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Evaluation of Antioxidant Activity of *Beta vulgaris* Root Extract in Rats

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The antioxidative activity of the mewthanolic extract of *Beta vulgaris* roots (MEB) was investigated in rats with carbon tetrachloride (CCl_4) induced erythrocyte damage. Simultaneous intraperitoneal administration of the crude extracts (100 and 200 mg/kg body weight/day) with CCl_4 (1 mL/kg of body weight) to rats for alternate days of two weeks prevented the loss of functional integrity and membrane lipid alteration in red blood cells induced by oxidative stress. *Beta vulgaris* extract inhibited the accumulation of lipid peroxidation products in the plasma as well as maintained the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. The extracts further had the ability to decrease the membrane fluidity induced by CCl_4. It can therefore be suggested that the root extract of *Beta vulgaris* possess an erythrocyte protective activity against drug induced oxidative stress.

Key Words: *Beta vulgaris*, Antioxidative activity, Carbon tetrachloride, Erythrocyte.

INTRODUCTION

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. It is widely accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias[1]. Free radicals such as hydroxy radicals, superoxide anion radicals and singlet oxygens are agents that attack the unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation and lipid peroxidation is also strongly associated with aging and carcinogenesis[1]. However, living systems are protected from active oxygen species by enzymes such as superoxide

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dismutase, glutathione peroxidase and catalase. These living systems have also been reported to receive non-enzymatic protection by endogenous antioxidants such as α-tocopherol, ascorbic acid, β-carotene and uric acid. Generally food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions[2].

Beta vulgaris, also known as red beet (F: chenopodiaceae), is a small sized plant, cultivated in many parts of India. The leaves of *Beta vulgaris* possess diuretic, purgative and antiinflammatory activity, seeds known to possess expectorant and carminative properties, roots possess sedative and emenogogue effects[3]. It is also used as a natural food colour in dairy and meat products[4].

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 vulgaxanthine I and vulgaxanthine II.

Chemically, the chromophore of betalaine is described as a protonated 1,2,4,4,7-penta substituted 1,7-diazaheptamethin system and are polyphenolic in nature[5]. Based on the chemical nature of pigments, in the present study, the antioxidant potential of betalaine in CCl_4 induced rat models of erythrocyte damage is determined using lipid peroxidation and the antioxidants superoxide dismutase (SOD) and catalase as biomarkers.

EXPERIMENTAL

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

Extraction of pigments[6]: Roots were washed, peeled and the edible portion was grated. The grits were homogenized in methanol using a servoll cell homogenizer and centrifuged at 10,000 xg at 4 ºC for 10 min. The supernatant was analyzed spectrophotometrically (Shimadzu, double beam) for betacyanin (Bc) and betaxanthin (Bx). Total betalaine content was quantified by summing the value of Bc and Bx. The beet extract was then concentrated under vacuum at 20 ºC to obtain a semi solid slurry containing 100 mg of betalaine/mL.

Animals: Mature albino male rats (Wistar) weighing 180-195 g were used in this study. They were supplied with standard pellet diet (Hindustan Lever) and water *ad libitum*. The rats were divided into six groups and housed in wire-meshed cages for 6 days to acclimatize them to the experimental environment before the start of the experiment.

Experimental design: Group-I animals served as control, each of them was intraperitonially (i.p.) administered by 2 mL of propylene glycol (PG)/kg body weight on the alternate days for 14 days. Group-II received the combination of CCl_4 and PG by i.p. injection on the alternate days for 14 days. Group III and IV

were the herb-treated ones which received CCl_4 by sub cutanious administration (s.c.) and i.p. injection twice a week as mentioned above. Each of them also received MEC daily at the dose of 70 mg/kg body weight (effective dose) in a suspension of 1 mL water, orally by intubation.

Propylene glycol (PG) was used as a carrier of methanolic extracts of *Beta vulgaris* (100 and 200 mg/kg body weight/day) as well as of CCl_4 (1 mL/kg body weight), administered i.p. on the alternate days for 14 days. The following experimental groups were used: Group I, II, III, IV, V and VI were received distilled water + PG (normal control), 100 mg MEB/kg + PG (plant control), MEB 200 mg/kg + PG (plant control), CCl_4 in PG, MEB 100 mg/kg + CCl_4 in PG, MEB 800 mg/kg + CCl_4 in PG, respectively. On the 15th day rats were kept fasting for 12 h and sacrificed by cervical dislocation. Blood was collected from the jugular vein into tubes containing heparin, centrifuged at 3000 rpm for 15 min and the resulting buffy coat was removed. The packed cells were washed three times with physiological saline (0.9 % NaCl), lyzed by suspending them in cold distilled water and then centrifuged at 7000 rpm for 30 min. The resulting pellet contained the erythrocyte membrane and the supernatant represented the haemolyzate.

Biochemical estimation: Plasma resulting from the initial centrifugation was used for measuring lipid peroxidation following the method[7], while the haemolyzate was used for the estimation of superoxide dismutase[8] and catalase activities[9].

Lipids from the erythrocyte membrane were extracted using the method[10]. The concentration of cholesterol and phospholipids were determined using previously established methods[11], the cholesterol/phospholipid ratio was then calculated.

Statistical analysis: The data, presented as means SD, were analyzed using ANOVA. Duncan's multiple range test (DMRT) was used to determine significant differences between means. The results were considered statistically significant if the P values were 0.05 or less.

RESULTS AND DISCUSSION

Table-1 shows the effect of methanolic extract of *Beta vulgaris* on CCl_4 induced oxidative stress. Treatments with the extracts significantly prevented the accumulation of lipid peroxidation products in the plasma. Intoxication of the rats with CCl_4 also led to significant increases in superoxide dismutase and catalase activities, while simultaneous administration of CCl_4 with the extracts decreased these activities.

Intoxication with CCl_4 brought about an increase in membrane cholesterol, a decrease in membrane phospholipid and a subsequent increase in the cholesterol to phospholipid ratio (Table-2). The results obtained in this study indicate the rigidity of the membranes. Administration of methanolic extract of *Beta vulgaris* prevented changes in membrane lipids as well as those in membrane fluidity.

It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases[12]. Under normal physiological conditions low concentrations of

lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed due to cell damage. Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase normally challenge oxidative stress. In this study, CCl_4 damage to erythrocytes was confirmed by the increase in lipid peroxidation products, superoxide dismutase and catalase activities and decrease in membrane fluidity. The increased superoxide dismutase activity resulted in the accumulation of hydrogen peroxide, which stimulated increase in catalase activity. Pre-treatment of experimental animals with the methanolic extract of *Beta vulgaris* exhibited an improved free radical scavenging resulting in decreased activities of superoxide dismutase and catalase and the concentration of lipid peroxidation products towards normal.

TABLE-1
EFFECTS OF *Beta vulgaris* EXTRACT ON LIPID PEROXIDATION PRODUCTS AND PRIMARY ANTIOXIDANT ENZYMES OF THE ERYTHROCYTES OF CCl_4-INTOXICATED RATS

Design of treatments	Lipid peroxidation $\times 10^{-6}$ (units)	Enzyme activities (units/mg protein)	
		Superoxide dismutase	Catalase
(Control, PG)	0.28 ± 0.03	192.6 ± 9.3	1.9 ± 0.2
(MEB 100 mg/kg + PG)	0.27 ± 0.05	193.2 ± 6.7	1.8 ± 0.1
(MEB 200 mg/kg + PG)	0.28 ± 0.02	190.7 ± 10.2	1.8 ± 0.2
(CCl_4 + PG)	0.47 ± 0.03^{c}	272.4 ± 1.7^{c}	4.7 ± 0.6c
(CCl_4 + MEB 100 mg/kg)	0.39 ± 0.04^{ce}	228.1 ± 2.3^{ce}	3.8 ± 0.2^{ce}
(CCl_4 + MEB 200 mg/kg)	0.34 ± 0.06^{ce}	214.0 ± 1.8^{ce}	3.3 ± 0.6^{ce}

Values are means ± standard deviation for 6 rats/group. Means in the same column having different superscript are significantly different ($p < 0.05$). MEB = Methanolic extract of *Beta vulgaris* root; PG = Propylene glycol.

TABLE-2
EFFECT OF *Beta vulgaris* EXTRACT ON ERYTHROCYTE MEMBRANE LIPIDS AND CHOLESTEROL/PHOSPHOLIPID RATIO OF CCl_4-INTOXICATED RATS

Design of treatments	Cholesterol (mg/100 µL)	Phospholipid (mg/100 µL)	Cholesterol/phospholipid
(Control, PG)	0.64 ± 0.03	1.08 ± 0.04	0.60 ± 0.04
(MEB 100 mg/kg + PG)	0.63 ± 0.02	1.09 ± 0.03	0.61 ± 0.02
(MEB 200 mg/kg + PG)	0.64 ± 0.04	1.07 ± 0.04	0.59 ± 0.05
(CCl_4 + PG)	0.83 ± 0.05^{c}	0.85 ± 0.02^{c}	0.98 ± 0.03^{c}
(CCl_4 + MEB 100 mg/kg)	0.72 ± 0.02^{ce}	0.94 ± 0.03^{ce}	0. 77 ± 0.02^{ce}
(CCl_4 + MEB 200 mg/kg)	0.67 ± 0.03 ^{ce}	0.99 ±0.02 ^{ce}	0.70 ± 0.04 ^{ce}

Values are means ± standard deviation for 6 rats/group. Means in the same column having different superscript are significantly different ($p < 0.05$). MEB = Methanolic extract of *Beta vulgaris* root; PG = Propylene glycol.

The cumulative effect of CCl_4 resulted in increases in erythrocyte membrane peroxidation, which might also lead to haemolytic changes. It has been shown that microviscosity of a membrane increases markedly with increases in cholesterol to

phospholipid ratio thus leading to cellular rigidity. Intoxication of experimental animals with CCl_4 altered membrane structure and function as shown by the increases in cholesterol and subsequent decreases in phospholipid concentrations. Hence increased cholesterol to phospholipid ratio reported that alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system[13]. Thus, *Beta vulgaris* plays a role in peroxidation by inhibiting the free radical attack on bio-membranes.

Previous phytochemical investigations on this plant demonstrated the presence of poly phenolic compounds. These constituents have been reported to protect lipids, blood and body fluids against the attack of reactive oxygen species like superoxide, peroxide and hydroxyl radicals. The presence of poly phenolic compounds in *Beta vulgaris* extract might be responsible for their observed antioxidant activity.

Since reactive oxygen species are involved in stress and pathogenesis of cancer, diabetes mellitus, atherosclerosis and dementia, the use of this plant may be beneficial in preventing initiation or progress of such disorders.

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REFERENCES

1. K. Yagi, *Chem. Phys. Lipids*., **78**, 8658 (1987).
2. J. Groff and S. Gropper, Advanced Nutrition and Human Metabolism, Belmont: Wadsworth, edn. 3, pp. 225-227 (2000).
3. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, Vol. III, Periodical Experts, Delhi, India, pp. 2310-2311 (1975).
4. H.P. Bias, R. Madhusudhan, N. Bhagyalakshmi, T. Rajashekaran, B.S. Ramesh and G.A. Ravishankar, *Acta Physiol. Plantarum*, **22**, 151 (2000).
5. J.H. Von Elbe, Current Aspects of Food Colourants, Basic Symposim Series, pp. 29-39 (1978).
6. M. Abeysekere, S.R. Sampathu and M.L. Shankaranarayana, *J. Food Sci. Tech*., **27**, 336 (1990).
7. J.M.C. Gutteridge and C. Wilkins, *FEBS Lett*., **137**, 327 (1982).
8. H.P. Misra and I. Fridovich, *J. Biol. Chem*., **247**, 3170 (1972).
9. R.F. Beers and I.W. Sizer, *J. Biol. Chem*., **195**, 133 (1952).
10. J. Folch, M. Lees and G.H.S. Stanley, *J. Biol. Chem*., **226**, 497 (1957).
11. H.V. Connerty and A.R. Briggs, *Clin. Chem. Acta*, **7**, 37 (1961).
12. G.R. Martin, D.B. Danner and N.J. Holbrook, *Planta Med.*, **64**, 120 (1998).
13. R.A. Cooper, J.R. Durocher and M.H. Leslie, *J. Clin. Invest*., **60**, 115 (1977).

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