Alleviatory effects of hydroalcoholic extract of
Brassica oleracea var. botrytis leaves against sodium fluoride
induced hepatotoxicity and oxidative stress on male Wistar rats

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Fluoride is one of the most common pollutants of potable water. Fluorosis is an endemic and global problem. Excessive intake of fluoride might accumulate and alter the functions of soft tissues including liver. The main objective of the present study was to investigate the alleviatory effects of hydroalcoholic extract of Brassica oleracea var. botrytis (BOB) leaves on sodium fluoride (NaF) induced hepatotoxicity. Thirty-six male Wistar albino rats were divided into six groups of six animals in each. Group I served as the normal control. Group II served as toxic control. Group III, IV, and V served as treatment groups received extract at three doses 100, 200, and 400 mg/kg respectively. Group VI served as plant control received a hydroalcoholic extract of BOB leaves 400 mg/kg. All groups except I, and VI, received NaF (100 ppm) through drinking water for 30 days. After the end of the study, serum profile and lipid peroxidation, reduced glutathione and catalase enzyme levels were measured in homogenates of the liver. The results of the present study suggested that BOB alleviates sodium fluoride-induced hepatotoxicity, probably via its antioxidant activity.

Keywords: Lipid peroxidation, Lipid profile, Liver biomarkers, Pancreatic enzymes, Sodium fluoride

The liver is one of the vital organs of vertebrates playing a central role in the body homeostasis through biotransformation of toxicants in the body. It is an accessory digestive gland and produces bile, which aids in the digestion of lipids by emulsification. Compared to western countries, India has been suffering from more percentage of liver diseases mainly due to many chemicals (inorganic and organic), medicinal preparations, chronic alcohol consumption and toxicants1,2.

Fluoride is a highly reactive electronegative ion and is the 13th most abundant naturally occurring element in the earth’s crust. It can easily cross cell membrane either by a simple or passive diffusion mechanism. The major and natural source of fluoride is soil rock and the other chief sources of fluoride include beverages, food, industries, medicines, cosmetics and fluoride pesticides. Fluorosis is the most widespread common health problem in the entire world. It is the most debilitating disease commonly seen where calcium deficiency and malnutrition are more prevalent. Fluoride often described as a double-edged sword because, in small doses, it can be considered as an essential trace element with significant protective effect in preventing dental caries, osteoporosis and bone fractures. On the other hand, many research reports clearly described that the excessive and/or prolonged intake of fluoride increased oxidative stress by generation of reactive oxygen species (ROS) and free radicals, increased lipid peroxidation, suppressed antioxidant enzymes level in soft tissues such as liver, kidney, brain, lung, and testes, which were considered as an important mechanism of intoxication3,4. Administration of fluoride also severely causes oxidation of macromolecules, membrane phospholipids breakdown, mitochondrial membrane depolarization and induces aberrations in various cellular processes such as gene expression, cell cycle, proliferation migration, respiration, ion transport, secretion, endocytosis, apoptosis, and necrosis in the body5,6.

The higher amount of fluoride disturbs the metabolic processes and detoxification capabilities of
liver and induce necrosis, modifications of membrane lipids and apoptosis in hepatocytes\textsuperscript{7,8}. The toxic effects were influenced by the amount of fluoride ingested, duration of exposure, and species of the animal \textit{etc}. In addition, an effect of fluoride is related to dosage form and the other factors that influence the animal’s physiological and anatomical responses\textsuperscript{9}.

Earlier reports have suggested that antioxidants and antioxidant-rich foods are useful for management of fluorosis\textsuperscript{10-13}. Thus, the purpose of present study is to find out the alleviatory effects of hydroalcoholic extract of \textit{Brassica oleracea} var. \textit{botrytis} (BOB), cauliflower leaves against sodium fluoride (NaF) induced toxicity in the liver.

**Materials and Methods**

**Collection and authentication of plant material**

The leaves of the BOB were collected from the local market of Kadapa, Kadapa district, Andhra Pradesh, India and were authenticated by Dr. Sunita Garg, Chief scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR; Voucher specimen was stored in the Department of Pharmacognosy, CMR College of Pharmacy, Hyderabad, Telangana state, India. The leaves were air-dried, ground to powder and stored in an airtight container.

**Preparation of the extract**

100 g of the BOB powder was mixed with various proportions of hydroalcoholic solvents (50:50, 30:70 and 70:30, respectively) and kept at 25°C for one week with occasional shaking. Thereafter, they were stirred for 20 min and filtered. The filtrates were dried in rotary evaporator (ROTA VAP) apparatus and suitable extract of plant material was selected based on the percentage of yield and stored in a refrigerator at 4°C for further studies.

**Phytochemical investigation**

The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents\textsuperscript{14}.

**Experimental animals**

Male Wistar albino rats (36) weighing in between 220-250 g were procured from Sai Thirumala Enterprises, Hyderabad, India. The animals were acclimatized for 10 days before starting the experiment. Rat feed was provided with water \textit{ad libitum} and maintained a photoperiod of 12 h light/dark cycle. The study was completed as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India, after approval from Institutional Animal Ethics Committee (IAEC no: CPCSEA/1657/IAEC/CMRCP/PhD-14/35).

**Acute Toxicity Studies**

An acute toxicity study was performed for aqueous extract according to the acute toxic class method described by OECD 423.

**Experimental design**

The dose of NaF was selected based on the previous study\textsuperscript{15}. The experimental animals were divided into six groups (n=6), where, Group I (normal control), animals received drinking water (F level <1.5 ppm); Group II (toxic control), NaF (100 ppm) was administrated through drinking water for 30 days; Group III, treatment with BOB extract at a dose of 100 mg/kg body wt/day for 30 days (p.o) + NaF through drinking water for 30 days; Group IV, treatment with BOB extract at a dose of 200 mg/kg body wt/day for 30 days (p.o) + NaF through drinking water for 30 days; Group V- Treatment with BOB extract at a dose of 400 mg/kg b.wt/day for 30 days (p.o) + NaF through drinking water for 30 days; and Group VI (plant control), treatment with BOB extract at a dose of 400 mg/kg body wt/day for 30 days (p.o) alone. After the treatment schedule, animals fasted overnight and blood was collected by puncturing the retro-orbital plexus. Blood samples were allowed to clot for approximately 1 h at room temperature and centrifuged at 2500 rpm for 15 min to obtain the serum used for estimation of various biochemical parameters like aspartate transaminase (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), direct and total bilirubin albumin, total protein, glucose, magnesium, total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), pancreatic amylase, and lipase using Coral diagnostic kits, India. The liver was homogenized and the post-mitochondrial supernatant was used for the estimation of lipid peroxidation and reduced glutathione (GSH), and catalase levels\textsuperscript{16-18}.

**Statistical Analysis**

The values were expressed as Mean ± SEM (n=6 in each group). The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparison tests using Graph pad prism 5.0. The values were significant at $P < 0.05$.

**Results**

**Percentage Yield**

Based on the percentage yield, we selected 30:70 (Water: Ethanol) extract of \textit{Brassica oleracea} Var.
Botrytis leaves (BOB). About 31.4% of semi-solid yield was obtained which was more compared to other two.

**Phytochemical screening**

The phytochemical screening of the hydroalcoholic extract of BOB leaves was found to consist of the following secondary metabolites: alkaloids, glycosides, flavonoids, and phenols.

**Acute toxicity studies**

Since no mortality was observed with hydroalcoholic extract of BOB at the dose of 2000 mg/kg, it was assumed that 2000 mg/kg was the cut-off dose. Therefore 1/20th (100 mg/kg), 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of cut-off dose were selected for screening alleviatory effects of BOB on NaF-induced hepatotoxicity.

**Clinical observations**

There were no significant changes in the appearance or behaviour of animals during the study. No mortality was recorded in all experimental groups at the end of the study. A significant decrease in the body weight was observed in group II when compared to the group I. An increase in the body weight was observed in all the groups (Group III-V) (Fig. 1).

**Effect of hydroalcoholic extract of BOB on hepatic biomarkers**

Chronic intake of fluoride increases the blood serum levels of AST, ALT, ALP, direct and total bilirubin, and decreased levels of total protein and albumin when compared to group I indicating hepatic dysfunction. Group III, IV, and V protected hepatic tissue against fluoride-induced toxicity and recovered its functioning as indicated by the decrease in hepatic biomarkers level (Table 1) and increase in the level of total protein and albumin in group III, IV, and V when compared to group II (Table 2).

**Effect of hydroalcoholic extract of BOB on serum glucose and magnesium level**

Increased serum glucose and decreased magnesium levels were observed in group II when compared to group I. Administration of hydroalcoholic extract of

![Fig. 1— Effects of hydroalcoholic extract of *Brassica oleracea* var. botrytis leaves on sodium fluoride induced changes in body weight of rats](image)

**TREATMENT GROUPS**

![Table 1 — Effects of hydroalcoholic extract of *Brassica oleracea* var. botrytis leaves on serum AST, ALT, ALP, direct bilirubin, and total bilirubin in sodium fluoride intoxicated rats](table1)

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/mL)</th>
<th>ALT (IU/mL)</th>
<th>ALP (KA/dL)</th>
<th>D. Bilirubin (mg/dL)</th>
<th>T. Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>31.71±4.19</td>
<td>21.76±4.04</td>
<td>28.28±10.91</td>
<td>0.14±0.01</td>
<td>1.25±0.12</td>
</tr>
<tr>
<td>Group II</td>
<td>100.9±15.9</td>
<td>109.9±28.0</td>
<td>70.30±18.24</td>
<td>0.31±0.05</td>
<td>4.04±0.46</td>
</tr>
<tr>
<td>Group III</td>
<td>93.71±9.16</td>
<td>52.07±13.25***</td>
<td>57.52±4.89***</td>
<td>0.25±0.01*</td>
<td>3.64±0.38</td>
</tr>
<tr>
<td>Group IV</td>
<td>56.8±8.56***</td>
<td>31.43±4.01***</td>
<td>44.53±2.05***</td>
<td>0.20±0.02***</td>
<td>1.46±0.08***</td>
</tr>
<tr>
<td>Group V</td>
<td>50.32±3.44***</td>
<td>30.43±3.43***</td>
<td>30.68±1.77***</td>
<td>0.17±0.02***</td>
<td>0.78±0.10***</td>
</tr>
<tr>
<td>Group VI</td>
<td>37.28±4.39***</td>
<td>27.67±2.57***</td>
<td>23.80±1.15***</td>
<td>0.13±0.01***</td>
<td>0.70±0.25***</td>
</tr>
</tbody>
</table>

[Values are represented as Mean ± SEM, n=6 in each group. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett’s multiple comparison test. Where, *P <0.05, *** P <-0.001 when compared to Group II]

**Table 2 — Effects of hydroalcoholic extract of *Brassica oleracea* var. botrytis leaves on serum total protein, albumin, glucose, and magnesium levels in sodium fluoride intoxicated rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/dL)</th>
<th>Albumin (mg/dL)</th>
<th>Glucose (mg/dL)</th>
<th>Magnesium (mEq/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.92±0.37</td>
<td>2.46±0.21</td>
<td>100.7±5.74</td>
<td>4.02±0.07</td>
</tr>
<tr>
<td>Group II</td>
<td>6.39±0.41</td>
<td>2.09±0.18</td>
<td>131.5±16.86</td>
<td>2.11±0.04</td>
</tr>
<tr>
<td>Group III</td>
<td>7.43±0.20**</td>
<td>2.93±0.10***</td>
<td>116.6±4.00***</td>
<td>3.66±0.11***</td>
</tr>
<tr>
<td>Group IV</td>
<td>7.70±0.29***</td>
<td>3.02±0.15***</td>
<td>97.59±2.13***</td>
<td>3.54±0.06***</td>
</tr>
<tr>
<td>Group V</td>
<td>7.94±0.32***</td>
<td>3.41±0.27***</td>
<td>92.23±2.35***</td>
<td>4.52±0.26***</td>
</tr>
<tr>
<td>Group VI</td>
<td>8.15±0.42***</td>
<td>4.75±0.04***</td>
<td>87.73±3.72***</td>
<td>4.92±0.38***</td>
</tr>
</tbody>
</table>

[Values are represented as Mean ± SEM, n=6 in each group. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett’s multiple comparison test. Where, ** P <0.01 and, *** P <0.001 when compared to Group II]
BOB leaves showed a significant increase in the level and V administered with hydroalcoholic extract of enzymes when compared to the group I. Group III, IV, serum levels of pancreatic alpha-amylase and lipase water for 30 days showed significant decrease in the **P <0.001 when compared to Group II**

Table 3 — Effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* leaves on serum total cholesterol, triglycerides, and HDL-Cholesterol levels in sodium fluoride intoxicated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL-cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>99.17±9.36</td>
<td>64.42±4.50</td>
<td>31.05±0.80</td>
</tr>
<tr>
<td>Group II</td>
<td>186.3±8.77</td>
<td>88.10±11.14</td>
<td>21.09±0.68</td>
</tr>
<tr>
<td>Group III</td>
<td>177.7±3.25</td>
<td>78.86±3.15</td>
<td>23.04±0.21</td>
</tr>
<tr>
<td>Group IV</td>
<td>159.9±5.34***</td>
<td>65.17±9.19***</td>
<td>24.59±0.79***</td>
</tr>
<tr>
<td>Group V</td>
<td>130.3±11.78***</td>
<td>57.78±5.60***</td>
<td>27.27±0.72***</td>
</tr>
<tr>
<td>Group VI</td>
<td>101.7±9.22***</td>
<td>45.15±4.87***</td>
<td>33.35±0.93***</td>
</tr>
</tbody>
</table>

[Values are represented as Mean ± SEM, n=6 in each group. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett’s multiple comparison tests. Where **P <0.01 and ***P <0.001 when compared to Group II]

Table 4 — Effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* leaves on serum pancreatic amylase, and lipase levels in sodium fluoride intoxicated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pancreatic amylase (U/L)</th>
<th>Pancreatic lipase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>654.0±124.5</td>
<td>153±10.2</td>
</tr>
<tr>
<td>Group II</td>
<td>88.17±9.30</td>
<td>25.7±3.63</td>
</tr>
<tr>
<td>Group III</td>
<td>305.5±99.33***</td>
<td>31.3±5.76</td>
</tr>
<tr>
<td>Group IV</td>
<td>484.4±60.05***</td>
<td>64.4±11.6***</td>
</tr>
<tr>
<td>Group V</td>
<td>726.0±67.58***</td>
<td>101±10.2***</td>
</tr>
<tr>
<td>Group VI</td>
<td>1017±132.8***</td>
<td>146±11.7***</td>
</tr>
</tbody>
</table>

[Values are represented as Mean ± SEM, n=6 in each group. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett’s multiple comparison tests. Where **P <0.01 and ***P <0.001 when compared to Group II]

BOB leave resulted from a significant reduction in serum glucose and a significant increase in the magnesium levels in group III, IV, and V when compared to group II (Table 2).

**Effect of hydroalcoholic extract of BOB on lipid profile**

Group II showed high levels of total cholesterol, triglycerides and decreased HDL-C when compared to the group I. Administration of hydroalcoholic extract of BOB leaves in group III, IV, and V significantly reduced the total cholesterol and triglycerides levels when compared to group II (Table 3).

**Effect of hydroalcoholic extract of BOB on pancreatic enzymes**

Administration of sodium fluoride through drinking water for 30 days showed significant decrease in the serum levels of pancreatic alpha-amylase and lipase enzymes when compared to the group I. Group III, IV, and V administered with hydroalcoholic extract of BOB leaves showed a significant increase in the level of both these enzymes when compared to group II (Table 4).

Table 5 — Effects of hydroalcoholic extract of *Brassica oleracea* var. *botrytis* leaves on liver lipid peroxidation, reduced glutathione, and catalase levels in sodium fluoride intoxicated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipidperoxidation (µM/mg of tissue)</th>
<th>Reduced glutathione (µM/mg of tissue)</th>
<th>Catalase (µM/Mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.21±0.63</td>
<td>14.2±2.66</td>
<td>2.09±0.17</td>
</tr>
<tr>
<td>Group II</td>
<td>10.20±0.50</td>
<td>6.01±1.09</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>Group III</td>
<td>8.66±0.81**</td>
<td>9.57±1.25**</td>
<td>0.46±0.08</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.79±0.85***</td>
<td>15.2±2.01***</td>
<td>0.81±0.07***</td>
</tr>
<tr>
<td>Group V</td>
<td>4.86±0.58***</td>
<td>16.3±1.22***</td>
<td>1.39±0.16***</td>
</tr>
<tr>
<td>Group VI</td>
<td>3.95±0.48***</td>
<td>21.0±1.90***</td>
<td>2.33±0.33***</td>
</tr>
</tbody>
</table>

[Values are represented as Mean ± SEM, n=6 in each group. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett’s multiple comparison tests. Where, **P <0.01 and ***P <0.001 when compared to Group II]

**Effect of hydroalcoholic extract of BOB lipid peroxidation and antioxidant profile of liver**

Chronic intake of fluoride caused a significant increase in the levels of liver lipid peroxidation and decreased the levels of reduced glutathione and catalase when compared to the group I. Administration of hydroalcoholic extract of BOB leaves showed significant reduction in lipid peroxidation level and increased level of reduced glutathione and catalase in group III, IV, and V when compared to Group II. Administration of hydroalcoholic extract of BOB leaves to group VI has significantly reduced lipidperoxidation level and increased the level of reduced glutathione and catalase (Table 5).

**Discussion**

The main route of entry of fluoride in a chronic exposure is through drinking water. Thus, in the present study fluoride was administered in rats through the same route. The body weight of the animals was decreased in group II compared to group I. Fluoride exposure caused a significant reduction in the body weight which could be attributed to atrophic gastritis and poor gastrointestinal absorption, suppressed appetite and disturbed nutrient digestibility that can eventually lead to the excessive breakdown of cellular macromolecules causing weight loss19. The rats treated with the hydroalcoholic extract of BOB leaves in group III, IV, and V showed significant recovery of body weight.

The increase in the concentration of aminotransferases (AST and ALT) and alkaline phosphatase (ALP) levels in serum was a clear indication of cellular leakage and a loss of functional integrity of hepatocytes cell membrane20. Previous studies supported that significant increase in AST and ALT levels in rats and mice in a dose-dependent manner
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The increase in the level of serum bilirubin reflected the severity of hepatotoxicity. Data obtained in the present study revealed that disturbed liver function in group II was reflected by the increased level of AST and ALT, ALP and direct bilirubin, and total bilirubin. Treatment with hydroalcoholic extract of BOB leaves in group III, IV, and V significantly normalized serum biomarkers and could indicate its alleviatory effects on liver against NaF.

The present results showed that NaF significantly altered protein metabolism and this was similar to the findings of earlier report. Treatment with hydroalcoholic extract of BOB increased serum levels of total proteins and albumin of NaF-intoxicated rats in group III, IV, and V. This indicates that suppression of NaF-induced liver damage with subsequent improvement in liver function following BOB treatment.

Since significant changes were observed in lipid profile (TC, TG, and HDL-C) of NaF-intoxicated rats of the earlier reports. The possible mechanisms for alternations in lipid profile may be due to the abnormal activities of lipases enzymes and phospholipases, which are the chief factors responsible for the rise in serum TG, TC and decreased the level of HDL-C indicating hyperlipidemia and atherogenesis.

Increased blood serum glucose and decreased magnesium levels indicate diabetic properties of NaF-intoxication. The possible mechanisms involved in the increased blood glucose level are i) dramatic changes in carbohydrate metabolism by inhibiting the key enzymes involved in glycolysis and TCA cycle, ii) decreased level secretion of insulin from pancreas, and iii) Hypomagnesaemia might worsen insulin resistance, a condition that often precedes diabetes, or it might be a consequence of insulin resistance. The results obtained are similar with that of the previous studies. Administration of hydroalcoholic extract of BOB leaves in group III, IV, and V showed the significant increase in the serum levels of pancreatic alpha-amylase, it indicates potential secretagogue effect of the plant extract.

Oxidative stress is a condition that indicates the imbalance between the pro-oxidants and antioxidants leading to the chemical injury to lipids, proteins, and DNA. No clear mechanism was available regarding the fluoride-induced oxidative stress in soft tissues. However, the following points strongly support for better understanding of the mechanisms of fluorosis: 1) Intake of fluoride can induce excessive production of oxygen free radicals and cause the decrease in biological activities of endogenous enzymatic and non-enzymatic levels viz., catalase, superoxide dismutase, xanthine oxidase and glutathione peroxidase which plays a central role in the elimination of free radicals and maintenance of antioxidant homeostasis, 2) Fluoride disturbs the carbohydrate, protein and lipid metabolism by impairing the activities of enzymes like alkaline Phosphatase, cholinesterase, and 3) It can also disturb the metabolism of nucleic acids, suppress the immune system and damage the parts of the body. Besides, it also causes oxidation of macromolecules, membrane phospholipid breakdown, lipid peroxidation, mitochondrial membrane depolarization, and apoptosis. Decreased levels of enzymatic and/or non-enzymatic antioxidant systems were unable to eliminate H2O2 and lipid peroxidation which damages the cell membranes.

In the present study, the administration of NaF resulted in increased lipid peroxidation level with decrease in reduced glutathione and catalase levels in liver. These findings were matched with the results of previous reports. Administration of hydroalcoholic extract of BOB leaves in group II, III, and IV showed a significant reduction in the lipid peroxidation level and normalized the reduced glutathione and catalase levels indicating its antioxidative ability.

Conclusion

In conclusion, the present study clearly observed that administration of hydroalcoholic extraction of BOB leaves alleviated the sodium fluoride-induced hepatotoxicity probably by reducing the level of free
radical production and/or enhancing the enzymatic and non-enzymatic antioxidants and improving the pancreatic function. It was effectively reduced fluoride-induced toxicity in dose-dependent manner. The administration of BOB was ascertained to alleviate the toxic effects of fluoride in the observed parameters.

References


