse side effects.

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