Antioxidant and alleviatory effects of hydroalcholic extract of cauliflower leaves against sodium fluoride-induced cardiotoxicity in Wistar male rats

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The objective of the present study was to explore the alleviatory effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* (*BOB*) leaves against sodium fluoride (NaF)-induced cardiotoxicity. Animals served as group I (normal control), group II (toxic control) and groups III–V (treatment groups) received extract at doses of 100, 200 and 400 mg/kg body wt respectively. Group VI served as plant control and received extract at a dose of 400 mg/kg body wt. All groups, except groups I and VI, received NaF (100 ppm) through drinking water for 30 days. Results showed that administration of extract significantly minimized elevated serum levels of CK-MB and LDH, decreased cardiac lipid peroxidation, increased levels of reduced glutathione content and catalase enzyme in a dose-dependent manner. The study revealed that *BOB* leaves show moderate antioxidant and alleviated sodium fluoride induced cardiotoxicity.

Keywords: Antioxidant activity, cardiac markers, cauliflower leaves, hydroalcoholic extract, sodium fluoride.

FLUORIDE is considered as a semi-essential anion which plays a key role in the treatment of dental problems. However, greater intake of fluoride can cause serious illness by inducing excessive production of free radicals and profound oxidative stress; this has been well-discussed in various biological systems, including cardiac tissue[1]. Fluoride inhibits the activity of various *in vivo* antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase, and decreases the glutathione content. Fluoride interacts with various types of cellular reactions such as gene expression, cell-cycle progression, migration, respiration, detoxification, transportation of ions, secretion, vesicular process and apoptosis, and also alters the mRNA expression of neural cell adhesion molecules in rat hippocampus neurons[2,3]. Therefore, fluoride-induced oxidant–antioxidant imbalance plays a decisive role in the progression of cardiac failure and ischaemia[4].

Diet plays an important role in healthcare management. It is widely agreed that natural diets are a rich source of vitamin C, polyphenols and nutrients. Previous studies have clearly suggested that natural diets potentially minimize fluoride-induced elevated lipid peroxidation and decrease antioxidant enzyme levels in cardiac tissue[5–7].

Brassica oleracea var. *Botrytis* (*BOB*), popularly known as cauliflower, is a widely available and most commonly used edible plant material throughout the world. It is a good source of polyphenols[8], which are reported to be well-known secondary metabolites for the treatment of various ailments, viz. hepatotoxicity[8], nephrotoxicity[9], diabetes mellitus[10], neoplasm[11] and coronary heart diseases[12] through their antioxidant properties and also elevate the antioxidant enzyme levels. The aim of the present study was to explore the alleviatory effects of hydroalcoholic extract of *BOB* leaves against sodium fluoride (NaF)-induced toxicity in cardiac muscle.

Materials and methods

Collection and authentication of plant material

Leaves of *BOB* were collected from the local market of Kadapa, YSR Kadapa district, Andhra Pradesh, India. Plant material was authenticated by Sunita Garg (Raw Material Herbarium and Museum, CSIR-NISCAIR, Delhi) and voucher specimen was stored in the Department of Pharmacognosy, CMR College of Pharmacy, Hyderabad.

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Preparation of the extract

Hundred grams of *BOB* powder was mixed with water–alcohol (30 : 70) in a round-bottom flask and left for seven days at room temperature with occasional stirring. After seven days, the filtrate was separated using Whatman No. 1 filter paper and evaporated with a rotary flash evaporator. The yield was calculated as 31.4%. *BOB* leaves extract was stored at 4°C for further studies.

In vitro antioxidant studies

The DPPH radical scavenging activity[13], ABTS radical scavenging activity[14], metal chelating assay[15], total antioxidant activity[16], reducing power assay[17], total flavonoid content[18] and total phenol content[19] were estimated according to standard protocols.

Preparation of 100 ppm fluoride water

To prepare fluoride water 221 mg of NaF was dissolved in 500 ml of tap water (<1 ppm F^-), and final volume was made up to 1 litre.

Experimental animals

Male Wistar albino rats (36) weighing between 220 and 250 g were procured from Sai Thirumala Enterprises, Hyderabad. The animals were acclimatized for 10 days before starting the experiment. Rat was fed with standard diet and water *ad libitum*. Animals were maintained at a photoperiod of 12 h light/dark cycle. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC no. CPCSSEA/1657/IAEC/CMRCP/PhD-14/35).

Acute toxicity studies

Acute toxicity study was performed for hydroalcoholic extract of *BOB* leaves according to OECD 125 guidelines.

Experimental design

The dose of NaF was selected based on previous studies[20]. After ten days of adaptation period, the rats were divided into six groups ($n = 6$) for treatment as follows:

Group I – normal control: the animals received drinking water.

Group II – toxic control: NaF (100 ppm) through drinking water for 30 days.

Group III – hydroalcoholic extract of *BOB* at an oral dose of 100 mg/kg body wt/day + NaF (100 ppm) through drinking water for 30 days.

Group IV – hydroalcoholic extract of *BOB* at an oral dose of 200 mg/kg body wt/day + NaF (100 ppm) through drinking water for 30 days.

Group V – hydroalcoholic extract of *BOB* at an oral dose of 400 mg/kg body wt/day + NaF (100 ppm) through drinking water for 30 days.

Group VI – plant control: hydroalcoholic extract of *BOB* at an oral dose of 400 mg/kg body wt/day for 30 days (p.o.) alone.

After the treatment schedule, animals were fasted overnight and approximately 1 ml of blood was collected through retro orbital plexus. Blood samples were centrifuged at 2500 rpm for 15 min to obtain the serum, for the estimation of various biochemical parameters like creatine kinase-MB and lactate dehydrogenase (LDH) using Coral Diagnostic Kits, India. Heart tissue homogenized and the post mitochondrial supernant were used for the estimation of lipid peroxidation, and reduced glutathione (GSH) and catalase levels[21–23].

Statistical analysis

The values were expressed as mean ± SEM, $n = 6$ in each group. Statistical analysis was performed using one-way ANOVA followed by post-hoc Dunnett's test. The values were significant at $P < 0.05$.

Results

In vitro antioxidant activity

The IC_{50} values for DPPH radical scavenging activity were found to be 6.8 and 380.09 μg/ml for the standard vitamin C and plant extract respectively. The IC_{50} values for ABTS radical scavenging activity were found to be 14.1 and 191.23 μg/ml for the standard vitamin C and plant extract respectively. The IC_{50} values for metal chelation assay were found to be 76.19 and 482.09 μg/ml for the standard EDTA and plant extract respectively. Total antioxidant activity of the hydroalcoholic extract of *BOB* leaves was found to be 194.10 ± 0.03 μg vitamin C equivalents per mg. The reducing power ability of the hydroalcoholic extract of *BOB* leaves was found to be 29.00 ± 0.093 μg vitamin C equivalents per mg. Total flavonoid content of the hydroalcoholic extract of *BOB* leaves was found to be 29.5 ± 0.007 μg quercetin equivalents per mg, and total phenol content was found to be 170.02 ± 0.052 μg gallic acid equivalents per mg.

Acute toxicity studies

Since no mortality was observed with hydroalcoholic extract of *BOB* leaves at a dose of 2000 mg/kg body wt, this was taken as the cut-off dose. Therefore, 1/20th (100 mg), 1/10th (200 mg) and 1/5th (400 mg) of the cut-off dose were selected for screening alleviatory effects of *BOB* on NaF-induced cardiotoxicity.

Effect on cardiac serum biomarker level

Chronic intake of fluoride increased the blood serum levels of CK-MB and LDH in group II, indicating cardiac toxicity. Administration of hydroalcoholic extract of *BOB* leaves showed significant reduction in CK-MB and LDH in groups III–V when compared to group II, suggesting its alleviating effect on cardiac tissue against fluoride-induced toxicity. No significant change was observed in serum CK-MB and LDH levels in group VI. Table 1 presents the results.

Effect on lipid peroxidation and antioxidant profile of cardiac tissue

Table 2 summarizes the effects of hydroalcoholic extract of *BOB* leaves on oxidative stress markers such as lipid peroxidation, reduced glutathione and catalase levels of cardiac tissue homogenate. There was significant increase in the lipid peroxidation level and decrease in the levels of reduced glutathione and catalase in group II. Treatment with hydroalcoholic extract of *BOB* leaves showed significant reduction in lipid peroxidation level and increased levels of reduced glutathione and catalase in groups III–V when compared to group II. Administration of hydroalcoholic extract of *BOB* leaves to group VI significantly decreased the lipid peroxidation level and increased the level of reduced glutathione and catalase.

Discussion

In this study, *in vitro* antioxidant activity of hydroalcoholic extract of *BOB* has been evaluated in terms of scavenging activity of DPPH radical, ABTS radical and metal chelation method, and reducing power assay, total flavonoids content and total phenol content. The results clearly indicate moderate antioxidant activity.

Globally, cardiac diseases are the common cause of death in both men and women, and are related to oxidant–antioxidant imbalance which is initiated by the reaction of free radicals with cell components such as proteins, lipids and nucleic acids[24]. It has been reported that more than normal intake of fluoride leads to excessive generation of free radicals and thereby increased lipid peroxidation, decreased glutathione levels and also diminishes the effect of antioxidant enzymes which play a primary role in the pathogenesis of fluoride induced cardiomyopathy[25]. Excessive formation of ROS leads to increased damage of biological molecules such as nucleic acids, proteins and membrane phospholipids by oxidative reactions[26].

Elevated lipid peroxidation is widely used as a biomarker for free radical-induced injury. Maximum induction of lipid peroxidation was observed in fluoride control group (group II). The hydroalcoholic extract of *BOB* extract decreased cardiac lipid peroxidation in a dose-dependent manner. Catalase is one of the main antioxidant enzymes which contains a transition metal as part of its cofactor or in its active site. Due to its chemical nature, fluoride has good affinity with metals and thus is capable of inhibiting the activity of the catalase enzyme by interrelating with its metal ion. In addition, it is reported that maximum cellular damage occurs after the depletion of glutathione and also impairs the cellular capacity in antioxidant defence system. Glutathione content is not as abundant in the heart, which is reflected by greater sensitivity of heart to fluoride-induced toxicity from free radicals. Increased level of glutathione-associated metabolism is a major concern for protection of cellular systems against agents which are generated through oxidative stress. In the present study, exposure to NaF was found to be reduced catalase enzyme level and reduction in reduced glutathione in the heart indicating an impaired function of the antioxidant defence system. These results are in agreement with those of earlier studies[5]. Treatment of NaF-induced rats with hydroalcoholic extract of *BOB* effectively improved the catalase level and reduced glutathione level in a dose-dependent manner, indicating its ability to restore antioxidant homeostasis in cardiac tissue.

Thus the study clearly suggests that intake of high fluoride-contaminated food and water shows characteristic signs of biochemical abnormalities, changes in the composition of plasma, urine and bone. Apart from these changes, fluoride is also known to cross the cell membrane passively and is likely to cause functional impairment of soft tissues. CK-MB and LDH enzymes are a common means of detecting myocardial infarction and muscular dystrophy[27]. Fluoride toxicity elevates the levels of above enzymes that result from oxidative damage of myocardial cell membrane by excessive production of superoxide anions and hydroxyl radicals in the presence of fluoride ions. Significant increase in the serum CK-MB and LDH levels in toxic control is in agreement with the results of previous studies[28]. In this study, hydroalcoholic extract of *BOB* leaves steadily decreased the fluoride-induced elevated CK-MB and LDH in a dose-dependent manner.

Table 1. Effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* (*BoB*) leaves on serum levels of creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in sodium fluoride-intoxicated rats

Group	CK-MB (IU/l)	LDH (IU/l)
I	214.1 ± 35.00	282.2 ± 15.51
II	1214 ± 12.95	1922 ± 117.6
III	1182 ± 35.42	1499 ± 77.18*
IV	1117 ± 36.30	1034 ± 33.03*
V	724.9 ± 35.64*	696.7 ± 27.47*
VI	254.5 ± 17.94	274.7 ± 16.79

*$P < 0.001$ when compared to group II.

Table 2. Effects of hydroalcoholic extract of *BoB* leaves on heart lipid peroxidation reduced glutathione and catalase levels in sodium fluoride-intoxicated rats

Group	Lipid peroxidation (μmol/mg of tissue)	Reduced glutathione (μmol/mg of tissue)	Catalase (μmol/mg of tissue)
I	2.38 ± 0.354	21.34 ± 1.710	1.53 ± 0.157
II	11.0 ± 0.791	8.24 ± 0.8460	0.17 ± 0.018
III	10.3 ± 0.383	10.02 ± 1.055	0.38 ± 0.055
IV	6.96 ± 0.325*	16.58 ± 0.978*	0.778 ± 0.029*
V	4.25 ± 0.368*	21.96 ± 0.859*	0.832 ± 0.037*
VI	2.97 ± 0.261	24.86 ± 0.454	1.54 ± 0.124

*$P < 0.001$ when compared to group II.

Conclusion

In conclusion, *BoB* leaves possess good *in vitro* antioxidant activity and moderate alleviatory effect against NaF-induced intoxication in cardiac tissue, which might be due to reduced production levels of free radicals and/or enhancing the enzymatic and non-enzymatic antioxidants. However, further detailed study is required to know the exact mechanism involved in its alleviatory effect. Results may be useful for further application of this natural product in herbal formulations and drug development.

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