PROTECTIVE EFFECT OF BEE POLLEN AGAINST SODIUM FLUORIDE-INDUCED HEPATONEPHROTOXICITY AND SERUM ELECTROLYTE CHANGES IN RATS

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ABSTRACT: The aim of the present study was to investigate the protective role of bee pollen against sodium fluoride (NaF)-induced hepatonephrotoxicity in the rat. Male Western albino rats were divided into four groups, each consisting of seven animals: (i) normal [control] group; (ii) rats treated with a chronic dose of NaF [5 mg/kg body weight (bw) for one month]; (iii) rats treated with bee pollen [250mg/kg bw for 30 days], and (iv) rats treated with bee pollen first [250 mg/kg bw for 30 days] followed by NaF [5 mg/kg bw for one month]. The level of serum electrolytes and enzymatic antioxidants in the liver and kidney were measured. We found a significant increase in magnesium (Mg) [$p<0.001$] whereas sodium (Na) was significantly [$p<0.05$] decreased in the serum of the NaF-exposed rats, as compared to the control group. Also, in the NaF-treated group, as compared to the control group, in both liver and kidney tissue, we found a significant ($p<0.05$) increase of lipid peroxidation and glutathione S-transferase (GST) while, remarkably, there was a significant ($p<0.05$) decrease in both glutathione (GSH) and vitamin C. The bee pollen administration did not show any significant toxic or negative effects on the serum electrolytes, the liver function, or the kidney function in the rats. The changes induced in the group treated with NaF alone were, remarkably, restored in the bee pollen+NaF treatment group. Thus, bee pollen exerts a protective property by ameliorating the NaF-induced serum electrolyte changes and the NaF-induced oxidative stress in the rat.

Keywords: Bee pollen; Hepatonephrotoxicity; Oxidative stress; Serum electrolytes; Sodium fluoride.

INTRODUCTION

Some of the universal health problems experienced currently are diseases which result from exposure to toxic chemicals. These chemicals mainly target and damage the liver and kidney as the liver is the main site of xenobiotic metabolism and the kidney plays a major role in plasma filtration and the maintenance of metabolic homeostasis.[1] The fluoride ion may be present in public drinking water, dietary choices, dental products, and industrial emissions and, when these levels are high, the total fluoride intake may exceed the safe dose.[2] Fluoride is not an essential trace mineral in the human body and is not necessary for the development of healthy teeth and bones.[3] An excessive intake induces many pathological changes and disturbs the functions of many tissues.[4] Fluoride in the body is readily absorbed by the intestine and, in high doses, may lead to hepatonephrotoxicity as it can alter the free radical metabolism in both the liver and kidney.[5] Bee pollen has been used in folk medicines for centuries as a remedy against various types of diseases. It is rich in protein, carbohydrates, fat, minerals, vitamins, ash, water, and other substances with high antioxidant activity in addition to a capacity for scavenging free radicals.[6] Bee pollen is also very rich in phenolic acids and flavonoids and about 70% of the substances in it are biologically active. Bee pollen thus possesses nutritive, antioxidative, hepatoprotective, nephroprotective, anti-inflammatory, antibacterial, anticancerogenic, and immunostimulant properties. In the present study, the effect of a chronic dose of sodium fluoride (NaF) in rats was assessed using oxidative stress

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biomarker assays on liver and kidney in addition to measuring serum electrolyte changes. Also, the possible protective role of bee pollen on the tissue damage in NaF-induced hepatonephrotoxicity was evaluated.

MATERIAL AND METHODS

Animals: Thirty-two male Western albino rats (100–150 g) were obtained from King Saud University, Riyadh, Kingdom of Saudi Arabia. The animals were kept under standard conditions of temperature and humidity. The rats were divided into four groups, each consisting of seven rats: (i): a normal control group who received phosphate buffered saline throughout the experiment; (ii) group two who received a chronic dose of NaF (5 mg/kg bw) for one month; (iii) a third group who received bee pollen (250 mg/ kg bw) for 30 days; and (iv) group four who received bee pollen (250 mg/ kg bw) for 30 days followed by a chronic dose of NaF (5 mg/kg) for one month.

Ethics approval: All the animal experiments were conducted with the approval of King Saud University.

SAMPLE COLLECTION:

Serum: Blood collected from the retro-orbital plexus was processed to obtain serum by centrifugation at 3,000 rpm for 10 min for the estimation of the serum electrolytes.

Tissue: Liver and kidney tissues were collected and washed with cold normal saline and then homogenized in ten volume/weight of buffer (50 mM Tris-HCl, 1.15% KCl pH 7.4). The homogenate was then centrifuged at 3,000 rpm for 10 min. The supernatant obtained was used for the various biochemical assays.

BIOCHEMICAL ANALYSES:

Serum sample: The potassium levels were measured in a protein-free alkaline medium by reaction with sodium tetraphenyl boron, which produced a colloidal suspension.[7] The sodium levels were assayed by the sodium-dependent galactosidase activity using ortho-nitrophenyl-β-galactoside (ONPG) as a substrate.[8] The magnesium level was assayed by the formation of a colored complex with magonsulfonate in alkaline solution.[9] The chlorine level was estimated by the quantitative displacement of thiocyanate by chlorine from mercuric thiocyanate and the subsequent formation of a red ferric thiocyanate complex.[10]

Liver tissue: Lipid oxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS) by the method of Ruiz-Larrea et al.[11] The assay of vitamin C was performed according to the method of Jagota and Dani.[12] Glutathione (GST) was assayed by the method of Beutler et al.[13] using 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) with sulfhydryl compounds to produce a relatively stable yellow color. The glutathione S-transferase activity (GST) activity was assessed using an assay kit (Biovision, USA) that was based upon the GST-catalyzed reaction between GSH, GST substrate, and CDNB (1-chloro-2,4-dinitrobenzene).[14]

Statistical analysis: The results were expressed as the mean±standard error of mean (SEM). The results were evaluated by using SPSS (version 12.0) and Origin 6 software and evaluated using one-way ANOVA. Differences were considered statistically significant at $p<0.05$.

RESULTS

The data on the serum electrolyte levels in the control rats and in all the treated groups are presented in Table 1.

Table 1. Serum electrolytes in the treated rats compared with the control group. (Values are mean± SEM)

Parameter	Group			
	Control	NaF	Pollen	Pollen +Na F
Mg (mg/dL)	2.161 ±0.02622	2.261 ±0.01469†	2.195 ±0.03311	2.125 ±0.01637
Cl (mmol/L)	87.22 ±0.7004	85.59 ±0.698	88.24 ±0.7716	88.71 ±1.149
Na (mmol/L)	88.29 ±3.476	75.29 ±3.663*	94.29 ±4.167	103.6 ±1.888†
K (mmol/L)	5.713 ±0.4509	6.117 ±0.09458	6.743 ±0.1251*	7.014 ±0.1405*

Compared to the control group: *p<0.05, †p<0.001)]

In the NaF group, as compared to the control group: (i) the magnesium (Mg) level was significantly ($p<0.001$) increased; (ii) the sodium (Na) level was significantly ($p<0.05$) decreased; and (iii) the chloride and potassium levels were not significantly different.The pollen treatment alone did not change the level of electrolytes except for the potassium which was significantly ($p<0.05$) increased. Remarkably, the pollen+NaF treatment restored the increase in the Mg and the decrease in the Na seen in the group treated with NaF alone back to values that did not differ significantly from those found in the control group (Table 1).

Tables 2 and 3 and Figures 1A and 1B show the lipid peroxidation levels (MDA) in the liver and kidney of the rats of all the treated groups.

Table 2. MDA, GSH, GST, and vitamin C in liver tissue in the treated rats compared with the control group. (Values are mean± SEM)

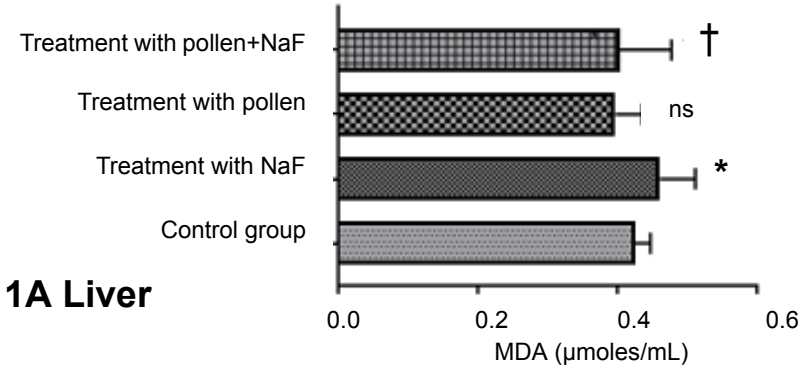
Parameter	Group			
	Control	NaF	Pollen	Pollen +Na F
MDA (µmoles/mL)	0.42 ±0.0075	0.46 ±0.0180*	0.39 ±0.0136	0.40 ±0.0261†
GSH (µg/mL)	2.718 ±0.1244	2.43 ±0.0846	3.33 ±0.0460†	2.93 ±0.1393*
GST. (U/mL)	73.83 ±1.582	75.33 ±0.853*	70.83 ±1.238	71.28 ±0755
Vitamin C (µg/mL)	23.05 ±0.02	22.00 ±0.01*	30.42 ±0.140‡	21.05 ±0.14

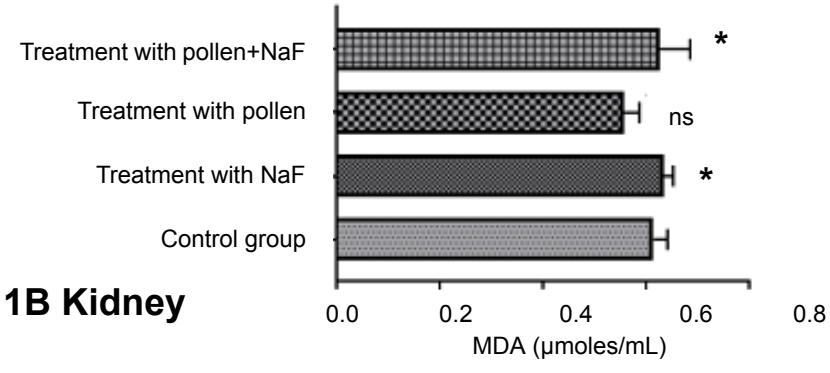
Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.]

Table 3. MDA, GSH, GST, and vitamin C in kidney tissue in the treated rats compared with the control group. (Values are mean± SEM)

Parameter	Group			
	Control	NaF	Pollen	Pollen +Na F
MDA (µmoles/mL)	0.61 ±0.0089	0.63 ±0.0059*	0.56 ± 0.0136	0.625± 0.0019*
GSH (µg/mL)	3.5 ±0.527	3.414 ±0.0301	4.238± 0.1224‡	3.422 ±1.1507†
GST. (U/mL)	16.55 ±1.566	20.68 ±1.374*	19.88 ±1.048*	17.16 ±1.355
Vitamin C (µg/mL)	63.64 ±2.792	60.33 ±4.472*	62.82 ±3.389	61.86 ±1.195

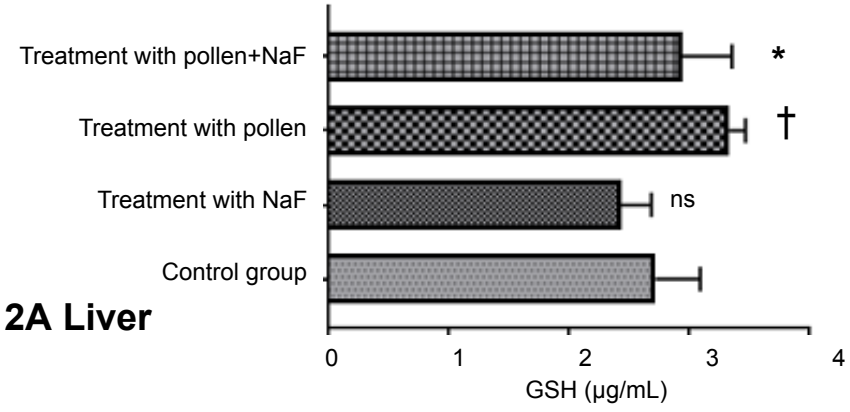
Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.

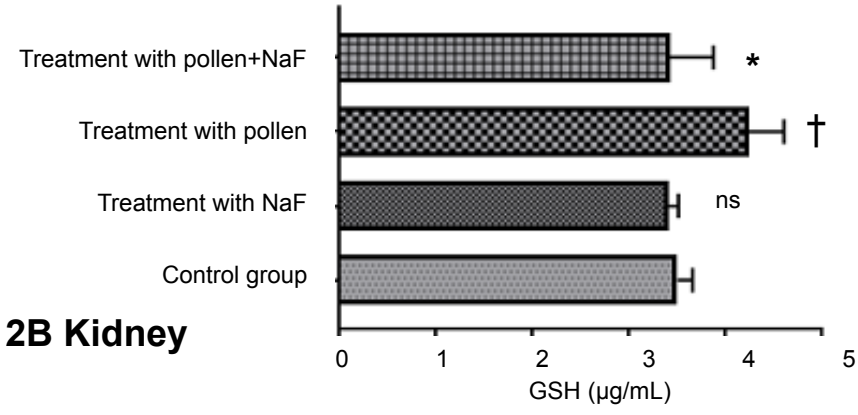




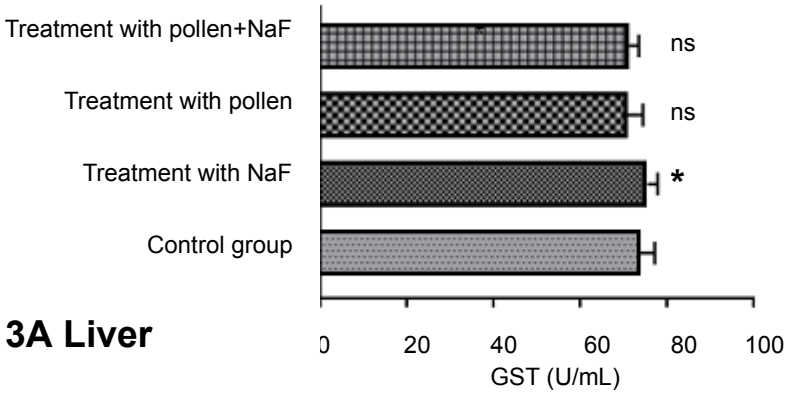
Figures 1A and 1B. Lipid peroxidation levels, MDA (µmoles/mL), in liver and kidney homogenates in the treated rats compared with the control group. 1A: Lipid peroxidation levels, MDA (µmoles/mL), in liver homogenate; 1B:1A: Lipid peroxidation levels, MDA (µmoles/mL), in kidney homogenate. Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.

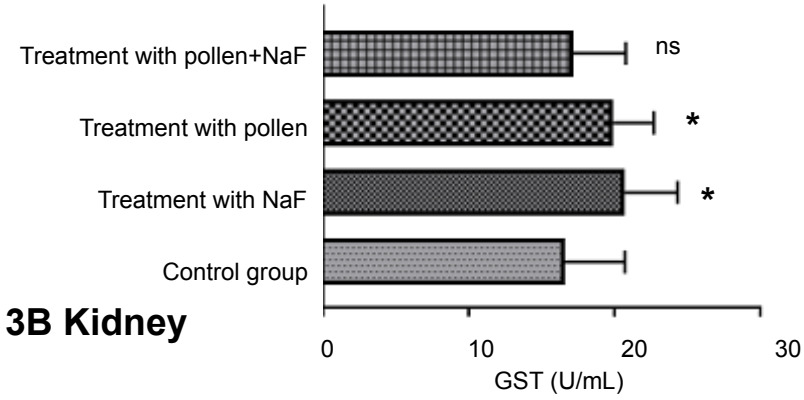
A chronic dose of NaF caused a significant ($p<0.05$) increase of lipid peroxidation while pollen alone resulted in a non-significant decrease in the levels in both the liver and kidney. The pollen+NaF treatment significantly reversed the NaF-induced increase in lipid peroxidation in both the liver ($p<0.001$) and kidney ($p<0.05$) tissues. Tables 2 and 3 and Figures 2A, 2B, 3A, 3B, 4A, and 4B show the levels of GSH, GST, and vitamin C, respectively, in the liver and kidney of all the treatment groups. The vitamin C level was significantly ($p<0.05$) decreased while GST was significantly ($p<0.05$) increased by the NaF treatment. Treatment with pollen+NaF treatment restored the vitamin C and GST levels back to levels not significantly different from those of the control group in both tissues.





Figures 2A and 2B. GSH (µg/mL), in liver and kidney homogenates in the treated rats compared with the control group. 2A: GSH (µg/mL) in liver homogenate; 2B:1A: GSH (µg/mL) in kidney homogenate. Compared to the control group: *$p<0.05$, †$p<0.001$, and ‡$p< p<0.001$.





Figures 3A and 3B. GST (U/mL), in liver and kidney homogenates in the treated rats compared with the control group. 2A: GST (U/mL) in liver homogenate; 2B:1A: GST (U/mL) in kidney homogenate. Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.

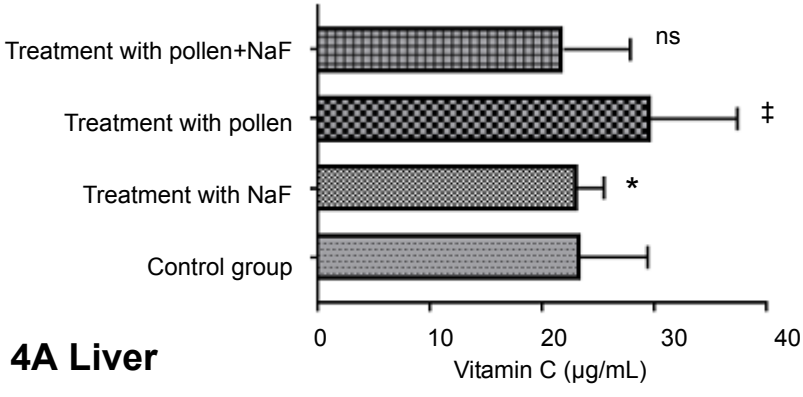


Figure 4A. Vitamin C (µg/mL) in liver homogenates in the treated rats compared with the control group. Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.

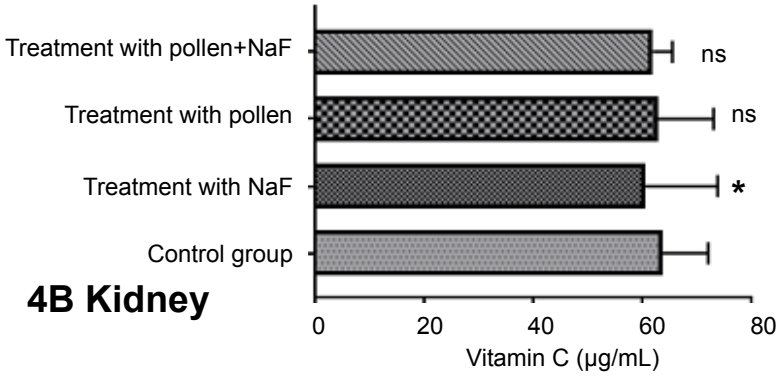


Figure 4B. Vitamin C (µg/mL) in kidney homogenates in the treated rats compared with the control group. Compared to the control group: *p<0.05, †p<0.001, and ‡p< p<0.001.

DISCUSSION

Our results revealed that a chronic dose of sodium fluoride induced toxicity, leading to significant changes in the serum electrolytes and the antioxidant enzymes in liver and kidney tissue. The administration of bee pollen helped to restore the levels of antioxidant enzymes and of some of the serum electrolytes alterations induced by the NaF.

Our results showed a significant increase in the Mg level in serum of the NaF-treated rats which indicates improper function of kidney as Mg is excreted only through the kidney.[15] When the serum levels of K and Na were analyzed, we found a significant decrease in the Na level which could also indicate impaired renal function because of impaired tubular reabsorption and osmotic disequilibrium between the luminal fluid and medullary interstitial fluid could impair dilution.[16] The non-significant increase of the K concentration caused by NaF, may indicate that the membrane channels are affected and that renal function is depressed. [17] The chloride ion levels were, however, non-significantly lower than the control values.

The administration to rats of a chronic dose of NaF for one month significantly increased lipid peroxidation which is the fundamental index of oxidative damage and is reflected in the increased MDA level both in the liver and kidney. NaF consumption results in the production of free radicals, which can react with polyunsaturated fatty acids to yield lipid hydroperoxides which in turn initiate a lipid-radical chain reaction leading to oxidative damage to the cell membrane. The pro-oxidative properties of fluoride ions were confirmed previously by Birkner et al.[18] In our work, the glutathione and vitamin C levels were decreased in both the liver and kidney by a chronic dose of NaF indicating an impaired function of the hepatic and nephritic antioxidant defense systems. The reduction of both parameters with fluoride administration might result from their utilization in redox reactions as some studies have concluded that fluoride can affect the activity of the enzymes involved in the cell antioxidative system.[19] GSH acts as an antioxidant and plays an important role in the cell defense system[20] and vitamin C plays an important physiological role in cells as an antioxidant and free radical scavenger.[21] GST protects cells from oxidative stress by detoxifying some of the secondary ROS produced when ROS

react with cellular constituents. A significant increase of GST in the liver and kidney in the NaF-treated group can be correlated with an increased resistance to oxidative stress.[22]

In the present study, when the results of the bee pollen-treated group of rats were evaluated and no significant toxic or negative effects on the serum electrolytes and the liver and kidney function were observed. Also, we observed a remarkable improvement in all the serum electrolytes, except for potassium, and in the oxidative stress markers of the group which was treated with bee pollen for one month before receiving a chronic dose of NaF for one month. This can be related to the potency of the bee pollen in restoring damage in tissues.[23] Bee pollen is a source of hydrophilic antioxidants which protect cells, organs, and extracellular fluid against oxidative structure damage. Bee pollen is known for its detoxification activity and it can remove metals and drugs.[24,25] The potassium level was significantly increased in the pollen as well as the pollen+NaF treated groups which may be due to the fact that bee pollen is very rich in potassium and, of all the elements, it occurs in the highest concentration in bee pollen.[23,26] However, further research is needed to verify the effect of a high chronic dose of bee pollen on the potassium level in serum for the reason that bee pollen is but one among the high potassium diets. Also, there is a need to be evaluate the dose of bee pollen which, when given chronically, has the most beneficial effects with a minimum of side effects.

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