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Hepatic and renal oxidative stress in acute toxicity of N-nitrosodiethylamine in rats

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Nitrosoamines such as N-nitrosodiethylamine (NDEA) produce oxidative stress due to generation of reactive oxygen species and may alter antioxidant defence system in the tissues. NDEA was administered ip as a single dose to rats in LD_{50} or in lower amounts and the animals were sacrificed after 0-48 hr of treatment. The results showed that lipid peroxidation in liver increased, however no significant increase in kidney LPO was observed after NDEA administration. Superoxide dismutase (SOD) and glutathione reductase (GSH-R) activity increased in liver, however, catalase (CAT) activity in liver was inhibited in NDEA treated rats. Kidney showed an increase in SOD activity after an initial decrease along with increase in GSH-R activity in NDEA treated rats. However, kidney CAT activity was not significantly altered in NDEA intoxicated rats. Serum transaminases, serum alkaline phosphatase blood urea nitrogen, serum creatinine and serum proteins were elevated in NDEA treated rats. The results indicate NDEA-induced oxidative stress and alteration in antioxidant enzymes in liver and kidney to neutralise oxidative stress.

The presence of nitroso compounds in diet together with the possibility of their endogenous formation in human body from precursors has been of concern as these compounds are known carcinogenic agents[1]. Nitrosoamines have been found in foods such as meat and dairy products[2] and in alcoholic beverages[3]. Industrial wastes from leather tanning, metal working and rubber and tyre manufacturing processes produce high concentrations of volatile nitrosoamines[4]. In humans, the average intake of volatile nitrosoamines from food is approximately 1 μg/day[5]. Nitrosoamines such as N-nitroso diethylamine (NDEA) has been suggested to produce oxidative stress and cellular injury due to involvement of reactive oxygen species (ROS)[6,7]. There are several reports where acute doses of NDEA, varying between 100 and 200 mg/kg body wt were used to show its carcinogenic effects[8,9]. However, there are limited studies to show the effect of acute toxicity of NDEA on hepatic and renal oxidative stress and changes in the antioxidant defence system in these tissues. Liver being the main site of NDEA metabolism and kidneys through which the metabolites are excreted may be the major organs affected by the administration of NDEA. The ROS deteriorate cell membranes and cause cellular injury and may impair the function of both of these important organs. Lipid peroxidation (LPO) in tissues was determined as a measure of cellular injury. The antioxidant defence enzymes such as Cu-Zn superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GSH-R) may alter due to increased oxidative stress. Other parameters such as serum transaminases (SGOT, SGPT), serum alkaline phosphatase (ALP), blood urea nitrogen (BUN) and serum creatinine were measured to assess the functioning of these organs in NDEA intoxicated rats.

Materials and Methods

Male Wistar rats weighing 150-180 g were allowed free access to drinking water and pellet diet (Godrej, India). The animals were treated with a single dose at LD_{50} of NDEA (Sigma Chemical Co, USA) (200 mg/kg body wt) and the animals were sacrificed after 0-48 hr of treatment. However, lower doses of NDEA (0.25 and 0.5 LD_{50}) were also administered and animals were sacrificed after 24 hr of treatment.

Liver, kidney and spleen were removed, washed in phosphate buffered saline (PBS 1:9), *p*H 7.4, blotted dry with filter paper and weighed. A 10% homogenate of liver and kidney tissues was prepared

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in PBS *p*H 7.4 with the motor driven teflon pestle glass homogeniser. The homogenate was centrifuged at 2000 *g* for 15 min at 4°C, then the supernatant was collected and centrifuged at 15,000 *g* for 30 min. at 4°C. The supernatant was used to determine LPO and antioxidant enzymes.

LPO was determined by the method of Ohkawa *et al.*[10] as modified by Jamall and Smith[11] by malonyldialdehyde-thiobarbituric acid (MDA-TBA) reaction. SOD activity was determined by the measurement of the ability of enzyme to inhibit the autoxidation of pyrogallol[12]. CAT activity was assayed by the decomposition of H_2O_2[13]. GSH-R activity was determined by the method of Carlberg and Mannervik[14].

Blood was collected in vials and was used for the determination of blood urea nitrogen (BUN), serum proteins, serum creatinine, serum alkaline phosphatase (ALP) and transaminases (SGOT, SGPT) using Auto pak reagent kits of Miles India, Ltd. in a computerised Semi-Autoanalyser SEAC CH-100 of Ames.

Statistical analysis was performed using Student's t-test and probability levels of less than 5% were considered significant.

Results

Specific organ weights—The animals treated with NDEA showed increased specific weight of liver at 24 hr and of spleen at 48 hr of treatment (Table 1). However, kidney showed no change in specific wt. within 48 hr of treatment.

Lipid peroxidation—LPO increased in liver at 12-48 hr of NDEA treatment at LD_{50} dose as compared to control (Table 2). Treatment with lower doses of NDEA (50 and 100 mg/ kg body wt.) also increased liver LPO at 24 hr as compared to control. However, kidney showed no significant change in LPO after NDEA treatment at all the doses (Table 3).

Antioxidant enzymes—The activity of liver SOD increased at 6-48 hr of NDEA treatment at LD_{50} dose as compared to control (Table 2). However, liver CAT activity decreased significantly after 12 hr of NDEA treatment. The liver GSH-R activity increased at 12-48 hr of NDEA treatment at LD_{50} dose. Treatment with lower doses of NDEA (50 and 100 mg/kg body wt.) showed no significant changes in liver SOD and CAT activity at 12-24 hr, however, mild increase in liver GSH-R activity was observed at 24 hr.

The activity of kidney SOD significantly increased at 48 hr after an initial decrease at 6 hr of NDEA treatment (Table 3). The kidney CAT activity decreased at 6 hr of NDEA treatment and thereafter, showed no significant change as compared to control. The kidney GSH-R activity increased significantly after 12 hr of NDEA treatment at LD_{50} dose. Treatment with lower doses of NDEA showed mild increase in kidney SOD activity, no change in CAT activity along with significant increase in GSH-R activity in kidney at 12-24 hr.

Serum enzymes, BUN and serum creatinine—Serum GOT, GPT and ALP were observed to be elevated at 6-48 hr of NDEA treatments (Table 4). BUN and serum creatinine increased initially after NDEA administration, however, both decreased towards control levels at 48 hr of NDEA administration. Treatment with lower doss of NDEA showed non significant changes in BUN, however, serum creatinine showed mild increase at 100 mg/kg body wt. dose of NDEA.

Serum proteins—The serum proteins increased after 12 hr of NDEA administration with the increase in both serum albumin and globulin without significantly affecting the A/G ratio (Table 5).

Table 1—Effect of NDEA on specific organ weights in rats

[Values are mean ± SE of 6 rats]

Time after treatment (hr)	Liver	Kidney (g/100 g body wt.)	Spleen
0	4.333±0.210	1.310±0.083	0.172±0.019
6	4.747±0.087	1.425±0.035	0.176±0.013
12	4.553±0.406	1.374±0.039	0.175±0.010
24	5.187±0.111**	1.375±0.050	0.193±0.009
48	5.623±0.160***	1.506±0.074	0.220±0.010***

NDEA was administered ip at a single dose of 200 mg/kg body wt and the animals were sacrificed at various time periods.
P values, **< 0.01, ***< 0.001 as compared to the control.

Discussion

Nitrosamines are known carcinogenic agents, however, the mechanism of carcinogenesis is not clear. One of the theories suggest that activated forms of oxygen, organic peroxides and other radicals may have a role in carcinogenesis[15]. These cellular proxidant states can initiate oxidation of lipids and of other biomolecules[16]. The biological consequences results in the formation of metabolic products which are able to alkylate the genetic material or proteins associated with these materials[17]. The metabolic activation of NDMA possibly produces ROS capable of initiating peroxidative damage in the cell. It has been shown that during the cytochrome P-450 mediated metabolism of xenobiotics there is formation of $O_2^{\bullet-}$ and H_2O_2[18]. The effects produced by NDMA *in vivo* took place rapidly. The peak was reached within 20-60 min after the NDMA administration and in most cases the changes were no more seen 24 hr after the dosing[19].

The increased oxidative stress due to ROS generation may alter the antioxidant defence system of the tissues which may be an important factor in nitrosamine-induced carcinogenesis. Liver showed increased LPO indicating increased oxidative stress with NDEA treatment. The increase in liver SOD activity though not exponential along with increase in LPO may be an adaptive response towards generation of $O_2^{\bullet-}$. However, liver CAT activity diminished after 12 hr of NDEA administration probably due to increased $O_2^{\bullet-}$ anions as $O_2^{\bullet-}$ anions have been shown to inhibit CAT activity[20]. The elevated liver GSH-R activity may increase glutathione (GSH) synthesis in order to counteract NDEA induced oxidative stress.

Table 2—Effect of NDEA on lipid peroxidation and antioxidant enzymes in liver

[Values are mean ±SE of 6 rats in each group]

Dose of NDEA (mg/ kg body wt)	Time after treatment (hr)	LPO (n moles of MDA formed/ mg protein/ hr)	SOD (units/ mg protein)	CAT (μmoles of H_2O_2 decomposed/ mg protein/ min)	GSH-R (nmoles of NADPH oxidised/mg protein/min)
0	0	138±9	5.0±0.4	131±9	38±2
50	24	182±11*	6.3±0.8	140±2	43±1
100	24	196±17*	7.4±1.0	133±3	51±3**
200	6	167±13	6.3±0.2*	123±6	41±2
200	12	188±18*	9.0±0.1***	110±5*	50±3***
200	24	222±15***	8.3±0.3***	108±2*	64±2***
200	48	243±19***	12.9±0.5***	89±3***	60±3***

NDEA was administered ip at a single dose and the rats were sacrificed after various time periods.
P values, * < 0.05, ** < 0.01, *** < 0.001 as compared to the control.

Table 3—Effect of NDEA on lipid peroxidation and antioxidant enzymes in kidney

[Values are mean ±SE of 6 rats in each group]

Dose of NDEA (mg/ kg body wt)	Time after treatment (hr)	LPO (nmoles of MDA formed/ mg protein/ hr)	SOD (units/ mg protein)	CAT (μmoles of H_2O_2 decomposed/ mg protein/min)	GSH-R (nmoles of NADPH oxidised/mg protein/ min)
0	0	149±12	5.3±0.2	86±3	45±2
50	24	152±13	6.7±0.4*	80±4	60±2***
100	24	159±17	6.2±0.5	88±2	65±1***
200	6	173±12	3.8±0.1***	76±2*	47±2
200	12	163±16	5.2±0.2	88±3	68±2***
200	24	151±16	5.7±0.3	88±5	72±3***
200	48	155±13	7.9±0.3***	84±5	82±2***

Legends are as shown in Table 2.

NDEA treatment induce oxidative stress, however, parallel changes between LPO and antioxidant enzymes were not observed.

Kidney, however, showed no significant increase in LPO with increase in SOD activity at 48 hr of NDEA treatment. CAT activity in kidney was initially inhibited at 6 hr of NDEA treatment with increase in GSH-R activity. The results suggest of mild oxidative stress in kidney during the period of NDEA treatment. Kidney showed alterations in antioxidant enzymes after NDEA treatment, however, oxidative stress was not exhibited as increase in LPO (Table 3). It may be probable that TBA reactive substances (TBARS) increased slower than other measures of LPO as well as TBA method measures end products rather than early events of LPO.

The NDEA intoxication, however, affect the renal function to some extent as observed by the increased BUN and serum creatinine at 6 hr of treatment. The increased serum enzymes indicate impairment of hepatic function. The increased serum levels of GOT, GPT and ALP has been used as an indicator of cellular damage, increased membrane permeability, or altered metabolism of these enzymes[21,22].

The increased spleen wt. could be attributed to the chemical intoxication and immunological response generated against the carcinogen[23]. The increase in total serum protein and serum albumin content in NDEA intoxicated rats may be an antioxidant function of serum proteins. The serum albumin bind to copper and iron and therefore, albumin is suggested to be an antioxidant[24]. Albumin is a carrier of free fatty acids (FFA) in the circulation and the FFA's associated with albumin may also be responsible for the antioxidant activity[25,26]. The protein act as antioxidant and have a sparing action on other antioxidants[27]. In conclusion, NDEA induced oxidative stress in liver and kidney and alter the antioxidant enzymes to counteract oxidative stress. The increase in serum enzymes, BUN and serum

Table 4—Effect of NDEA on serum enzymes, BUN and serum creatinine

[Values are mean ±SE of 6 rats in each group.]

Dose of NDEA (mg/kg body wt)	Time after treatment (hr)	GOT (units/lt)	GPT (units/lt)	ALP (IU/L)	BUN (mg%)	Creatinine (mg%)
0	0	50±1	60±3	129±3	21.7±0.3	0.68±0.07
50	24	83±3***	62±3***	174±4***	22.2±0.8	0.85±0.08
100	24	107±3***	76±5**	222±7***	23.3±0.7	1.0±0.1*
200	6	57±2*	76±3***	154±5***	31±1.3***	1.3±0.1***
200	12	177±7***	79±2***	237±5***	25.7±1.0*	1.2±0.1***
200	24	173±2***	94±2***	248±2***	22.2±1.7	0.95±0.06*
200	48	201±6***	147±5***	386±5***	23.4±1.0	0.85±0.08

Legends are as shown in Table 2.

Table 5—Effect of NDEA on serum proteins

[Values are mean ±SE of 6 rats in each group]

Dose of NDEA (mg/ kg body wt)	Time after treatment (hr)	Total protein	Albumin (A) g%	Globulin (G) g%	A/G ratio
0	0	6.37±0.26	2.92±0.20	3.46±0.36	0.97±0.19
50	24	6.08±0.11	2.87±0.07	3.22±0.04	0.89±0.02
100	24	6.30±0.14	3.13±0.11	3.17±0.12	1.00±0.06
200	6	6.40±0.26	2.95±0.10	3.45±0.28	0.85±0.07
200	12	7.53±0.28*	3.42±0.21	4.33±0.15*	0.83±0.05
200	24	7.20±0.13*	3.90±0.09***	3.30±0.26	1.18±0.06
200	48	7.20±0.12*	3.48±0.10	3.72±0.25	0.93±0.07

Legends are as shown in Table 2.

creatinine also indicate NDEA-induced oxidative stress in these tissues.

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