

Anti-Inflammatory Effect of Ethanolic Extract of *Boerhavia diffusa* leaves in Wistar rats

Santhoshkumar Muthu¹, Sivaganesh M¹, Shibi Ashir¹, Tamilselvan A² and Shiba Sakeer^{1*}

¹Department of Biochemistry, RVS College of Arts and Science, Coimbatore-641402, Tamil Nadu, India.

²Department of Pharmacology, Teegala Krishna Reddy College of Pharmacy, Hyderabad-97, Andhra Pradesh,

*For correspondence: shibanamsheed@gmail.com

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ABSTRACT

The present study aimed to evaluate the *in-vitro* and *in-vivo* anti-inflammatory activity of ethanol extract of *Boerhavia diffusa* leaves (EBDL). Nitric Oxide free radical scavenging assay and protein denaturation inhibition assay were carried out to evaluate the *in-vitro* anti-inflammatory effect. Carrageenan induced hind paw edema and Cotton pellet induced granuloma in Wistar rats were employed to test the *in-vivo* anti-inflammatory potential. The extract at the concentration of 200 mg/kg body weight (BW) and 400 mg/kg.BW were administered orally to the treatment groups and indomethacin used as standard drug. Preliminary phytochemical evaluation revealed the presence of alkaloids, flavonoids, phenols, tannins, carbohydrates, saponins, glycosides, and proteins. *In-vitro* anti-inflammatory effect of ethanol extract at various concentrations was confirmed by the results obtained in nitric oxide free scavenging activity, protein denaturation and proteinase inhibition assays. The reduced thickness of the paw volume measured at different intervals in treated group exhibited the *in-vivo* anti-inflammatory effect of EBDL in both carrageenan induced inflammation and cotton pellet induced granuloma. These experimental results suggests that *B. diffusa* extract displayed significant potency in anti-inflammatory action in dose dependent manner.

Keywords: *Boerhavia diffusa*, Carrageenan, cotton pellet, indomethacin, anti-inflammatory

1. INTRODUCTION

Several medicinal plants possess anti-inflammatory property proven through the extensive investigations in the past few decades. Scientific validations support that drugs from medicinal plants were able to reduce inflammation [1]. However, use of anti-inflammatory drugs provokes adverse side effects and toxicity though they tentatively relieve inflammation and pain. One of the most common side effects includes gastrointestinal complication. Traditionally, plenty of medicinal plants were exercised to treat various

ailments including inflammation and gastric ulcers [2].

Boerhaavia diffusa Linn (Family: Nyctaginaceae) popularly known as chicken-weed is an herbaceous perennial plant distributed in the tropics and subtropics [3]. Pharmacological studies have disclosed its diaphoretic, laxative, snake venom neutralizing, to cure stomach ache, treat anemia and expectorant properties [4, 5]. A wide range of phytochemicals such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins were found to be present

in *B. diffusa* [6]. The alkaloids namely, punarnavine and punernavoside constitute 0.04 % major among the total alkaloids present in this particular plant. Hence, the name “punarnava” has been given to this plant in Ayurvedic system of medicine. An oily substance of potassium nitrate and ursolic acid constitute about 6% in its total phytoconstituents [7]. In addition, the plant was practiced in the treatment of nephrotic syndrome, hepatitis, gall bladder abnormalities, and urinary disorders [8, 9]. Flowers and seeds are used as contraceptive and the roots were reported to use for treatment of asthma and hepatitis [10, 11]. *B. diffusa* has been scientifically proven to possess antispasmodic, anticonvulsant and pain-relieving efficacy [12]. Previous studies reported, *B. diffusa* aqueous extract was reported to possess anti-inflammatory activity in rats as well as no mortality within the experimental period at the tested doses ranging from 40 to 320 mg/kg.BW [13]. The present study aimed to evaluate the *in-vivo* anti-inflammatory activity of *B.diffusa leaves* on carrageenan induced- and cotton pellet induced paw edema in murine models. Further, *in-vitro* anti-inflammatory potential was assessed by radical scavenging activity, protein denaturation inhibition assay and proteinase inhibitory action.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

Fresh leaves of *B. diffusa* were collected in the month of January 2013 from the Coimbatore district in India and were authenticated and deposited in herbarium of Manian Institute of Science and Technology, Coimbatore. The leaves were dried under shade at room temperature. Then shade dried leaves were finely ground using mechanical blender. Finally, the powder obtained was subjected to prepare ethanol extraction.

2.2. Preparation of the Ethanol extracts

The air dried powdered *B. diffusa* leaves were extracted in soxhlet extractor with 80% ethanol (25g in 450 ml) for 6 hours. The ethanol extract obtained were concentrated using rotatory evaporator and were dried to remove the final traces of solvent. The percentage yield was calculated. EBDL was used for evaluating anti-inflammatory activity by dissolving it in distilled water before use. Phytochemicals in the extract were screened for alkaloids, flavonoids, phenols, tannins, steroids, sterols, carbohydrates, saponins, glycosides, protein and amino acids as described using standard methods [14,15,16].

Carrageenan and Indomethacin were purchased from Sigma Aldrich, India. All other chemicals used were of analytical grade.

2.3. In-vitro anti-inflammatory activity

2.3.1. Nitric oxide radical scavenging efficacy

The nitric oxide scavenging activity of EBDL was evaluated according to the method of Sreejayan and Rao [17]. 3 ml of 10 mM sodium Nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with varying concentrations ranging from (100-500 µg/ml) of extract and incubated at room temperature for 150 mins. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylene diamine dihydrochloride (NNED) in 2% H₃PO₄) was added. The absorbance of the chromophore formed in the reaction was measured at 546 nm. Percentage radical scavenging activity of the leaf extract was calculated as follows,

$$\% \text{ NO radical scavenging activity} = \left[\frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \right] \times 100$$

The analysis was performed in triplicate. The concentration of the extract providing 50% inhibition (IC₅₀) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.2. Inhibition of protein denaturation

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and different concentrations of EBDL ranging from 100-500 µg/ml (pH 6.3). The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured in spectrophotometry at 660 nm [18, 19]. For control tests 0.05ml of distilled water was used instead of extract, the product control tests lacked bovine serum albumin. The analysis was done in triplicate. The percentage inhibition of protein denaturation was calculated as follows,

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{O.D of test} - \text{O.D of product control}}{\text{O.D of Control}} \right] \times 100$$

2.3.3. Proteinase inhibitory action

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml of 25 mM tris-HCl buffer (pH 7.4) and different concentrations of EBDL (100-500 µg/ml). The mixtures were incubated at 37°C for 5min and then 1.0 ml of 0.8% (w/v) casein was

added. The mixtures were incubated for an additional 20 min. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction [20]. The cloudy suspension was centrifuged and the absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated as follows,

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{O.D of test} - \text{O.D of product control}}{\text{O.D of Control}} \right] \times 100$$

2.4. Evaluation of in-vivo anti-inflammatory effect

2.4.1. Animals

Female albino Wistar rats (150-200 g) used in the present study were procured from the small animals breeding station, Kerala Agricultural University, Mannuthy, Kerala. They were housed in polypropylene cages (38×23×10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14 h dark /10 h light cycles; temp 24 ± 2°C; 35-60% humidity) and were fed with standard pellet diet with fresh water *ad libitum* (M/s. Hindustan Lever Ltd, Mumbai, India). The animals were acclimatized to the environment for two weeks prior to experimental procedure. Animals were fasted over night before the experimental schedule, but had free access for water *ad libitum*. All experiments were performed based on the rules and regulations assigned by Institutional Animal Ethical Committee (1454/po/c/11/CPCSEA).

2.4.2. Carrageenan regimen

Female Wistar rats (120–150 g weight) were distributed into five groups consists of six animals each (n=6). Control, Induced, Standard drug treatment, extract at dose of 200 mg/kg.BW and 400 mg/kg.BW groups were assigned for the study. The animals were fasted overnight prior to the start of the experiment, and water *ad libitum*. The animals were orally pretreated with the drugs (standard drug indomethacin / EBDL extracts) 60 minutes prior to the administration of 0.1 ml of 1% carrageenan (in 1% CMC (Carboxy methyl cellulose) w/v) into the sub-plantar tissue of the right hind paw. Indomethacin (10 mg/kg p.o.) drug was administered orally to standard group animal. The progression of edema (mm) was evaluated by measuring the paw with the help of Vernier caliper (Model-2061, Mututoyo Digimatic Caliper, Japan) immediately at 30, 60, 120, 180 and 240 minutes intervals after the carrageenan injection [21]. Animals in control group received only distilled water (10 ml/kg p.o.). Test Groups IV and V orally received ethanol extract of 200

mg/kg.BW and 400 mg/kg.BW respectively. Mean values of hind paw edema in treated groups were compared with those of control group and using statistical methods.

2.5. Cotton pellet-induced granuloma

Female Wistar rats (120–150 g) were divided into four groups consists of six animals each (n=6). The animals were fasted overnight prior to the start of the experiment but had free access to water *ad libitum*. Cotton pellets, weighing 30 mg each, were sterilized. Under ether anesthesia, the pellets were introduced subcutaneously through a skin incision in the thigh of the animals [22]. All the groups were treated orally (as per above mentioned treatment regimen) for 7 days and the treatment started 30 min after cotton pellet implantation. On the eighth day, the animals were sacrificed with chloroform, the granulomas were removed, dried for 24 h at 60°C and the dry weights were determined. The difference between the initial and final dry weight were considered to be the weight of the amount of granulomatous tissue produced [23].

2.6. Statistical analysis

All the data values were expressed as mean ± SD. Statistical significance of differences between the treated and control groups was determined by one way ANOVA followed by least significant difference (LSD) test. *P* values of <0.05 is considered significantly different from control. All statistical tests were performed using SPSS version 17.

3. RESULTS

3.1. Phytochemicals analysis

The preliminary analysis indicated that ethanolic extract of *B. diffusa* leaves showed presence of glycosides, alkaloids, protein, phenolics and tannins, saponins, flavonoids and carbohydrates as depicted in Table 1. The results are in accordance with the previous studies reported in *B. diffusa* [7].

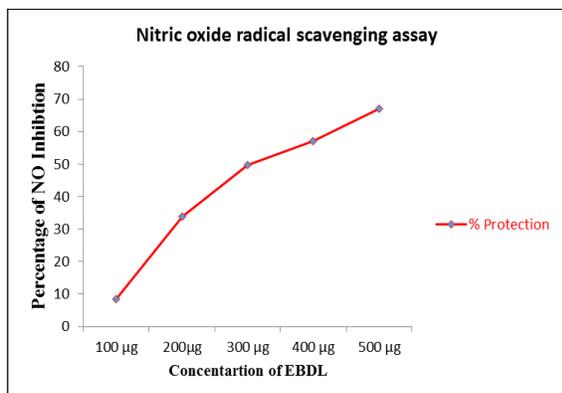
3.2. In vitro anti-inflammatory activity

3.2.1. NO Radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, platelet aggregation and regulation of cell mediated toxicity [24]. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other

pathological conditions [25]. Ethanol extract of *B. diffusa* leaves exhibited a significant nitric oxide inhibition (IC_{50} 370.47±7.33) and the capacity to scavenge NO free radicals was found to be increased while increasing the concentration of the extract (Figure 1). It is suggested that *B. diffusa* may have a role in preventing free radical mediated chain reactions in inflammation.

Figure 1. Nitric oxide radical scavenging effect of Ethanol extract of *B. diffusa* leaves



3.2.2. Inhibition of protein denaturation

Protein denaturation is commonly known as any non-covalent change in the structure of a protein. This change may alter the secondary, tertiary or quaternary structure of the molecules. Most of biological proteins lose their biological function when they get denatured. For instance, enzymes lose their activity, because the substrates can no longer bind to the active site [26]. Protein denaturation assay of *B. diffusa* extract proved its significant inhibitory action against protein denaturation (IC_{50} 356.29±1.46). The inhibitory action increased in concentration dependent manner. Therefore, the result confirms that EBDL is capable of controlling protein denaturation during inflammation (Figure 2).

3.2.3. Proteinase Inhibition assay

Proteinases are enzymes which catalyze the hydrolysis of the peptide bonds formatting the primary structure of proteins [27]. Overall Proteinases are responsible for the modification of proteins. Proteinase inhibition assay was carried out with *B. diffusa* extract and IC_{50} value was found to be 348.84±1.40. When the concentration of extract was increased, the activity of inhibiting protein degrading enzyme was observed to increase. It is evident that the herbal extract inhibits proteinase activity thereby

preventing the protein hydrolysis of protein structure (Figure 3).

Figure 2. Effect of Ethanol extract of *B. diffusa* on *in-vitro* protein denaturation

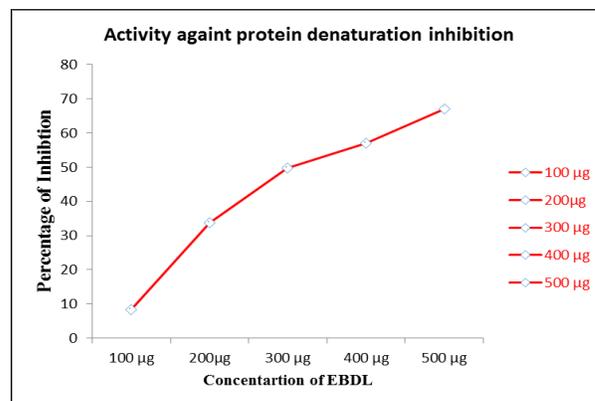


Figure 3. Effect of ethanol extract of *B. diffusa* on *in-vitro* proteinase activity

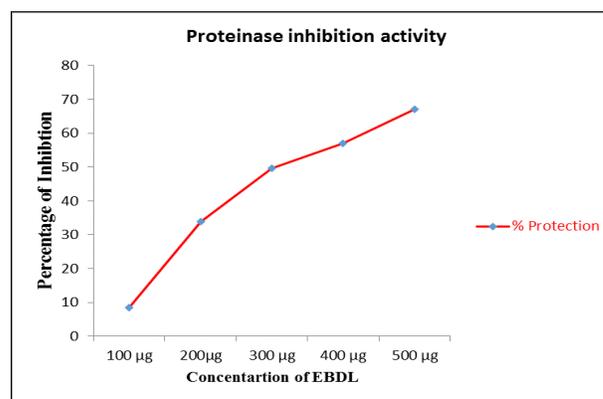


Table 1. Preliminary phytochemical screening of ethanolic extract of *B. diffusa* (+ present; - absent)

Phytochemical constituent	Name of the test	Ethanolic extract of <i>B. diffusa</i>
Alkaloids	Wagner's test	+
Flavonoids	Shinoda	-
	Lead acetate	+
Phenolics & tannins	Lead acetate	+
	Ferric chloride	+
	Sodium hydroxide	+
Steroids & sterols	Salkowski's test	-
Carbohydrates	Fehling's test	+
	Benedict's test	+
Saponins	Honey comb test	+
	Foam test	+
Glycosides	Glycoside test	+
	Protein	Biuret test
	Ninhydrin test	+

Table 2. Effect of ethanolic extract of *B. diffusa* on carrageenan induced paw edema

Group	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Control	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08	3.85 ± 0.08
Induced	3.75 ± 0.03	5.84 ± 0.15	5.95 ± 0.18	6.12 ± 0.23	6.33 ± 0.17	6.57 ± 0.14	6.48 ± 0.13	6.30 ± 0.11	6.02 ± 0.10
Indomethacin (200mg/kg.BW)	3.70 ± 0.03	5.42 ± 0.23	5.58 ± 0.21	5.66 ± 0.23	5.64 ± 0.18	5.36 ± 0.12	4.99 ± 0.08	4.73 ± 0.24	4.49 ± 0.17
(400mg/kg.BW)	3.66 ± 0.10	5.53 ± 0.26	5.79 ± 0.25	5.95 ± 0.17	6.15 ± 0.21	5.95 ± 0.19	5.81 ± 0.24	5.53 ± 0.24	5.32 ± 0.21
(400mg/kg.BW)	3.69 ± 0.15	5.45 ± 0.14	5.78 ± 0.12	5.99 ± 0.10	6.14 ± 0.11	6.02 ± 0.17	5.85 ± 0.13	5.33 ± 0.15	5.08 ± 0.09

Paw volume in (mm). All the data values are expressed as mean ± SD (n=6 animals in each group).

Table 3. Percentage protection of ethanolic extract of *B. diffusa* against carrageenan induced paw edema

Group	Initial paw volume (mm)	Final paw volume (mm)	Difference	Percentage protection (%)
Control	3.85 ± 0.08	3.85 ± 0.08	---	----
Induced	3.75 ± 0.03	6.02 ± 0.10	2.27 ± 0.13	----
Indomethacin (200mg/kg.BW)	3.70 ± 0.03	4.49 ± 0.17	0.78 ± 0.18	65.34
(400mg/kg.BW)	3.66 ± 0.10	5.32 ± 0.21	1.65 ± 0.19	27.16
(400mg/kg.BW)	3.69 ± 0.15	5.08 ± 0.09	1.39 ± 0.16	38.39

Paw volume in (mm).
All the data values are expressed as mean ± SD (n=6 animals in each group).

Table 4. Percentage protection of ethanolic extract of *B. diffusa* against cotton pellet induced granuloma

Group	Initial pellet weight (mg)	Final pellet weight (mg)	Difference	Percentage protection (%)
Induced	30	108.60 ± 5.41	78.60 ± 5.41	---
Indomethacin	30	41.60 ± 1.07	11.60 ± 1.07	85.24
(200mg/kg.BW)	30	84.66 ± 1.67	54.66 ± 1.67	30.44
(400mg/kg.BW)	30	57.10 ± 2.01	27.10 ± 2.01	65.52

All the data values are expressed as mean ± SD (n=6 animals in each group).

3.3. In vitro anti-inflammatory activity

3.3.1. Carrageenan induced rat paw edema

Carrageenan induced hind paw edema is a multimediated phenomenon that liberates diversity of mediators. The increased paw volume after 30 min, 60 min, 90 min, 120 min, 150 min, 180 min 210 min, and 240 min was calculated as percentage compared with volume measured immediately after the injection of carrageenan for each animal. The difference of average volume between control and test groups in time intervals was statistically evaluated. Ethanol extract of *B. diffusa* (200 mg/kg.BW) has shown significant difference with control at 30 min, 60 min, 90 min, 120 min, 150 min, 180 min 210 min, 240 min. Whereas, groups treated with 400 mg/kg.BW and standard drug showed significant anti-inflammatory effect up to 240 min (Table 2). In case of percentage protection of ethanol extract of *B. diffusa* (EBDL) against carrageenan induced paw edema, the dose at concentration of 400 mg/kg.BW of EBDL provided 38.39% protection which was significant as that of standard (65.34 % protection). Dose at 200 mg/kg.BW concentration also exhibited 27.16% protection (Table 3).

3.3.2. Cotton pellet induced granuloma

In cotton pellet induced granuloma model, *B. diffusa* extract presented significant percentage protection against inflammation (Table 4). The dose at concentration of 400 mg/kg.BW of EBDL

exhibited a better percentage protection (65.52%) when compared to Dose at 200 mg/kg.BW concentration (30.44%). In traditional system of medicine, certain herbs are claimed to provide pain relief and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. Inflammation is a complex process in which reactive oxygen species (ROS) play an important role in the pathogenesis of inflammation [28]. Thus antioxidants which can scavenge free radicals formed in the system are expected to improve the condition of inflammation and related diseases.

4. DISCUSSION

Secondary metabolites in medicinal plants possessing medicinal values have been extensively investigated for their essential biological activities and disease prevention in the past years [28-29]. *B. diffusa* plant reported to possess flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins [30, 31]. Phytochemical screening in the present study reported the presence of major phytoconstituents as evidenced by the previous investigations. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [32]. Inflammation and free radicals have the relationship of mutual promotion. The

inflammation can cause the increase of free radicals. Meanwhile, free radicals such as the hydroxyl radicals ($\cdot\text{OH}$) and peroxynitrogen ($\text{ONOO}\cdot$) play an important role in the progress of inflammation, and inhibiting these free radicals can reduce the severity of inflammation. H_2 molecule has a very strong covalent bond, so it cannot easily react with all the oxidants. However, in recent studies, it has been found that molecular hydrogen can specifically reduce $\cdot\text{OH}$ and $\text{ONOO}\cdot$ in vitro and induce a therapeutic antioxidant effect in an animal model [33]. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes [34]. Excess concentration of NO is associated with several diseases including inflammation [35]. NO is generated in biological tissues by specific nitric oxide synthase (NOSs), which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction [36]. These compounds are responsible for altering the structural and functional behavior of many cellular components. Free radical scavenging effect of *B. diffusa* against NO radicals was proved in the present investigation. This could evidence the *in-vitro* anti-inflammatory activity of the extract.

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Heat denatured proteins are as effective as native proteins in provoking delayed hypersensitivity reaction [26]. Conventional NSAIDs like phenylbutazone and indomethacin do not act only by the inhibition of endogenous prostaglandins production and blocking COX enzyme but also by prevention of denaturation of proteins [31]. Denaturation of protein has been identified to be one of the major causes of inflammation [32]. Hence, the ability of EBDL extract to inhibit protein denaturation was evaluated delineate the mechanism of *in vitro* anti-inflammatory activity as a part of the study. Maximum inhibition (76.61%) against protein denaturation was exhibited at 500 $\mu\text{g}/\text{ml}$ dose. IC_{50} value found to be 356.29 ± 1.46 in this anti-denaturation assay.

Proteinase activity is associated with hydrolysis of peptide bonds that takes place during protein denaturation process in arthritic reaction. Proteinases occur in all organisms and are involved in a multitude of physiological reactions from simple digestion of

food proteins to highly regulated cascades such as the blood-clotting cascade, the complement system, and apoptosis pathways. Proteinases can either break specific peptide bonds (limited proteolysis), depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids [33]. The activity can be a destructive change, abolishing a protein's function or digesting it to its principal components; it can be an activation of a function, or it can be a signal in a signaling pathway [34]. Neutrophils serve as rich source of proteinase enzymes which are packed inside the lysosomal granules. Serine proteinase plays an important role in degrading peptide bonds in protein confirmation [35]. Hence, proteinase inhibitors are employed in the treatment of inflammation so as to inhibit tissue damage caused by proteinase enzymes. EBDL extract at 500 $\mu\text{g}/\text{ml}$ concentration revealed the maximum inhibition (67%) with IC_{50} value of 348.84 ± 1.40 . Sachin *et al* (2010) performed similar study with methanol extract of *Oxalis corniculata* and evaluated its *in-vitro* anti-inflammatory potential and it was found to be inhibit protein denaturation ($288.04 \pm 2.78 \mu\text{g}/\text{m}$) and proteinase activity with IC_{50} value of $435.28 \pm 5.82 \mu\text{g}/\text{ml}$ [36].

Carrageenan induced inflammation is easy method for determining the anti-inflammatory efficacy of orally administered drugs [37]. Development of paw edema in carrageenan induced inflammatory response is mainly due to the release of kinins and polymorpho leukocytes that include proinflammatory mediators such as prostaglandins [38-39]. The mechanism behind carrageenan induced edematous paw is biphasic in nature [40]. Histamine and serotonin are released in the first one hour and then prostaglandins are produced in 3-4 hours [22, 41]. Cyclooxygenase enzyme that leads to the generation of prostaglandins is also released in 6 hours after the carrageenan mediated inflammation [42]. In addition, carrageenan induced inflammation is associated with neutrophil infiltration along with the production of neutrophil mediated free radicals. Nitric oxide, superoxide and hydroxyl free radical species are predominantly released due to neutrophil mediated inflammatory reaction [43]. Carrageenan injection, in particular, causes the release of prostaglandins and free radicals including NO [44]. This has promoted the use of carrageenan induced inflammation model among the researcher engaged in inflammation research. In the present study, ethanol extract of EBDL was tested for its effect on paw edema volume after carrageenan injection and was observed that treatment with EBDL extract provided protection against hind paw edema in rat models. The observation says that plant extract might down-regulated the expression of

proinflammatory kinins and COX enzyme. Ramesh et al (2010) carried out similar study with methanol extract of *Pandanus odoratissimus* in carrageenan induced rat models and was found to inhibit the paw edema by 68% at 100 mg/kg dose level [45].

Cotton pellet induced granuloma model is widely preferred to evaluate the transudatory and proliferative components in chronic inflammation condition. Increase in the wet weight of the pellet in 3 hours is attributed to the transudative phase. Release of plasma from blood stream around the granuloma is witnessed in about 3-72 hours during exudatory phase of the inflammation. Dry weight of the granuloma increases in 3- 6 hours during proliferative phase [46]. Anti-inflammatory effect of EBDL extract against cotton pellet induced granuloma was well evidenced from the results obtained in this study. Granuloma inhibitory action of EBDL might be due to the control of fibroblast synthesis of collagen and mucopolysaccharide during granuloma formation. Cotton pellet induced glaucoma also was found to be reduced upon treatment with *B. diffusa* extract. Similarly, the anti-inflammatory effect of herbal formulation was evaluated by Mahesh et al (2007) and found to be inhibitory concentration ranging from 400 mg/kg and 800 mg/kg BW [47].

Conclusion

The present preliminary investigations of ethanolic extract of *B. diffusa* reveal that the plant has wide range of phytochemical constituents and possesses free radical scavenging effect. *B. diffusa* leaves also exhibit *in-vitro* and *in-vivo* anti-inflammatory potentials providing a scientific basis for anti-inflammatory effect in inflammatory diseases and support traditional claims. The underlying molecular mechanism behind its anti-inflammatory action is to be delineated in order to validate its potential use. Future and ongoing studies will seek to ascertain which compounds present in the *B. diffusa* could be responsible for the protective effect reported.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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