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ENHANCEMENT OF KIDNEY AND LIVER RESPIRATORY ACTIVITY BY QUERCETIN SULFONATES IN RATS CHRONICALLY EXPOSED TO AMMONIUM FLUORIDE

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Summary: The respiratory activity of liver and kidney slices of rats chronically exposed to ammonium fluoride was studied. It was found that a mixture of quercetin sulfonates stimulated tissue metabolism and exerted a protective effect in NH_4F intoxication through normalization of respiratory activity.

Keywords: Ammonium fluoride, Fluoride in rats, Kidney respiration, Liver respiration, Quercetin sulfonates.

INTRODUCTION

Fluorides in the atmosphere are the most hazardous pollutants when and where their concentrations exceed admittedly very low levels. Their high toxicity and continuous build-up in the environment have been a growing threat to mankind.[1-3]

One of the most serious effects of fluorine intoxication is impairment of the energy system of the organism, affecting both glycolysis and oxidative processes of cells.[2,4] Morphological, histochemical, and biochemical studies on the toxicity of fluorine compounds have revealed lesions to parenchymatous organs like kidneys and liver.[3,5,6-9]

Our previous work demonstrated a beneficial effect of quercetin on disturbances of lipid metabolism.[5] A protective effect of pollen (*Cernitin*) on the respiratory activity of rats tissues in ammonium fluoride intoxication was also proven.[10] We have now decided to continue the search for easily accessible and safe pharmaceuticals effective in reducing the toxic effects of fluorides.

MATERIAL AND METHODS

The experiment was performed on 96 male Wistar rats with an initial body weight of ca. 300 g. The animals were exposed in a 1-m^3 toxicological chamber to ammonium fluoride, NH_4F, as an aerosol at an average concentration of 2 mg F/m^3 (twice the work-period MAC) for 3 months (13 weeks) or 6 months (26 weeks) for 6 hr/day for 5 days each week. The rats were randomly divided into 12 groups with 6 to 10 rats per group that were treated as shown in the following Table.

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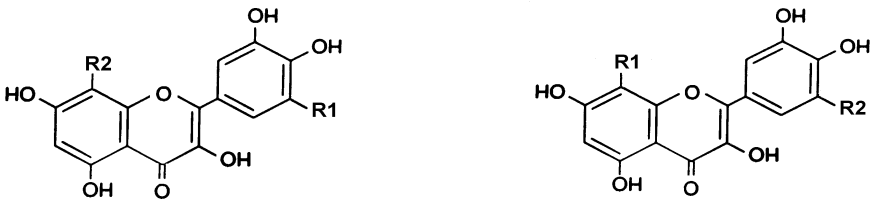
Table. Treatment protocol for the rat experiments

Groups 3 Mo.	6 Mo.	Treatment
IA	IB	Non-exposed control
IIA	IIB	Quercetin 5 mg/kg bw/24 hr
IIIA	IIIB	Quercetin 20 mg/kg bw/24 hr
IVA	IVB	NH_4F 2 mg/m^3 (Exposed control)
VA	VB	NH_4F 2 mg/m^3 + Quercetin 5 mg/kg bw/24 hr
VIA	VIB	NH_4F 2 mg/m^3 + Quercetin 20 mg/kg bw/24 hr

A mixture containing 1:1 quercetin 5',8-disulfonic (Na_2QDSA) and monosulfonic acid sodium salts (NaQSA-5'/NaQSA-8) synthesized at the Department of Inorganic Chemistry, Technical University of Rzeszów (Figure 1), was mixed with standard chow pellets containing 7 mg F/kg. Full consumption of quercetin pellets was ascertained before the animals were returned to normal chow. Rats had free access to tap water containing 0.2 mg F/L.



A R1 = SO_3H R2 = SO_3Na B

Figure 1. Quercetin 8,5'-disulfonic acid sodium salts: NaQSA-8 (A) and NaQSA-5' (B).

As in our previous work,[5] an aerosol of NH_4F was led into the 1-m^3 toxicological chamber by an atomizer inhaler (Ogarit, JV-S). Rats were kept at constant conditions: humidity (60%), temperature (20 deg C), air flow (10 m^3 /hr), and a 12/12-hr light/dark cycle. The concentration of fluoride ion in the chamber was measured once a week using an ion-selective fluorine electrode according to PN-83/Z-04093.07 norm. The average value from 12

measurements (3-month exposure period) deviated from 2 mg F/m^3 by 6.0%, with individual values ranging from 1.6 to 2.4 mg F/m^3. The average value from 24 measurements (6-month exposure period) deviated from 2 mg F/m^3 by 4.8%, with individual values ranging from 1.6 to 2.5 mg F/m^3. Operation of the fluorine dosing system was continuously monitored by checking the volume of NH_4F solution fed to the chamber.

Rats were weighed every two weeks. At the end of the experiment the animals were lethally anaesthetized with ether. Liver and kidneys were removed, frozen immediately at -70°C, and stored for up to one month. Tissue respiration rates were monitored using Clark's oxygen electrode (5331 Standard Oxygen Probe, YSI Instrument Co., Ohio, USA) in 40-80 mg tissue slices suspended in 5 cm^3 of medium containing 50 mM KCl, 2 μM rotenone (inhibitor of complex I of the respiratory chain), and 50 mM phosphate buffer at pH 7.3 and 37°C. Respiration was started by adding 100 mg potassium succinate to the incubation chamber. This amount of respiratory substrate grossly exceeded the normal cellular level and served to maintain maximal respiratory rate.[11] Oxygen consumption was expressed in nanoatoms/hr/mg dry tissue. The initial concentration of oxygen was taken as 199 μM. Dry tissue mass was determined by drying for 5 hr at 100°C and weighing on a precision balance. Statistical analysis was done using Student's t-test for unpaired results and taking the level of significance as $p < 0.05$.

RESULTS

A decrease of 10% in the body mass of rats exposed for 6 months to ammonium fluoride as compared with controls (group IVB *vs.* IB) was noted. Differences in body mass for controls receiving and not receiving quercetin (IA-B and IVA-B) were insignificant. Changes in oxygen consumption rates of liver and kidney tissue slices are given in Figures 2a and 2b, respectively.

Oxygen consumption rates of kidney slices taken from animals not exposed to NH_4F were increased by quercetin sulfonates administered during 6 months. The increase was 76% at 5 and 39% at 20 mg/kg bw/24 hr (groups IIB and IIIB, respectively *vs.* IB) (Figure 2b).

After the rats were exposed to HN_4F for 3 or 6 months, the following changes in tissue respiratory activity were observed: an exposure for 3 months led to a 29% increase in the respiratory activity in the liver slices (Figure 2a) and a 25% decrease in the kidney slices (Figure 2b). After 6 months, the respiratory activity of the liver tissue decreased by 22% (Figure 2a), while that of kidney tissue increased by 71% (Figure 2b). The differences were statistically significant, except for the decrease in the respiratory rate of liver after 6 months (groups IVA *vs.* IA and IVB *vs.* IB).

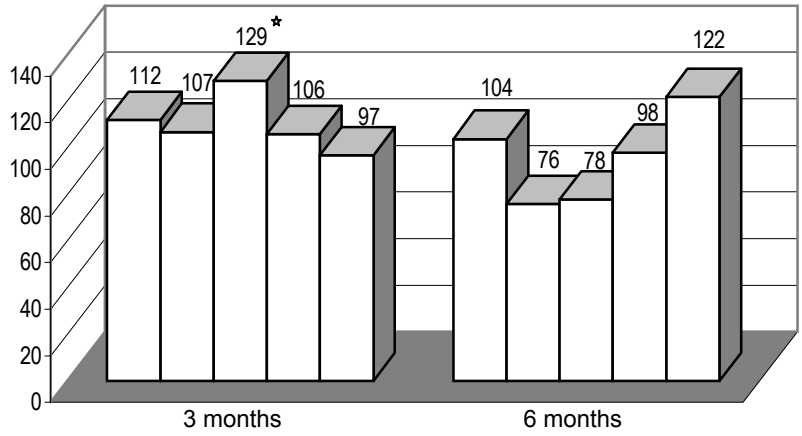


Figure 2a. Respiratory activity (control = 100%) of liver tissue of rats after 3 months (A groups) and after 6 months (B groups) of the experiment.

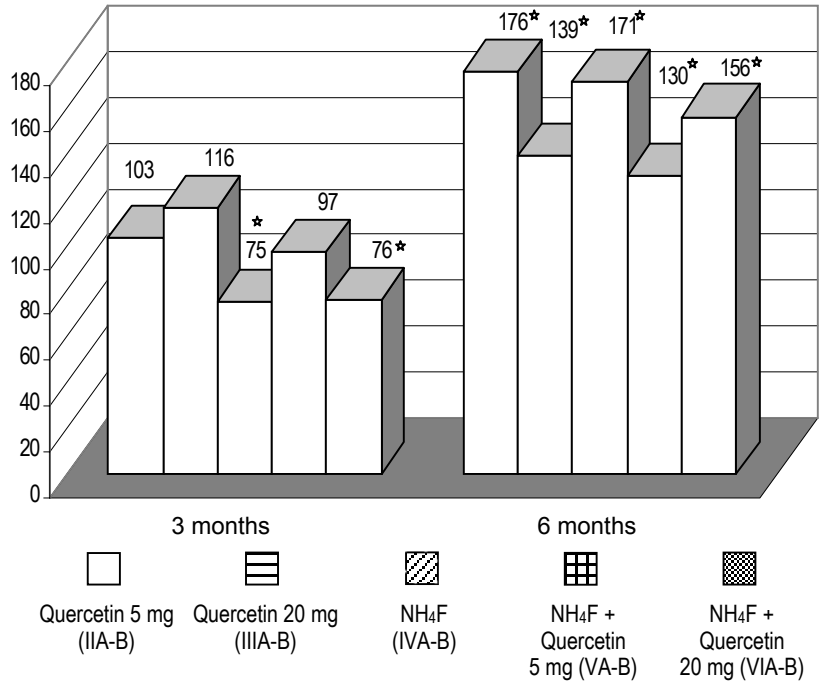


Figure 2b. Respiratory activity (control = 100%) of kidney tissue of rats after 3 months (A groups) and after 6 months (B groups) of the experiment.

*Statistically significant ($p<0.05$) in comparision with the respective control Group IA or IB taken as 100%.

As also seen in Figures 2a and 2b, in the 3-month exposure groups, 5 mg/kg bw/24 hr quercetin sulfonates normalized the rate of oxygen consumption of both liver and kidney slices (groups VA *vs.* IA). Quercetin sulfonates at 20 mg/kg bw/24 hr normalized oxygen consumption of liver slices and decreased it by 24% in the case of kidney tissue (groups VIA *vs.* IA). When exposure to HN_4F was for 6 months, quercetin sulfonates at 5 mg/kg bw/24 hr normalized the rate of oxygen consumption of liver slices and increased the kidney rate by 30% (groups VB *vs.* IB). The higher dose of quercetin sulfonates increased oxygen consumption of kidney slices by 56%. An insignificant increase of 22% was noted for the liver (groups VIB *vs.* IB).

DISCUSSION

The aim of the present work was to study the effect of ammonium fluoride and a mixture of quercetin sulfonates on the respiratory activity of rat liver and kidney. Under conditions of the present experiment with maximal activation of succinate dehydrogenase, oxygen consumption rates can be regarded as an index of the metabolic activity of the cell.[11]

The overall condition of animals assessed on the basis of body weight deteriorated only in the case of exposure to NH_4F for 6 months as evidenced by a 10% weight loss in relation to the control group (groups IVB *vs.* IB). This finding is in line with other reports on the toxic properties of fluorine compounds.[1,2] No significant changes in body weight were observed when animals exposed to NH_4F were simultaneously given quercetin sulfonates. We are inclined to attribute this result to the protective action of quercetin. The weight of animals in the remaining groups did not change in comparison with the controls (groups IA and IB).

Changes in the respiratory activity of the liver and kidney clearly show that both organs play a complementary role in NH_4F detoxification. The respiratory activity of liver tissue was increased after 3 months of exposure to NH_4F and decreased after 6 months. A reverse pattern was observed for kidney tissue: respiratory rates were reduced after 3 months of exposure and increased after 6 months. Apparently, the role of kidneys becomes more important in prolonged exposure to ammonium fluoride.

When assessing the toxic effect of NH_4F one has to remember that both components, *i.e.*, fluoride ion and ammonium ion may contribute to the properties of this compound. In present study NH_4^+ ion concentration applied as an aerosol was 1.9 mg/m^3. There is an evidence, that NH_4^+ ion given as a NH_4Cl salt inhibited tissue respiration in rats, but at higher concentrations than these used in the present study, *i.e.*, 0.6 g/kg bw.[12] No further data on effects of NH_4^+ ion on tissue respiration in animals chronically exposed to NH_4^+ are available.

Plant products containing quercetin have long been exploited to stimulate bile excretion, protect the liver, enhance renal filtration, and increase the excretion of xenobiotics. Furthermore, the toxicity of fluorine compounds has been traced to the stimulation of peroxidation of cellular lipids.[13,14] The antioxidative action of flavonoids like quercetin is due to the presence of hydroxyl groups, particularly in ring B (Figure 1), which may contain two (catechol) or three (pyrogallol) hydroxyls.[15] An additional feature of quercetin sulfonates used by us is their enhanced solubility in water without any loss of antioxidative properties and metal chelating strength.[16]

In the present work, either 5 or 20 mg/kg bw/24 hr of quercetin sulfonates given to rats during NH_4F exposure (3 or 6 months) normalized the respiratory activity of liver tissue. Normalization of the respiratory activity of kidneys was observed only for the lower dose and shorter exposure period. The effect was particularly sharp in the case of kidney slices from rats given quercetin sulfonates (5 or 20 mg/kg bw/24 hr) during 6 months. We attribute these findings to stimulation by sulfonate derivates of quercetin of cellular respiratory activity.

As already mentioned, the present work is a continuation of the search for safe and easily accessible pharmaceuticals that are effective in reducing the toxic effects of fluorides. In our previous study, a protective effect of quercetin sulfonates on hepatic metabolism of lipids in rats exposed to ammonium fluoride was observed. Quercetin salts normalized lipid content in serum, liver homogenates, and liver microsomal fraction in rats exposed to NH_4F for 3 months.[5] The results of the present work confirm beneficial properties of quercetin salts. The mixture of quercetin sulfonates stimulated and normalized tissue respiratory activity and thus could be applied as an prophylactic remedy in fluoride intoxication of people who are exposed to excessive fluoride emission.

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