PROTECTIVE EFFECT OF CHRYSIN IN RATS SUBCHRONICALLY EXPOSED TO SODIUM FLUORIDE

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SUMMARY: The aim of this study was to examine the influence of the flavonoid chrysin on the morphology and histochemistry of rat liver and kidneys during subchronic exposure to sodium fluoride. Fluoride intoxication produced hepatocellular and focal necrosis with distension of central veins and sinusoids, necrosis of the renal cortex, and lesions in renal tubules in the form of vacuolar degeneration, metaplasia, and hyperplasia. Histochemical evidence for inhibition of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in the liver, as well as SDH and alkaline phosphatase (AlP) in kidneys was obtained. Chrysin, particularly at 20 mg/kg body weight, offered protection against lesions and normalized enzyme activities in the liver and kidneys of rats intoxicated with fluoride.

Keywords: Chrysin; Flavonoids; Kidney histochemistry; Liver histochemistry; Rats; Sodium fluoride hepatic and renal toxicity.

INTRODUCTION

Morphological, histochemical, and biochemical studies have demonstrated that fluoride compounds produce lesions in the liver and kidneys.[1-5] Disorders of energy metabolism in the form of abnormal anaerobic and aerobic respiration are among the key effects of fluoride intoxication.[6,7]

Flavonoids are ubiquitous plant-derived compounds with pigment-like properties. The chemical and biological heterogeneity of flavonoids implies multifarious activities for this group of compounds. Many flavonoids display antioxidative, spasmolytic, anti-inflammatory, anti-allergic, hepatoprotective, and antitumor action [8-12].

The flavonoid chrysin (5,7-dihydroxyflavone) of the polyhydroxyflavone group is present in relatively large quantities in several plant products (*e.g.* pine wood, poplar buds, propolis).[13] Chrysin exhibits strong complexing activity which potentiates its physiological activity.[14] Previous studies have demonstrated the protective action of chrysin in rats subchronically exposed to sodium fluoride as evidenced by an attenuated rise in the activity of serum aminotransferase.[15]

The aim of the present study was to examine the influence of chrysin on organ morphology and activities of some enzymes in the liver and kidneys of rats subchronically exposed to sodium fluoride to determine whether this flavonoid might be a candidate agent for combating fluoride toxicity in humans.

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MATERIAL AND METHODS

Sixty male Wistar rats weighing 180–220 g were randomly divided into six groups with 10 rats in each group. The experimental protocol is presented in the Table.

Table. Three-month rat treatment protocol

Group	Treatment	No. of rats
I	Control	10
II	Chrysin 10 mg/kg bw/day	10
III	Chrysin 20 mg/kg bw/day	10
IV	NaF[a]	10
V	NaF[a] + chrysin 10 mg/kg bw/day	10
VI	NaF[a] + chrysin 20 mg/kg bw/day	10

[a]Estimated mean intake of 8.82 mg F⁻/kg bw/day from 100 mg F⁻/L in drinking water.

Group I (control) was maintained on standard chow and water *ad libitum*. Groups II and III received chrysin at 10 and 20 mg/kg bw/day, respectively. Groups IV, V, and VI were exposed to NaF in their drinking water at a concentration of 100 mg F⁻/L. Groups V and VI were also administered chrysin at 10 and 20 mg/kg bw/day, respectively. Chrysin (5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one; Merck, Germany) was mixed with standard granulated chow having a mean fluoride content of 0.7 mg/kg. Besides the above daily intake of chrysin by each rat, the volume of NaF solution averaged 15.1 mL/day, equivalent to 8.82 mg F⁻/kg bw/day. Rats were kept at 20 ºC in a room with a 12-hr light/dark cycle.

After three months of the experiment, the rats were anesthetised with sodium pentabarbital intraperitoneally (200 mg/kg bw). Liver and kidney slices were fixed with Bouin's solution embedded in paraffin and stained with hematoxylin and eosin (HE). Liver slices were also stained with periodic acid-Schiff reagent (PAS).

Frozen, unfixed liver and kidney slices were used in the histochemistry part of the experiment to determine the activity of succinate dehydrogenase (SDH) (EC 1.3.99.1) according to Nachlas with sodium succinate as substrate.[16] NAD-dependent lactate dehydrogenase (LDH) (EC 1.1.1.27) activity in the liver was determined with sodium lactate.[17] Alkaline phosphatase (AlP) (EC 3.1.3.1) activity in the kidney was studied according to Gomori with sodium beta-glycerophosphate as substrate.[18]

RESULTS

MICROSCOPIC ANATOMY

Normal histology of the liver and kidneys was ascertained in the control group. No significant changes were evident in group II, other than the presence in porto-

biliary spaces and scattered infiltrates composed of mononuclear cells around central veins. Acidophilic degeneration was found in hepatocytes of group III. Histopathologic changes in group IV included distension of the sinusoids and central veins, venous congestion, and pyknosis of hepatocyte nuclei, as well as hepatocellular and focal necrosis. Infiltrates of lymphoid cells were seen chiefly in the portobiliary and central vein fields (Figure 1).
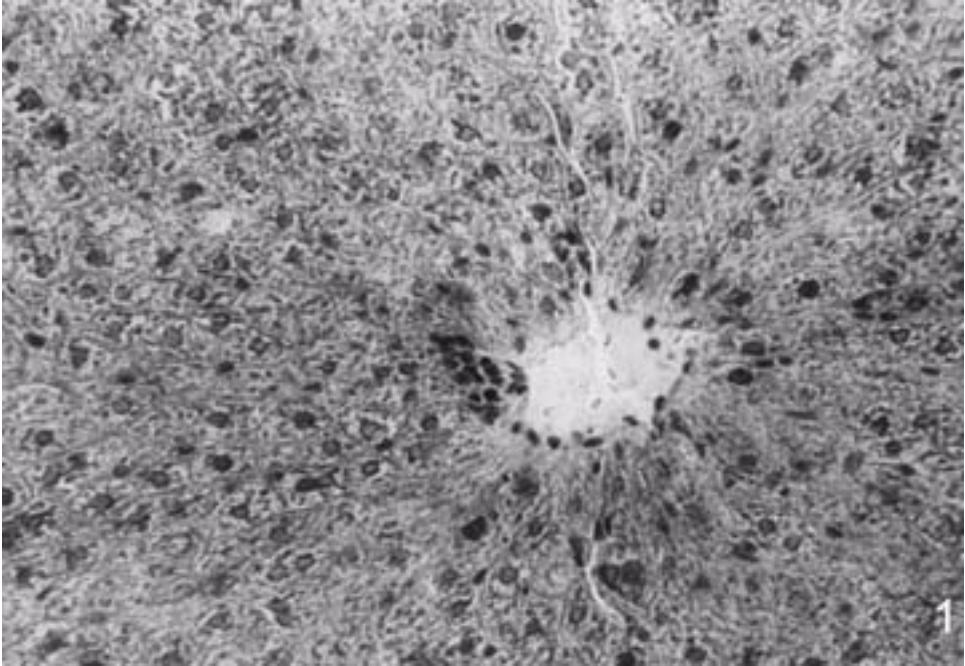

Figure 1. Liver of rat treated with NaF (group IV; x400, HE).

Group V exhibited hepatic degeneration, hepatocellular and focal necrosis, nuclear pyknosis, and distension of the sinusoids (Figure 2).Changes in group VI were mild and limited to venous congestion, along with dilation of the sinusoids and foci of necrosis and hepatocyte degeneration (Figure 3).

Histopathologic changes in kidneys of groups II and III were limited to mild venous congestion affecting the cortex. In group IV, extensive lesions in the renal cortex were present. Glomeruli were often hypertrophic with loss of lobular structure (Figure 4), and atrophy of some was seen. Epithelial cells of proximal and distal tubules showed evidence of micro- and macrovacuolar degeneration, metaplasia, and hyperplasia, as well as loss of the brush border. Necrosis of most tubules and some glomeruli was evident in 50% of rats in this group (Figure 4). The dominant finding in group V was microvacuolar degeneration of proximal tubules. Sporadically, necrotic tubules were seen, but glomerular morphology was otherwise normal. Renal histology in group VI (Figure 5) did not deviate from the control group.
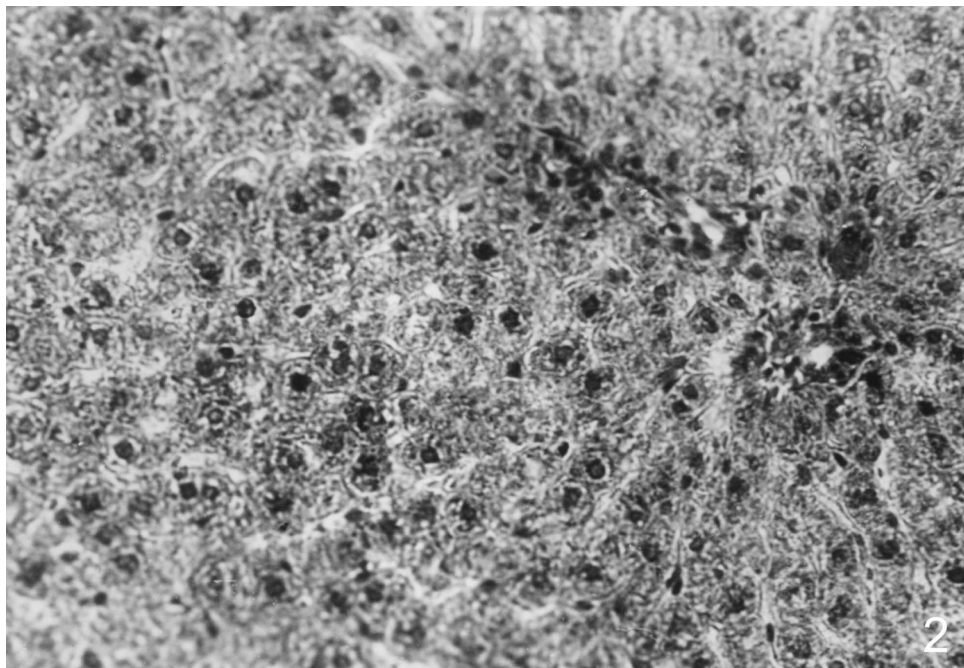
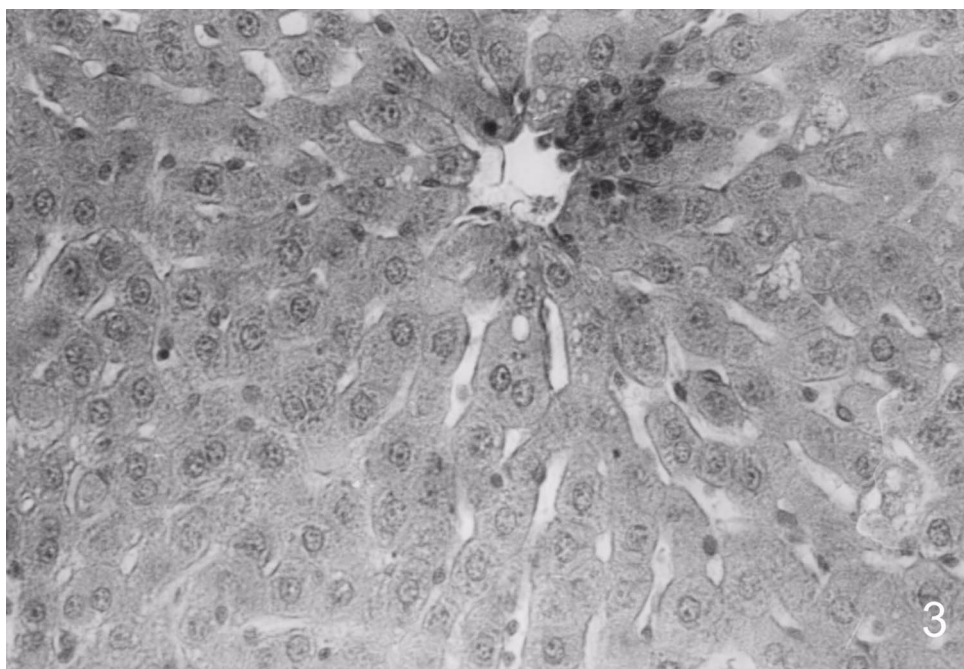**Figure 2.** Liver of rat treated with NaF and chrysin (group V; x400, HE).

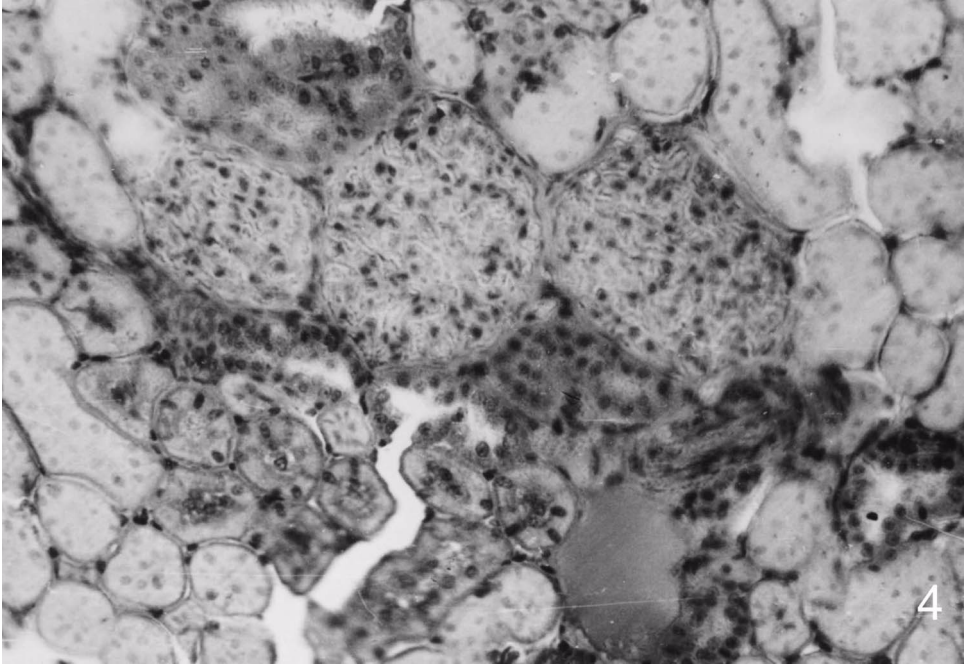**Figure 3.** Liver of rat treated with NaF and chrysin (group VI; x400, PAS).
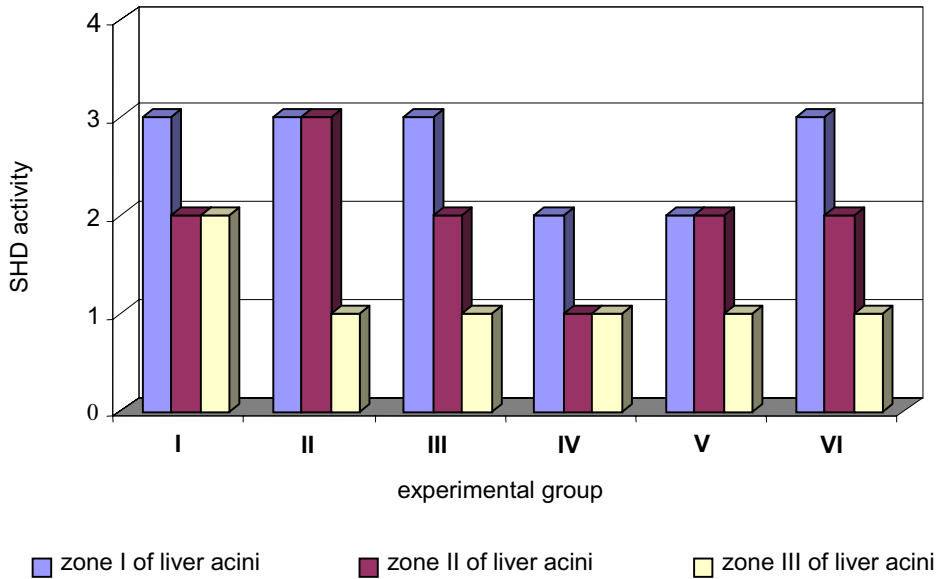
Figure 4. Kidney of rat treated with NaF (group IV; x400, HE).

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Figure 5. Kidney of rat treated with NaF and chrysin (group VI; x400, HE).

HISTOCHEMISTRY

Succinate dehydrogenase (SDH): Changes in the amount and location of SDH activity in hepatic acini are presented in Figure 6.

I – control group, **II-VI** – experimental groups.
Reaction strength: 1- weak; 2 – moderate; 3 – strong; 4 – very strong

Figure 6. Reaction to SDH in control and experimental rat livers.

Rat liver from the control group revealed moderate SDH activity in zones II and III of hepatic acini with zone I demonstrating a somewhat stronger activity. In group II, enhanced SDH activity was noticeable in zones I and II, as opposed to zone III, which revealed a weak activity. SDH activity and location of the reaction product in the liver of group III resembled that of the control group with the exception of zone III hepatocytes showing a weak reaction. In group IV, the decrease in SDH activity was evident. Few formazan grains could be seen in zones II and III of acini, albeit the reaction was somewhat stronger in hepatocytes located near the portobiliary spaces. The activity was moderate in zones I and II of group V, as opposed to a weak reaction in the periportal field. SDH activity in group VI was similar to that in the control group, except for a weak reaction in zone III.

Kidneys of control rats showed moderate SDH activities in the epithelium of renal tubules and weak activities in cells of glomerular capillaries. No deviation from control could be noticed in groups II and III. In group IV, the reaction was weak or negative in the epithelium of renal tubules and glomerular capillaries alike. Moderate SDH activities were observed in the tubular epithelium of groups V and VI.

Lactate dehydrogenase (LDH): Changes in the activity and location of LDH product in hepatic acini are shown in Figure 7.
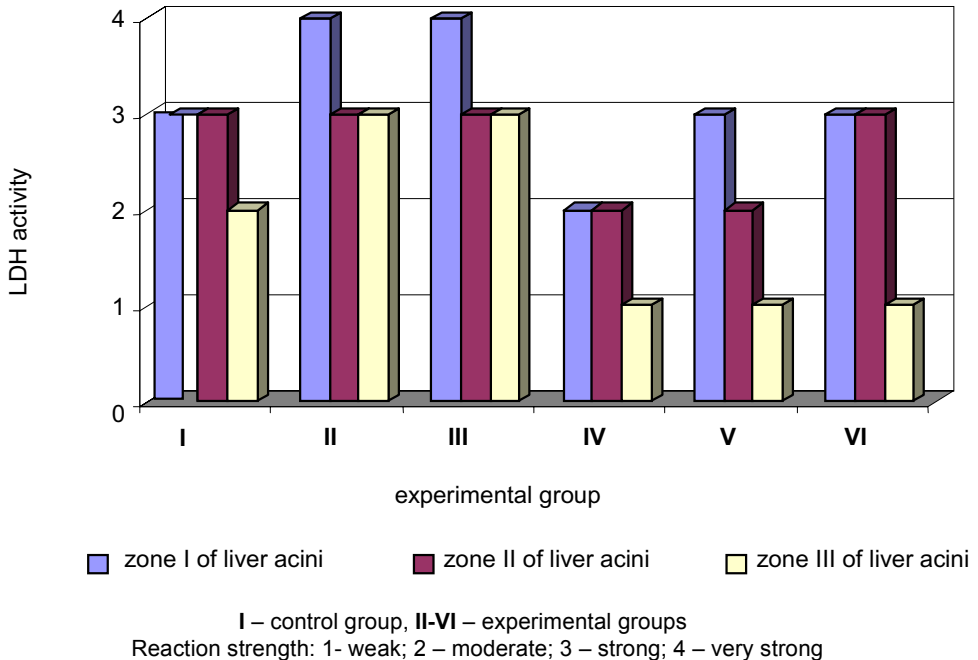

Figure 7. Reaction to LDH in control and experimental rat livers.

I – control group, **II-VI** – experimental groups
Reaction strength: 1- weak; 2 – moderate; 3 – strong; 4 – very strong

Control livers demonstrated strong LDH activities except for hepatocytes adjacent to the central vein where the activities were moderate. Livers of groups II and III showed enhanced LDH activities and unchanged distribution of the reaction product. Very strong activities were observed in zone I and strong activities in the remaining zones. Activities in group IV were moderate in zones I and II and weak in zone III. In comparison to group IV, a slight increase in LDH activity, evidenced by a strong reaction in the portobiliary space, was noticed in group V. No other differences between both groups were observed. LDH activities in group VI resembled those of the control group, with strong reaction in zones I and II and weak reaction in zone III.

Alkaline phosphatase (AlP): AlP activities in the tubular epithelium of control rats were strong, particularly in the microvillar area (Figure 8). Activities in the glomeruli were weak. Rats of groups II and III demonstrated very strong activities in tubules of the renal cortex. The reaction to AlP was generally weak in tubules of group IV, and in only a few tubules was the reaction strong (Figure 9).
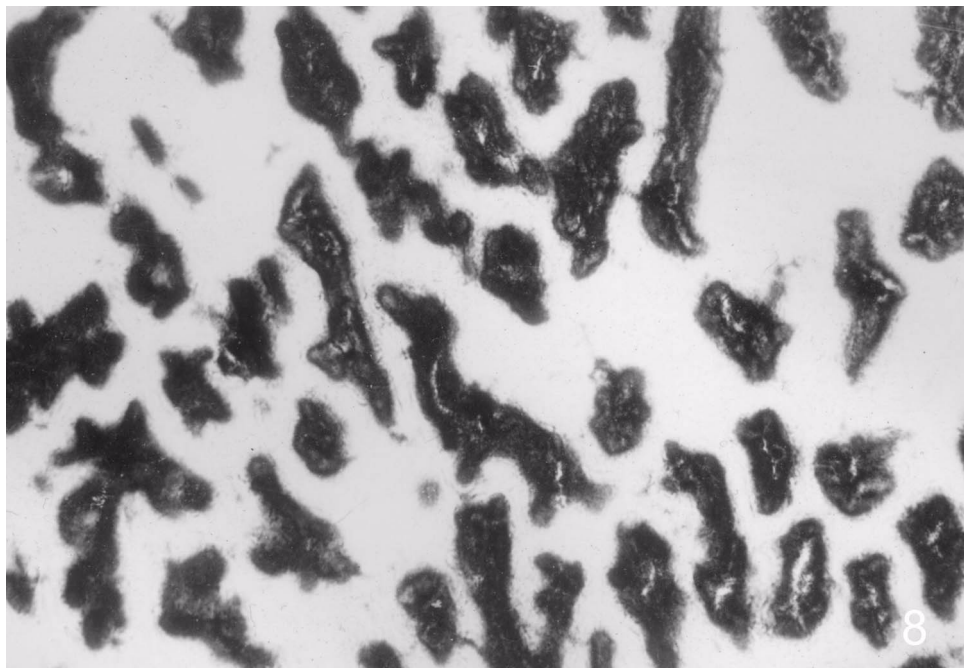
Figure 8. Moderate AlP reaction in the kidney of a rat of control group (group I; x 200).
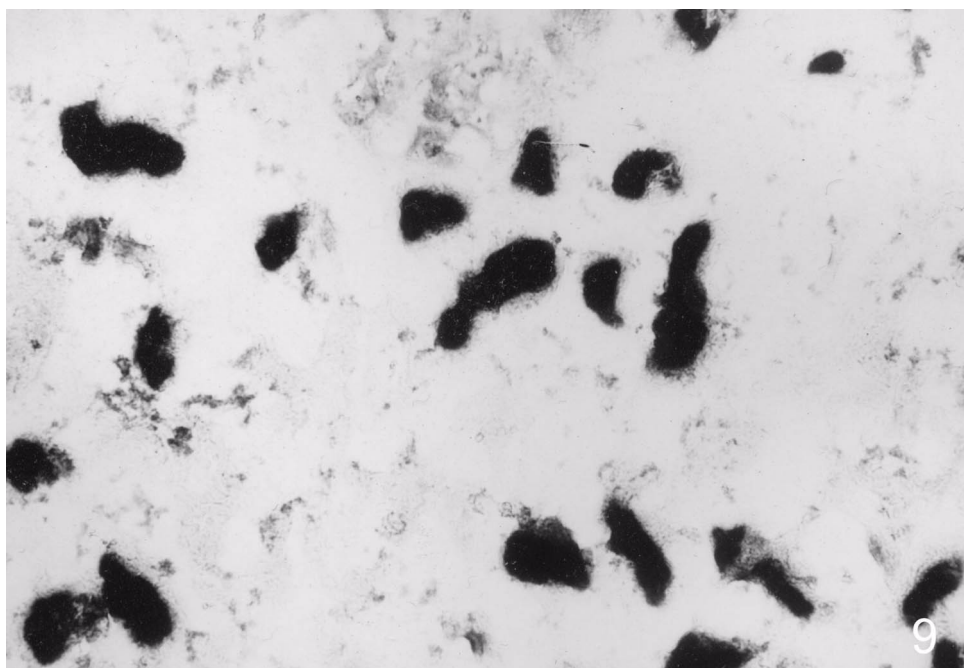

Figure 9. Negative or strong AlP reactions in the kidney treated with NaF
(group IV; x 200).

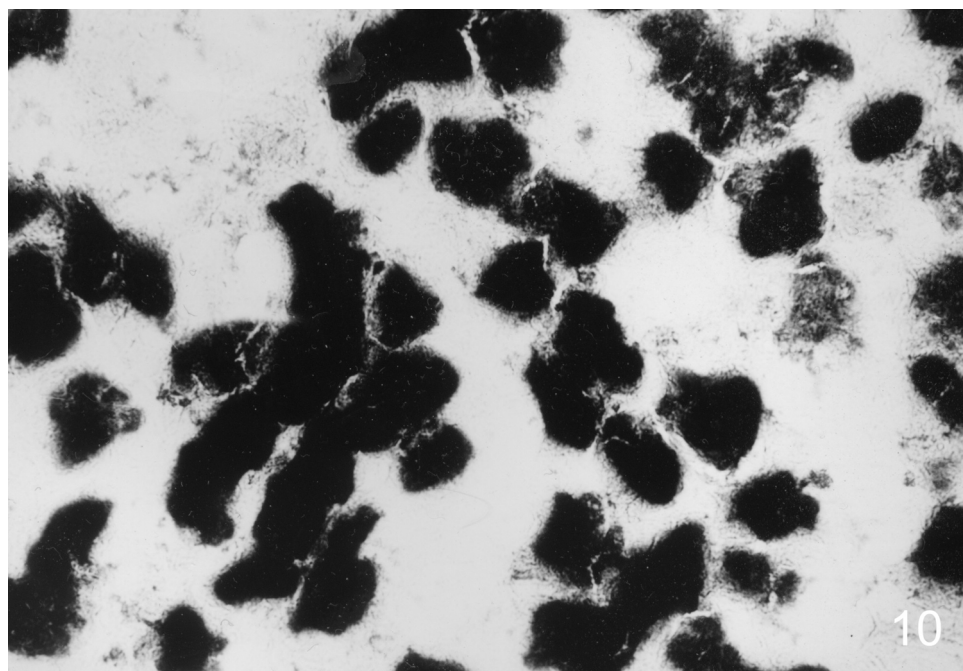In groups V and VI, the reaction in epithelial cells of renal tubules was strong or very strong (Figure 10).

Figure 10. Strong AIP reaction in the kidney treated with NaF and chrysin (group VI; x 200).

DISCUSSION

Numerous studies have corroborated the hepatotoxic and nephrotoxic properties of fluorine. The extent of structural and functional alterations in various organs depends on the dose and duration of exposure. In the present study, histologic lesions in the liver induced by NaF were mainly in the form of hepatocellular and focal necrosis, as well as distension of sinusoids and central veins. Varying degrees of degenerative and inflammatory changes in the liver of laboratory animals exposed to fluorine have been reported by others.[19-22]

Enzyme alterations induced by NaF in rat livers that we observed were in the form of reduced LDH and SDH activities. Histoenzymatic tests of the liver revealed that supplementation with chrysin ameliorates symptoms of fluoride intoxication as reflected by increase in LDH activity in zone I of liver acini of group V, as well as zones I and II of group VI (Figure 7). Our findings are in accord with the fact that hepatocytes are recognized for their functional and structural heterogeneity. The decreased LDH activity in zone III of liver acini (around the central vein) of groups V and VI, despite chrysin supplementation may be attributed to depressed hepatocellular function in this zone affecting predominantly storage of glycogen and lipids and biotransfomation of xenobiotics. Chinoy et al[23] demonstrated disturbances in carbohydrate metabolism in the form of glycogen accumulation in liver of rats treated with NaF. On the other hand,

chrysin at a dose of 10 or 20 mg/kg bw/day in unexposed rats (groups II and III), compared with control, enhanced LDH activity in zones I and III of hepatic acini.

The present study of the liver of rats treated with NaF revealed disorders in aerobic respiration processes by a decrease of SDH activity — a marker for the tricarboxylic acid cycle. Low SDH activities in tissues of experimental animals exposed to fluoride have been reported by others.[25-30] Ultrastructural studies have confirmed the role of fluorine ions in the disintegration and swelling of mitochondrial cristae.[27,31,32] Chrysin supplementation (especially at the higher dose) alleviated symptoms of fluoride intoxication in exposed rats, as evidenced by the increase of SDH activity in zones I and II of liver acini (Figure 6). Hepatocytes in the zone associated with the portobiliary space (zone I) are known for high metabolic rates linked with gluconeogenesis and cellular respiration processes.

Histopathologic lesions found by us in the kidneys were most evident in the cortex in the form of necrosis and partial degeneration of glomeruli and tubules (vacuolar degeneration of the epithelium, metaplasia, and atrophy of microvilli). Except for necrosis, similar changes have been observed by others.[5,24]

Reduced SDH activities were observed in renal tubules of rats exposed to NaF. This finding can be interpreted as an outcome of abnormal cellular respiration. As AlP participates in active transport through cellular membranes, depressed activity leads to dysfunction of renal tubules interfering in particular with resorption. SDH and AlP activities in renal tubules of groups V and VI resembled the control group, suggesting that supplementation with chrysin normalizes cellular respiration and resorption in rats exposed to NaF.

Fluorine ions are known to stimulate the respiratory burst and generation of superoxide radicals.[33] Research in recent years has demonstrated that fluoride interferes with antioxidative mechanisms of the cell[34] producing changes in the activities of antioxidative enzymes and enhancing lipid peroxidation processes.[21,35-38] Symptoms of fluoride intoxication can be alleviated by vitamins E, D,[21] and C,[23] or by selenium.[30]

Chrysin is a flavonoid with particularly potent complexing properties due to the presence of a hydroxyl group at carbon 5. Studies on the antioxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, antioxidative properties of polyphenols depend on hydroxylation of ring B. Nevertheless, meta hydroxyl groups in ring A at positions 5 and 7 play a minor role in antioxidative activity of chrysin.[39]

The present results corroborate the protective action of chrysin in fluorine intoxication of rats, particularly noticeable with the higher dose used by us (20 mg/kg bw/day). Supplementation with this flavonoid ameliorated structural changes and normalized enzyme activities in the liver and kidneys of rats subchronically exposed to sodium fluoride.

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