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NOTE

Free Radical Scavenging Activity of Betalaine of *Beta vulgaris*

ASHOKA BABU V.L.*, SHANAZ BANU†, DHANAPAL R.,
VRUSHABENDRA SWAMY B.M., G.S. KUMAR and SRIDHAR CHANDANAM
Department of Pharmaceutical Chemistry
Rural College of Pharmacy, Devanahalli, Bangalore-562 110, India
Email: ababu007us@yahoo.com

The present study deals with the antioxidant potential of betalaines. The acidified aqueous extract of the plant (root) was investigated for *in vivo* effect on the production of body enzymes, *i.e.*, catalase and superoxide dismutase. The extract has shown significant anti-oxidant activity.

Key Words: Betaxanthin, Betacyanin, Free radical scavenging, Catalase superoxide dismutase.

Beta vulgaris, also known as red beet (family Chenopodiaceae) is a small sized plant, cultivated in many parts of India. The leaves of *Beta vulgaris* possess diuretic, purgative and antiinflammatory activity, seeds are known to possess expectorant and carminative properties and roots possess sedative and emenogogue effects[1]. It is also used as a natural food colour in dairy and meat products[2].

Betalaine comprises of two main groups, the red violet betacyanin group and the yellow betaxanthine group. The betalaine group contains about 50 red pigments and 20 yellow pigments. Betanine accounts for *ca.* 75–90% of total betacyanin content and betaxanthnin comprises of vulgaxanthine-I and vulgaxanthine-II.

Chemically, the chromophore of betalaine is described as a protonated 1,2,4,4,7-penta-substituted 1,7-diazaheptamethine system and is polyphenolic in nature[3]. Based on the chemical nature of pigments, in the present study, we determined the anti-oxidant potential of betalaine.

Red beet root of variety "Asoka" obtained from local market (Bangalore, India) was used to extract beet colour. The root was identified by the Botanist of Rural College of Pharmacy, Devanahalli. The voucher specimen (BCSF) was kept in the laboratory for future reference.

†Department of Pharmacognosy, Dayanand Sagar College of Pharmacy, Bangalore-562 078, India.

Extraction of pigments[4]: Roots were washed, peeled and the edible portion was grated. The grits were homogenized in glass distilled water (adjusted to pH 5.2 using 0.1 N HCl) using a servoll cell homogenizer and centrifuged at 10,000 xg at 4°C for 10 min. The supernatant was analyzed spectrophotometrically (Shimadzu, double beam) for betacyanin (Bc) and betaxanthin (Bx). Total betalaine content was quantified by summing up the values of Bc and Bx. The beet extract was then concentrated in vacuum at 20°C to obtain a semi-solid slurry containing 100 mg of betalaine per mL.

Animals: Male wistar albino rats (250–300 g) were obtained from Animal House, Rural College of Pharmacy, Devanahalli maintained under standardized environmental conditions (22–28°C, 60–70% relative humidity) at 12 h dark/light cycle and fed with standard rat feed and water *ad libitum*.

***In vivo* antioxidant evaluation**

Animals were divided into three groups of six animals each. One group was kept as control and given only vehicle. Group 2 and 3 animals received two concentrations, *i.e.*, 100 mg/kg body weight and 200 mg/kg body weight, respectively. The animals were given drug for 5 days continuously and on 6th day 1 h after administration of the drug by oral route, the rats were sacrificed for biochemical estimation.

Isolation of blood serum: The animals were anaesthetized with diethyl ether and blood was collected through sino-ocular vein. The blood was centrifuged at 2500 rpm for 20 min to get clear supernatant solution which was used for analysis.

Preparation of the brain homogenate: Rats were sacrificed with euthanasia and brains were removed after decapitation, weighed and homogenized immediately with teflon plunger in ice-chilled 10% KCl solution (3 mL/g tissue). After centrifugation at 2000 rpm for 10 min, the clear supernatant liquid was used for determination of enzyme levels.

Catalase assay[5]: 2.25 mL of potassium phosphate buffer (65 mM, pH 7.8) and 0.1 mL of brain homogenate (3 mL/gm tissue in 10% KCl solution) or blood serum (sucrose for blank) were incubated at 25°C for 30 min. 0.65 mL of H_2O_2 (7.5 mM) mL was then added to initiate the reaction and change in absorption at 240 nm was measured for 3 min. Results are expressed as CAT units/0.1 mL of the sample.

Superoxide dismutase assay[6]: Pipetted out 2.8 mL of carbonate buffer, 0.1 mL brain homogenate or blood serum (sucrose for blank) incubated for 30 min. Absorbance was set at zero and then added 0.1 mL of adrenaline solution to the sample cells. Readings were taken at 480 nm at 1 min intervals for 12 min. Standard calibration curve was prepared for comparison. Results are expressed as units of SOD activity/0.1 mL of sample.

From Table-1, it is clear that the plant extract has significantly raised the catalase and SOD at both dosage schedules, indicating the significant antioxidant activity of betalaine.

TABLE-1
EFFECT OF BETALAINE EXTRACT ON CATALASE AND SUPEROXIDE DISMUTASE

S. No.	Group	Catalase		SOD	
		Blood	Brain	Blood	Brain
1.	Control	3.70 ± 0.51	2.133 ± 0.439	0.911 ± 0.201	0.727 ± 0.509
2.	Plant extract (100 mg/kg bw)	4.98 ± 0.235[a]	3.365 ± 0.351[b]	0.937 ± 0.001	0.735 ± 0.004
3.	Plant extract (200 mg/kg bw)	5.69 ± 0.308[c]	3.67 ± 0.188[c]	1.487 ± 0.09[a]	1.22 ± 0.165[b]

Data are expressed as mean ± S.D. (n = 6), a = $p < 0.5$, b = $p < 0.1$, c = $p < 0.01$.
bw = body weight.

The results suggest that the antioxidant action is attributed to the free radical scavenging activity of the extracts. The activity may be attributed to the polyphenolic nature of betalaine. But many researches showed that the stability of betalaine is greatly affected by temperature, light, air and pH[7, 8], which reveals that efforts should be made to improve the stability of betalaine for the better utilization of their antioxidant potentials.

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