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Protective Role of Capsaicin against Sodium Fluoride-induced Oxidative Damage of Liver in Rats

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Abstract

Capsaicin, a bioactive component of hot chili pepper, is a potent antioxidant and effective in ailments of diverse health problems. The present study evaluated the possible protective effectiveness of capsaicin (Caps) against sodium fluoride (NaF) - induced oxidative damage in liver tissue. Rats treated with NaF (10.3mg/kg b.wt/day) orally for five weeks showed marked elevation of serum level of ALT, AST and bilirubin which are the biochemical markers of liver lesions. Results also showed that NaF exposure significantly elevated LPO level and NO generation in the liver with a parallel significant decline in activities of SOD, CAT, and content of GSH. While, Caps supplementation (0.5 mg/kg b.wt/day) was significantly reversed the NaF-induced oxidative stress; amended AST, ALT levels and bilirubin content; and restored normal histology of the liver. Therefore, it can be concluded that Caps plays a protective role against hepatic toxicity produced by NaF exposure.

Keywords: Antioxidant, Capsaicin, Fluoride, Hepatotoxicity, NaF, Oxidative Stress

1. Introduction

Fluoride contamination in drinking water is an alarming ecological issue of present reality. In India, about sixty-five million peoples including six million children are at danger of fluorosis because of the presence of abundant fluoride in groundwater beyond the level of permissible limit of 1.5 mg/l in drinking water[1]. Besides, notable adverse impacts on the bone and teeth[2], fluorosis can likewise harmfully affect delicate tissues, for example, the liver, spleen, intestine and kidneys[3–5]. Researches revealed that there is a positive correlation between fluoride exposure and hepatic damage. A plethora of literature reported that fluoride-induced oxidative stress is the well thought pathological mechanism of fluoride intoxication[6]. Fluoride can penetrate soft tissues by diffusion where it develops oxidative stress by the generation of oxygen free radicals and by constraining the actions of primary antioxidant enzymes such as SOD, CAT and antioxidant like GSH[7]. Researchers also showed that fluoride-induced oxidative stress caused oxidative damage of liver by promoting lipid peroxidation and apoptosis of liver cells[8,9]. Furthermore, recent studies confirmed the harmful impact of excess fluoride in hepatic and renal functions in human beings living in endemic fluorosis areas[10].

Medicinal use of plant products, particularly those that are available in normal diets, has been of enthusiasm in recent years since they are natural, inexpensive, and less toxic. Capsaicin (trans-8-methyl-N vanillyl-6-noneamide) is a bioactive compound of chili peppers. This phytochemical belongs to vanilloid family and confers their biological activity by binding to the Transient Receptor Potential receptor Variant (TRPV). Several beneficial properties of capsaicin (Caps) including antioxidant, anti-inflammatory,

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anti-apoptotic, and anti-malignant activities have made it an attractive nutraceutical[11–13]. Furthermore, capsaicin is found to be an effective natural antidote against numerous disorders such as hyperalgesia, inflammation, and gastric ulcers. Interestingly, the oxidative damaging effects of various toxic chemicals e.g. CCl_4, benzo(a) pyrene can be successfully counteracted by its free radicals scavenging property which make Caps a potent natural antioxidant compound[11,14].

However, a health beneficial property of Caps against fluoride intoxication has not been reported so far. Therefore, the present study aimed to investigate the possible protective role of naturally occurring Caps on the toxic effects of fluoride on the liver in adult rats. The data of the present study might also help to develop a natural antidote against fluoride toxicity.

2. Material and Methods

2.1 Chemicals and Reagents

NaF and Capsaicin were obtained from Sigma-Aldrich. Sulfanilamide, phosphoric acid, Bovine Serum Albumin (BSA), Thiobarbituric Acid (TBA), Napthyl Ethylene Diamine Dihydrochlorine (NEDH), Nitroblue Tetrazolium (NBT), xanthine oxidase was purchased from Merck (Darmstadt, Germany). All other chemical reagents were of graded systematically.

2.2 Animal Maintenance and Ethical Clearance

Twenty four adult male albino rats (Wister strain) weighing 110–125 gm, were obtained from CPCSEA (The Committee for Control and Supervision of Experiments on Animals) approved registered breeder for experimental purpose. The rats were housed in an environmentally controlled animal house at a constant temperature of 21 ± 2°C, fed standard pellet diets with purified water available ad libitum and kept under a 12-h light/dark cycle (lights on at 8:00 am) with 10% relative humidity. The animals were acclimatized in the new environment for about 7 days before the experiment. All animal experiments were performed according to the guidelines of the Institutional Animal Ethical Committee of Serampore College, [Registration Number-1946/PO/Re/18/CPCSEA] Serampore, West Bengal, India, with the approval No: 02/P/S/Sc/IAEC/2017.

2.3 Experimental Regimen

After two weeks of acclimation, rats were randomly divided into four equal groups' six rats each (n = 6). The experimental regimen of present study was tabulated in Table 1. Fluoride in the form of Sodium fluoride (NaF) and the phytochemical capsaicin (Cap) were administered orally for five weeks. The doses and the administration route of NaF were decided on the basis of previous established study[7] and also on the basis of laboratory observations. The dose of capsaicin was selected on the basis of previous published report[15] and duration of the phytochemical co-treatment was modified according to the experimental observations conducted in our own laboratory. At the end of the experimental duration, animals were euthanized.

Table 1. Grouping and treatment schedule of mice in exper imental regimen

Group number	N = 6 in each group	Treatment
Group 1	Control vehicle only	Olive oil
Group 2	NaF	10.3 mg/kg b.w.(oral administration)
Group 3	Caps	0.5 mg/kg b.w.(oral administration)
Group 4	NaF+Caps	10.3 mg/kg b.w and 0.5 mg/kg b.w.(oral administration)

2.4 Evaluation of Hepatic Toxicity Markers

Rats of each group were euthanized at the end of treatment period. Blood samples were collected by cardiac puncture and serum samples were obtained by centrifugation at 860× g for 20 min and stored at −60°C till measurements. The serum was used to evaluate liver function by estimating the activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and the level of total bilirubin by the methods of Reitman and Frankel[16].

2.5 Preparation of Liver Tissue Extract

Liver tissue was collected by opening the abdomen without delay. It was then washed using a chilled saline solution. The collected sample was dissolved in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) and was centrifuged (10% w/v) by a Potter–Elvehjem type homogenizer at 10,000× g for 20 min at 4°C. The resultant supernatant was set aside for further analysis.

2.6 Estimation of Protein in Crude Extract

The total protein content was measured according to Lowry (1951) method[17]. Bovine serum albumin was used as standard to measure protein content of liver tissue homogenate. Blue color formed was read at 640 nm in a Shimadzu spectrophotometer and a standard graph was obtained with different concentrations of bovine serum albumin solution. The values were expressed as mg/ml.

2.7 Estimation of Nitric Oxide Production (NO) and MDA Level

Tissue nitrite acts as an indicator of NO production because nitric oxide decomposes rapidly to nitrite. In the present study, nitrite accumulation in liver homogenate was measured by Griess reaction[18].

Also hepatic content of MDA was measured based on the reaction of Thiobarbituric Acid (TBA) test[19]. The amount of MDA formed was quantitated with TBA and used as an index of lipid peroxidation.

2.8 Estimation of Antioxidant Enzymes and Molecule

The Superoxide Dismutase (SOD) activity of tissue homogenates was spectrophotometrically measured according to the Nitroblue Tetrazolium (NBT) method of Beauchamp and Fridovich[20]. The Catalase (CAT) activity was also assessed by spectrophometrically by measuring decomposition of H_2O_2 at 240 nm according to the method of Beers et al[21]. The levels of reduced Glutathione (GSH) of liver tissue were determined according to earlier described method of Ellman[22].

2.9 Preparation of Permanent Slides for Histo logical Studies

Permanent slides of liver tissue of all experimental groups were prepared and stained with eosin-hematoxylin for histological evaluation under a microscope (Carl Zeiss, Primoster model).

2.10 Statistics

In the present study, results were expressed as Mean ± standard deviation (SD). Kruskal-Wallis non-parametric ANOVA test was used to find out whether or not the scores of different groups differ significantly and the significant difference between intergroup was assessed by Mann-Whitney multiple comparisons test using Minitab software and p<0.05 was considered as statistically significant.

3. Results

3.1 Effect of Capsaicin on Hepatosomatic Index

The current study demonstrated the hepatoprotective role of Caps against NaF induced liver damage. NaF treatment for 5 weeks significantly increased the final relative liver weight in NaF-exposed rats (Group B) compared to control animals. Treatment with capsaicin alone did not alter the hepatosomatic index. However, oral administration of Caps along with NaF significantly diminished the relative liver weight compared to the NaF-treated group (P<0.05) (Figure 1).

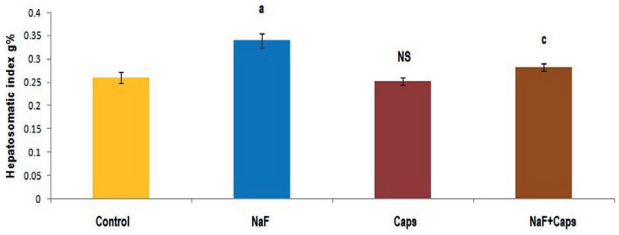


Figure 1. Effect of Caps on relative liver weight to total body weight. Error bar represent Mean±SE (n = 6). Significant based on Kruskal Wallis non-parametric ANOVA test ### (p=0.0021). Significant based on Mann Whitney: [a]Control vs. NaF (P<0.05); [b]control vs. Caps (P<0.05); [c]NaF vs. Fluoride+Caps (P<0.05). NaF: sodium fluoride; caps: capsaicin.

3.2 Liver-function Markers

The increase of serum level of ALT, AST and bilirubin are regular biochemical markers of liver disease and damage. Herein, all these biochemical markers were shown to be significantly increased in rats of NaF-treated group which indicated the harmful impacts of fluoride in liver functions (Figures 2A to 2C). Co-treatment of capsaicin to NaF-treated rats significantly (P<0.05) restricted the harmful effects of NaF. The results showed that NaF exposure increased the serum level of ALT, AST, and bilirubin to 144 ± 1.19 IU/l, 205.1 ± 1.4 IU/l and 6.7 ± 0.33 mg/L respectively (P<0.05) while Caps co-treatment decreased the serum of ALT, AST and bilirubin to 45.3 ± 1.14 IU/l 62.1 ± 0.79 IU/l and 0.37 ± 0.03 mg/l respectively.

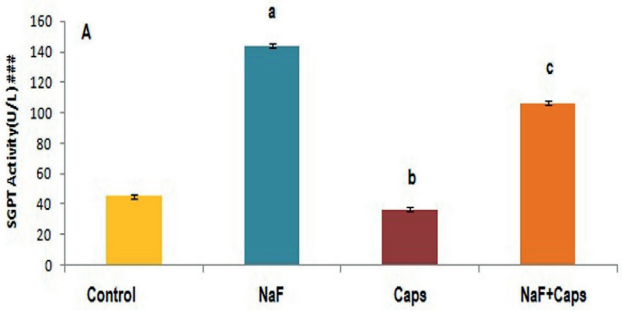


Figure 2A. Protective effect of Caps on serum level of ALT (SGPT). Error bar represents Mean±SE (n = 6). Significant based on Kruskal Wallis non-parametric ANOVA test ###(p<0.0001). Significant based on Mann Whitney: [a]Control vs. NaF (P<0.05); [b]control vs. Caps (P<0.05); [c]NaF vs. Fluoride+Caps (P<0.05). NaF: sodium fluoride; caps: capsaicin.

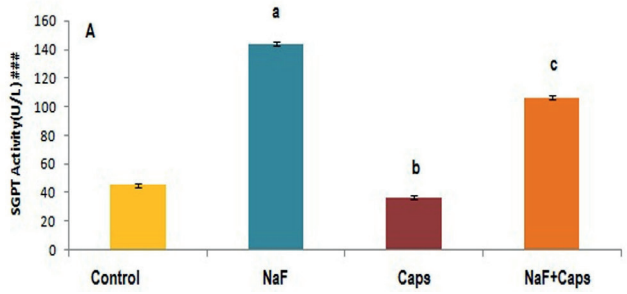


Figure 2B. Effect of Caps on NaF induced changes in serum AST (SGOT) level. Error bar represents Mean±SE (n = 6). Significant based on Kruskal Wallis non-parametric ANOVA test ### (p<0.0001). Significant based on Mann Witney; [a]Control vs. NaF (P<0.05); [b]control vs. Caps (P<0.05); [c]NaF vs. Fluoride+Caps (P<0.05). NaF: sodium fluoride; caps: capsaicin.

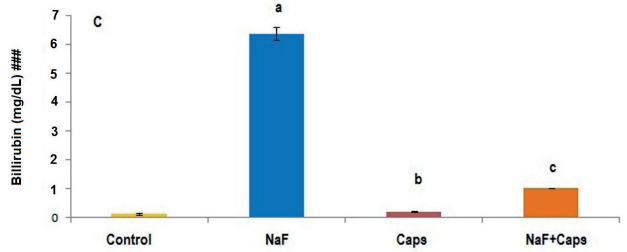


Figure 2C. Effect of Caps supplementation on NaF induced changes in total bilirubin content. Error bar represent Mean Error bar represent Mean±SE (n = 6). Significant based on Kruskal Wallis non-parametric ANOVA test ###(p<0.0001). Significant based on Mann Whitney: [a]Control vs. NaF (P<0.05); [b]control vs. Caps (P<0.05); [c]NaF vs. Fluoride+Caps (P<0.05). NaF: sodium fluoride; caps: capsaicin.

3.3 Status of Oxidative Stress Markers

The generation of MDA (a marker of lipid peroxidation) and NO production were significantly (P<0.05) enhanced by NaF exposure. These augmentations, the MDA level and NO production induced by NaF treatment was significantly decreased by capsaicin supplementation (Table 2).

Results further demonstrated the marked declination of the endogenous antioxidant defense system of the liver in NaF treated rats (Table 3). Five weeks of exposure to NaF significantly lowered the antioxidant enzymes activities of SOD and CAT and level of GSH in comparison to rats of the control group, indicating that NaF exposure caused oxidative stress in the liver. Treatment of Caps with NaF significantly prevented these alterations and restored these antioxidant enzymes activities nearly to those present in normal hepatocytes, demonstrating the antioxidant properties of Caps.

Table 2. Level of the parameters as the index of lipid peroxidation and NO production in the hepatocytes

Parameters	Control	NaF	Capsaicin	capsaicin+ NaF	Significance Level		
					KruskalWallis Test	Mann-Whitney Test	
						Control vs NaF	Control vs Caps	NaF vs caps+ NaF
MDA	24.38± 0.77	51.93± 0.23	25.36 ± 0.51	33.92 ± 0.91	P<0.05	P<0.05	P<0.05	P<0.05
NO	85.53± 1.85	43.62 ± 1.63	87.54 ± 1.1	74.89± 0.34	P<0.05	P<0.05	NS	P<0.05

Values are expressed as mean ± SE. n = 6. NaF: sodium fluoride; caps: capsaicin.

Table 3. Effect of NaF and extract of Capsaicin on enzymatic and non enzymatic antioxidant status in rat hepatocytes

Parameters	Control	NaF	Capsaicin	capsaicin+ NaF	Significance Level			
					KruskalWallis Test	Mann-Whitney Test		
						Control vs NaF	Control vs Caps	NaF vs caps+ NaF
SOD	56.37± 1.05	38.7 ± 2.56	56.7 ± 1.01	52.51 ± 0.83	P<0.05	P<0.05	NS	P<0.05
CAT	76.41± 1.07	38.73± 2.54	79.79 ± 0.95	65.19 ± 1.14	P<0.05	P<0.05	P<0.05	P<0.05
GSH	22.6 ± 0.5	11.74± 0.37	23.13 ± 0.18	16.12 ± 0.4	P<0.05	P<0.05	P<0.05	P<0.05

Values are expressed as mean ± SE. n = 6. NaF: sodium fluoride; caps: capsaicin.

3.4 Histopathology of Liver

The histology of liver sections of experimental rats is presented in Figure 3. Hematoxylin and eosin staining exhibited the normal structural features without any abnormalities in the liver tissue of the control group under light microscope (Figure 3A). Oral Administration of NaF caused marked deformation of liver histology manifested by decreased cellularity, distorted and spare nuclei, and increased intercellular matrix and unstained vacuoles (Figure 3B). While, supplementation of Caps along with NaF, reversed the NaF-induced histological alteration (Figure 3D).

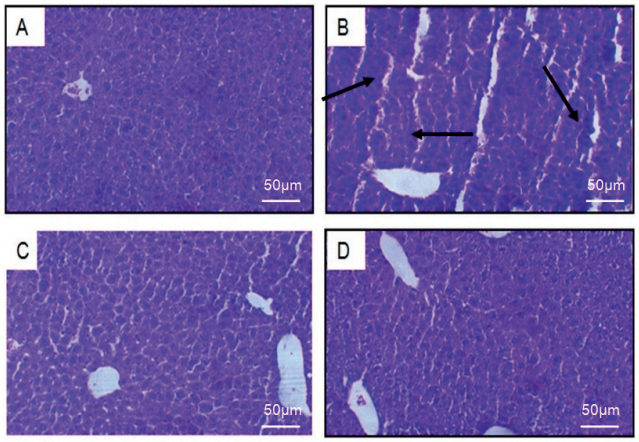


Figure 3. Representative photo micrograph of hematoxylin and eosin stained section (H&E) of liver tissues collected from the rats treated with NaF and Caps [**A**] Control: no histological changes were noticed; [**B**] NaF: marked degenerative changes (⟶) were seen; [**C**] Caps alone: normal histology of liver was noticed; [**D**] combination of Caps and NaF: restoration of NaF-induced degenerative changes in the liver structure.

4. Discussion

One of the factors affecting people's vulnerability to liver disease is the intake of toxic chemicals or drugs since the liver is badly injured in doing its detoxification role. Several studies have reported that excess fluoride consumption leads to injurious effects in the liver by inducing oxidative stress[23,24]. Further, various experimental evidences have confirmed that fluoride-induced oxidative injury was the key underline mechanism in the pathogenesis of fluoride toxicity and its complication in several tissues including liver[9,25,26]. In the present study, the toxic effects of NaF were indicated by a significant increase of free radicals generations, depletion of antioxidant defense system, change in serum AST, ALT and bilirubin level in fluoride-treated rats.

Nonetheless, we examined that these toxic effects of NaF on the liver could be reversed by supplementation of Caps along with NaF. These results were further well supported by histological examination of liver.

Oxidative stress is a condition whereby the homeostasis of the redox status of the cell is distorted. Free radical generation is a natural process and overproduction of oxygen free radicals and decreased antioxidant defense system lead to oxidative stress. The enzymes SOD and CAT act as an endogenous antioxidant defense system and afford first-line defense against oxidative damage by detoxifying toxic superoxide, H_2O_2, and OH^- radicals[27,28]. In the present study, exposure to NaF significantly induced ROS production and lipid peroxidation in hepatic tissue of rats. The earlier studies established that NaF treatment caused lipid peroxidation in rat hepatic tissue [24-25] which confirmed our findings. Besides, a significant reduction in SOD and CAT activities in previous studies is similar to our results in NaF-treated hepatic cell[7,29].

The toxic effects of fluoride on the liver and other tissues led the researchers to supplemental processes that could combat the damaging effect on the liver. The therapeutic use of bioactive compounds has been increasing gradually since they are natural and have less toxic effects. Thus, traditional medicine will serve as an important search engine to rediscover new drugs from natural sources[30]. Capsaicin is present in hot and red chili peppers and acts as a potent natural antioxidant[14]. Previous researches demonstrated that this phytochemical could efficiently diminish oxidative stress by detoxifying ROS[11] and can prevent lipid peroxidation[12–14].These reports are well in agreement with the results obtained in the present study. Our results indicated that Caps protected the liver from NaF-induced oxidative damage by elevating the activities of SOD, CAT and the level of GSH.

Superoxide anion (O_2^-), produced by Kuffer cell and hepatocyte, is a potential mediator of hepatitis[31] and also promote apoptosis. Antioxidant enzyme SOD protects the cells by scavenging these toxic superoxide anions[32]. The significant reduction in SOD activity in previous studies is similar to our results in NaF-treated liver cells[7,8]. We found that Caps co-treatment significantly restored the SOD activity.

Catalase (CAT) is an iron-dependent antioxidant enzyme that protects the cell by degrading H_2O_2 to water[33]. Fluoride is believed to inhibit CAT activity[7]. On Caps co-treatment, the activity of this enzyme was elevated in rats treated of NaF plus Caps group.

GSH is a tripeptide and is an important component of the endogenous redox buffer system, which protects cells by detoxifying ROS. In the present study, a decline was observed in the GSH level in NaF-treated rats. The level of GSH in rats challenged with NaF was markedly increased upon Caps co-treatment.

The results of previous studies demonstrated that fluoride-induced oxidative stress damage liver histoarchitecture such as disruption of hepatic cord, Kupffer cell proliferation[34,35]. However, Caps supplementation in the present study was able to restore normal histoarchitecture of liver. At the same time, it was suggested by many investigators that fluoride-induced histological damages of the liver significantly elevated the serum AST and ALT activities[36,37]. We observed that Caps successfully prevented the leakage of AST and ALT from liver cells, a protective effect of Caps, partly owing to above mentioned antioxidant property. It is generally acknowledged that flavonoids-rich diet is a good source of antioxidant and Caps is one of such flavonoid whose potential antioxidant and antiapoptotic role has confirmed. The result of the present investigation is in concurrence with past discovering[14,38] which demonstrated that Caps supplementation were successful against oxidative damage by scavenging ROS and by improving the antioxidant defense system. Henceforth, the role of Caps as a free radical quencher and its role in maintaining tissue's redox status are clear from our examination.

5. Conclusion

Therefore, it can be concluded from this investigation that fluoride (NaF) intoxication caused severe oxidative damage in the liver of rats by increasing free radicals generation. Furthermore, Caps supplementation in NaF-treated rats showed marked amelioration, indicating its protective role against fluoride-induced liver injury. Capsaicin, an active principle of red pepper, may, therefore, be used as a natural safeguard of liver in human beings of the community where fluorosis concerns.

6. Acknowledgements

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7. Conflict of Interest

Authors declare that they have no conflict of interest.

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