NEGLIGIBLE AMELIORATIVE EFFECT OF ALUMINIUM SULPHATE ON OXIDATIVE STRESS PARAMETERS IN GOATS DURING SUBACUTE FLUORIDE INTOXICATION

Vinay Kant,a Anil K Srivastava,b Pawan K Verma,c Rajinder Raina,c Nrip K Pankajc

R.S. Pura, Jammu, India

SUMMARY: Four healthy goats were administered 20 mg NaF/kg bw/day for 30 days. Oxidative stress occurring in the blood was indicated by a significant increase in catalase activity (p<0.01) and a decrease in superoxide dismutase (SOD) activity (p<0.05) along with a significant increase in lipid peroxidation (p<0.01). Concurrent administration of 150 mg Al2(SO4)3•16H2O/kg bw/day in another group of four healthy goats failed to reverse the F-induced oxidative stress.

Keywords: Aluminium sulphate; Catalase; Fluoride intoxication; Goats; Lipid peroxidation; Oxidative stress; Superoxide dismutase.

INTRODUCTION

Excessive exposure to fluorine (F, as fluoride ion, F –) is one of the most important public health problems throughout much of the world.1 In mammals F ingestion/intoxication leads to characteristic changes in teeth, bones and soft tissues.2 It inhibits many enzymes such as those involved in the pentose pathway, antioxidant defense systems, and the myosin-ATPase pathway.3-5 Extensive information in the literature is available on the role of F in cellular respiratory processes and free-radical-induced degenerative alterations in human and animals.6,7 F intoxication inhibits the activity of superoxide dismutases leading to increases in the intracellular level of super-oxide radicals.8 Further various studies also suggested that intoxication elevates nitric oxide synthase (NOS) activity which lead to excess formation of intracellular nitric oxide (NO).5 Excess NO interacts with super-oxide radicals forming very reactive peroxynitrite radicals incriminated in various neurodegenerative effects.9,10 Tao et al.11 reported that oral feeding of Mg-Al mixed oxides (magnesium-aluminum) significantly alleviated F intoxication in pigs fed a diet containing excessive F by increasing excretion of F through urine and feces. Other salts like aluminium sulphate, calcium carbonate, and sodium acid phosphate have been tried to minimize the adverse effects of F intoxication in animals.12,13 However, studies on the use of aluminium sulphate to overcome F-induced oxidative stress in ruminants are limited. Therefore, the present study was undertaken to evaluate the ameliorative effect of aluminium sulphate on oxidative stress resulting from subacute toxicity of F in goats.

MATERIALS AND METHODS

Experimental Design: Eight healthy cross-bred goats 1.5-2.0 years in age were divided into two groups of four in each group. In Group 1, sodium fluoride (NaF) was administered at a dosage of 20 mg/kg bw (providing 9 mg/kg bw fluorine)
daily for 30 days. In Group 2, the same dose of NaF along with aluminium sulphate hexadecahydrate \([\text{Al}_2(\text{SO}_4)_3\cdot16\text{H}_2\text{O}]\) at a dosage of 150 mg/kg bw (providing 12.78 mg/kg bw aluminium) was administered orally daily for 30 days. Both the salts were dissolved separately in 100 mL of distilled water and administered orally. The aluminium sulphate was provided 30 minutes before the administration of NaF. All the animals were weighed weekly and dosages of NaF and \(\text{Al}_2(\text{SO}_4)_3\) were corrected accordingly.

**Sample collection, enzyme assaying, and statistical analysis:** Blood samples were collected by jugular vein puncture in thoroughly cleaned heparinized glass vials on day 0, 1, 3, 7, 14, 21, 28, 30 of treatment and on day 3, 7, 14 after termination of exposure. The washed red blood cells were used for preparing a 1% haemolysate for catalase and superoxide dismutase (SOD) determinations and a 33% haemolysate for lipid peroxidation analysis by measurement of the level of malondialdehyde (MDA). Lipid peroxidation, SOD and catalase were assayed in erythrocytes by the methods of Ohkawa et al\(^{14}\), Marklund and Marklund,\(^{15}\) and Aebi,\(^{16}\) respectively. Values at different days were compared to pre-exposure (0 day) values of the same group by using Student’s paired-t test and a probability levels of \(p<0.05\) and \(p<0.01\) were considered statistically significant.

### RESULTS

The effect of repeated oral administration of NaF alone and in combination with \(\text{Al}_2(\text{SO}_4)_3\) on erythrocyte catalase, superoxide dismutase (SOD), and lipid peroxidation is presented in the Table.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Treatment days (n=4)</th>
<th>Post-treatment days (n=4)</th>
<th>3 7 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (µmol H(_2)O(_2) utilized/min/mg of protein)</td>
<td>1</td>
<td>42.94 ±4.13</td>
<td>90.24 ±23.16</td>
<td>121.60 * ±35.63</td>
</tr>
<tr>
<td>SOD (Units/gm protein)</td>
<td>1</td>
<td>66.45 ±3.13</td>
<td>57.92 ±12.16</td>
<td>33.06 ±15.06</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol MDA formed/mL erythrocytes)</td>
<td>1</td>
<td>3.68 ±0.62</td>
<td>5.56 ±0.82</td>
<td>4.71 ±0.78</td>
</tr>
</tbody>
</table>

| *Mean of three animals; ne = not estimated. Compared to pre-exposure (day 0) control value of the same group: *\(p<0.05\), †\(p<0.01\). |

In Group 1, the catalase activity increased significantly (\(p<0.05\)) on day 3 of exposure and remained elevated but somewhat less until day 30. After termination of exposure, the catalase activity showed a decreasing activity before returning to normal. However, in Group 2, the only significant increase (\(p<0.05\)) in catalase activity was observed on day 30. The SOD activity showed decreasing pattern during exposure days in Group 1, and the activity decreased significantly (\(p<0.05\)) on day 7 after termination of exposure. But in Group 2, the SOD activity decreased significantly (\(p<0.01\)) on day 1 of exposure and remained significantly lower than the pre-exposure level.
In Group 1, the values of lipid peroxidation increased significantly (p<0.05) on day 7 of exposure and remained elevated during the exposure days. After termination of exposure, the values showed a decreasing trend but remained significantly higher until the end of the experiment. Also in Group 2, the values of lipid peroxidation exhibited a significant increase (p<0.01) on different days of exposure.

DISCUSSION

Oxidative stress resulting from excessive production of free radicals and reactive oxygen species (ROS) during F intoxication. F adversely affects the activity or production of free radical scavengers such as SOD, catalase, glutathione-peroxidase, and blood glutathione. Studies also suggested the inhibitory effects of fluoride on the secretion of insulin may be due to damage of the β cells of pancreas further provoked the oxidative stress in animals. SOD is the first line of defense against the action of •O2− and ROS. Three types of SOD are currently known: iron-containing FeSOD, manganese-containing MnSOD, and copper- and zinc-containing CuZnSOD. In the present study a significant decrease in the activity of SOD was observed with sub acute F-intoxication that may be due to competitive inhibition by the F ion for binding to the active site of Cu, Fe, or Mn on SOD. Our findings corroborate those of earlier reports of reduced SOD activity with F intoxication by Tao et al. in pigs, Lawson and Yu in earthworms, Shivarajashankara et al. in rats, and Shivarajashankara et al. in children. Similarly, Chlubek et al. also reported that a decrease in SOD activity can be due to a direct action of fluoride on the enzyme rather than to increased free radicals generation by fluoride intoxication. Differing from our findings that showed a significant increase in catalase activity with F intoxication that attenuated somewhat with time, some studies have indicated that F decreases the activity of catalase in pigs and in rat brain. On the other-hand, Reddy et al. reported they did not find any significant changes in catalase activity from F in human and rabbit blood. In the present study, the increase in catalase activity may be due to reduced detoxification of H2O2 generated by F-induced oxidative stress.

Excessive production of free radicals or ROS is mainly responsible for peroxidation of cell membrane lipids, and malondialdehyde (MDA) is a terminal product of the lipid peroxidation process. Determination of MDA levels provides a good measure of lipid peroxidation, which is among the chief mechanisms of cell damage leading to necrosis or apoptosis. In the present study, increased MDA concentration was observed in fluorotic goats that is correlated with enhanced lipid peroxidation in them. Similar findings were observed by Shivarajashankara et al. in RBC, liver, and brain of rats, Liu et al. in chick serum, and Shivarajashankara et al. in erythrocytes in children.

In the present study, elevation in the activity of catalase and reduction in the activity of SOD suggested that oxidative stress was produced by F intoxication. Due to reduced antioxidant activity, excess free radicals attack the polyunsaturated fatty acid (PUFA) chain of phospholipids of biological membranes resulting in deterioration of membrane function. Free-radical-induced lipid peroxidation
extends and amplifies oxidative damage through different free radicals and ROS resulting in the deterioration of biological membranes.9,30

CONCLUSION

Alteration in stress parameters indicating disruption in pro-oxidant/antioxidant balance leads to oxidative stress in cells during subacute F intoxication. However, concurrent administration of aluminium sulphate failed to provide any appreciable reversal of F-induced oxidative stress parameters in goats.

REFERENCES