

Evaluation of anti-inflammatory effect of beetroot extract in animal models

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ABSTRACT

Background: Inflammation is complex biological response of body tissues to foreign particles or pathogens. However, adverse effects of anti-inflammatory drugs, necessitate the search for a novel compound from herbal origin. Beta vulgaris rubra or red beetroot, a vegetable plant, is known to possess various beneficial effects. Present study aimed to evaluate the anti inflammatory effect of beet roots in animal models.

Methods: Carrageenan-induced and Complete Freund's adjuvant-induced rat paw edema model were used to assess the acute and chronic anti-inflammatory potential of the Beetroot as compared to standard drug, Diclofenac Sodium.

Results: Oral administration of Beetroot extract showed carrageenan-induced rat paw edema inhibition by 26.9%, 34.6% and 50% for beetroot 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively, at the end of four hours. In vivo results noted that percentage inhibition of paw volume and ankle diameter of the Complete Freund's adjuvant-induced rats were significantly higher for the groups consuming 100 and 200mg/Kg Beetroot extracts. Paw volume of the rats in these two groups reduced by 35% and 30% respectively as compared to the control.

Conclusions: Thus, beetroot was found to possess anti-inflammatory property in animals. Further investigations are essential to validate the bioactive components responsible for this property.

Keywords: Anti-inflammation, *Beta vulgaris*, Carrageenan, Complete Freund's adjuvant

INTRODUCTION

Inflammation is involved in the body's defense system which helps in removal and repairing of damaged tissues and neutralization of harmful agents. Inflammation may release or generate a diverse population of pro-inflammatory mediators like bradykinins, serotonin,

histamines, prostaglandins and nitric oxide.¹ Chronic inflammation can also lead to a number of diseases such as hay fever, rheumatoid arthritis, arteriosclerosis, cardiovascular diseases, diabetes, obesity, pulmonary diseases, neurologic diseases and cancer.² Numerous anti-inflammatory drugs like non-steroidal anti-inflammatory drugs (NSAIDs) are available commercially. NSAIDs

inhibit the production of prostaglandins and thereby reduce pain.³ Diclofenac, the most commonly used NSAIDs, is employed in the treatment of inflammation and also as pain killer. However, various adverse effects like hepatic and renal disorders are reported with these drugs.⁴ Potential harmful effects of synthetic drugs have encouraged researchers, all over the world, to hunt for remedies from natural sources. Apart from having less side effects, natural products are cost-effective as compared to the synthetic drugs. According to the estimate given by World Health Organization (WHO), 80% of the world population is dependent on alternate system of medicine and natural health products.⁵

Beetroot (*Beta vulgaris* L.) is a vegetable plant, belonging to the family Amaranthaceae. Beets are high in vitamin C, fiber and also contain some essential minerals like manganese and potassium. Potassium is essential for healthy nerve and muscle function. Beetroot also contains vitamin B which helps in detoxification of blood and liver. It contains natural minerals that provide strength to bones. For these reasons, beetroot has long used in traditional Arab medicine for treatment of wide variety of disease. It has powerful antioxidant property. In ancient times, it was believed that beet root helps to enhance human sex hormone and was used as an aphrodisiac. So, the juice of beetroot was consumed as a natural remedy for sexual weakness. This is also used to treatment of kidney and bladder stones.⁶ Beetroot extracts also have an important effect on reduction of tumor formation in various animal model when administered in drinking water.⁷ Beetroot also help to reduce the effect of dementia by increasing the blood flow to the brain. Beetroot was also found to improve loss of memory in elderly population.⁸ Previous studies have reported that Beetroot possesses strong antioxidant property. Beetroot also showed abilities to protect protein from denaturation indicating its relevant therapeutic properties.⁹ Present study thus tried to estimate the anti-inflammatory potential of the Beetroot as compared to standard drug, Diclofenac Sodium in various in vivo models.

METHODS

Maintenance of animals

All rats were maintained following Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines at the animal laboratory of R.G Kar Medical College, Kolkata, after getting clearance from Institutional Animal Ethics Committee.¹⁰

Preparation of plant extract

Fresh roots of *Beta vulgaris* L. were obtained from local market and identified by Botanical Survey of India, West Bengal [CNH/2016/Tech II/69/5]. Fresh roots were cut into small pieces, crushed with deionized water (1:2 w/v), filtered and lyophilized to powder.

Carrageenan-induced hind paw edema in rats

Carrageenan-induced hind paw edema in rats were used to estimate acute anti-inflammatory effect of the extract in vivo models.¹¹ 0.1ml of 1% Carrageenan prepared in normal saline (0.9%) were injected subcutaneously into the subplantar region of right hind paw of all animals to induce edema. Rats were divided into six groups; each group consisting of six animals. Group 1 were treated as control, group 2 were induced with carrageenan but without treatment, group 3 were treated with standard drug Diclofenac Sodium (10mg/Kg), group 4, 5 and 6 were treated with aqueous extract of Beetroot, 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively.¹² Doses were selected based on pilot study. The change in paw volume was measured by plethysmometer at 0, 1, 2, 3 and 4 hours respectively. The percentage inhibition in edema was calculated as below:

$$\text{Percentage inhibition} = \left(1 - \frac{\text{Absorbance of Test group}}{\text{Absorbance of Control group}}\right) \times 100$$

Adjuvant induced arthritis Model

This model was used to evaluate the chronic anti-inflammatory effect of beetroot extract in animal models. Initially, body weight of all rats was measured and then 0.1 ml complete Freund's-adjuvants (CFA) was injected into the intraplantar region of right hind paw of rats.¹³ Rats were divided into five groups; each group consisting of six animals. Group 1 were treated as control, Group 2 were treated with standard drug Diclofenac Sodium (10mg/Kg), group 3, 4 and 5 were treated with aqueous extract of Beetroot, 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively.¹²

Doses were selected based on pilot study. Paw volume and ankle diameter of each rat was measured by using Plethysmometer on 0th, 1st, 3rd, 7th, 14th and 21st day. Secondary lesions on ear, nose, tail, fore paw, left hind paw were also estimated.

The scores were shown in Table 1.

Motility test

The movement of rats were determined by this test.

The score is given below:

- Rats avoiding touch with ground- 0
- Rats walking difficulty but toes touching ground- 1
- Rats walking easily- 2.¹⁴

Statistical analysis

Statistical analysis was done by One Way ANOVA followed by Dunnet Test with the help of SPSS version 20.

Table 1: Scores for secondary lesions in animals.

Parameters	Score
Ears	
Absence of nodules and redness	0
Present of nodules and redness	1
Nose	
No swelling of connective tissue	0
Swelling of connective tissue	1
Tail	
Absence of nodules	0
Presence of nodules	1
Fore paw	
Absence of inflammation	0
Presence of inflammation	1
Left Hind paw	
Absence of inflammation	0
Slight inflammation	1
Moderate inflammation	2
Marked inflammation	3

RESULTS

Carrageenan-induced hind paw edema in rats

Acute inflammation induced by injecting Carrageenan in rats were found to be inhibited by 8.3%, 0%, 25% and 25% for Diclofenac, Beetroot extracts 100 mg/Kg, 200 mg/Kg and 400 mg/Kg, respectively, at the end of one hour. At the end of 2nd hour, the inhibitions were 29.6%, 18.5%, 33.3% and 40.7% for the standard drugs and Beetroot extracts 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively. After 3 hours, the inhibitions amount to 38.5%, 17.5%, 32% and 38.4% for the standard drugs and Beetroot extracts 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively. It was observed that at the end of 4th hour, the percentage inhibitions were 42.3% for the standard drug and 26.9%, 34.6% and 50% for beetroot 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively (Figure 1).

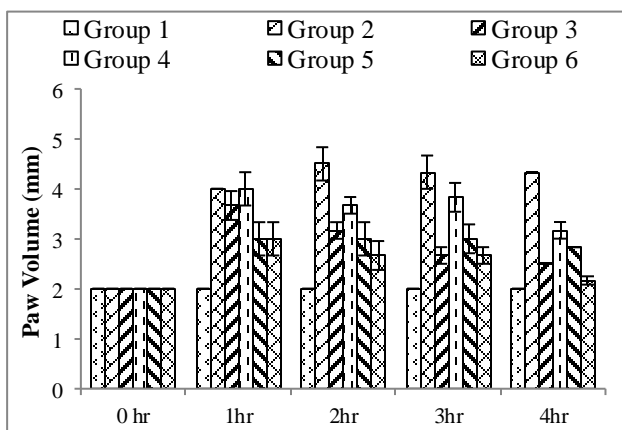
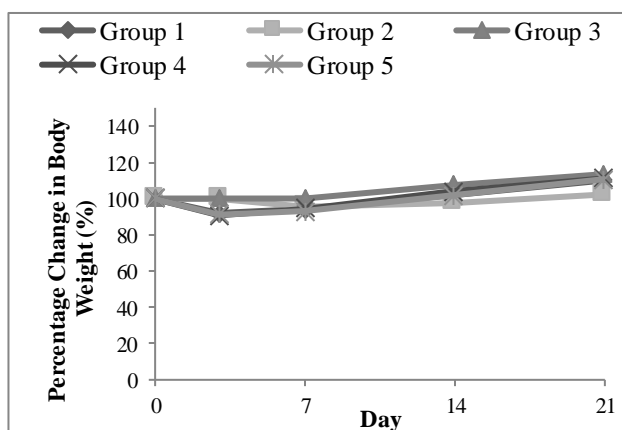


Figure 1: Distribution of acute anti-inflammatory activity of Beetroot (BR) extract with Carrageenan-induced rat paw edema in comparison with standard drug, Diclofenac sodium.

Thus, a dose dependent increase in anti-inflammatory activity was observed in beetroot extracts at 100mg/Kg, 200mg/Kg and 400mg/Kg.

Adjuvant induced arthritis Model

Chronic inflammation was induced in the rats with the help of Complete Freund’s Adjuvant. Figure 2 represented the increase in body weight of all the rats during the study period. It was observed that there was an increase by 10% on average in control group rats. However, the increase in the standard and BR 100mg/Kg, 200mg/Kg and 400mg/Kg, were respectively 2.27%, 13.5%, 11.1% and 10.7%.



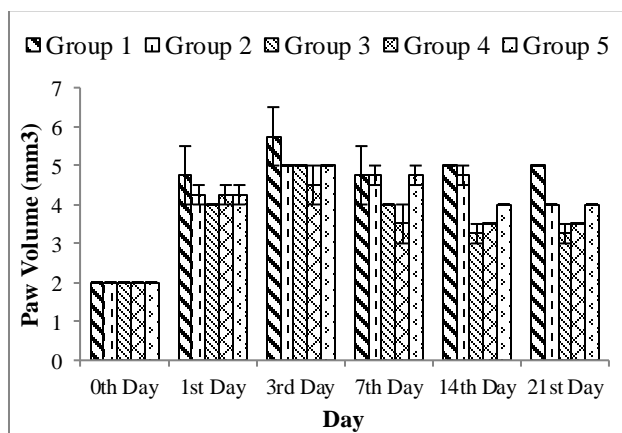
mean±S.E.M

Figure 2: Percentage change in body weight of Freund-induced rats treated with standard drug and Beetroot extracts, at 0th, 7th, 14th and 21st day.

Paw volume of the rats were increased on induction with Freund’s reagent. Present study tried to estimate the reduction in paw volume on treatment with standard drug and beetroot extracts as compared to the control. Figure 3 represented the change in the paw volume in the study groups during the entire study period. After 24 hours, paw volume of control group without treatment, groups receiving standard, 100mg/kg, 200mg/kg, and 400mg/kg of beetroot extracts were respectively 4.75, 4.25, 4, 4.25 and 4.25mm³. After 7th day, the percentage inhibition of paw volume of rats receiving standard, 100 mg/kg, 200mg/kg and 400mg/kg of beetroot extracts were respectively 0 %, 15.7%, 26.3% and 0%, as compared to the control. After 21 days, the percentage inhibition was found to be 20%, 35%, 30% and 20%, respectively for the rats receiving standard, 100 mg/kg, 200 mg/kg and 400 mg/kg of beetroot extracts. Thus, it was observed that there was a gradual decrease in paw volume after treatment with beetroot extracts. However, the decrease was significantly low for the rats under Diclofenac therapy as compared to that of the Beetroot extracts.

Oral administration of Beetroot extracts at doses 100mg/Kg, 200mg/Kg and 400mg/Kg, inhibited the ankle diameter by 5.08%, 8.47% and 0%, after 7 days and

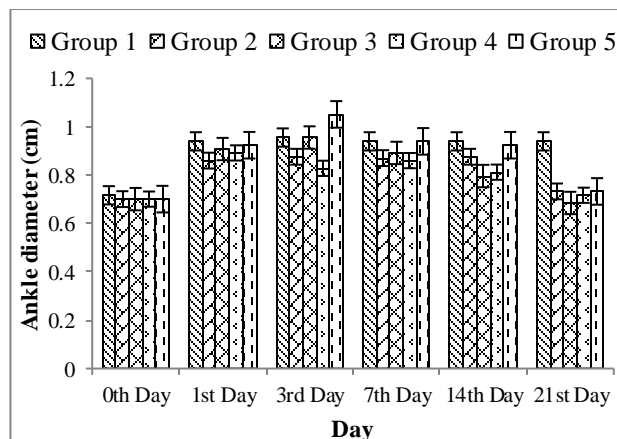
27.11%, 23.72%, 22.03%, after 21 days, respectively, as compared to the control (Figure 4). Thus it was evident that Beetroot extract was effective in reducing paw volume and ankle diameter in Freund's induced paw edema (Figure 5).



mean±S.E.M

Figure 3: Distribution of change in paw volume of Freund-induced rats treated with standard drug and Beetroot extracts, at 0th, 1st, 3rd, 7th, 14th and 21st day.

It was observed from the motility scores (Table 2) that all the rats had difficulty in walking just after induction with Freund's reagent. However, after treatment with standard and beetroot extract, their walking capacity improved as compared to the control rats.



mean±S.E.M

Figure 4: Distribution of change in ankle diameter of Freund-induced rats treated with standard drug and Beetroot extracts, at 0th, 1st, 3rd, 7th, 14th and 21st day.

Table 2: Motility Test score of the rats during the study period.

Group	0 th Day	1 st Day	3 rd Day	7 th Day	14 th Day	21 st Day
Group 1	2	0.5	0	0.5	0.5	1
Group 2	2	0.5	0.5	0.5	1	1
Group 3	2	0	0.5	1	1	1
Group 4	2	0.5	1	1	1	1
Group 5	2	0	0	0.5	1	1

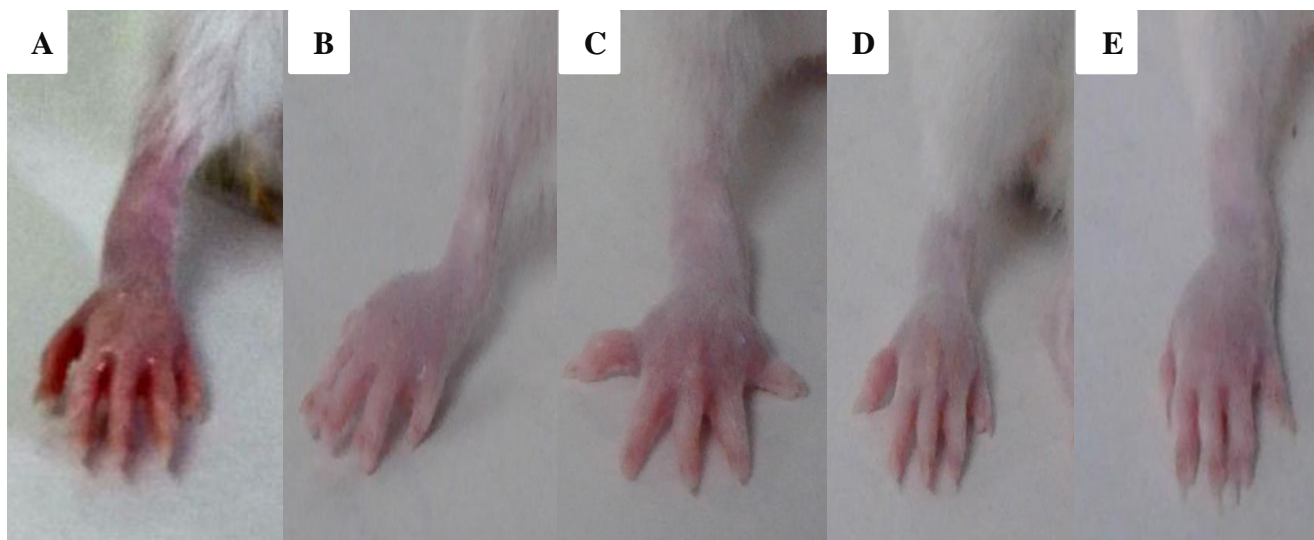


Figure 5: The inhibition of Freund's adjuvant induced paw edema in rats after 21 days of treatment. A, B, C, D and E represent Freund's adjuvant induced control (untreated) paw, standard drug Diclofenac sodium treated paw, Beetroot extracts 100mg/Kg treated paw, 200mg/Kg treated paw and 400mg/Kg treated paw, respectively.

DISCUSSION

Beta vulgaris rubra or red beetroot, a vegetable plant, has been found to possess various health benefits. Present

study aimed to evaluate the anti-inflammatory effect of Beetroot extract with the help of Carrageenan-induced and Complete Freund's Adjuvant induced paw edema model in rats. Rat paw edema induced by carrageenan was found

to be inhibited on oral administration of Beetroot extracts. It was observed that at the end of 4th hour, the percentage inhibitions were 42.5% for the standard drug and 25.83%, 33.75% and 50% for beetroot at 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively.

The body weight of the rats induced with Complete Freund's Adjuvant was estimated for 21 days. It was revealed from this study that body weight of the rats consuming Beetroot increased significantly as compared to the rats under Diclofenac therapy. After 21 days, the increase in body weight was observed to be 2.27%, 13.5%, 11.1% and 10.7%, for Diclofenac and Beetroot doses of 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively.

The increase in paw volume on induction with Freund's reagent could be arrested on treatment with standard drug and Beetroot extract. After 21 days, the inhibition, as compared to the control group, was found to be 35% for 100mg/Kg, 30% for 200mg/Kg whereas 20% for 400mg/Kg of Beetroot extract, whereas for that of Diclofenac was 20%. Inhibition of ankle diameter was 27.11%, 23.72% and 22.03% for 100mg/Kg, 200mg/Kg and 400mg/Kg, respectively. Beetroot extract was thus found to be effective in both acute and chronic inflammation. Anti-inflammatory potential was also evaluated in the leaves of *Beta vulgaris* by Jain et al.¹⁵ Betaine or trimethylglycine, is one of the major components of Beetroot. It protects cells under stress and also is involved in various biological pathways. In vitro experiments deciphered that Betaine possesses significant anti-inflammatory effect. It inhibits Nitric oxide release from activated microglial cells.¹⁶ In vivo research observed that betaine suppressed NF-kappaB and its related gene expressions of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), vascular cell adhesion molecule-1 (VCAM-1), and intracellular cell adhesion molecule-1 (ICAM-1) in aged kidney.¹⁷ Thus high betaine content might be responsible for the anti-inflammatory property of Beetroot. HPLC (High Pressure Liquid Chromatography) analysis of this beetroot extract, confirmed the presence of bioactive polyphenols such as quercetin, sinapic acid, p-coumaric acid, syringic acid, gallic acid, coumarin, caffeic acid, chlorogenic acid and catechin.⁹ Literature survey suggested anti-inflammatory activity of quercetin, coumarin, caffeic acid and chlorogenic acid were evident in in vitro and in vivo systems.¹⁸⁻²¹ However, further investigations are essential to find out the bioactive components involved in the anti-inflammatory activity. Secondary lesions were not much observed in the rat groups. The motility test also confirmed that Beetroot treatment improved the walking capacity of the rats as compared to the normal rats.

Present study evaluated the effect of beetroot in acute and chronic anti-inflammatory rat models. These studies thus support the traditional use of this herb for medicinal purposes. However further investigation is required to validate the effect of Beetroot in inflammation. The biological compounds responsible for this anti-

inflammatory potential of Beetroot also need to be elucidated.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee of R. G. Kar Medical College, Kolkata, India

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