Antioxidant and Anti-Inflammatory potential of *Barleria grandiflora* leaves extract

Revendra Parganiha[1]*, Dr. Gaurav Tiwari[1], Dr. Neeraj Sharma[1]

[1]Patel college of Pharmacy, MPU, Bhopal.

Corresponding author detail

Revendra Parganiha

Patel college of Pharmacy, MPUniversity, Bhopal, M.P.

Email.id – parganiharevendra@gmail.com

Contact No- 9826676860

Abstract:

Ancient literature also mentioned herbal medicines for age related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders etc. for which no modern medicine is available. The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, natural distribution and biological function. In the current study, the extracts of *Barleria grandiflora* leaves in different solvents have been shown to possess antioxidant activity to fair extent. Various extract of *Barleria grandiflora* leaves exhibit DPPH and nitric oxide scavenging capacity, comparable even to the standard antioxidant, ascorbic acid. The hydroxyl radicals scavenging activity of hydro alcoholic as well as ethanol extracts of the *Barleria grandiflora* leaves is comparable to the standard antioxidant. *Barleriagrandiflora* leaves extracts were analysed using various free radical scavenging models. All models showed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, Nitric oxide, Hydroxyl radical to non-radical form. The Ascorbic acid used as positive control in all model. The reduction capability of the DPPH radical was determined by decrease in its absorbance at 517 nm, induced by antioxidants. Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Ethanol extract of *Barleria grandiflora* leaves show significant anti-inflammatory activity.

Introduction: Plants have been utilized as medicines for thousands of years**.** These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. Isolation and characterization of

pharmacologically active compounds from medicinal plants involving aschemo preventive drug [1,2]. Numerous methods have been utilized to acquire compounds for drug discovery including isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry, and molecular modelling [3]. Drug discovery from medicinal plants engage a multifaceted approach in current timecombining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain [4]. Natural products will remain a reliable source for drug discovery. The key to drug discovery from natural products is to find the suitable strategy. The drug discovery of natural products becomes more and more difficult. We should pay more attention to the new strategies and technologies of drug discoveries. In this process, careful observation, deep thinking and innovation are the key factors for the potential success [5]. Drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists [6]. Several known compounds isolated from traditionally used medicinal plants have already been shown to act on newly validated molecular targets, as exemplified by indirubin, which selectively inhibits cyclin dependent kinases and kamebakaurin, which has been shown to inhibit NF- nB [7, 8]. In ancient time of facing the challenge of unknown disease, the natural products were discovered by errors and trials. The ancient written documents originated from North Africa, India and China showed the earliest record of clinical practices [9,10]. An animal model is an important method and means to explore the essence of human disease and the evaluation of the effectiveness of new drugs. Animal models include spontaneous animal models and induced or experimental categories [11]. *Barleria grandiflora* is a shrub belonging to the family Acanthacea which is widely distributed in Amravati district in Maharashtra, Durg district in Chhattisgarh, shivamogga and Uttar kannad district in Karnataka and is native in India [12,13]. It is commonly known as Shwetkeshariain Chhattisgarh, Dev korantiin Maharashtra region.*Barleria grandiflora* traditionally regarded to posse's antioxidant and antiinflammatory activity alongwith use in skin disorder [14]. The present study aimed to investigate the potential of leaf extracts of *Barleria grandiflora* as antioxidants and also for reducing inflammation.

Material and Methods:

Collection of plant material

Fresh leaves of *Barleria grandiflora* were collected from the local area of Raipur (Chhattisgarh), India. The plant was authenticated by Dr. Praveen Joshi Professor and Head Govt. Ayurvedic college Raipur, Chhattisgarh. The collected samples were washed with water and shade-dried. The dried samples were then ground into powder. The powdered and the whole dried samples were stored in air tight containers along with silica.

Preparation of extracts –

The leaves of *Barleria grandiflora* were chopped into fine pieces and air dried. The air dried material was further dried in an oven at 40°C and pulverized in electric grinder. The powder thus obtained was defatted with petroleum ether (60-80oC) in soxhlet

apparatus, air- dried and subsequently extracted with water. The extract was dried under vacuum by evaporation [15,16].

In-Vitro Antioxidant Activities of the Extracts

DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay: Principle: DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging is one of the simplest and the most commonly used method for analyzing the antioxidant activity of the test compounds. The test extract and the DPPH solution are mixed with each other and absorbance of the solution is recorded after specific time period. DPPH is a stable free radical. On mixing DPPH solution with a substance that can donate an electron and a hydrogen atom (anti-oxidant compound) it gives rise to the reduced form of DPPH, which shows a strong absorption band at 517 nm. More the potential of antioxidant compound, greater is the discoloration [17].

For preparation of DPPH stock solution, 24 mg of DPPH was dissolved in 100 ml methanol. The working solution was prepared by diluting DPPH solution with methanol to attain an absorbance of around 0.98 ± 0.02 at 517 nm. Three ml of DPPH solution was mixed with 100µl of the sample. The reaction mixture was shaken well and incubated in dark for 30 minutes at room temperature. Absorbance was measured at 517 nm. The control was prepared as above except for the addition of distilled water in place of sample. Ascorbic acid was chosen as a known DPPH scavenger to compare the potential DPPH scavenging activity of the herbal extracts**.**The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following formula [18].

Scavenging effect (%) = (Absorbance of Control –Absorbance of Sample)/ Absorbance of Control X 100

Nitric oxide scavenging assay: Principle: In aqueous solution, sodium nitroprusside has a tendency to spontaneously generate nitric oxide, which interacts with oxygen to produce nitric oxide free radicals that can be estimated spectrophotometrically at 546 nm. Antioxidant compounds proportionally reduce the formation of nitric oxide radicals**.**

Sodium nitroprusside (1ml of 10mM) was added to 1ml solution of the extract and curcumin (as standard) in phosphate buffer (pH 7.4). The reaction mixture was incubated at 25°C for 150 min. 1ml solution was taken out from the mixture and 1 ml of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride) was added to it. Absorbance of the solution was measured at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the formula [19].

Nitric oxide scavenging effect (%) = (Absorbance of Control –Absorbance of Sample)/ Absorbance of Control X 100.

Hydroxyl radical scavenging potential: Principle: Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Klein et al.. The reaction mixture contained 1.0 mL of different concentration of extracts (2–10 mg/mL), 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1.0 mL of DMSO (0.85% in 0.1 mol/L phosphate buffer pH 7.4) and 0.5 mL of 0.22% ascorbic acid. The tubes were capped tightly and

heated in a water bath at 80–90 °C for 15 min, the reaction was terminated by adding 1.0 mL of ice-cold TCA (17.5%). To the above reaction mixture 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 min for color development. The intensity of the yellow color formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards. The percentage of inhibition was determined by comparing test with standard [20].

% of inhibition = (Absorbance of control – Absorbance of extract) / Absorbance of control × 100

Pharmacological screening

Animal Housing and environmental condition: Animalswistar rats weighing between 180-200 gm were selected for anti-inflammatory activity of extracts of *Barleria Grandiflora* leaves. All the animals were segregated into groups of six animal rats each. All animals were housed in air-conditioned rooms with 10-15 air circulation cycles per hour. The relative humidity was maintained between 30-70%, temperature between 22-250°C and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark. In each of the polypropylene cages with stainless steel grill top (32.5cm x 21cm), facilities for food and water bottle and bedding of clean paddy husk, the animals were kept in the groups of six. Standard pelleted basal diet and purified water were provided ad libitum to the animals. All the animals were acclimatized to the laboratory conditions before they were used in the experiments. Experimental protocol was approved by Institutional Animal Ethics Committee (Reg.No.PCP/CPCSEA/2021/06) and ethical norms were strictly followed during all experimental procedures.

Anti-Inflammatory Activity: The anti-inflammatory activity of suspension of extract of selected plantswas evaluated by the carrageenan-induced rat hind paw edema method.

Animals Wistar albino rats (150-200g) of either sex were used for experimental study. The animals were housed in cages at 25 ± 2ºC, and relative humidity (50 ± 5%) with 12 hours light, and 12 hours dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water ad libitum [21].

Carrageenan induced hind paw edema: The initial paw volume was measured using vernier callipers at each individual group of animals. The specific dose of drug Indomethacin 10mg/kg while extract of *Barleria grandiflora* leaves at doses of 200 mg/kgvia orally administered to animals. Now, 0.1 ml of 1% w/v carrageenan was injected in the right hind leg after 30 minutes addition of drug. The edema formed in the paw was measured by digital vernier callipers after 3 hours. The degree of swelling provoke was assess by the proportion of the degree of hind paw previous to after carrageenan treatment. The percentage inhibition was resolute by allowing for edema induced by carrageenan alone was as 100% induction. The statistical as mean ± SEM was performed by one way analyses ofvariance (ANOVA) with GraphPad, The $P<0.05$ was considered as statistically significant**.** Edema was expressed as the increment in paw thickness due to carrageenan administration. The paw volume was measured using

a vernier callipers at the time intervals of 60, 120, 180 and 240, minutes after administration of carrageenan. Percent inhibition of edema volume between treated and control group was calculated as follows:

Percentage inhibition = $\frac{Vc - Vt}{Vc} \times 100$

Where, Vc and Vt represented mean increase in paw volume in control and treated groups respectively.

Result and Discussion:

In-Vitro Antioxidant Activities of the Extracts: In-vitro antioxidant activity has been evaluated with DPPH radical scavenging assay, nitric oxide radical scavenging assay, and hydroxyl ion radical scavenging activity. The current study the in-vitro antioxidant potential of *Barleria grandiflora leaves* has been analysed. In the current study, the extracts of *Barleria grandiflora leaves* in different solvents have been shown to possess antioxidant activity to fair extent.

Various extract of *Barleria grandiflora leaves* exhibit DPPH, hydroxyl ion and nitric oxide scavenging capacity, comparable even to the standard antioxidant, ascorbic acid. The hydroxyl ionradicals scavenging activity of ethanol as well as aqueous extracts of the *Barleria grandiflora leaves* is comparable to the standard antioxidant.

In-vitro antioxidant Activity: Extracts*Barleriagrandiflora* leaves were analysed using various free radical scavenging models. All models showed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, Nitric oxide and Hydroxyl radical to non-radical form. The Ascorbic acid, used as positive control in all model.

Diphenyl-picryl-hydrazyl (DPPH) radical scavenging activity: The reduction capability of the DPPH radical was determined by decrease in its absorbance at 517nm, induced by antioxidants. The absorbance maximum of a stable DPPH radical in methanol was measured at 517nm. The decrease in absorbance of DPPH caused by ascorbic acid, hydro alcoholic, ethanol, and aqueous extracts of *Barleriagrandiflora* leaves because of the reaction which results in the scavenging of free radical by hydrogen donation by changing the colour from purple to yellow measured at 517nm. Significant decrease in the concentration of DPPH radicals due to the scavenging ability of hydro alcoholic, ethanol, and aqueous extracts of *Barleriagrandiflora* leaves extract and Ascorbic acid as standard.

Nitric oxide radical scavenging activity: Hydro alcoholic, ethanol, and aqueous extracts of *Barleriagrandiflora* leaves were subjected to nitric oxide radical scavenging activity.Different groups were subjected to reaction; reaction mixture alone was kept as control, ascorbic acid was used as standard and extract (100, 200, 300 400 and 500µg/ml) of *Barleriagrandiflora* leaves extract were used. Absorbance of all groups was determined before and after the reaction at 697 nm. Percentage inhibition in their absorbance with different groups is shown in Table 2.So it was clear that hydro alcoholic and ethanol extracts of *Barleriagrandiflora* leaves had demonstrated concentration dependent inhibition in the nitric oxide anion scavenging activity. However aqueous extracts of *Barleriagrandiflora* leaves showed lesser inhibition than standard.

Hydroxyl radical scavenging model: The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of the selected plant extracts were assessed by means of the iron (II)- dependent DNA damage assay. All the results showed hydroxyl radical scavenging activity in a dose dependent manner. hydro alcoholic, ethanol extract of Barleriagrandiflora leaves at different concentrations showed notable inhibitory effect of Hydroxyl radical scavenging activity as compared to aqueous extract. The hydro alcoholic extract of *Barleriagrandiflora* leaves showed more inhibitory effect of Hydroxyl radical scavenging activity compared other extract.

Carageenan-Induced Rat Paw Edema Method: Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical use for the release of inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc.).

The course of acute inflammation is biphasic. First phase starts with the release of histamine, serotonin, and kinins after the injection of phlogistic agent in the first few hours. While the second phase is related to the release of prostaglandins like substances in 2-3 hours. Second phase is sensitive to both the clinically useful steroidal and non-steroidal anti-inflammatory agent. Table 4 shows the effect of *Barleriagrandiflora* leaves extract and standard drug as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier calliper. Hydro alcoholic extract administered at a dose of 200 mg/kg p.o prevented carrageenan- induced paw edema with a percentage inhibition of 25.92%, 34.89%, 51.00%, 57.23% at 1, 2, 3,and 4 hour, respectively, while ethanol extract showed 20.00%, 29.48%, 40.93% and 47.38% edema inhibition at a dose of 200 mg/kg p.o. at 1, 2, 3.and 4 hour, respectively. Aqueous extract administered at a dose of 200 mg/kg p.o showed carrageenan-induced paw edema with a percentage inhibition of 14.07%, 27.23%, 32.05%, 38.76% at 1, 2, 3,and 4 hour, respectively. Indomethacin at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition of 54.81%, 61.53%, 69.58%, and 72.30% at 1, 2, 3.and 4 hour, respectively. The present results suggest that hydro alcoholic,ethanoland aqueous extracts of *Barleria grandiflora* leaves suppresses the carrageenan-induced paw edema. Hydro alcoholic extract cause significant reduction in rat paw edema than other extract of *Barleria grandiflora* leaves. The present study showed that hydro alcoholic, and ethanol extract of *Barleria grandiflora* leaves have good anti-inflammatory properties.

Summary and conclusion:

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. It therefore became the aim to produce the active principles of all medicinal plants as far as possible as pure substances, which could then be investigated in the same way as clearly-definable chemical compounds. A new trend developed where such native substances were looked for, designating those phytopharmaceuticals. In-vitro antioxidant activity has been evaluated with DPPH radical scavenging assay, nitric oxide radical scavenging assay, hydroxyl ion radical scavenging assayactivity. The current study the in-vitro antioxidant potential of *Barleria grandiflora leaves* has been analysed. The present results suggest that hydro alcoholic extract show high antioxidant activity than ethanol extract. The aqueous extract show less anti oxidant activity as compare to others. Similarly hydro alcoholic extract show high anti-inflammatory activity and suppresses the carrageenan-induced paw edema in rat. Ethanol extract cause significant reduction in rat paw edema than aqueous extract of *Barleria grandiflora* leaves. The present study showed that hydro alcoholic, ethanol and aqueous extract of *Barleria grandiflora* leaves have anti oxidant and anti-inflammatory properties.

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Table 1: Effect of Extract of Barleria Grandiflora leaves on percent edema inhibition in Carageenan- induced rat paw edema model.

Treatment (Plant extract)	Dose	1h	2 h	3 h	4 h
		Edema Inhibition (%)	Edema Inhibition (%)	Edema Inhibition (%)	Edema Inhibition (%)
Control		-	-	-	-
Indomethacin	10 mg/kg	54.81	61.53	69.58	72.30
AQBG	200 mg/kg	14.07	27.23	32.05	38.76

EABG	200 mg/kg	20	29.48	40.93	47.38
HAEBG	200 mg/kg	25.92	34.89	51	57.23

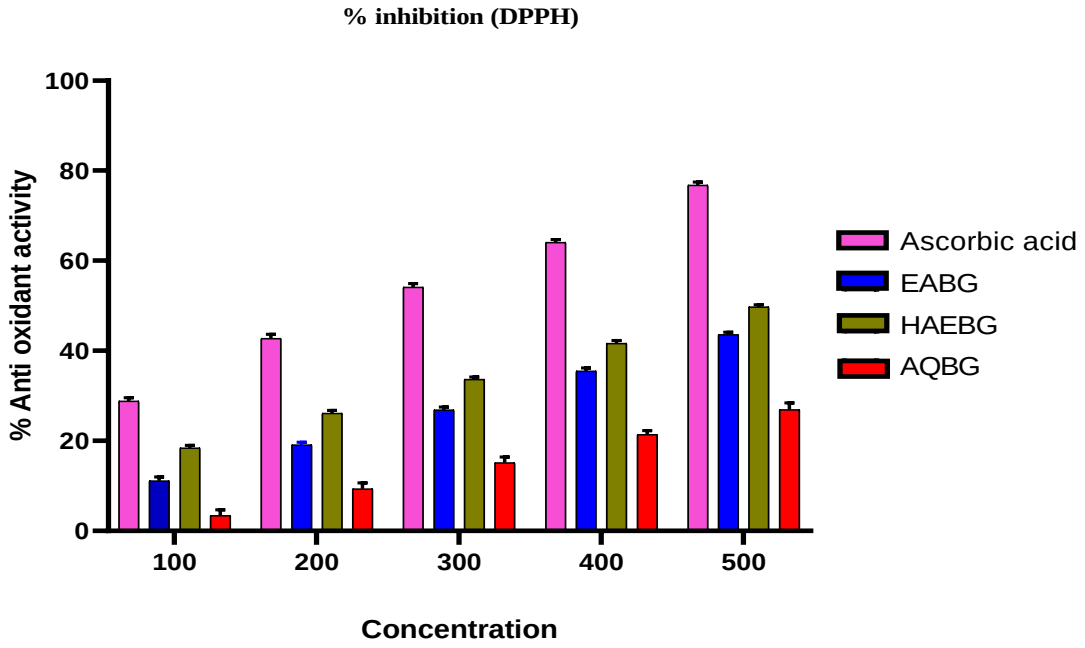

Figure 1: Effect of *Barleriagrandiflora* leaves extracts on DPPH radical scavenging model.

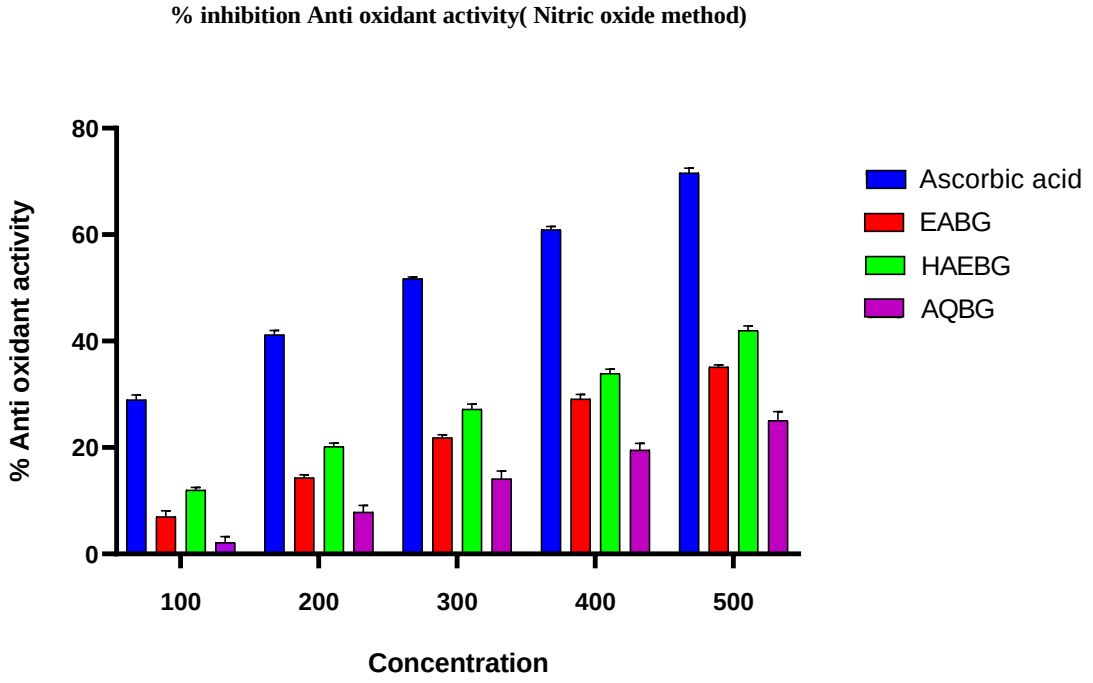

Figure 2: Effect of *Barleriagrandiflora* leaves extracts on Nitric oxide radical scavenging model

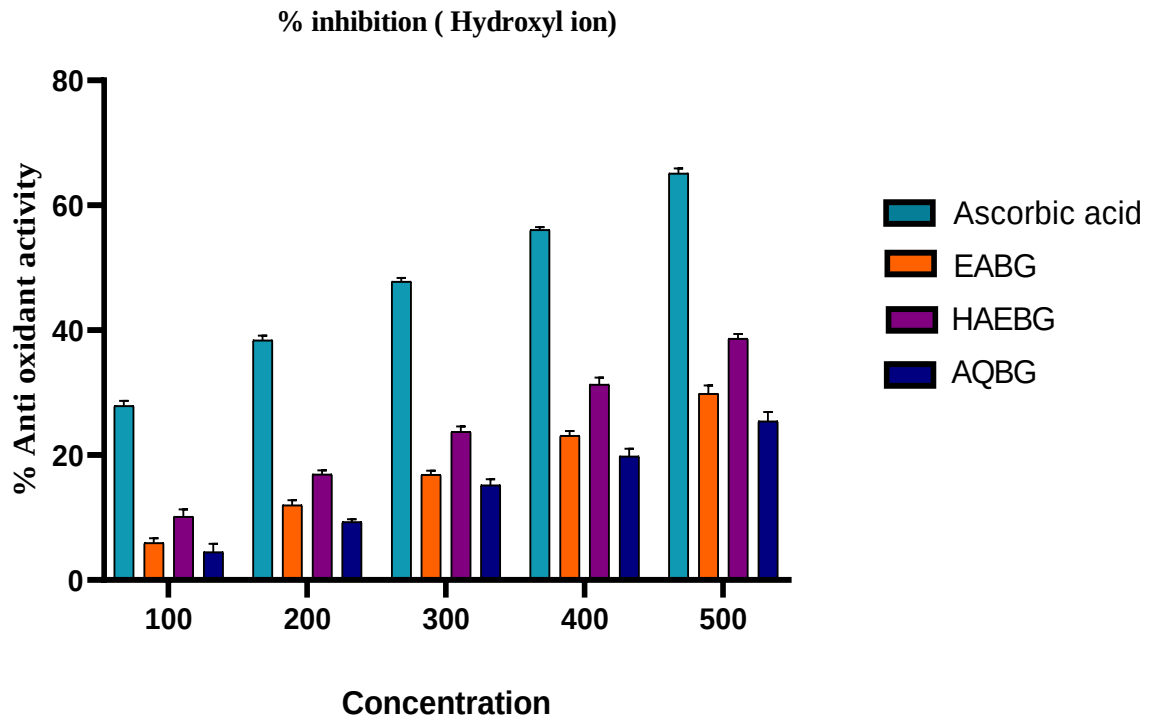

Figure 3: Effect of *Barleria grandiflora*leaves extracts on Hydroxyl radical scavenging model

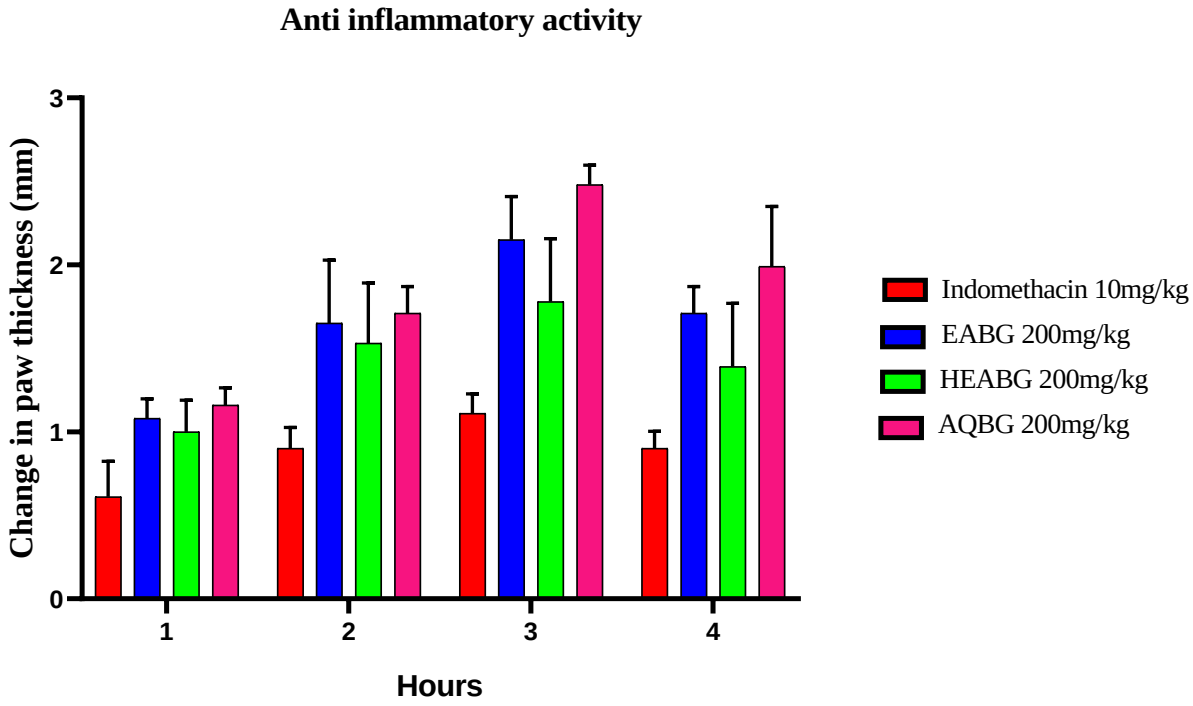

Figure 4: Effect of Extract of Barleria Grandiflora leaves on edema volume in Carageenan-induced rat paw edema model

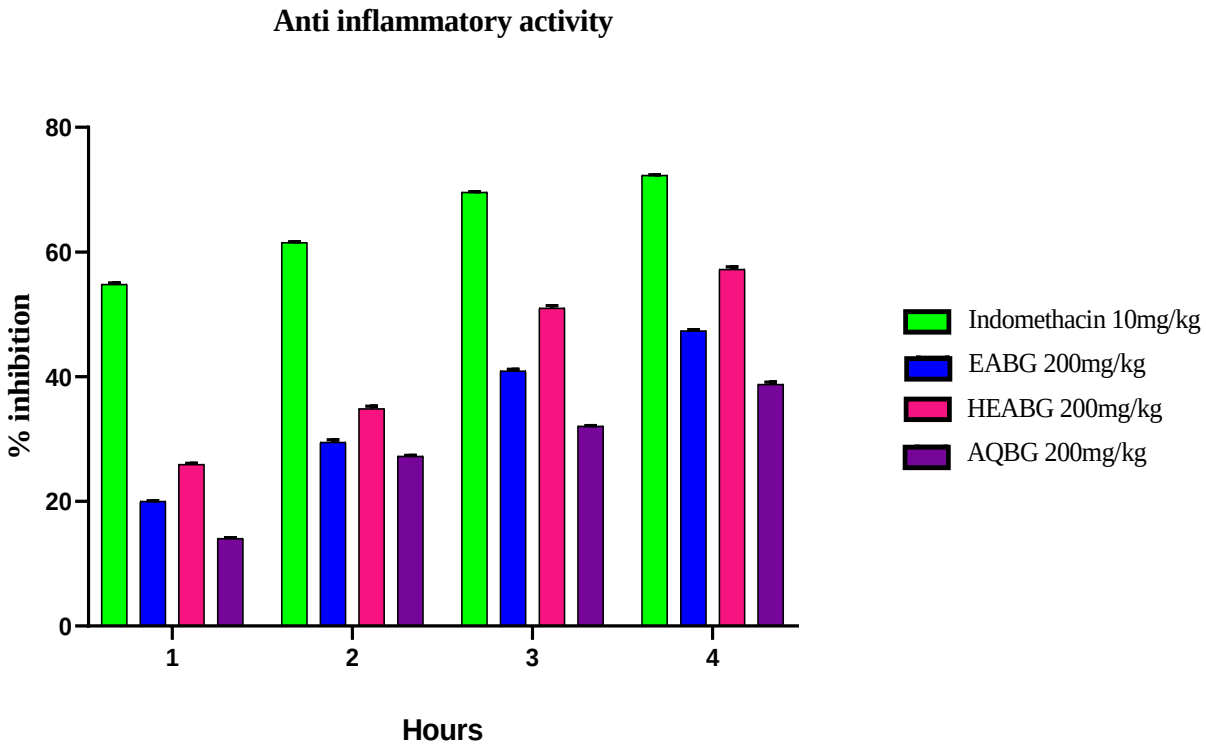

Figure 5: Effect of Extract of Barleria Grandiflora leaves on percent edema inhibition in Carageenan-induced rat paw edema model