ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF BETA VULGARIS LINN. ROOTS

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Abstract

The present study deals with evaluation of antioxidant and anti-inflammatory activity of ethanolic extract of Beta Vulgaris roots. The ethanolic extract was subjected to screen for antioxidant activity using DPPH radical scavenging method. The anti-inflammatory activity was carried out by using carageenan induced rat paw edema method. The tested extract of different dilutions in range 200 µg/ml to 1000 µg/ml shows activity in range of 4.34% to 18.55%. The extract shows prominent anti-inflammatory activity as compared to that of standard (Ibuprofen gel). The extract shows good anti-inflammatory activity on carageenan induced rat paw edema method.

Keywords: Beta Vulgaris, Anti-inflammatory activity, Antioxidant activity

Introduction

Beta vulgaris (Linn.) is a native of South Europe, extensively cultivated as an article of food and especially for the production of sugar, and presents many varieties. Different beet root compounds, e.g., betalains, became especially important for phytomedicine: betalains (betacyanins and betaxanthis) have been detected only in red-violet-, orange-and yellow-pigmented botanical species belonging to closely related families of the order caryophyllales ¹. Betalain pigments have specifically been shown to possess various antioxidant functions². Antioxidant and phase II enzyme (quinine reductase)-inducing activities of beetroot were enriched in regulate and high-pigment red phenotypes compared to orange and white phenotypes³. Antioxidants, which in many cases are free radical scavengers or quenchers of activated states, comprise a vast number of classes of organic molecules including most prominently the phenolics. About 0.5-1% of extracted beet juice solids are betalains. In addition to the pigments, the root contains about 700-800 mg oxalic acid and 5-6 mg ascorbic acid per 100 g beetroot ⁴. The Beetroot contains about a tenth portion of pure sugar, which is one of the glucoses or fruit sugars and is very wholesome. It is softer than cane sugar and does not crystallize as well as the latter. There is a treacle principle in it, but this renders it all the more nutritious.
Canesugar has to be converted by the digestive juices into fruit sugar, before the body can absorb it, but the sugar present in the Beetroot is already in the more easily assimilated form, thus making the Beet a valuable food. Its sugar is a force-giver and an energy creator, a source of vitality to the human body. Besides its tenth portion of pure sugar, Beetroot has as much as a third of its weight in starch and gum. Paste has been used for application to ulcer, inflammation and skin troubles. Beets also contain small amounts of calcium, Vitamin A, Vitamin C, potassium, magnesium, copper, folate, phosphorus, and iron. Anti-inflammatory agents are the agents which normally inhibit the release of these inflammatory mediators. The present study deals with traditionally claimed use of Beta Vulgaris. The in vitro antioxidant activity was screened by DPPH method. Anti-inflammatory study was screened by carageenan induced rat paw edema method.

Material and Methods

Plant material and Preparation of Herbal Extract: Beta Vulgaris (BV) roots were purchased from the local market and authenticated in Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University Campus, Nagpur. Initially these roots were washed with fresh water to remove adhering dirt and foreign particles. Excess of water shake off and dried at 35 - 40°C in an oven for 24 hours. The dried roots were subjected to crushed and grinded and the coarsed powder mass of roots weighed and allow to undergo continuous heat extraction.

The shade dried and a powdered root of Beta Vulgaris (1 kg) was extracted exhaustively with 95% ethanol in a soxhlet apparatus by continuous heat extraction. The ethanol extract was concentrated to a small volume and then evaporated to dryness. The percent yield of Beta Vulgaris roots extract is 23.26%. The dry extracts were subjected to various chemical tests to detect presence of different photochemical constituents.

Animals: In the present study male wistar rats (150-200g) were used for the study. They were individually housed and maintained on normal standard diet (Gold Muhor Brand, Lipton India limited) and water ad libitum. Temperature was maintained at 23±1°C with 12hr light and 12hr dark cycle throughout the course of the study.

Antioxidant activity: The DPPH (1,1-diphenyl-2-picryl hydrazyl) method was used for the determination of in vitro antioxidant activity for crude extract. DPPH is stable free radicals scavenge by antioxidant present in test solution.
To 1.0mL of 0.01mM Solution of DPPH, 1.0mL of ethanolic solution and 0.95mL of HCl buffer of 0.05M was added. To this solution 50µl of extract of specific strength was added. It was kept for 20 min for reaction at room temperature and then absorbance was recorded at 517 nm. Control solution was also carried out without extract. Percent scavenging of DPPH radical was calculated by comparing absorbance between the test and diluted control mixture10, 11.

**Anti-inflammatory Study:** Anti-inflammatory activity of BV extract was studied by carrageenan induced paw edema method. The animals were divided into three groups containing six animals in each group. Group 1 (control) untreated group, Group 2 topical application with BV extract, Group 3 topical application of Ibuprofen gel. Group 2 and 3 received topical application of BV extract and Ibuprofen gel for comparison of anti-inflammatory activity. One hour after the application of BV extract 0.1 ml of carrageenan (1%) was injected into subplanar region of hind paw of rat. The BV extract was applied on the palm of the paw of the rat. Measurement of paw volume (ml) were done by mercury displacing techniques using plethysmometer immediately before and 1, 2, 3 and 4 hr after carrageenan injection. Percentage inhibition of inflammation after 1,2,3 and 4 hr was calculated by Newbould’s method 12. Statistical difference between the groups were evaluated using one way analysis of variance (ANOVA) followed by Turkey’s Kramer Multiple comparison test (P < 0.001).

**Results**

The present study deals with evaluation of antioxidant and anti-inflammatory activity of ethanolic extract of roots of *Beta Vulgaris*. The method used to determine the peroxide value or scavenging activity is based on decomposition or scavenging of DPPH which is a very stable free radical. The comparative percent peroxide value of BV extract is shown in Figure 1 Percent peroxide value for BV extract is in the range of 4.34% to 18.55% for the extract with 200 µg/ml to 1000 µg/ml strength extract.

The anti-inflammatory study was performed by carrageenan induced rat paw edema method. The acute toxicity study of ethanolic extract of roots of *Beta Vulgaris* do not show any signs of toxicity up to 3g/kg body weight. Since there was no mortality at higher dose 1/10th of maximum dose of extract tested for acute toxicity was screened for evaluation of wound healing activity i.e., 300mg/kg.
The topical anti-inflammatory activity of BV extract was carried out. The percentage protection (inhibition) of edema for BV extract and Ibuprofen gel was found to be 12.56 (1hr), 71.49 (2hr), 37.91 (3hr) 47.05 (4hr) and 36.54 (1hr), 64.85 (2hr), 63.83 (3hr), 67.62 (4hr) respectively. The tested extract showed significant anti-inflammatory activity when the results are compared with untreated group and decreased in inflammation by Ibuprofen gel was taken as reference standard. The results are highly significant (p< 0.001) and are shown in Table 1 and 2.

Discussion

The determination of anti-inflammatory activity is based on plethysmographic measurement of edema produce by sub planer injection of carageenan in hind paw of rat. The increase edema in animal treated standard (Ibuprofen gel) and BV extract were composed with increase in edema of untreated control animals at constant interval of 1, 2,3 and 4hrs. The percentage inhibition of edema at known interval in treated animals was used for the purpose of calculating the percent inhibition of edema of control. The present study revealed that the BV extract showed significant inhibition of edema. The maximum activity showed during 2nd and 3rd hrs, the results are highly significant (p<0.001) as compared to standard. The anti-inflammatory activity may be due to inhibition of release of histamine, serotonin and kinins in first hour after injection of carageenan and also retard the release of prostaglandin and like substances in 2-4hr 13, shows anti-inflammatory activity of BV extract.

Conclusion

The plant extracts also shows antioxidant activity i.e., free radical scavenging activity which might be also due to presence of terpenoids. Which reduces lipid peroxidation, may not only prevent or slows down the onset of necrosis but also improve vascularity 14. Thus it may be concluded that the root of Beta Vulgaris shows significant antioxidant and anti-inflammatory activity.

REFERENCES


Figure-1

Comparative antioxidant activity of ethanolic extract of Beet Vulgaris roots.

![Graph showing antioxidant activity](image)

Table-1

Anti-inflammatory activity of ethanolic extract of Beta Vulgaris roots.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Weight of Animals (mg/kg)</th>
<th>Dose (mg/kg)</th>
<th>Mean value ± SEM of edema volume at different interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3156 ± 3.03</td>
<td>200</td>
<td>0.7576 ± 7.75</td>
</tr>
<tr>
<td>2</td>
<td>0.2267 ± 3.70</td>
<td>300</td>
<td>0.4265 ± 4.21</td>
</tr>
<tr>
<td>3</td>
<td>0.2014 ± 1.77</td>
<td>300</td>
<td>0.2488 ± 1.78</td>
</tr>
</tbody>
</table>

F values

(2,15) = 4.141, (2,15) = 6.759, (2,15) = 1.829, (2,15) = 2.246

Values are mean ± S.E.M., *P<0.001 vs. control; Student’s t-test.

a N = 6, b topical application
Table 2

Effect of ethanolic extract of *Beta Vulgaris* roots on percent inhibition of paw volume.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard b</td>
<td>300</td>
<td>36.54±1.76</td>
<td>64.85±1.76</td>
<td>63.83±1.58</td>
<td>67.62±1.33</td>
</tr>
<tr>
<td>(Ibuprofen gel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test b</td>
<td>300</td>
<td>19.56±2.12</td>
<td>59.49±2.22</td>
<td>55.91±1.67</td>
<td>46.05±3.44</td>
</tr>
<tr>
<td>(BV extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., *P<0.001 vs. control; Student’s t-test.

a n = 6, b topical application