

## THE INFLUENCE OF QUERCETIN ON SOME PARAMETERS OF LIPID METABOLISM IN RATS CHRONICALLY EXPOSED TO AMMONIUM FLUORIDE

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**SUMMARY:** Male Wistar rats were exposed to ammonium fluoride vapours in a toxicological chamber for 3 months. A mixture of sulfonic acid sodium salts of quercetin at a dose of 5 or 20 mg/kg body weight/24 h was given to some of the animals. It was found that quercetin salts alleviate changes in hepatic metabolism of lipids caused by ammonium fluoride.

Keywords: Ammonium fluoride, Fluoride toxicity, Fluoride vapours, Lipid metabolism, Quercetin.

### INTRODUCTION

The aim of this work was to examine the influence of quercetin on chronic intoxication of rats with ammonium fluoride. A 1:1 mixture of quercetin 8,5' disulfonic acid sodium salts (Na<sub>2</sub>QDSA): NaQSA-5' and NaQSA-8 (Figure 1), synthesized at the Department of Inorganic Chemistry, Technical University of Rzeszów, was used.

Pharmacological investigations have demonstrated the diverse activity of flavonoids, including rutin and its aglucon – quercetin.<sup>1,2</sup> These compounds act as coenzymes, take part in cardiac carbohydrate metabolism, are helpful in the treatment of hypertension, slow down the process of atherosclerosis in blood vessels (Figure 1) and protect the liver from various harmful factors.<sup>3-5</sup>

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

### MATERIALS AND METHODS

The experiment was performed in 60 male Wistar rats with an initial body weight of approx. 300 g, divided into six groups of 10 animals:

- Group I Control
- Group II Quercetin 5 mg/kg b.w./24 h (Q-1)
- Group III Quercetin 20 mg/kg b.w./24 h (Q-2)
- Group IV NH<sub>4</sub>F 2 mg/m<sup>3</sup> in air (HN<sub>4</sub>F)
- Group V NH<sub>4</sub>F 2 mg/m<sup>3</sup> and quercetin 5 mg/kg bw/24 h (NH<sub>4</sub>F + Q-1)
- Group VI NH<sub>4</sub>F 2 mg/m<sup>3</sup> and quercetin 20 mg/kg bw/24 h (NH<sub>4</sub>F + Q-2)

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Quercetin was added to standard chow in the form of globules. The rats had free access to standard chow and water and were weighed every two weeks. Exposure to  $\text{NH}_4\text{F}$  was for 3 months, 6 hours per day and 5 days per week, in a toxicological chamber. Humidity (60%), temperature ( $20^\circ\text{C}$ ) and air flow ( $10\text{ m}^3/\text{h}$ ) were kept constant.  $\text{NH}_4\text{F}$  at a concentration of  $2\text{ mg}/\text{m}^3$  was administered in the form of aerosol in air. The concentration of fluoride ion in the chamber was monitored using an ion-selective fluorine electrode according to Polish norm PN-83/z-04093.07.

At the end of the experiment the animals were anaesthetised with ether. Blood was obtained by cardiac puncture. The liver was excised, weighed, placed immediately in ice-cold, pH 7.4 0.1 M phosphate buffer, cut into pieces, and rinsed free of blood. Livers were homogenised in a Potter-Elvehjem glass homogeniser with a teflon pestle. The homogenate was centrifuged at 700 g for 10 min at  $3\text{-}5^\circ\text{C}$ . The supernatant was poured off and centrifuged at 9,000 g for 20 min at  $3\text{-}5^\circ\text{C}$ . The resulting supernatant was centrifuged at 100,000 g for 80 min and the sedimented microsomal pellet was resuspended by gentle homogenisation in 0.1m pH 7.4 phosphate buffer with 20% glycerol. The following biochemical tests were performed:

*Serum*: total lipids according to Chromy,<sup>6</sup> cholesterol according to Błaszczyzyn,<sup>7</sup> and triglycerides according to Chromy.<sup>6</sup>

*Liver*: total cholesterol according to Rudel and Morriss<sup>8</sup> and triglycerides according to Chromy.<sup>6</sup>

*Hepatic microsomal fraction*: total cholesterol according to Rudel and Morriss,<sup>8</sup> and total phospholipids according to Barlett.<sup>9</sup>

Statistical analysis was done using Student's t-test for unpaired results. The level of significance was taken as  $p < 0.05$ .<sup>10,11</sup>

## RESULTS

No statistically significant differences as to body mass, mortality, peripheral blood count and urinalysis were found between the groups.

### Biochemical tests in serum (Figure 2)

*Total lipids*:  $\text{NH}_4\text{F}$  caused an increase of 37% in total lipids, as compared with controls. When 20 mg/kg b.m. quercetin was given to exposed rats a decrease of 17% in total lipids, in comparison to the  $\text{NH}_4\text{F}$  group, was observed.

*Total cholesterol*: The serum level of cholesterol in the  $\text{NH}_4\text{F}$  group rose by 29%. When quercetin was administered at either dose the level of cholesterol returned to near-control level.

*Triglycerides*: Triglycerides in the exposed group exceeded control level by 26%. Treatment with quercetin sharply reduced triglyceride levels to values lower than in controls.

**Figure 2.** Effect of quercetin on some lipids in serum of rats chronically exposed to ammonium-fluoride vapours

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|  | <b>total lipids</b>                         | <b>triglycerides</b>                            | <b>total cholesterol</b>               |
|--|---|---|--|
| *Statistical significance to the control group |   | **Statistical significance to the control group |  |
| <b>Legend:</b>                                 | <sup>1</sup> Control group                  | <sup>2</sup> Quercetin 1                        | <sup>3</sup> Quercetin 2               |
|  | <sup>5</sup> Quercetin 1+ NH <sub>4</sub> F | <sup>6</sup> Quercetin 2 + NH <sub>4</sub> F    | <sup>4</sup> NH <sub>4</sub> F-exposed |

#### **Biochemical tests in liver homogenate (Figure 3)**

*Total cholesterol:* Ammonium fluoride caused a rise of 65% in cholesterol levels. Quercetin at either dose markedly reduced the content of cholesterol in liver.

*Triglycerides:* Triglycerides in liver homogenate were increased by 61% in the NH<sub>4</sub>F group. When quercetin was administered the level of triglycerides fell to near-normal values.

#### **Biochemical tests in hepatic microsomal fraction (Figure 4)**

*Total cholesterol:* The level of cholesterol in the exposed group was higher by 27%. This increase was much smaller when either high and low dose of quercetin was given.

*Phospholipids:* The amount of phospholipids was slightly reduced by NH<sub>4</sub>F. In the presence of quercetin phospholipid values rose by 14%.

#### **DISCUSSION**

In this work chronic exposure of rats to ammonium fluoride led to a sharp increase in the concentration of total lipids, triglycerides, and cholesterol in

serum. The content of cholesterol in the microsomal fraction rose, while the content of phospholipids fell.

**Figure 3.** Influence of quercetin on some lipids in liver homogenate of rats chronically exposed to ammonium fluoride

|                |  |  |   |
|----------------|--|--|---|
|                | <b>triglycerides</b>                           |  | <b>cholesterol</b>                              |
|                | *Statistical significance to the control group |  | **Statistical significance to the control group |
| <b>Legend:</b> | <sup>1</sup> Control group                     | <sup>2</sup> Quercetin 1                     | <sup>3</sup> Quercetin 2                        |
|                | <sup>5</sup> Quercetin 1+ NH <sub>4</sub> F    | <sup>6</sup> Quercetin 2 + NH <sub>4</sub> F | <sup>4</sup> NH <sub>4</sub> F-exposed          |

Abnormal enzyme activities seem to be one of the chief factors responsible for the rise in serum triglycerides and cholesterol. It appears that enzymes inhibited by fluoride, such as triglyceride lipase, unspecific esterases, and pyrophosphatase, were involved.<sup>10,11</sup> Opinions as to the influence of fluorides on cholesterol levels are controversial. Some authors did not observe any changes after fluoride exposure,<sup>12,13</sup> while others have confirmed the hypercholesterolemic effect of fluorine compounds.<sup>11,14,15</sup>

Modern phytotherapy has benefited greatly from the use of pure compounds present in plants. This is due to the fact that galenics, such as infusions, lose approximately 40% of their active substances. Quercetin is a constituent of plant preparations used for the dilatation of kidney blood vessels. This property improves kidney perfusion, inhibits resorption in renal tubules, and accelerates the elimination of toxins.<sup>16</sup> Such preparations also stimulate the secretion of bile and protect the liver from harmful action of exogenous factors.

**Figure 4.** Influence of quercetin on some lipids in liver microsome of rats chronically exposed to ammonium fluoride

|                |  |  |   |
|----------------|--|--|---|
|                | <b>total phospholipides</b>                    |  | <b>total cholesterol</b>                        |
|                | *Statistical significance to the control group |  | **Statistical significance to the control group |
| <b>Legend:</b> | <sup>1</sup> Control group                     | <sup>2</sup> Quercetin 1                     | <sup>3</sup> Quercetin 2                        |
|                | <sup>5</sup> Quercetin 1+ NH <sub>4</sub> F    | <sup>6</sup> Quercetin 2 + NH <sub>4</sub> F | <sup>4</sup> NH <sub>4</sub> F-exposed          |

Flavonoids reduce the level of cholesterol in plasma and thus slow down the process of atherosclerosis in blood vessels. They possess anti-inflammatory, antibacterial, and antidotal properties.<sup>17,18</sup> The present results support the beneficial effect of quercetin on lipid metabolism. Serum cholesterol levels, triglycerides, and total lipids fell in the exposed and non-exposed groups. Cholesterol content in liver homogenate and microsomal fraction returned to normal values.

Our study in rats is useful for the prevention of fluoride toxicity in humans. Taking into account similarities in homeostasis between rat and man, it could be inferred that the pathomechanism of changes in humans may be similar. Quercetin is a non-toxic compound that ameliorates changes caused by chronic exposure to fluoride and thus might be helpful for the prevention of fluoride toxicity in persons who are exposed to excessive fluoride emissions.

#### CONCLUSIONS

- Chronic exposure to ammonium fluoride vapours leads to changes in lipid metabolism in experimental animals.
- Quercetin reduced biochemical changes caused by fluorine compounds and thus might be helpful for the prevention of fluoride toxicity in people who are exposed to excessive fluoride emissions.

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