



Antibacterial and Anti-inflammatory Potential *Bergenia ligulata*

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Abstract

Bergenia ligulata Wall., family *Saxifragaceae*, is an Indian folk medicine used for a variety of pharmacological effects. In this study, evidence is provided in animal model to demonstrate the role of aqueous as well as 50% ethanolic extract of *B. ligulata* in inflammation and as antibacterial agent. Oral administration of the extract at a dose level of 1 gm/kg bw showed anti-inflammatory and free radical scavenging activity as evaluated using pharmacological and biochemical parameters. The effect was studied on biochemical parameters reportedly perturbed in inflammation. While the extract treatment could alleviate the level of succinate dehydrogenase and xanthine oxidase, which increase in inflammation, the level of superoxide dismutase increased following the treatment with the extract as well as the diclofenac. Role of oxygen free radicals/peroxides was evaluated by measuring lipid peroxidation and glutathione. Treatment with the extract could significantly decrease the enhanced level of lipid peroxidation in inflammation, and increased the level of glutathione. Further, the antibacterial activity of various fractions was tested *in vitro* using cultures of *Escherichia coli*, *Bacillus subtilis*, and *S. aureus*, and the fractions were found to be antibacterial. The antifungal activity was also tested using the culture of *Saccharomyces*. However, the drug was ineffective in inhibiting fungal growth. Results provide evidence suggesting the anti-inflammatory as well as the antibacterial role of *B. ligulata*, thus implicating the plant extract in treatment against the bacterial infection and inflammation.

Keywords: *Bergenia ligulata*; anti-inflammatory; antibacterial.

1. Introduction

Bergenia ligulata Wall. (Syn. *Saxifraga ligulata*), family *Saxifragaceae*, is found in South and East Asia. In India, it grows at high altitudes

in the Himalayas usually in rocky areas and in the Kashmir valley where it is popularly known as *Paashaanbhed*. The rhizomes of *Paashaanbhed* have been used for centuries in Indian System of Medicine [1] and the effects of the herb on kidney

stone formation [2] and on influenza virus [3] have been validated. Chemical investigations have shown the presence of β -sitosterol, β -sitosterol-D-glucoside, bergenin [4] and paashaanolactone [5]. Bergenin and β -sitosterol are well known for pharmacological actions. In this study, the antiinflammatory, and antimicrobial activity of the crude plant extracts prepared from the rhizomes of *B. ligulata* is described. The role of free radicals that are important in inflammatory processes such as in the activation of NF- κ B (Ali and Mann, 2004), which induces the transcription of inflammatory cytokines and cyclooxygenase, is also investigated in this study in terms of biochemical parameters.

2. Materials and methods

2.1. Animals

Pathogen free adult male Wistar strain of rat (220-260 g) was used throughout the study. The animals were procured from the Central Animal House Facility of Jamia Hamdard, grouped randomly (six in each group) and were acclimatized for one week before the actual experiment. The animals were kept in polypropylene cages in an environmentally controlled room with a 12 h light-12 h dark cycle at $24 \pm 2^{\circ}\text{C}$, and 60% (± 15) relative humidity. Animals were allowed free access to pellet diet (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. Guidelines issued by the CPCSEA for the care and uses of laboratory animals were strictly followed. The study had the approval of the animal ethics committee.

2.2. Plant material and other consumables

Bergenia ligulata was collected from the Kashmir valley in India, and authenticated by a Taxonomist. All the chemicals and culture media used in this study were of analytical grade and were procured from the standard commercial sources in India. Thiobarbituric acid was procured from Sigma Chem. Co. The control allopathic drug diclofenac sodium (brand name voveran) was obtained from the local pharmacy.

2.3. Preparation of the aqueous and alcoholic extracts

Freshly collected rhizomes were chopped, dried, crushed and powdered in a grinder. To prepare the aqueous extract, 10 gm crude powder was mixed well in 50 ml of warm distilled water, and the mixture was left overnight in a conical flask. Following day, the mixture was filtered through muslin cloth, and the aliquot was kept separately in a pre-sterilized glass container and stored in refrigerator. The residue left after the first extraction was subjected to re-extraction in 50 ml water, and the mixture was kept for another 12 hour. The second aliquot obtained after filtering the mixture through muslin cloth was mixed with the first aliquot and stored. This final aliquot, the aqueous extract was used for testing the antibacterial and antiinflammatory activity. To avoid contamination, the aliquot was stored in refrigerator in autoclaved screw capped vials. Difference in the weight of the crude powder and the weight of the residue left after obtaining the aqueous extract was used to calculate the amount of the crude drug present in the aqueous extract. For preparing a 50% aqueous-ethanolic extract, Soxhlet apparatus was used. The extract was dried under reduced pressure using a rotatory flash evaporator. This extract was also stored under similar conditions as described for the aqueous extract. The percent yield for the aqueous extract and for the 50% ethanolic extract, respectively, was 45% and 50%. The extracts were used within two days of preparation.

2.4. Experimental protocol

For evaluating the effect of the aqueous or alcoholic extracts on carrageenan-induced inflammation model in rats, the animals were divided into different groups. Group-I rats (Normal Control, NC) received normal saline and group-II (Carrageenan, C) rats were injected with carrageenan in their hind paw, as described below, to induce inflammation. Group-III (Carrageenan + Aqueous extract, C+Aq) and group-IV rats (Carrageenan + 50%ethanolic extract, C+E-A), respectively, were treated with the aqueous extract and 50% ethanolic extract of *B. ligulata* at a dose corresponding to 1 g/kg body weight, p.o. In group-V (Carrageenan + Diclofenac, C+D), diclofenac was administered as an allopathic control to compare the efficacy of the herbal

extract under investigation. Diclofenac was administered orally at a dose level of 5 mg/kg bw. In groups III to V, the test compound/ control were administered one hour before injecting carrageenan.

2.5. Pharmacological evaluation of the anti-inflammatory activity

Antiinflammatory activity of *B. ligulata* was determined according to the method described by Winter and colleagues [7]. Briefly, 0.1 ml of 1% carrageenan solution, prepared by suspending 100 mg carrageenan in 10 ml normal saline solution, was injected into the left hind paw of rat. The swelling (oedema) produced by carrageenan injection was measured by measuring the increase in paw volume every hour for three hours using a plathysmometer (Ugo Basile, Italy). The percent inhibition was calculated as follows: $V_c - V_t / V_c \times 100$, where V_c is the volume of edema measured in the hind paw, and V_t is the volume of edema in the drug treated group. Volume of edema was derived by taking the difference in the volume of left hind paw receiving carrageenan injection minus the volume of right hind paw, which was not injected with carrageenan.

2.6. Biochemical analysis

The biochemical estimations were carried out from the tissue samples obtained from the drug treated and control groups. Biological samples were processed as described earlier [8]. Following biochemical estimations were made from the hepatic tissue samples: succinate dehydrogenase [9], superoxidase dismutase [10], xanthine oxidase [11], lipid peroxidation [12], and glutathione [13]. For biochemical analysis, animals were sacrificed four hours after administering carrageenan. All analyses were carried on the fresh samples, and throughout the period of analysis samples were kept at 0 to 4°C.

2.7. Antibacterial activity

The antibacterial activity was tested using the diffusion method [14]. A loop of bacteria from the agar-slant stock was cultured in the nutrient broth overnight and spread with a sterile cotton swap into the Petri plates containing 20 ml of nutrient agar. Wells (9 mm in diameter) were made in

nutrient agar and impregnated with a fixed volume of plant extract (10 mg/ml, 25mg/ml or 50 mg/ml). The culture plates were incubated at 37 °C for 24 hours. Antibiotic ciprofloxacin (25 µg/ml or 50 µg/ml) was used as positive control. Following 24 h incubation, the diameter in mm of the inhibitory or clear zones around the well was measured. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the well.

2.8. Statistical analysis

Mean \pm S.D. of all determinations in a group was taken, and the percent increase or decrease in some parameters was also calculated assuming the mean value of the control group as 100%.

3. Results and Discussion

Evaluation of the anti-inflammatory activity measuring carrageenan-induced paw edema is one of the widely used pharmacological methods. It measures the ability of a compound to reduce local oedema produced by injecting carrageenan in rat hind paw. The formation of oedema is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase the flow of blood [15]. It is a biphasic event. The early phase is observed around 1h after carrageenan injection and is attributed to the release of histamine and serotonin. In the late phase, molecules such as prostaglandins are released [16]. In this study, aqueous and 50% ethanolic extracts of the rhizomes of *Bergenia ligulata* are reported to attenuate the inflammatory response as determined by pharmacological and biochemical measurements. The treatment significantly decreased the inflammation as can be seen in figure 1. The activity level of succinate dehydrogenase (SDH), which has been reported to rise in inflammation [17], decreased in rats receiving the extract treatment (Figure 2). SDH is a key inner mitochondrial membrane enzyme linked with the energy yielding citric acid cycle in living cells. An increase in SDH would mean an increased supply of ATP to liver and possibly other tissues including the inflamed tissue.

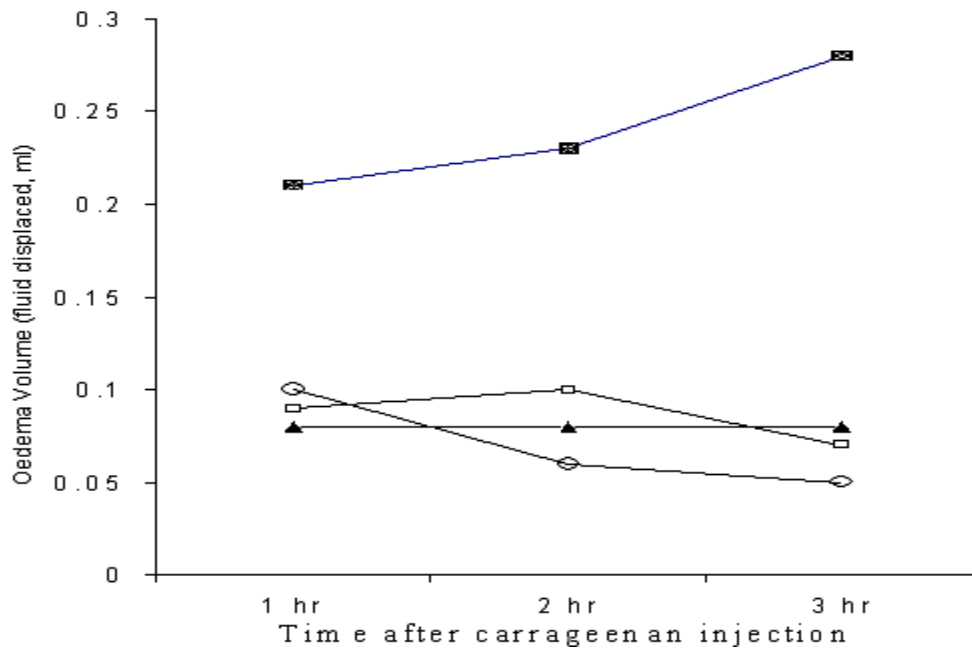


Figure 1: Effect of *Bergenia ligulata* on carrageenan induced oedema. ×: Paw volume measured following the injection of carrageenan in rat hind paw; □: Paw volume in rats treated with the aqueous extract in inflammation model; Δ: Group treated with 50% ethanolic extract; and o: Rats treated with diclofenac. In all groups, the paw volume was less in comparison to the carrageenan alone treated group.

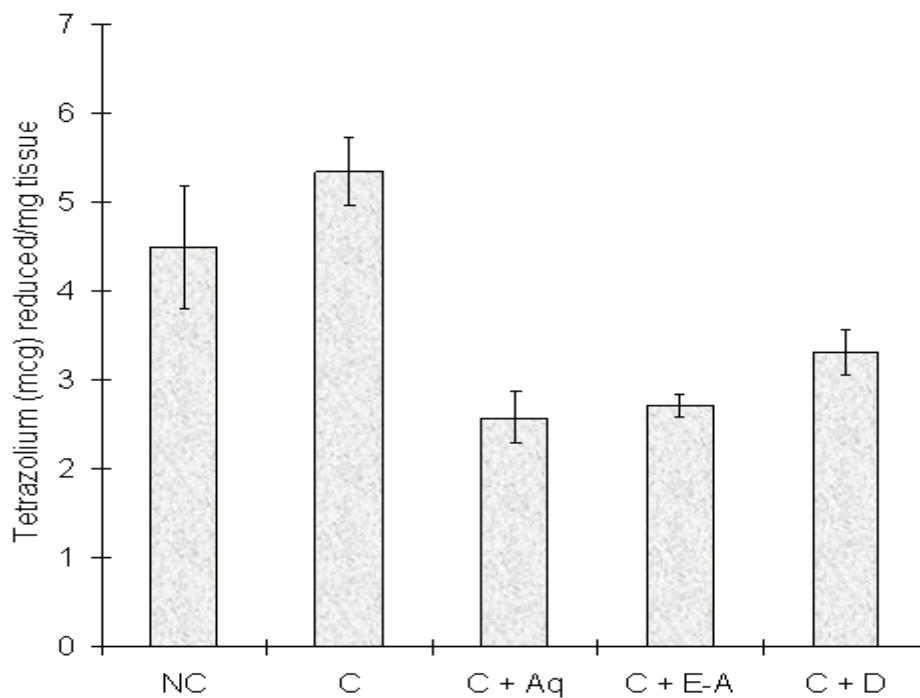


Figure 2: Succinate dehydrogenase in rats treated with *Bergenia ligulata* for treating inflammation. NC: normal control receiving the vehicle alone, C: animal group receiving carrageenan injection, C+Aq: treated with aqueous extract, C+E-A: treated with 50% ethanolic extract, and C+D: treated with diclofenac. Succinate dehydrogenase has

been reported to increase in animal model of inflammation. Extract treatment could significantly bring down the increased value.

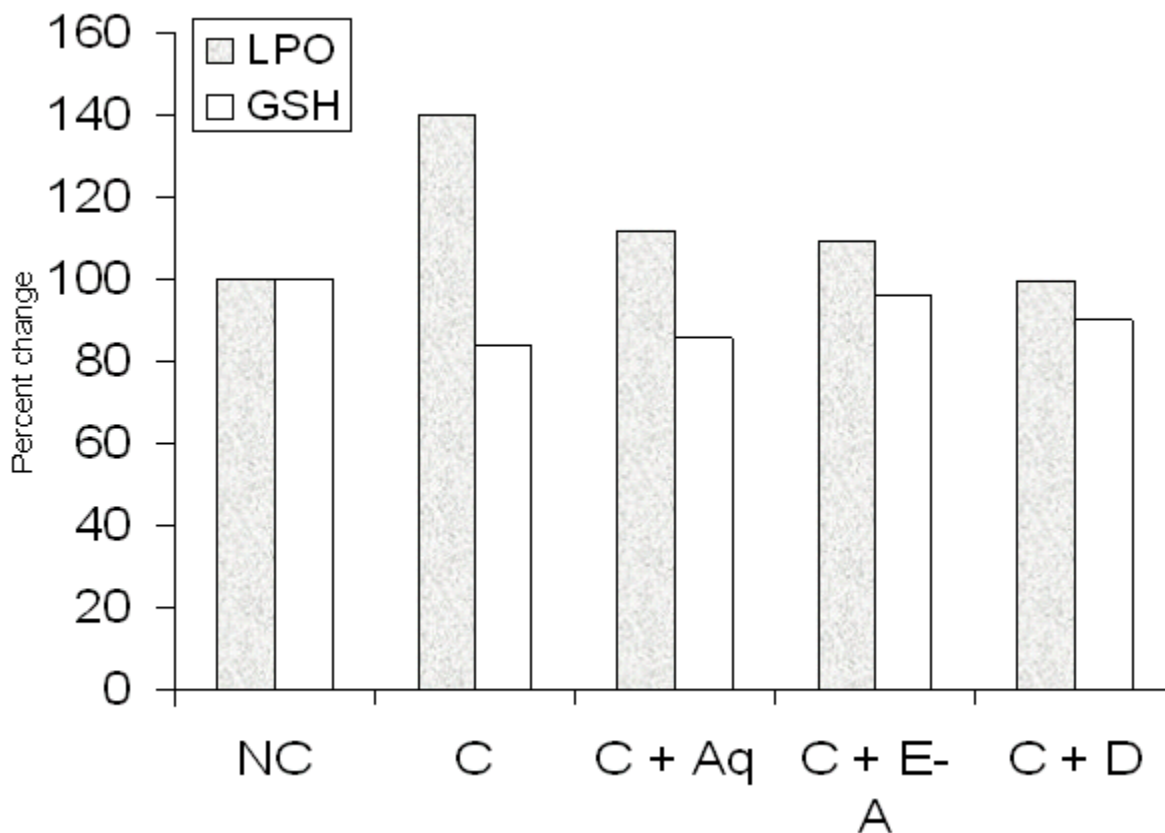


Figure 3: Lipid peroxidation and glutathione in rats following the treatment with *B. ligulata* in inflammation. NC: normal control receiving the vehicle alone, C: the group receiving carrageenan injection, C+Aq: treated with aqueous extract, C+E-A: treated with 50% ethanolic extract, and C+D: treated with diclofenac. Following inflammation, lipid peroxidation value shows a marked increase, while glutathione is reduced. Treatment with the extract is clearly shown to reverse the effect on the respective parameters.

The delayed onset of the carrageenan induced oedema has been linked to neutrophil infiltration, the release of neutrophil derived-mediators and free radicals [18]. The role of oxygen-derived free radicals and the oxidants in inflammation has been documented [19] and a large body of evidence is available linking the reactive oxygen species (ROS) to the pathophysiology of tissue damage associated with the inflammatory response. Free radicals cause the peroxidation of membrane lipids, and lead to the depletion of tissue glutathione by various mechanisms. In this study, we observed a significant increase in lipid peroxidation in the group of rats receiving carrageenan injection (Figure 3). Treatment with the extract significantly brought down the increased lipid peroxidation values. In states of oxidative stress, glutathione is depleted leading to

further peroxidation of lipid molecules [20]. In carrageenan-injected rats, we observe that the tissue glutathione concentration gets decreased. However, in rats receiving the 50% ethanolic extract, glutathione concentration was almost similar to the control rats (Figure 3). It is interesting to note that only the alcoholic extract was shown to increase the level of glutathione in comparison to the aqueous extract that exhibited little or no effect on glutathione concentration. A possible explanation for this could be attributed to the action of bergenin, a constituent of *B. ligulata*. Bergenin is a C-glucoside of 4-O-methyl gallic acid that has been isolated from the alcoholic extract of *Bergenia* and other plant species [21], and is well known for its therapeutic effects on the gastrointestinal diseases and antiinflammatory effects. Studies have reported that bergenin, over a

concentration range from 1 to 300 μM , could recover the decreased levels of glutathione in a dose dependent manner in hepatocytes [21]. Bergenin has been isolated from the methanolic extract of *B. ligulata*, and therefore, the antiinflammatory activity of the alcoholic extract of *B. ligulata* seen in this study can be attributed in part to the effect of bergenin. Literature has further suggested that the effects of bergenin can be related to its free radical suppressing activity

[21]. In this study, we also observed the activity of xanthine oxidase (Figure 4), a superoxide-generating enzyme, and superoxide dismutase (Figure 5), a superoxide-scavenging enzyme in carrageenan as well as extract treated rats. The results suggest that the extract attenuated the activity of the superoxide-generating enzyme (xanthine oxidase) and also enhanced the activity of superoxide scavenger (superoxide dismutase).

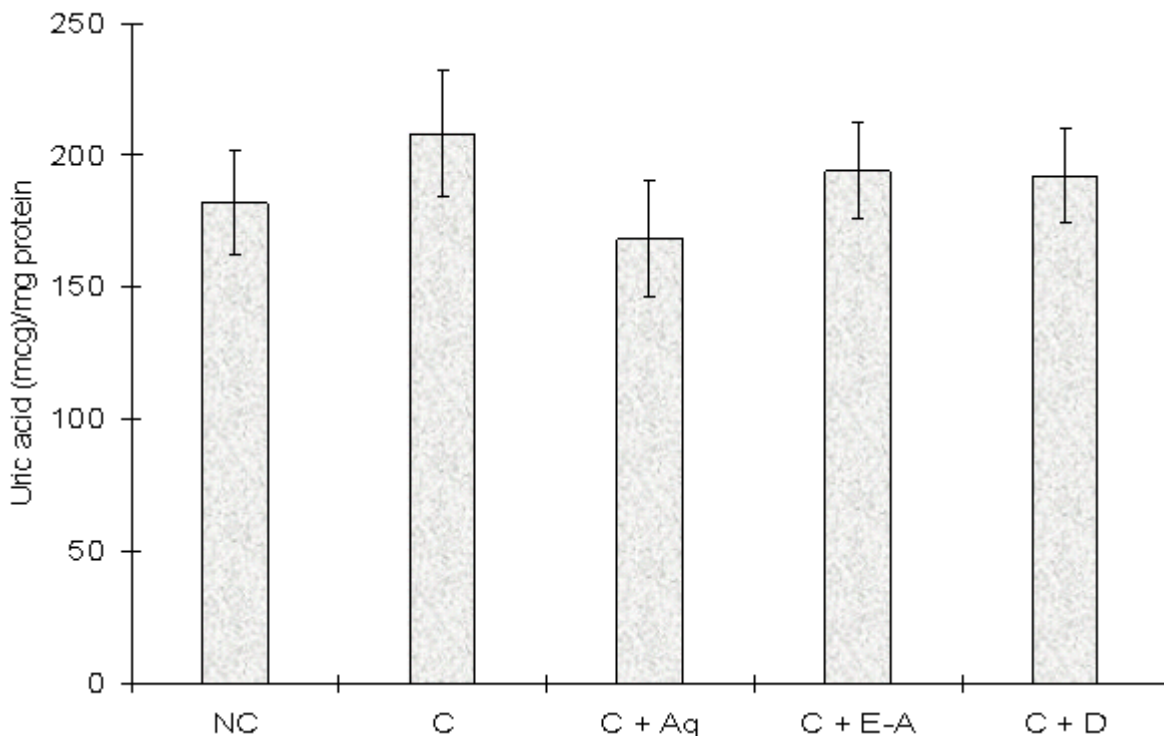


Figure 4: Effect of the treatment of *B. ligulata* on xanthine oxidase in rat model of inflammation. NC: normal control receiving the vehicle alone, C: the group receiving carrageenan injection, C+Aq: treated with aqueous extract, C+E-A: treated with 50% ethanolic extract, and C+D: treated with diclofenac. Xanthine oxidase is reported to be a pro-oxidant enzyme, causing an increase in the generation of superoxide. Treatment with the 50% ethanolic extract could alleviate the increased activity of xanthine oxidase.

As shown in Table I, aqueous, 50% ethanolic and methanolic extracts of *B. ligulata* rhizomes were tested for their ability to inhibit the growth of *E. coli*, *B. subtilis*, and *S. aureus* at the dose levels of 10, 25 or 50 mg/ml for each extract. At a dose level of 50 mg/ml, the antibacterial effect was most significant. Incidentally, the antibacterial effect of the extracts at this level was comparable to ciprofloxacin (25 mcg/ml). The results clearly

suggest that *B. ligulata* possesses a strong antibacterial activity.

The antiinflammatory and the antibacterial effects of *B. ligulata* can be attributed to the presence of sterols, glycosides and other chemical constituents present in *Bergenia* spp. In the roots of *B. ligulata* and *B. strecheyi*, bergenin and β -sitosterol [4] and recently paashaanolactone (1) have been isolated [22]. Simpler phenolic derivatives such as arbutin, 2-O-galloylarbutin, 6-

O-galloylarbutin and *p*-galloyloxy- β -D-glucoside have also been identified in the *Bergenia ligulata*. The antiinflammatory and the antibacterial activity of *B. ligulata*, as reported in this study, can be attributed, in part, to these and other chemical moieties. An important constituent of the extract, β -sitosterol (a phytosterol) that has been isolated from *Bergenia* species and other plant species has been documented for beneficial effects. Approximately 80% of the total phytosterol content of higher plants is composed of β -sitosterol [23] that differs from the major animal sterol (cholesterol) only by an extra ethyl group in its side chain. The β -sitosterol possesses several profound biological effects that also include the antiinflammatory effect [24]. In a study by Bouic [25], β -sitosterol and its glucoside have been shown to enhance T-cell proliferative responses and the release of IL-2 and γ -interferon, and

enhance the *in vitro* NK-cell activity. The β -sitosterol and its glucoside exert their effects on T-cells in hormonal concentration (picograms to femtograms) and therefore can be effective even if their absorption from the gastro-intestinal tract is poor; in humans no more than 5% of the daily intake is absorbed [26]. Thus, it is concluded that the antiinflammatory and antibacterial effects of *B. ligulata* can be due to the synergistic effect of pro-inflammatroy enzyme inhibitors, free radical scavengers or because of the corticoid like effects of some ingredients present in the extract. In conclusion, this study reports the antiinflammatory and antibacterial activity of *B. ligulata* extracts. Besides these activities, the study reports the radical scavenging activity of the rhizomes of *B. ligulata*, and establishes the therapeutic rationale of using *B. ligulata* in India System of Medicine.

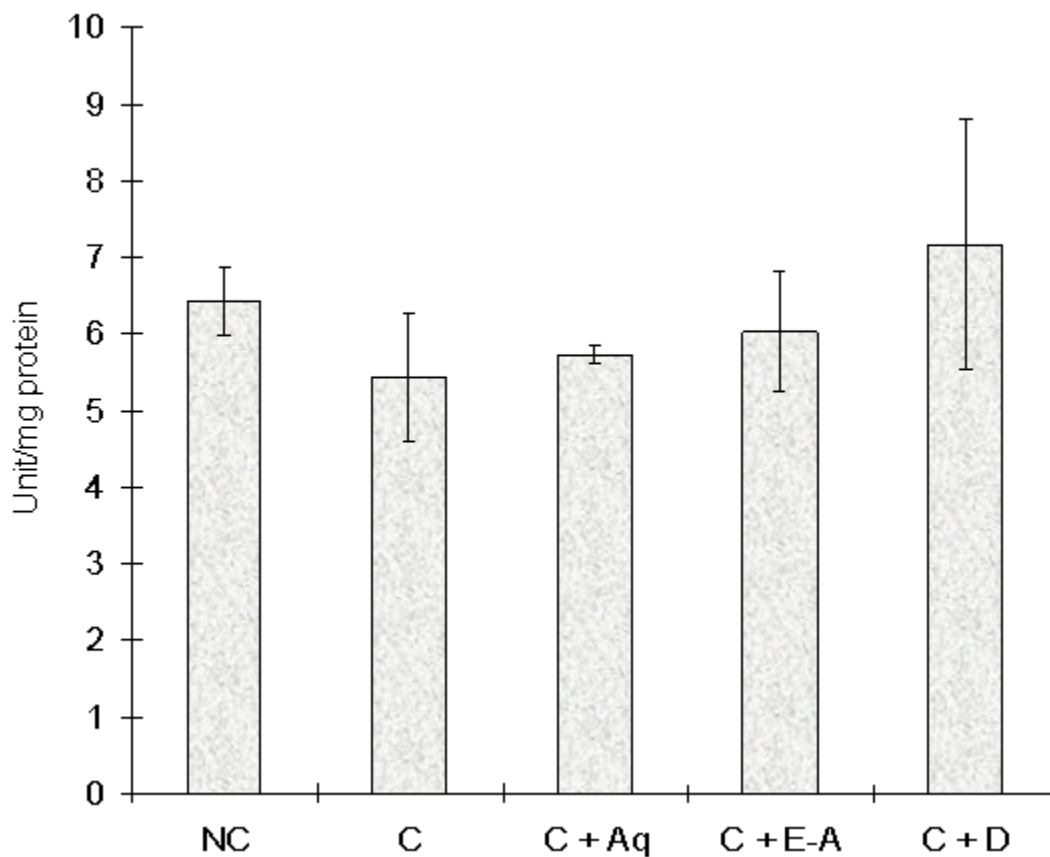


Figure 5: Superoxide dismutase in rats treated with *B. ligulata*. NC: normal control receiving the vehicle alone, C: the group receiving carrageenan injection, C+Aq: treated with aqueous extract, C+ E-A: treated with 50% ethanolic extract, and C+D: treated with diclofenac. This enzyme scavenges the superoxide anion radical. In animal model of

inflammation, superoxide dismutase showed a significant decrease. The enzyme level increased following the treatment with the extract as well as the diclofenac.

Acknowledgments

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