**Hepatoprotective effect of vitamin C on sodium nitrite-induced lipid peroxidation in albino rats**

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The possible protective role of vitamin C on liver antioxidant enzymes of albino rats in sodium nitrite induced lipid peroxidation (LPO) was investigated. Sodium nitrite and vitamin C were administered orally through intragastric tube. Sodium nitrite (300 mg/kg body wt) significantly increased the LPO and the activities of liver marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase and lactate dehydrogenase (LDH), and decreased the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) level in LPO-induced rats; the remarkable changes in the enzyme activities was due to hepatotoxicity of nitrite. The vitamin C (300 mg/kg body wt) significantly decreased the LPO level and the activities of liver enzymes and increased antioxidant enzymes activities, thus exerts ameliorating effect on sodium nitrite-induced lipid peroxidation.

**Keywords:** Sodium nitrite, Vitamin C, Lipid peroxidation, Antioxidant, Marker enzymes.

Nitrite (NO\(_2^-\)) and nitrate (NO\(_3^-\)) are the naturally occurring molecules in the environment and are products of oxidation of nitrogen by microorganisms in plants, soils and water. Nitrite is a contaminant of some water supplies, extensive use of nitrogenous fertilizer, decomposition of plants and sewage wastes, followed by leaching of nitrates into ground water\(^1\). Nitrite is thought to be responsible for most of the toxic effects observed with excess nitrate ingestion\(^2,3\). Nitric oxide free radical (NO\(^\cdot\)) is generated from the nitrite by non-enzymatic method\(^4\) and O\(^\cdot\) formation is found in acidic environments such as the stomach\(^5,6\) and oral cavity. Peroxy nitrite (ONOO\(^-\)), the reaction product formed between nitric oxide (NO\(^\cdot\)) and superoxide plays a critical role in the induction of inflammatory reaction and apoptosis. O\(^\cdot\) is also associated with tumor promotion and/or progression\(^7\). High level of NO inhibits hepatocyte mitochondrial respiration in vitro\(^8,9\). Nitric oxide (free radicals) can cause DNA damage by inhibiting DNA synthesis and cell cycle arrest\(^10\).

In the present study, we have investigated the protective role of vitamin C against nitrite-induced toxicity in the liver of albino rats by investigating the LPO, liver marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase and lactate dehydrogenase (LDH), and activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) level.

**Materials and Methods**

**Animals and treatment**

Adult Wistar rats weighing 170–200 g of either sex were maintained in large polypropylene cages in a well-ventilated room temperature with natural day-night cycle, and fed balanced rodent pellet diet and water ad libitum throughout the experimental period. They were quarantined for 1 week, prior to the experiments to acclimatize them to laboratory conditions. The study protocol was approved by the IAEC (Institutional Animal Ethics Committee of CPCSEA, New Delhi, Govt. of India).

The rats were divided into 3 groups of 6 animals each. Group I served as control, group II rats were administered sodium nitrite at the rate of 300 mg/kg body wt and, group III rats received 300 mg of sodium nitrite/kg body wt along with 300 mg of vit. C/kg body wt. All the doses were administered orally through intragastric tubes at every 24 h interval. Animals were sacrificed by decapitation under light ether anesthesia on the 30th day. Immediately after sacrifice, the liver was dissected out, washed in ice-cold saline, and the homogenate was prepared in 0.1 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was used for the assay of marker enzymes.

**Biochemical parameters**

Lipid peroxidation (LPO) was estimated in the
liver sample by thiobarbutric acid-reactive substance\textsuperscript{11}. Antioxidant enzymes SOD\textsuperscript{12}, catalase\textsuperscript{13}, and reduced GSH\textsuperscript{14} were estimated as described. AST and ALT activities\textsuperscript{15} in serum and LDH activity\textsuperscript{16} were estimated as described. Acid phosphatase activity was determined by measuring liberated inorganic phosphate\textsuperscript{17,18}.

Statistical analysis

The experimental data were analyzed by Students “t” test. The significance level was set at \( P<0.05 \).

Results and Discussion

Oral administration of sodium nitrite 300 mg/kg body wt for 30 days caused changes in the level of LPO and antioxidant enzymes (Table 1). The LPO level and the activities of liver marker enzymes AST, ALT, acid phosphatase and LDH increased significantly \((P<0.05)\), whereas antioxidant enzyme SOD activity decreased 13.4% in sodium nitrate-treated rats and 7.2% in sodium nitrate + vit. C treated rats. CAT activity decreased significantly (59.1%) in sodium nitrate-treated rats and 2.95% in sodium nitrite + vit. C administered rats. GSH level also decreased significantly (77.9%) in sodium nitrite administered rats and only 13.1% in sodium nitrite + vit. C administered rats. Rats treated with vit. C (300 mg/kg body wt) plus sodium nitrite showed comparatively reduced (94.4%) LPO and liver marker enzymes activity and higher levels of antioxidant enzymes.

Increase in the marker enzymes such as AST, ALT, acid phosphatase and LDH in sodium nitrite-treated rats indicate hepatic damage in experimental animals\textsuperscript{19,20} due to significant increase of LPO in liver. Earlier report\textsuperscript{21} also demonstrated the increase in the level of AST, ALT, acid phosphatase and LDH in ammonium nitrate-treated rats due to the formation of free radical (ONOO\textsuperscript{−}) from nitric oxide. Both NO and oxygen radicals could react further to produce other oxidants and nitro compounds such as peroxynitrite to induce liver injury\textsuperscript{22,23} and it plays an important role in death of liver cells\textsuperscript{24}. The high levels of AST and ALT in serum are usually indicative of liver damage in animals\textsuperscript{25}.

The decrease in the activities of liver marker enzymes such as AST, ALT, acid phosphatase and LDH in vit. C and sodium nitrite-treated rats compared to nitrite-treated rats may be due to the antioxidant effect of vit. C, which is reported to protect the liver from damage\textsuperscript{19,25}. Vit. C may protect lipids and lipoproteins in cellular membranes against oxidative damage caused by toxic free radicals, thus may prevent certain types of hepatic cellular damage\textsuperscript{20}.

SOD and CAT are the scavenging enzymes that remove the toxic free radicals\textsuperscript{26}. In the enzymatic antioxidant defense system, SOD is one of the most important enzymes and scavenges O\textsubscript{2}· anion to form \( \text{H}_2\text{O}_2 \), thus diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions\textsuperscript{27}. Results of the present study corroborate the earlier reports which suggest that chronic exposure of hepatocytes to reactive nitrogen species leads to functional and morphological alterations in the hepatocytes\textsuperscript{7,28}. Therefore, the cytotoxic and cytostatic activity of nitrite may be due to the NO and peroxynitrite (ONOO\textsuperscript{−}) formed from the nitrite.

The targets of NO\textsuperscript{·} include cytochrome c oxidase and SOD\textsuperscript{29}. The peroxynitrite causes oxidation of thiol groups and induces peroxidation\textsuperscript{30}. It is well accepted that NO with its one unpaired electron will react avidly with oxygen, superoxide anion radical (O\textsubscript{2}{·}) and transition metals\textsuperscript{31}. The peroxynitrite produced from nitric oxide, a potent oxidant\textsuperscript{32} may increase the LPO. The vit. C administered rats

| Table 1—Changes in the concentration of LPO, marker enzymes and antioxidant enzymes of liver of treated and control rats |
|-----------------|-----------------|-----------------|
| Groups          | Control         | Sodium nitrite  |
| LPO (nm/100g wet tissue) | 0.992 ± 0.08 | 1.80 ± 0.29* |
| AST (IU/l)      | 26.63 ± 3.31   | 61.33 ± 3.466* |
| ALT (IU/l)      | 11.20 ± 0.34   | 12.83 ± 0.46* |
| Acid phosphatase (KA units/dl) | 1.80 ± 0.17 | 2.76 ± 0.24* |
| LDH (IU/l)      | 162.40 ± 5.4   | 320.76 ± 12.2* |
| SOD (Units/mg protein) | 1.05 ± 0.04 | 0.910 ± 0.01* |
| CAT (µmoles of \text{H}_2\text{O}_2 consumed/min/ mg protein) | 0.684 ± 0.04 | 0.280 ± 0.03* |
| GSH (Units/mg protein) | 4.75 ± 0.57 | 1.08 ± 0.14* |

*\( P<0.05 \) (Control vs treatment)
have shown a significant reduction in the LPO and a significant increase in antioxidant enzymes activities.

Vitamin C is hydrophilic and is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxidative damage. It is also reported to be an excellent source of electrons and, therefore, can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity. The increase in SOD, CAT activities and GSH level reveals the better recovery of hepatic cells from the oxidative stress induced by nitrite toxicity. As glutathione is the major cellular nucleophile, it provides an efficient detoxification pathway for a variety of electrophilic reactive metabolites. The results of present study are in agreement with earlier report which suggests that vit. C, vit. E and carotene elevate hepatic SOD activity in diabetic rats. Thus, the present results conclude that vit. C may play a role in the prevention of hepatic cellular injury by its antioxidant capacity.

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References

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