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Anti-inflammatory and antioxidant activity of ethanolic extract of *Bauhinia purpurea* bark.

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Abstract

The aim of this study was to evaluate the anti-inflammatory and antioxidant efficacy of ethanolic extract of Bauhinia purpurea bark (EBP) in Sprague Dawley (SD) rats. Carrageenan induced paw edema and air pouch inflammation, arachidonic acid induced ear edema and complete freund's adjuvant (CFA) induced hematological alterations were studied in control and experimental rats. Administration of EBP (200mg, 400mg, 600 mg/kg body weight) showed a significant and dose dependent inhibition of paw edema and ear edema and also reversed the hematological alterations near to normalcy. DPPH assay and measurement of MDA levels showed potent antioxidant activity of EBP. With increase in concentration of EBP, improvement in walking scores of motility test confirmed the anti-inflammatory activity of EBP. Acute toxicity studies demonstrated non toxic nature of EBP even at higher doses. Together, our results demonstrate that EBP has potent anti-inflammatory as well as potent antioxidant properties validating the folk medicinal use of this species.

Keywords: *Bauhinia purpurea*, Anti- inflammatory, paw edema, ear edema, antioxidant activity

Introduction

Plants have been a valuable source of new bioactive factors and are considered as an efficient alternative strategy for the development of newer therapeutics. A good number of plants have been reported in traditional medicine to alleviate various inflammatory disorders. However, there has been little scientific effort to confirm these anecdotal. *Bauhinia purpurea* is a medium sized deciduous tree belongs to Leguminaceae family [1]. It is found in most types of vegetation ranging from evergreen lower lands, rain forests to mountain forests up to 2000-3000 m altitude and also in savanna scrub and dry deciduous forests to swamp forests on various soils. *Bauhinia purpurea* shows several pharmacological activities and research has been carried out on different parts of *Bauhinia purpurea* extract [2]. Previous reports state that the plant showed antidiarrhoeal [3], antispasmodic antimyocardial [4], antinociceptive, antioxidant and antipyretic [5-6] antimicrobial, cytotoxic and antimycobacterial [7-8] wound healing [9] antinephrotoxicity [10] activities. Different parts of *Bauhinia purpurea* were reported to possess such phytochemicals as glycosides, flavanoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols [6].

Inflammation is a complex set of interactions that allow tissue to respond against injury or infection. It involves the participation of various cellular types expressing and reacting to diverse mediators along a very precise sequence of events [11]. Non steroidal anti inflammatory drugs (NSAIDs) are among the commonly used medications to treat pain and inflammatory conditions. However, due to undesirable side effects attributed to the prolonged use of NSAIDs and the ineffectiveness in some cases, the control of inflammatory pain is still a major challenge. Therefore, the search for more effective anti-inflammatory drugs is still very relevant. Hence, the present study was aimed to evaluate the anti-inflammatory potential of ethanolic extract of *Bauhinia purpurea* bark in SD rats.

Materials and methods

Plant material and extract preparation

The bark of *Bauhinia pupurea* was collected from lower slopes of Seshachalam hills, Tirupati, shade dried, coarsely powdered and extracted with ethanol as per I.P (Indian Pharmacopeia) guidelines for 7 days with intermittent shaking. The filtrate obtained was evaporated to dryness at 50-65^{0}C in a rotary vacuum evaporator to obtain a dark colored molten mass. The residue was stored in a refrigerator at 4^{0}C until use. Phytochemical analysis of ethanolic extract of *Bauhinia purpurea* was carried out to find the nature of bioactive compounds present [12].

Animals

Experiments were conducted at Department of Biochemistry, Sri Venkateswara University, Tirupati, India. Adult Sprague Dawley

rats (190±20 g) and standard normal diet were purchased from National Institute of Nutrition (NIN), Hyderabad, India. Rats were housed in polypropylene cages (photoperiod 12 h light/12 h dark cycle, 27±3^{0}C, Humidity 30-60%). Animals were allowed to free access of diet and water ad libitum. Rats were acclimatized in laboratory condition for 7 days before performing experiments. All procedures involving laboratory animals were in accordance with the Institute Animal Ethics Committee regulations approved by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Fasting was applied prior to all assays as standard drugs and plant extracts were administered orally.

In vivo anti-inflammatory activity

Acute toxicity studies

The acute toxicity study of the plant extract (EBP) was performed with administration of two doses (1250 or 2500 mg/kg body weight). Rats were fasted for 12hr prior to the administration of plant extract. The animals in each group were observed for 2hr after treatment for immediate signs of toxicity. The mortality rate within 24hr was noted. Animals which survived were allowed free access to food and water and observed for over 5 days for signs of delayed acute toxicity including death [13].

Carragenan induced paw edema

Carragenan induced paw edema was produced according to the method of Winter et al (1962) with some modifications. Animals were divided in to five groups of six each, starved overnight with water prior to the day of experiments. Group-I was Carrageenan control, Group-II was Carrageenan+ Diclofenac treated, Group-III Carrageenan + EBP (200 mg /kg), Group-IV Carragenan + EBP (400 mg /kg) and Group-V Carragenan + EBP (600 mg /kg) treated. Left paw was marked with ink at the level of lateral malleolus, basal paw volume was measured plethysmo graphically by volume displacement method [14].

The plant extract i.e, EBP or Diclofenac were administered orally one hour before injection of carrageenan. One hour after dosing, the rats were challenged by a subcutaneous injection of 0.1 ml of 1 % solution of carrageenan in to the sub-plantar side of the left hind paw. The paw volume was measured again at 1, 2, 3, 4 and 5 hr after challenge. The increase in paw volume was calculated as percentage compared with the basal volume. The difference of average values between the treated animals and control group was calculated for each time interval and evaluated statistically. The percentage inhibition was 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.

Induction of ear edema

Rats were divided in to five groups of six each. Group-I was Arachidonic acid control, Group-II was Arachidonic acid+ Diclofenac treated, Group-III Arachidonic acid + EBP (200 mg /kg), Group-IV Arachidonic acid + EBP (400 mg /kg) and Group-V Arachidonic acid + EBP (600 mg /kg) treated. Arachidonic acid (2mg /ear) was subcutaneously injected into the right ear of rats. Thickness of the ears was measured 1 hr before and 1,3 hr after the induction of inflammation (Young et al.., 1987) using a digital caliper. The caliper was applied near tip of the ear just distal to the cartilaginous ridges and the thickness was recorded in µg. Edema was expressed as the increase in ear thickness due to the inflammatory challenge [15].

Carrageenan-induced air pouch inflammation in rats

The air pouch was created by injecting 20 ml of sterile air on day 1st and 3rd . Group-1 was carrageenan control, Group -2 was carrageena + Diclofenace (10 mg/kg, p.o), Group -3 was carrageenan + EBP (200 mg/kg, p.o), Group- 4 was carrageenan + EBP (400 mg/kg, p.o), Group-5 was carrageenan + EBP (600 mg/kg). On 5 th day, 1 ml of 1% w/v carrageenan was injected to all the animals (except normal control) directly into the pouch. After 6hr, the animals were sacrificed and 5 ml of ice-cold saline was injected into the pouch and the exudates were collected. Total leukocyte and granulocyte count of lavage fluid was estimated using cell counter [16].

Complete freund's adjuvant (CFA) induced hematological alterations in rats

CFA (0.1 ml) was injected subplantar (each ml contains 1 mg of mycobacterium tuberculosis strain H37Ra), heat killed and dried, 0.85 ml paraffin oil and 0.15 ml of mannide monooleate) procured from Sigma-Aldrich (Kalia et al., 2007). The rats were randomly divided into five groups viz., CFA control, DF (5mg/kg, p.o) treated, CFA + EBP (200 mg/kg, p.o) , CFA + EBP (400 mg/kg, p.o) and CFA + EBP (600 mg / kg, p.o) [17].

Motility Test

The normal control rats which walked comfortably without any difficulty were given a score of 5, the carrageenan induced paw edema rats which walked with great difficulty, avoiding touching the toes of the inflamed paw to the floor scored 1. If the rats improved their walking ability because of administration of either diclofenac or EBP, they got scores between 1 and 5 [18-19].

Antioxidant assay

The effect of EBP on DPPH radical was estimated following the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with different concentrations of EBP (25, 50, 100, 200, 400µg/ml). The reaction mixture was thoroughly shaken and left on the bench at room temperature for 30 min for complete reaction. The absorbance was measured at 517nm using a spectrophotometer. Ascorbic acid was used as standard. All determinations were done in triplicate. The ability of the sample to scavenge radicals was calculated by the following equation

DPPH radical scavenging activity (%) = absorbance control-absorbance sample/absorbance of control X 100 [20].

Assessment of Lipid Peroxidation

The liver tissue was collected and homogenised in 0.9% ice cold saline in teflon homogenizer. The homogenate was centrifuged at 800g for 10 min and the supernatant was again centrifuged at 12000 g for 15 min. The supernatant was used to measure the level of lipid peroxidation which was measured by the thiobarbituric acid (TBA) method [21].

Results

Acute toxicity studies

No abnormal behavior or death was noticed with rats administered with EBP of 1250 mg/kg body weight. However rats administered with EBP of 2500 mg/kg body weight showed restlessness, paw chewing for some time but became adjusted later.

Carrageenan induced Paw edema

Administration of EBP, 1 hr before the injection of carrageenan caused a considerable and dose dependant inhibition of increase in paw edema in a time dependant manner as shown in figure-1. The inhibitory effect was almost 90% of the standard Diclofenac treatment.

Arachidonic acid induced ear edema in rats

There was about 20% increase in ear weight in arachidonic acid induced group of rats when compared to control group. In a concentration dependent fashion, EBP significantly inhibited ear edema caused by arachidonic acid. The alleviation in ear weight in the presence of EBP was 4-8%, while that of Diclofenac was only 3-5 % when compared to arachidonic acid controls (figure-2). This shows that plant extract is showing efficient anti-inflammatory effect against ear edema.

Carrageenan induced air pouch inflammation in rats

Carragenan caused raise in leucocytes and granulocytes counts in air pouch exudates. Treatment with EBP at doses of 200, 400 and 600 mg/kg significantly and dose-dependently reduced total leukocytes count and granulocyte count in airpouch exudates when compared to carragenanan controls. EBP at 600 mg /kg showed similar results as that of Diclofenac (figure-3).

CFA- induced Haematological alterations in rats

CFA treatment enhanced WBC and ESR but reduced RBC and Hb per cent in experimental rats. However, EBP treatment significantly and dose-dependently restored the altered levels of the above parameters, the maximum effect being noticed with 600 mg /kg of EBP (table-1).

Motility test

Walking ability of the rats to climb the staircase at the time of peak inflammation was checked by motility score. Carrageenan-induced paw edema rats scored 1, because they could walk with great difficulty, avoiding touching the toes of the inflamed paw to the floor. Diclofenac treatment attenuated the paw edema substantially and the rats walked with little difficulty and were scored 4. Treatment with 200 mg/kg, 400 mg/kg, 600mg/ kg bodyweight of EBP gradually reduced the paw edema and rats could improve their walk and scored 2, 3, 4 respectively.

DPPH scavenging assay

The scavenging effect of EBP and ascorbic acid on the DPPH radicals is illustrated in figure 4. With increasing concentration of EBP from 25-400µg/ml, significant and concentration dependent scavenging effect on the DPPH was noticed. At 400µg/ml, the activity of EBP was on par with ascorbic acid.

Effect of EBP on lipid peroxidation

Carragenan induced inflammation caused substantial increases in MDA levels indicating enhanced lipid peroxidation. Oral administration of EBP at three doses (200 mg, 400 mg and 600 mg /kg body weight) resulted in significant and dose dependent decreases in LPO as evidenced by lowered MDA levels (figure-5).

Discussion

Carrageenan-induced rat paw edema is a prominent inflammatory model to evaluate anti-inflammatory effect of compounds. Inflammatory reactions are generally triggered off by exogenous or endogenous aggressions which are characterized by vascular and cellular events. These reactions play pivotal role in the expression of cyclooxygenases (COX) which enhance the production of inflammatory mediators such as leukotrienes and prostaglandins. Inflammation involves two phases of events. The first phase is due to release of histamine, serotonin and kinin like substance with in 3 hr and second accelerating phase of swelling is attributed to prostaglandins release after 3 hr. In our study, EBP (200, 400, and 600 mg/kg, p.o.) substantially reduced carrageenan induced paw edema in a dose dependent manner (fig-1). In carrageenan-induced air pouch inflammation, EBP significantly inhibited the cellular infilteration (neutrophils and granulocytes) in to the air pouch fluid (fig-3). Our results suggest that EBP possibly may have its inhibitory effect on the prostaglandin (PG) mediated inflammatory pathway or may act by inhibiting the release of histamine and serotonin [22].

Study of arachadonic acid(AA) induced ear edema has been one of the sensitive tests for detecting the anti-inflammatory activity of lipoxygenase and cyclooxygenase inhibitors. Our results showed that EBP at concentrations of 400 mg /kg and above had attenuated the AA-induced ear edema to a great extent (fig-2).

CFC-induced alterations in WBC, RBC counts, ESR and Hb per cent were substantially controlled by EBP treatments (table-1).

Reduction in Hb per cent and RBC count in inflammation could be due to decreased response of the erythropoietin and premature destruction of red blood cells. Elevated WBC levels are an indication of inflammation or infection. Erythrocyte sedimentation rate (ESR), is influenced by an increase in the plasma concentration of acute phase reactant proteins in response to the inflammation [23]. Our results showed that higher concentration of EBP (>400 mg/kg) had effectively normalized the CFC-induced hematological changes and thus confirmed its anti-inflammatory activity.

The ability to walk and climb the staircase is considered as one of the critical tests to measure the intensity of paw edema and inflammation. In our study, with increase in the concentration of EBP, paw edema rats could improve their walking ability gradually

Table.1 CFA induced haematological alterations in rats

Treatment groups	Haemotological parameters			
	RBC (10^6ml/mm^3)	WBC(X 10^3cell/mm^3)	ESR (mm/h)	Hb(mg%)
CFA control	7.96±0.23	12.98±0.8	13.76±0.22	11.94±0.54
Diclofenac	10.42±0.38	7.62±0.37	9.94±0.34	16.14±0.72
EBP (200 mg/kg)	7.92±0.19	11.42±0.64	13.12±0.66	12.01±0.92
EBP (400 mg/kg)	8.59±0.12	10.36±0.39	12.12±0.12	13.86±0.49
EBP (600 mg/kg)	9.26±0.24	7.91±0.56	10.44±0.31	15.38±0.83

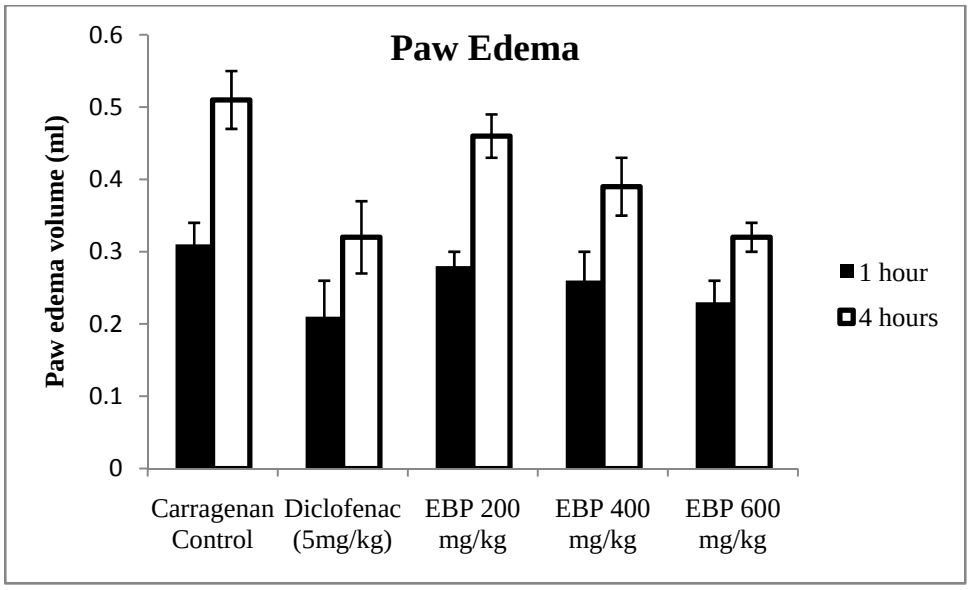

Figure. 1 Carrageenan induced paw edema in rats

The data are expressed as the mean±S.E.M (n=6). Values are statistically Significant at $P < 0.05$

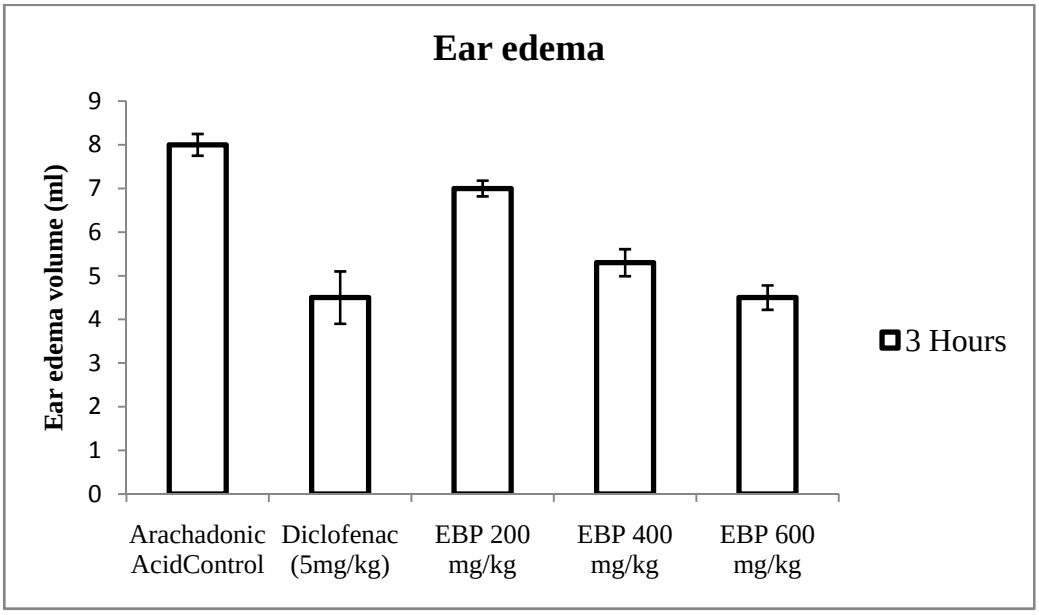

Figure.2 Arachidonic acid induced ear edema in rats

The data are expressed as the mean±S.E.M (*n*=6). Values are statistically Significant at P < 0.05

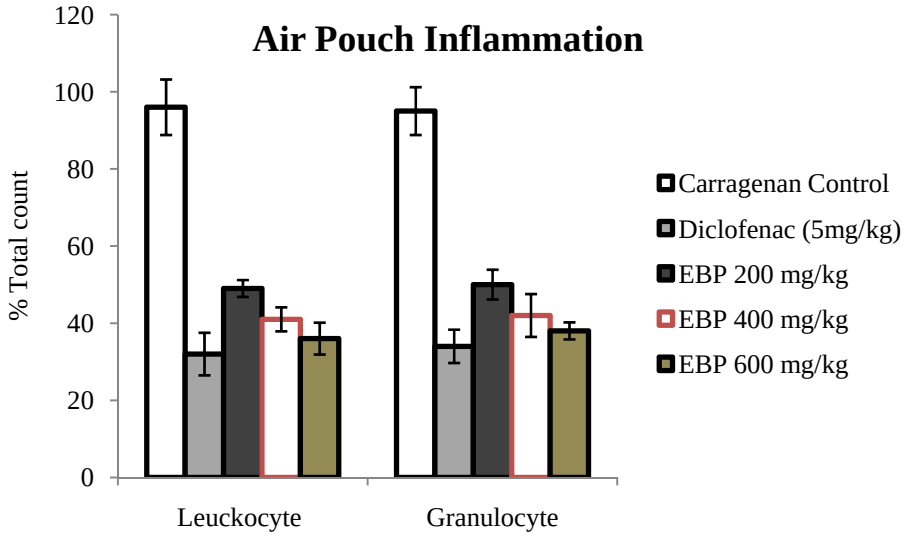

Figure.3 Carragenan induced air pouch inflammation in rats

The data are expressed as the mean±S.E.M (*n*=6). Values are statistically Significant at P < 0.05

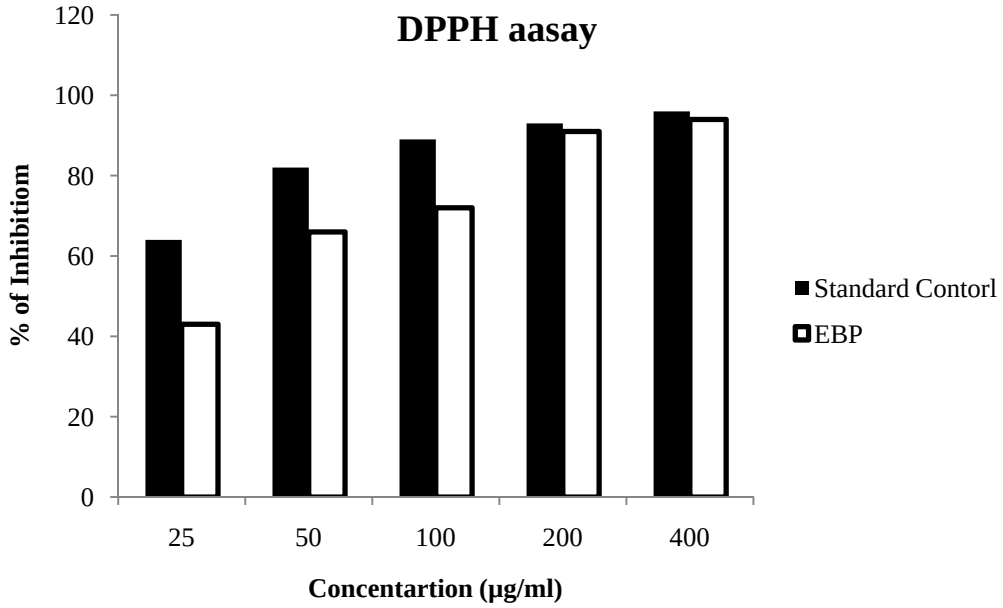

Figure.4 DPPH Scavenging assay

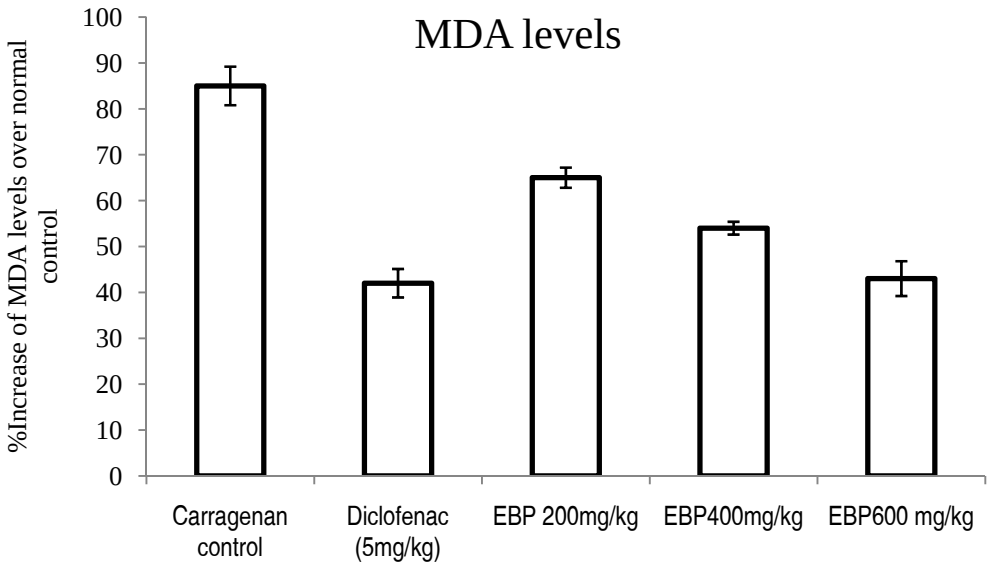

Figure.5 Effect of EBP on lipid peroxidation in rats

The data are expressed as the mean±S.E.M (n=6). Values are statistically Significant at $P < 0.05$

and at 600 mg /kg of EBP, they walked comfortably as evidenced by their achieved score 4 .

In this study, the antioxidant activity of EBP was evaluated using the DPPH and lipid peroxidation assay methods. DPPH is a simple and effective method regularly used to assess the radical scavenging ability of plant extracts in vivo [24]. EBP showed potent antioxidant activity in a dose dependent manner as that of standard ascorbic acid (fig-4). The lipid peroxidation assay is an indication of oxidative stress induced damage of membranes. Estimation of MDA levels gives information on the free radical scavenging ability of the plant extract. Our results demonstrated that, carrageenan induced lipid peroxidation was considerably mitigated by treatment with EBP in a dose dependent manner (fig-5). Together, our findings clearly demonstrated that, EBP has potent anti-inflammatory and antioxidant activity and provides scientific evidence for its use in folk and traditional medicine.

Conflict of interest

We declare that we have no conflict of interest.

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