Original Article

Evaluation of anti-inflammatory effect of natural dietary supplement *Beta vulgaris* (Beet root) in animal models of inflammation

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Objective: *Beta vulgaris* is an important part of daily diet and is consumed in salads. Current study was conducted to investigate the anti-inflammatory potential of lyophilized beet root powder.

Methodology: Albino rats weighing 140-200gm were chosen for this study. They were divided into six equal groups. For ethanoic acid induced paw edema model, group I was labeled as control and was orally given 1ml distilled water; groups II was given Ibuprofen as standard (400mg/70kg) and group III was given aqueous solution of lyophilized *Beta vulgaris* at dose of 500mg/kg. 30 minutes after giving these treatments 0.1 ml of 1% w/v ethanoic acid was administered in the subplantar tissue of rat paw. For carrrageenan induced paw edema method, group I (a), group II (a) and group III (a) were given same treatment as above divided groups; only difference was that they were administered 0.1ml of 1% carrageenan in subplantar tissue of rat paw after oral treatment. Swelling of paw was measured using Plethysmometer.

Results: *Beta vulgaris* had highly significant anti-inflammatory activity ($p<0.001$). In ethanoic acid paw edema method, the highest percentage inhibition was 26.9% and it was observed 2 hours after administration of ethanoic acid. In carrageenan induced paw edema, *Beta vulgaris* showed highest inhibitory percentage (41.4%), which was seen after 2 hours of administration of carrageenan.

Conclusion: *Beta vulgaris* had significant anti-inflammatory effects that may be of potential benefit in different inflammatory conditions. The anti-inflammatory effect is observed due to presence of betalain. (Rawal Med J 201;42:385-389)

KEY WORDS: *Beta vulgaris,* carrageenan, ethanoic acid, inflammation.

INTRODUCTION

Inflammation is considered as body's defensive response on exposure to injurious stimuli such as pathogens, irritants and chemicals.[1] Classic signs of inflammation include pain, swelling, local redness and edema.[2] Inflammation is mainly treated by Nonsteroidal anti-inflammatory drugs (NSAIDS), however their prolonged use leads to side effects and higher chances of reccurrence of symptoms after discontinuation.[3] Because of these reasons evaluation and screening of new compounds for anti-inflammatory activity is still ongoing. Currently, focus has been shifted towards plants and herbal therapy.[4]

Vegetables are already known as a source of nutrition, however based on the constituents present in them, they can be consumed as therapeutic agent as well. *Beta vulgaris* comes under family Chenopodiaceae. It is popularly known as Beetroot or garden beet. It has good nutritional value and is rich in vitamins and minerals. The main constituents of beet root are its pigments i.e betalains, which gives it red color. From pharmacological point of view, beet root extract is used as diuretic and inhibitor of calcium oxalate crystal formation.[5] It has also shown to possess antiproliferative activity.[6] More over, lyophilized aqueous extracts of *Beta vulgaris* has been shown to reduce cholesterol and increase HDL levels.[7] Aim of this Study was to explore the anti-inflammatory effect of lyophilized *Beta vulgaris* using two different models of inflammation.

METHODOLOGY

Rodents were taken from the animal house of Department of Pharmacology, University of Karachi with weight of 140-200gm adult albino rat of either sex were selected. They were given free access to water and regular diet and were housed as two rodents per cage at room temperature 22±2°C

and 12hour/12hour light and dark cycle. They were divided into 6 groups and each group comprised of 10 rodents. The study was approved by Karachi University Board of Advanced Studies and Research vide Resolution No10 (P) 18. They were handled as per specification provided in Helsinki Resolution 1964.

Lyophilized powder of *Beta vulgaris* was purchased from Sun Rise Nutra Chem Group with Lot # Ctc 2015 0320. The powder was packed and stored in zip lock plastic bag enclosed in aluminum foil to protect from sunlight. For ethanoic acid induced paw edema method, control group was labeled as Group I and administered 1ml distilled water orally. Group II was labeled as Standard and given Ibuprofen 400mg/70kg orally.[8] Group III was labeled as treated and administered aqueous solution of 500mg/kg *Beta vulgaris* orally.[9] For model of paw edema induction by carrageenan, group I (a) was labeled control and given distilled water 1 ml orally. Group II (a) was labeled standard and administered Ibuprofen 400mg/70kg orally. Group III (a) was administered aqueous solutions of 500mg/kg beet root orally.

1% w/v ethanoic acid was used to induce edema in rat's paw. 0.1ml prepared ethanoic acid was administered in sub-plantar tissue of rats paw 30 minutes following oral treatment.[10] Edema was induced by administering 0.1ml of 1% carrageenan. 30 minutes after the groups received treatment, carrageenan was given parentally in sub-plantar tissue of animal's paw.[11] Plethysmometer (Ugo, Basil, Italy)[10,11] was used for measuring the paw swelling. The following formula was used for calculating the % inhibition: % Inhibition = D_0-D_t / D_0 x 100 Where D_0 represents average paw edema of control rodent at a specific time and D_t represents average paw edema of treated group at same time (treated here will refer to Ibuprofen as well as *Beta vulgaris*).[9]

Data were analyzed by using SPSS version 20.0. Analysis of variance (Two way Anova) was applied followed by post hoc Tukey's test for multiple comparisons. P<0.001 was considered as highly significant.

RESULTS

There was highly significant (p<0.001) reduction in paw swelling by both Ibuprofen and *Beta vulgaris* after 1,2,3 and 4 hours administration of acetic acid as compared to control. *Beta vulgaris* highly significantly (p<0.001) reduced paw swelling as compared to Ibuprofen. However, after 2 hour of administration of ethanoic acid insignificant difference was observed between Ibuprofen and *Beta vulgaris*. Moreover, after 3h of acetic acid administration, *Beta vulgaris* did not show better result than Ibuprofen. The reduction in paw volume was highly significant (p<0.001) by *Beta vulgaris* after 4 hours when compared with Ibuprofen (Table 1). Maximum percent of inhibition by *Beta vulgaris* was observed after 2 hours (26.9%).

Table 1. Anti-Inflammatory effect of *Beta vulgaris* by ethanoic acid model of inflammation.

Groups	Displacement in ml		Displacement in ml & percent of inhibition			
	Baseline	0 hour	1 hour after Ethanoic acid administration	2 hour	3 hour	4 hour
Ethanoic acid + Distilled water	3.54±0.1	3.77± 0.08	4.16 ± 0.03	4.38 ± 0.2	4.18 ± 0.16	4.65 ± 0.87
Ethanoic acid + Ibuprofen 400mg/70kg	3.06 ± 0.4	3.18 ± 0.51	3.56 ± 0.22 (14.4 %) ***	3.13 ± 0.08 (28.5%) ***	2.82 ± 0.12 (32.5%) ***	4.13 ±0.33 (11.1%) ***
Ethanoic acid +*Beta vulgaris* 500mg/kg	2.72 ± 0.54	2.83 ± 0.11	3.33 ± 0.31 (19.9%) ***, ###	3.2 ± 0.09 (26.9%) ***	3.07 ± 0.84 (26.5%) ***, ###	3.5 ± 0.14 (24.7%) ***, ###

Values are Mean ± S.D. Data was analyzed by two way Anova followed by Post hoc analysis using Tukey's test. *** p≤0.001 is considered highly significant as compared to control. ### p≤ 0.001 is considered highly significant as compared to standard

Table 2: Anti-Inflammatory effect of *Beta vulgaris* by Carrageenan induced model of Inflammation.

Groups	Displacement in ml		Displacement in ml & percent of inhibition			
	Baseline	30 minutes after Ethanoic acid administration	1 hour after Ethanoic acid administration	2hour	3hour	4 hour
1% carrageenan + Distilled water	3.02±0.21	3.22± 0.63	3.51 ± 0.04	3.62 ± 0.17	3.81 ± 0.09	4.03 ± 0.77
1% carrageenan+ Ibuprofen 400mg/70kg	2.17 ± 0.36	2.37 ± 0.29	2.83 ± 0.42 (19.3 %) ***	2.53 ± 0.13 (30.1%) ***	2.41± 0.07 (36.7%) ***	3.4 ±0.14 (15.6%) ***
1% carrageenan+ *Beta vulgaris* 500mg/kg	2.04 ± 0.46	2.33 ± 0.16	2.86 ± 0.27 (18.5%) ***	2.12 ± 0.85 (41.4%) ***, ###	2.31 ± 0.36 (39.3%) *** #	3.33 ± 0.19 (17.3%) *** #

Values are Mean ± S.D. Data was analyzed by two way Anova followed by Post hoc analysis using Tukey's test. ***$p \leq 0.001$ is considered highly significant as compared to control. #$p \leq 0.01$, ###$p \leq 0.001$ are considered significant and highly significant as compared to standard

Our results show highly significant ($p<0.001$) decrease in paw swelling by *Beta vulgaris* as well as Ibuprofen after 1,2, 3 and 4 hours of administration of carrageenan as compared to control. *Beta vulgaris* and Ibuprofen showed similar effects in decreasing paw swelling. After 2 hour of administration of carrageenan, highly significant decrease ($p<0.001$) in paw volume was observed by *Beta vulgaris as* compared to Ibuprofen. *Beta vulgaris* showed significant ($p<0.05$) decrease in paw swelling after 3 and 4 hours of administration as compared to Ibuprofen (Table 2). Maximum percent of inhibition (41.4%) was observed by *Beta vulgaris* after 2 hours of carrageenan administration.

DISCUSSION

Ethanoic acid induces inflammation by releasing inflammatory mediators in two phases. First phase is considered as acute phase and is marked by release of serotonin (5HT), histamine and platelet activating factor. This phase lasts for 90 minutes.[12] The second phase is mediated by release of lysosomes, proteases, prostaglandins (PG'S) and kinins.[13] Formation of interleukin-1 (IL-1), IL-2, tumor necrosis factor along with prostaglandin E_2 and F_2 leads to free radical formation which induces inflammation and pain by stimulating the nociceptors.[14] Ethanoic acid may further stimulate cyclooxygenase-2 (COX-2) and induce nitric oxide synthase which further promotes oxidative stress in inflamed tissue of rat's paw.[15] Edema is said to be mediated due to release of histamine, bradykinin and 5HT.[16] Main mechanism by which NSAIDS act is by reduction of synthesis of PG's by inhibiting COX's pathway. This shows NSAIDS act at later stages of inflammation.[17]

Carrageenan induced paw edema method was also incorporated in this study to confirm the anti-inflammatory activity of *Beta vulgaris.*[18] Edema produced by carrageenan is due to biphasic response. Its first phase is mediated by release of kinins, histamine and serotonin, which causes vascular permeability during first 2 hours after administration of carrageenan. The second phase is related with the elevated production of prostaglandins, leukotrienes, free radicals and production of inducible cyclooxygenase.[19]

Beta vulgaris 500mg/kg dose showed maximum percentage inhibition after 2 hours in both models. This shows that anti-inflammatory effect of *Beta vulgaris* is mainly because of inhibition of histamine, serotonin and kinins. However, the inhibition was also observed in the later hours showing that *Beta vulgaris* can also inhibit PG's and cyclooxygenase. However, when compared with Ibuprofen (NSAID), *Beta vulgaris* showed highly significant anti-inflammatory effects after 1 hour showing that dominant mechanism is by suppression of PG'S and cyclooxygenase.

Beta vulgaris is considered as one of the richest

source of anti-oxidants.[20] Nitrogen pigments called betalains are the main source of anti-oxidant activity of beet root.[21] Betalains also reduce the formation of prostaglandins by suppressing and inhibiting cyclooxygenase-2 (COX-2). *Beta vulgaris* has shown marked anti-inflammatory effects in both animal models of inflammation probably because of the presence of betalain.[22] Besides this, *Beta vulgaris* also contains active compounds such as carotenoids,[23] glycine, betaine, saponins,[24] folates, betanin, polyphenols and flavonoids. These compounds are also responsible for free radical scavenging and anti-oxidant activity of beet root. Anti-oxidant activity promotes the anti-inflammatory effect.

CONCLUSION

Beta vulgaris showed positive anti-inflammatory potential. Since it is a daily dietary intake of many people, it can be used for treating acute symptoms of inflammation. However, for evaluation of the exact molecular mechanism of anti-inflammatory action of *Beta vulgaris* further studies need to be conducted.

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