ANTIOXIDANT POTENTIAL OF SEASONAL FRUITS IN FLUORIDE TOXICITY: AN IN VITRO STUDY

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Abstract: The present study was undertaken to investigate the antioxidant potential of ethanolic extracts of four fruits - Indian gooseberry, mango, star fruit and wood apple on the oxidative changes in hepatic tissue exposed to sodium fluoride in vitro. Liver homogenates were exposed to NaF and subsequently treated with ethanolic extracts of fruits and the levels of antioxidants (ascorbic acid, superoxide dismutase, catalase, glutathione, glutathione peroxidase) and lipid peroxidation (TBARS) were determined. Results indicated that of the four fruits tested, gooseberry exhibited a maximum potency in reducing the lipid peroxidation (56.70%) and increased the antioxidants - TAA, SOD, CAT, GsH and GPX where as wood apple was found to possess a comparatively low antioxidant potential.

Key words: Fluoride toxicity, Fruits antioxidants

INTRODUCTION

Many people are exposed to high levels of fluoride (more than 1 ppm) through drinking water, food and air in different parts of the world [1]. Chronic fluorosis is slow, progressive and debilitating particularly the musculoskeletal and dental systems. Metabolic, functional, and structural damages caused by chronic fluorosis have been reported in many tissues-myocardium, kidney, liver, ovary, testis, brain and various soft tissues of the body. It is also known that fluoride toxicity causes oxidative stress [2-8].

As there are no treatment modalities available to tackle fluorosis (other than defluoridation techniques for water purification), the present investigation was aimed at evaluating the protective mechanisms offered by biologically active compounds present in certain fruits such as Emblica officinalis (G) (Gooseberry, F: Euphorbiaceae), Mangifera indica (L) (Mango, F: Anacardiaceae), Averrhoa carambola (L) (Star fruit, F: Oxalidaceae) and Limonia acidissima (L) (Wood apple, F: Rutaceae). Gooseberry fruits are anabolic, antibacterial and resistance building with expectorant, cardiotonic, antipyretic, antioxidative, antiviral and anti-emetic properties. Besides, the fruit is cooling, refrigerant, diuretic and laxative [9]. Mango fruits are well-known in India eaten when ripe and pickled when raw. The decoction and powder of mango fruit are used as vermifuge, astringents in diarrhea, treating hemorrhages and bleeding hemorrhoids. Extracts of unripe mango fruits and of bark, stems and leaves have shown antibiotic property [10]. Star fruit is useful in counteracting fever, as a diuretic in kidney and bladder complaints, in treating eczema and used to halt hemorrhages and to relieve bleeding hemorrhoids. A conserve of star fruit is said to allay biliousness and diarrhea. A salve made of the fruit is employed to relieve eye afflictions [10]. Wood apple is much used in India as a liver and cardiac tonic and when unripe, as an astringent to halt diarrhea and dysentery and is effective in treatment of hiccough, sore throat and diseases of the gums. The fruit is also used as antiscorbutic. The pulp is used as poultice, applied on bites and stings of venomous...
insects, as is the powdered rind [10]. The present study was undertaken in order to investigate the efficacy of these fruits in improving the antioxidant profiles of hepatic tissue when exposed to sodium fluoride toxicity in in vitro conditions.

**MATERIALS AND METHODS**

**Fruit powder preparation and analysis:** All four fruits were procured from local market, brought to laboratory, shade dried, ground to powder and stored in airtight containers. The fruit powders were subjected to soxhlet extraction using ethanol and the extracts were evaporated at room temperature and stored at 4 °C until used and yields were calculated. The yields of the fruits are: Gooseberry- 29.50 %w/w, Mango- 22.65 %w/w, Star fruit- 12.45 %w/w and Wood apple- 19.75 %w/w. Phytochemical analyses of the fruits were carried out to quantify phytosterols [11], saponins [12], polyphenols and flavonoids [13] and total ascorbic acid [14]. The total antioxidant power of the fruits in terms of FRAP was also determined using TPTZ reagent [15].

**Animals:** Colony bred adult male Albino rats (Charles Foster, 250-300 gm bw), maintained on commercial diet, housed in polypropylene cages with ad libitum access to water (26 ± 2°C, humidity 60%) were used for the present investigation. Animals were sacrificed after light anesthesia (diethyl ether) and liver was quickly excised, washed with ice-cold normal saline. The care and procedures adopted for the present investigation were in accordance with the approval of Institutional Animal Ethics Committee.

**Determination of effective concentration of NaF:** Liver homogenates were prepared in accordance with homogenate requirements for TBARS [16], total ascorbic acid (TAA) [14], super oxide dismutase (SOD) [17], catalase (CAT) [18], reduced glutathione (GSH) [19] and glutathione peroxidase (GPx) [20] and exposed to one milliliter of sodium fluoride (NaF) at 1.5, 2.5 and 3.5 ppm concentrations and incubated for 60 min. These homogenates were used to determine the most effective concentration of NaF that would cause highest lipid peroxidation and show lowest levels of antioxidants.

**Determination of antioxidant potential of fruits:** After determining the most effective concentration of NaF, the homogenates with NaF were incubated with 25 and 50 mg/ml of Ethanolic extracts of the fruits for 60 min. After the incubation TBARS, TAA, SOD, CAT, GSH and GPx levels were assayed in the homogenates.

**Statistical Evaluation:** One-way analysis of variance (ANOVA) with Tukey’s significant difference post hoc test was used to compare differences among groups. Data were statistically handled by Graph Pad Prism 3.0 statistical software. P values <0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

Among the concentrations tested, 3.5ppm NaF was found to be most effective in decreasing antioxidant profiles and increasing the lipid peroxidation maximally. Therefore, 3.5ppm NaF was selected for further experimentation and two random doses of the ethanolic fruit extracts (25 and 50 mg/ml) were selected (Figs 1-6).

Exposure of liver homogenates to different concentrations of NaF resulted in a concentration-dependent increase in lipid peroxidation and decrease in antioxidants. Determination of MDA levels provide a good measure of peroxidation which is one of the chief mechanisms of cell damage leading to necrosis or apoptosis [21]. A significant decrease in lipid peroxidation occurred when NaF treated liver homogenates were exposed to ethanolic fruit extracts in a dose-dependent manner (Fig 1). Sodium fluoride with all concentrations significantly decreased the levels of TAA, SOD, CAT, GSH and GPx activities and the treatment with fruit extracts showed an elevation in antioxidant status (Figs 2-6). Vitamin C
Fig. 1: TBARS levels in fluoride exposed homogenates and treatment with fruit extracts
Fig. 2: TAA contents in fluoride exposed homogenates and treatment with fruit extracts
Fig. 3: SOD activity in fluoride exposed homogenates and treatment with fruit extracts
Fig. 4: CAT activity in fluoride exposed homogenates and treatment with fruit extracts
Fig. 5: GSH content in fluoride exposed homogenates and treatment with fruit extracts
Fig. 6: GPX activity in fluoride exposed homogenates and treatment with fruit extracts
is a well-known antioxidant as it reduces free radicals and the fluoride levels in the body [22].

The antioxidant enzymes superoxide dismutase and catalase are also known to play important roles in reducing the cellular stress [23, 24]. Glutathione (GSH), normally present at high concentrations in cells possesses the major reducing capacity and protects the cells against toxic effects of lipid peroxidation [25]. Glutathione peroxidase (GPX) acts on reduced glutathione and H₂O₂ to produce oxidized glutathione and H₂O. It provides second line of defense against hydro-peroxides. A reduced GPX activity with a concurrent reduction in GSH therefore indicates that GPX activity is dependent on GSH content [26].

The observed antioxidant potential of the fruits tested in the present investigation could be attributed to their phytochemical constituents i.e., phytosterols, polyphenols, flavonoids, saponins and ascorbic acid (Table 1). Although the phytosterols are recognized as important components of diets that help reduce hypercholesterolemia [27], they are also reported to possess antioxidant property as they decrease the oxidation of LDL as well as lipid peroxidation [28, 29]. Polyphenols and flavonoids are known to be hepato-protective, anti-oxidative and antihyperlipidemic [30, 31]. Ascorbic acid as mentioned earlier is a well-known antioxidant that quenches free radicals and conjugates with cytotoxic, genotoxic and lipid peroxidation products to eliminate them [22, 32]. Saponins have also been shown to improve the antioxidant potential and protect the hepatocytes from fluoride induced cytotoxicity and necrotic death [33].

In conclusion, the results of the present study indicate that all the tested fruit extracts improve the antioxidant status and reduce the lipid peroxidation in liver homogenates exposed to sodium fluoride. The increased antioxidant profiles in liver tissue homogenates correlate well with the antioxidant profiles of the fruit extracts: for instance, higher antioxidant levels in Gooseberry correspond closely to the increase in these levels in tissue homogenates. Similarly, the high FRAP value of Gooseberry extract also corresponds to overall increase in the antioxidant potential in liver homogenates. These findings suggest that the fruits used in this investigation could be useful as rich natural sources of antioxidants to overcome the oxidative stress induced by fluoride toxicity.

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Foot notes:

C- control, F 1.5 to 3.5 liver homogenates exposed to 1.5 to 3.5 ppm NaF, EO- Emblica officinalis (I- 25 mg/ml; II- 50 mg/ml), Mi- Mangifera indica (I- 25 mg/ml; II- 50 mg/ml), Ac- Averrhoa carambola (I- 25 mg/ml; II- 50 mg/ml) and La- Limonia acidissima (I- 25 mg/ml; II- 50 mg/ml).

Comparisons were made between control and fluoride exposed liver homogenates. Fruit extract treated groups were compared with fluoride 3.5 ppm control group. * indicates statistically significance at P < 0.05

REFERENCES