

Changes in Antioxidant Status and Lipid Peroxidation at Liver and Kidney Tissues of the Rats That Were Given Diethyl Nitrosamine

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Purpose: In this study, the effects of diethylnitrosamine (DEN) on superoxide dismutase (SOD) and malondialdehyde (MDA) were investigated. The protective effects of reduced glutathione (GSH) on this mechanism were also examined.

Methods: Three groups were investigated: The control group (n=8), the DEN given group (n=8) and the DEN+GSH given group (n=8). After these animals were executed, their liver and kidney tissues were taken.

Results: The MDA levels increased in the rat hepatic tissue more in the other groups than in the control group ($p<0.05$). SOD activities decreased in the DEN given group when compared to the control group ($p<0.001$). While no difference in the MDA levels was observed in the renal tissues as compared to the control group, the SOD activity decreased in the DEN given group to the control group ($p<0.01$) and increased in the DEN+GSH given group to the DEN given group ($p<0.001$).

Conclusions: These findings seem to reinforce the idea that DEN has an important effect on lipid peroxidation and SOD, and GSH could play a protective role in this peroxidation. *Ann Med Sci* 2001;10:50-54

Key words: Diethylnitrosamine, malondialdehyde, superoxide dismutase, reduced glutathione

It has been determined that DEN, a chemical carcinogen, is formed out of insecticides, agricultural chemicals and nitrate. DEN exists in cigarette smoke and also in nutrition, and it comes into existence when nitrate reacts with secondary and tertiary amines in the stomach¹⁻⁴.

DEN is converted into mutagenic active intermediary products by means of the microsomal mixed-function oxidases in the tissues. DEN causes alkylation in DNA and RNA, and it inhibits protein synthesis. Diethyl nitrosamines which are electrophilic substances, join to the nucleic acids and to the nucleophilic atoms that are found in proteins^{5,6}.

Aerobic organisms produce reactive oxygen species such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide and hydroxyl radicals through the chain reactions that start with the reduction of

molecular oxygen. These free radicals lead to a variety of cellular damages. Lipid peroxidation is one of these, and it is a process that is formed by means of the oxidation of polyunsaturated fatty acids. MDA is one of the final products of lipid peroxidation⁷⁻¹⁰.

There are various kinds of cytoplasmic scavengers and enzymes that protect cells from free radical damage. SOD is an enzyme which protects the cells from the injurious effects of free radicals, and it catalyses $O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$. However, as the formed H_2O_2 is a considerably toxic material for organisms, it is removed by catalase (CAT) and peroxidase enzyme systems. As for GSH, it is a nonenzymatic free radical scavenger that removes reactive oxygen products. Reduced glutathione has a protective role in the -SH groups of lipoproteins existing in the cellular membrane, as well as in hemoglobin and catalase in the erythrocyte metabolism¹⁰⁻¹².

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Premalignant rat liver nodules produced in the resistant hepatocyte model, by exposure to carcinogenic chemicals such as diethyl nitrosamine. It is considered that reactive oxygen species (ROS) are generated in both resistant hepatocyte models resulting in lipid peroxidation¹³.

In this study, whether the diethylnitrosamine, a chemical carcinogen, leads to an oxidative injury or not, and how it effects cellular antioxidant systems have been investigated. For this purpose, DEN was given to a group of rats. At the same time, in order to investigate the protective effects of GSH, it was, together with DEN, injected into another group of rats. The levels of SOD and MDA have been measured in these animals' blood and hepatic and kidney tissues.

Materials and Methods

This study was carried out on three groups of male wistar rats that included eight animals weighing between 150-250 g. The first group was the control group. Second group received a single intraperitoneal injection of 200 mg/kg of DEN dissolved into % 0.9 NaCl solution. The third one was the treatment group and the animals in this group were injected with 200 mg/kg DEN plus 300 mg/kg GSH. The control group was given equal amounts of %0.9 NaCl solution as in the other groups. All substances were injected intraperitoneally¹⁴. DEN and GSH were obtained from Sigma Chemical Co. (St. Louis, MO). These animals were killed by decapitation. Their liver and kidney tissues were removed, pieces from liver and kidney tissue were fixed in formol (%10) and kept for histological investigation. After these tissues were washed with saline three times and the rest were divided into smaller parts, they were homogenized for biochemical studies. In these homogenates, MDA, SOD and protein content were determined. Protein content was measured according to the Biuret method¹⁵.

MDA was measured by the method described by Okhawa et al¹⁶. SOD levels were determined according to the method of Winternbourn et al¹⁷.

All data is expressed as means $\pm$ SEM. The data was analyzed using the one-way variance analysis and the Tukey- α test together.

Results

Hepatic MDA and SOD levels were shown in table I. There was an important increase in the

levels of rat hepatic MDA in the DEN and DEN+GSH given groups when compared to the control group ($p<0.05$). The SOD activity in the DEN given group decreased in comparison with the control group ($p<0.001$). In the DEN+GSH given group, SOD activity was also as compared to DEN group ($p<0.001$).

Table 1. The statistical values of MDA and SOD in the hepatic tissue (Mean $\pm$ SEM).

GROUPS	n	MDA (nmol/mg prot.)	SOD (U/mg prot.)
CONTRO L	8	0.66 $\pm$ 0.07	5.67 $\pm$ 0.2
DEN given	8	0.98 $\pm$ 0.07*	3.81 $\pm$ 0.2**
DEN+GS H	8	0.92 $\pm$ 0.08*	5.51 $\pm$ 0.1†

*: $p<0.05$ as compared to control group

**: $p<0.001$ as compared to control group

†: $p<0.001$ as compared to DEN group

Table 2. The statistical values of MDA and SOD in the renal tissue.(Mean $\pm$ SEM).

GROUPS	n	MDA (nmol/mg prot.)	SOD (U/mg prot.)
CONTRO L	8	0.55 $\pm$ 0.05	4.90 $\pm$ 0.13
DEN given	8	0.67 $\pm$ 0.04	3.51 $\pm$ 0.14*
DEN+GS H	8	0.53 $\pm$ 0.03	5.82 $\pm$ 0.15*.†

*: $p<0.01$ as compared to control group

†: $p<0.001$ as compared to DEN group

Kidney tissues MDA and SOD levels were shown in table II. There was no significant differences in the MDA levels of renal tissues across the groups. As for the SOD data, a decrease was observed in the DEN given group when compared to the control group ($p<0.01$), and an increase was observed in the DEN+GSH given group to the control group ($p<0.01$). There was a significant increase in the DEN+GSH given group to the DEN given group ($p<0.001$).

Histological observations:

Histologic examination of liver tissue:

Control group: Sections of control animals displayed normal liver tissue characteristics (Figure-1).

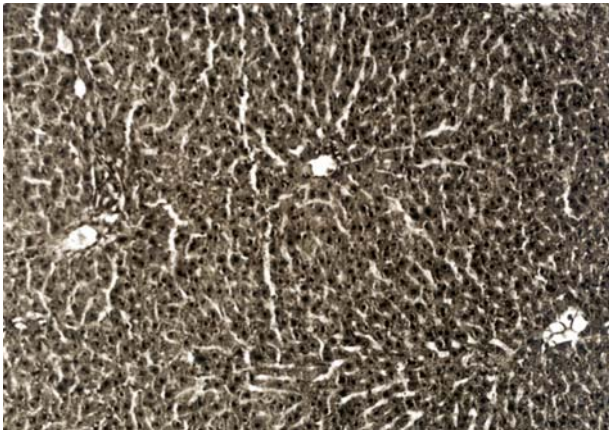


Figure 1. Normal liver histology in control group (H.E. X 33).

DEN given group: Significant changes were observed in the livers of this group. Most of the hepatocytes showed severe hydropic degeneration, pyknosis and focal necrosis. Many hepatocytes only displayed severe fatty changes. A mild infiltration of mononuclear cells is also present. Neither fibrosis nor interlobular bile duct damage was observed in the portal tract. Hyperemic blood vessels were seen in some liver sections. In the present study, the most degenerative liver was observed in the DEN given group (Figure- 2,3).

DEN + GSH given group: In this group, a decrease in the fatty change of hepatocytes was observed. In addition, there was a moderate degree of hydropic degeneration, cell infiltration and hyperemia. Results indicates that, GSH is not effective for full-preservation of liver tissue after DEN caused degeneration (Figure- 4).

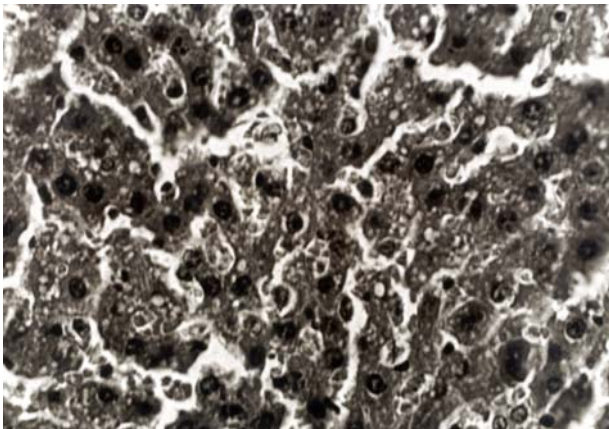


Figure 2. DEN given group: Liver cells contain lipid vacuoles (H.E. X 132).

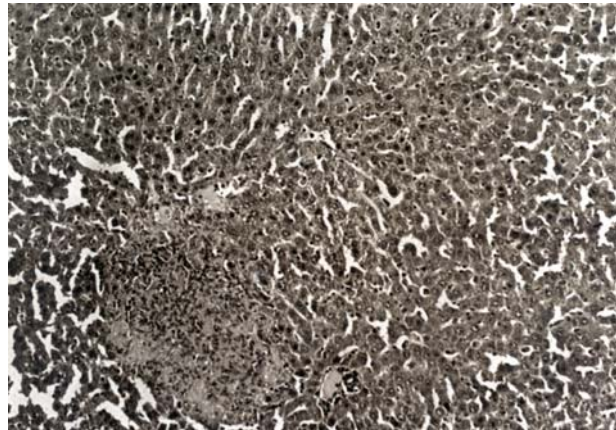


Figure 3. DEN given group: Focal necrosis in liver tissue (H.E. X 66).

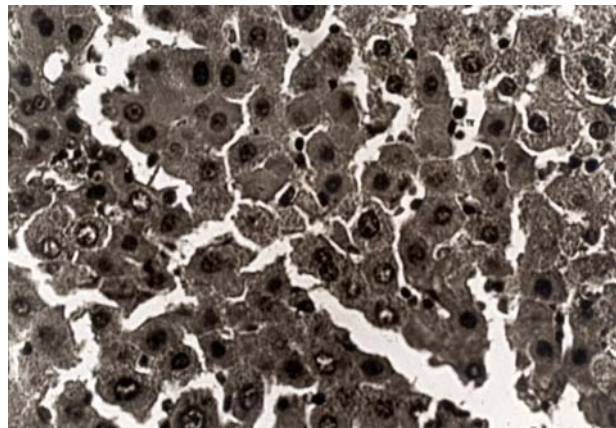


Figure 4. DEN+ GSH given group: Minimally degenerated liver tissue (H.E. X 132).

Histologic examination of kidney tissue:

Control group: No degeneration was observed in the kidneys of the control group (Figure-5).

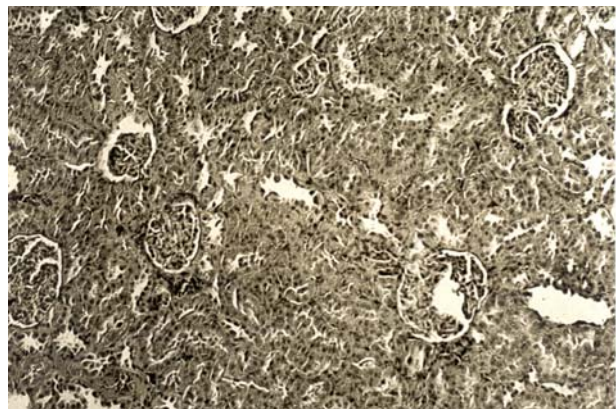


Figure 5. Normal kidney histology in control group (H.E. X 66).

DEN given rats: Following DEN injection, all zones of kidney tissue displayed almost normal renal histology. But, in some kidneys minimal necrotic tubular damage was also observed. Except minimal dilatation of distal tubules, the most striking feature was cortical and medullary congestion (Figure-6).



Figure 6. DEN given group: Highly dilated and also necrotic tubules are present (H.E.X 66).

DEN+GSH given group: No degeneration was observed in this group of rat kidneys. Renal histology showed normal architecture in all zones (Figure-7).

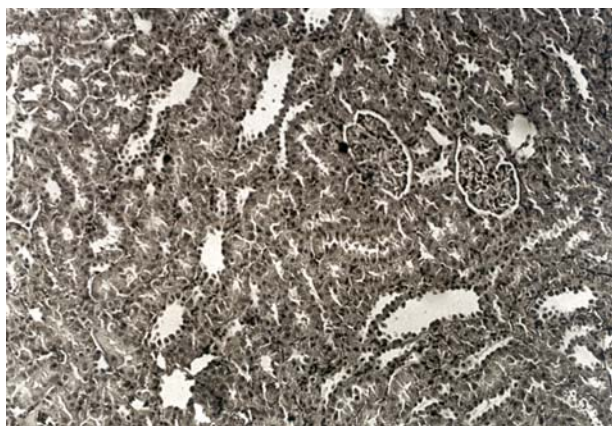


Figure 7. DEN+GSH given group: Normal histologic structure in kidney tissue (H.E. X 66).

Discussion

In the various studies carried out with nitrosamine, it has been shown that they are hepatotoxic substances and initiate liver carcinogenesis. In addition, they also inhibit many enzymes utilized by other various organs and tissues¹⁸.

In recent years, it has been shown that some xenobiotics cause oxidative-damage by formation of reactive oxygen products¹⁹. GSH, as an anti-

oxidant compound, is effective in removing the reactive oxygen products. Furthermore, it has an important role in removing the reactive metabolites which are formed during the biotransformation of xenobiotics¹².

In our study, The MDA levels were increased significantly in the hepatic tissues in the DEN and DEN+GSH group as compared to the control group ($p < 0.05$). As for the kidney tissue, a moderate increase has been seen, but was not statistically significant. These findings prove that DEN has a toxic effect particularly in the liver depending on the lipid peroxidation increase. Our histological findings seem to reinforce that.

SOD enzyme has a direct protective function against oxygen radicals, the toxic and reactive products of molecular oxygen within the cell. SOD catalyses the dismutation reaction between two superoxide radicals^{10,20}.

In our study, SOD enzyme decreased significantly in the liver and kidney tissues in the DEN groups as compared to the control groups ($p < 0.001$, 0.01) respectively. GSH, given for treatment purposes increased SOD enzyme.

It has been suggested that the tissue enzymes are generally inhibited in various animal groups to which DEN is applied²¹. In our study, the low SOD activity that we found in the liver and kidney tissues may depend on this phenomenon. Besides, the low SOD activities seem to indicate that DEN may cause the increase of the concentration of the superoxide radicals in the tissues. The fact that DEN decreases SOD, an antioxidant enzyme, may also cause an oxidative stress as depending on the depletion of the capacity of the cellular antioxidant. The liver tumor initiator was diethyl nitrosamine (DEN)²². Ito et al reported that there are harmful effects at liver of DEN²³.

Bhagvat et al have investigated the in vivo effects of the tobacco specific toxins nicotine and 4- (N- methyl-N-nitrosamine)-1- (3-pyridyl)-1 butanone (NNK) on antioxidant defense systems in the liver. Nitrosamines induced oxidative damage in the fraction of liver tissue. They invented that reactive oxygen species also increased significantly ($p < 0.001$) in liver tissues²⁴.

GSH protected the decrease of the SOD activity, which was formed through DEN. This situation may depend on the fact that GSH is an antioxidant compound and has a scavenger effect on the reactive metabolites of xenobiotics. Glutathione and antioxidant enzymes provide protection against free radical and reactive oxygen species induced lipid peroxidation and the resultant tissue degeneration²⁵.

In conclusion, our findings have shown that DEN might cause oxidative-damage particularly in the liver tissue, because DEN created the increase of the lipid peroxidation and decreased the SOD activity, which is an antioxidant en-

zyme. In addition, our findings have shown that GSH could be effective in the protection of the DEN-mediated oxidative stress.

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