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In vitro and *In vivo* metabolism of N-Nitrosodimethylamine using male Rats

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ABSTRACT

The research is to investigate the denitrosation of N-Nitrosodimethylamine (NDMA) by the mitochondrial fractions (MF) and post-mitochondrial fractions (PMF) of liver, kidney, spleen and testes of rats and to determine the level of toxicity in these organs. In the first part of the work, a group of male albino rats (5 rats) was used to obtain the organs whose homogenates were used for the Kinetic experiments. In the second part, two groups were used. One group was group was treated with 2.5mg/kg NDMA twice per week for four weeks, while the other group was the control. The organs obtained from the rats were used for histology and biochemical parameters (ALT, AST and GGT). In the four organs, the level of nitrite was found to be higher in the MF than PMF. The (Km) values of denitrosation were lower in the MF than the PMF. The Km values for the MF of the kidney, spleen and testes were calculated to be 0.005, 0.004, 0.004 and 0.005 μM, respectively, while the Km values for the PMF were calculated to be 0.013, 0.025, 0.007 and 0.033 μM, respectively. The activities of ALT, AST and GGT enzymes in serum and liver were significantly higher ($p < 0.05$) in the NDMA-treated rats than controls. Histology showed the presence of shrunken hepatocytes in liver, interstitial cellular infiltration in kidney, lymphoid depletion in spleen, and severe subcapsular congestion in the testes of NDMA-treated rats, compared with controls. This research shows that NDMA denitrosating enzymes in the MF have greater affinity for NDMA than the same enzymes in the PMF, It also shows that NDMA at a low dose could induce toxicity in the spleen, kidney, testes, as well as liver.

Introduction

N- Nitrosamines are activated by microsomal CYP 450 based on α-hydroxylation of the compounds, in which hydrogen atom is transferred instead of removal of an electron due to the high oxidation potential of the nitrogen carrying the nitroso group [1,2]. The α-hydroxynitrosamine being formed becomes converted to an aldehyde and an alkyl diazohydroxides. The aldehyde is finally oxidized to carboxylic acid, while the diazohydroxide is converted to an alkyl nitrenium ion or a carbocation, which are DNA alkylating agents [3]. Dimethylnitrosamine (DMN) yields formaldehyde, while dimethylnitrosamine (DEN) yields acetaldehyde on oxidation. CYP450 2A6 and 2E1 oxidize both DMN and DEN, however, 2A6 shows higher activity for DEN than that of 2E1 [4,5]. Certain studies revealed that acetyltransferase (NAT) enzymes could catalyze the acetylation of alkyl diazohydroxides. This is evident by the high sensitivity of acetyltransferase-overexpressing strain of Salmonella typhimurium to nitrosamine exposure and the concomitant presence of N-nitrosomethylacetoxymethylamine in the organism [4].

The metabolic activation of nitrosamines by CYP2E1 is most likely the route to ROS generation, which occurs by redox cycling [6-8]. Apart from denitrosation, microsomal CYPs could also catalyze dealkylation of nitrosamines [9]. However, at low concentration, nitrosamines could spontaneously denitrosated, a reaction similar to UV light-induced denitrosation [10-12].

Oxidative stress due to nitric oxide effect on Caco-2 has immunoregulatory functions such as stimulation of cytokine production [13-15] Since nitrosamines are denitrosated to form nitric oxide radical (NO*), this radical could be responsible for immunoregulation. In the other way, the oxidative stress as a result of nitrosamine-induced ROS formation could also be implicated in immunoregulation [16].

The administration of dimethylnitrosamine (NDMA) at doses of 20 – 40mg/kg body weight to rats, dogs, rabbits and guinea pigs resulted in severe liver damage [17]. They also reported the toxic effect of a single dose of NDMA (20mg/kg) administered to rats orally or by injection, which caused the hepatocytes in the centrilobular and mid –zonal areas to be pale, when examined under light or electron microscopy. After about 18 hours, the cytoplasm of the liver cells became vacuolated and amorphous. After 24 hours, there was centrilobular necrosis with hemorrhage. Hepatic recovery process was observed after 72 hours which was near completion in a period of about 3 weeks. A co-administration of sodium nitrite (100 or 150mg/kg) with dimethylamine (500 – 2500mg/kg) and methylbenzylamine (800 – 1600mg/kg) was studied by Asahina *et al* [18], and they reported that there was hepatic lesion similar to the one produced by either dimethylnitrosamine or nitrosomethylbenzylamine. Moreover, similar effect was observed when DMA was administered about 3 hours before nitrite, whereas, administration of nitrite prior to DMA did not produce the same level of toxicity.

Several studies are available on the carcinogenicity of N – Nitrosamines in many species of animals, including mammals, which point out the organ specificity of these compounds. N –

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Nitrosamines cause carcinogenesis in organs, such as the liver, oesophagus, lungs and kidney. The development of carcinogenesis in an organ due to nitrosamine is dose – dependent. This has been demonstrated by Magee and Barnes [17], who found that low doses of NDMA on a long- term basis caused liver carcinogenesis, whereas large doses or even a single large dose, produced kidney carcinogenesis. A treatment of pregnant rats with a single dose of nitrosamines caused carcinogenesis in the first three generations [19].

In earlier studies by Druckrey *et al.*[20], the carcinogenesis of nitrosamines (particularly NDEA) was found to be related to daily dose (d) and induction time (t) .The product of the quantities is usually constant, which can be expressed as $d \times t^{2.3}$ = constant. Transplacental carcinogenesis, due to N-nitrosamines, in many species of animals has been found to be dependent on the time of treatment, during pregnancy. In early period of gestation, the metabolic system in the fetus is highly inefficient, hence the compounds are not metabolically activated to cause tumor. However, tumors can only occur in the offspring, when the compounds are administered in the last period of gestation [21]. This study is to investigate the abilities of liver, kidney, testes and spleen of rats in metabolizing NDMA and to determine the level of toxicity in the organs.

Materials and Methods

Chemical reagents

N-nitrosodimethylamine (NDMA) was purchased from Sigma Inc. USA. All other reagents were of analytical grades.

Animal Treatment (I)

Male albino rats (average weight of 140g) were purchased from the animal house at the University of Ibadan. They were acclimatized in cages, for 7 days, in a room (24-25°c and 40-50% humidity) exposed to a 12 hours light-dark cycles. The animals were weighed and then randomized into 2 groups (5 rats each) and fed for 4 weeks as indicated below, Water was added ad libitum in both groups:

Group 1 (Control) Rats fed with normal rat pellets alone

Group 2 - Rats fed with 2.5mg/kg NDMA and normal rat pellets

Collection of organs (I)

After 4 weeks, the rats were starved overnight and then sacrificed by cervical dislocation. Blood was collected by cardiac puncture and subjected to centrifugation at 2000 x g for 10 minutes to obtain serum, which was later refrigerated at 4°c until use. Liver was excised and homogenized with Teflon glass at 2000xg for 10 minutes, and the homogenates were kept at 4^0c to be subsequently used for enzyme assays. The liver, kidney, testes and spleen were excised and washed with 1.15% KCl (BDH chemicals) and used for histopathology.

Animal Treatment (II) – Collection of organs and preparation of mitochondrial and post-mitochondrial fractions

5 male albino rats (average weight of 160g) were purchased from the animal house at the University of Ibadan and sacrificed by cervical dislocation. Four organs (liver, kidney, testes and spleen) were harvested and washed with 1.15% KCl and then homogenized using Teflon homogenizer. Each of the homogenates was divided into two portions. One portion was centrifuged at 800 x g for 10 minutes in an MSE high speed refrigerated centrifuge, to obtain the mitochondrial fractions, which was then subjected to 70% ammonium sulphate precipitation and dialyzed overnight against 0.06M phosphate buffer (pH 7.4). The second portion of each homogenate was subjected to centrifugation at 10,000 x g for 30minutes in an MSE high speed refrigerated centrifuge, to obtain the post-mitochondrial fractions (containing microsomal enzymes). The protein concentrations of both mitochondrial and post-mitochondrial fractions were determined by the method of Lowry *et al.* [22].

Determination of the Time-course of denitrosation of NDMA by mitochondrial and post-mitochondrial fractions of homogenates of rat organs

A mixture containing 0.5ml sample (mitochondrial and post-mitochondrial fractions separately), 0.2ml NDMA (0.15mM) and 0.6µM NADPH in a total volume of 5ml made up with 0.06M phosphate buffer (pH 7.4) was incubated with shaking for 5, 10, 15, 20, 25 and 30mins at 37^0c in a water bath. The reaction was stopped by the sequential addition of 100µl of 10% $ZnSO_4.7H_2O$ and 100µl $Ba(OH)_2.8H_2O$ and centrifuged at 2000 x g for 5 minutes to obtain a supernatant (Chowdhury *et* al., 2010). The concentration of nitrite was determined by sequential addition of 0.5ml 0.5% Na_2CO_3, 1.5ml sulphanilic acid and 1.5ml Naphthylethylene diamine to the supernatant. The reaction mixture was allowed to stand for 15mins and the absorbance of the resulting pink solution was read at 550nm with a spectrophotometer [23].

Determination of the Km and Vmax of denitrosation of NDMA by mitochondrial and post-mitochondrial fractions of homogenates of rat organs

A mixture containing 0.5ml sample (mitochondrial and post-mitochondrial fractions separately), 0.2ml NDMA (0.05, 0.10, 0.15, 0.20 and 0.25mM) and 0.6µM NADPH in a total volume of 5ml made up with phosphate buffer (pH 7.2) was incubated with shaking for 15mins at 37^0c in a water bath. The reaction was stopped by the sequential addition of 100µl of 10% $ZnSO_4.7H_2O$ and 100µl of Ba $(OH)_2.8H_2O$ and centrifuged at 2000 x g for 5 minutes, to obtain a supernatant (Chowdhury *et al.*, 2010).

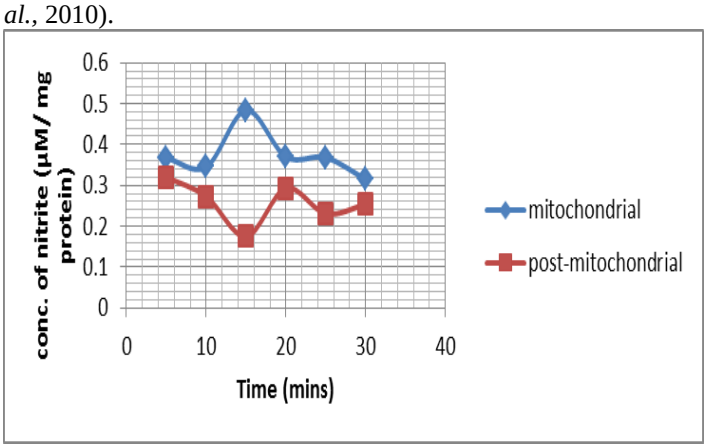


Fig 1b: Time course of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat liver homogenates

The concentration of nitrite was determined by sequential addition of 0.5ml 0.5% Na_2CO_3, 1.5ml sulphanilic acid and 1.5ml Naphthylethylene diamine to 1ml of the supernatant. The reaction mixture was allowed to stand for 15mins and the absorbance of the resulting pink solution was read at 550nm with a spectrophotometer [23]. The Km and Vmax values were determined by using Lineweaver-Burk (double reciprocal) plots.

Determinations of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma- glutamyl transferase (GGT) activities

The activities of ALT, AST and GGT enzymes in the serum and liver of the rats under animal treatment (I) were determined using the assay kits (RANDOX kits).

Statistical analysis

The data were expressed as mean ± Standard error of mean. $P < 0.05$ was considered statistically significant for differences in mean values.

Histopathology of organs

The liver, kidney, spleen and testes (harvested from animal experiment I) were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. The sections were stained with Eosin and Hematoxyline (E&H).

Results

In figures 1a-1d, the levels of Nitrite in the mitochondrial fractions were higher than the post-mitochondrial fractions.

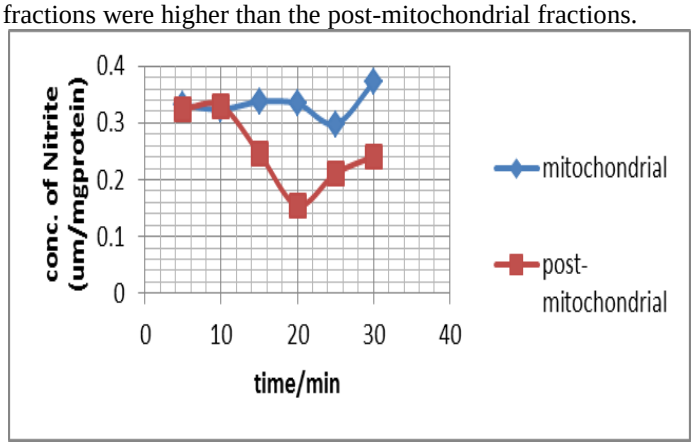


Fig 1b: Time course of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat kidney homogenates

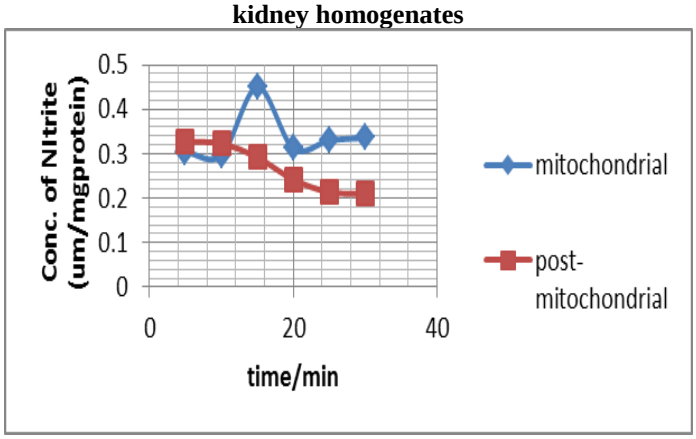


Fig 1c: Time course of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat spleen homogenates

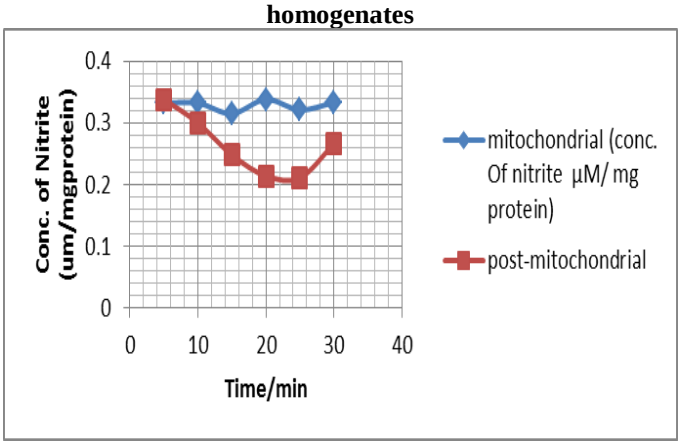


Fig 1d: Time course of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat testes homogenates

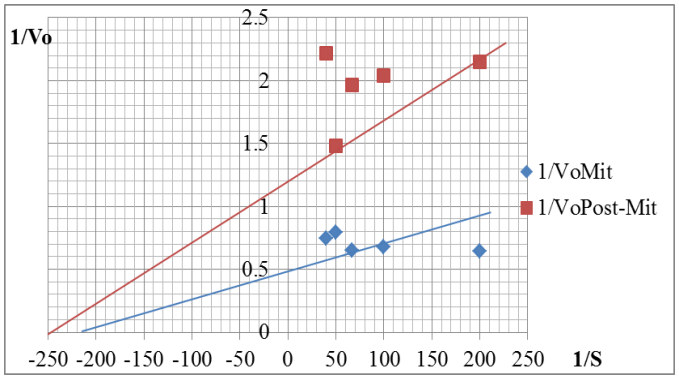


Fig 2a: Lineweaver-Burk plots of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat liver homogenates

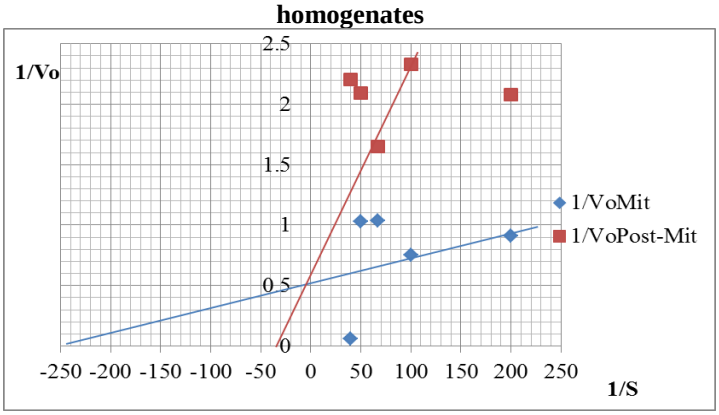


Fig 2b: Lineweaver-Burk plots of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat kidney homogenates

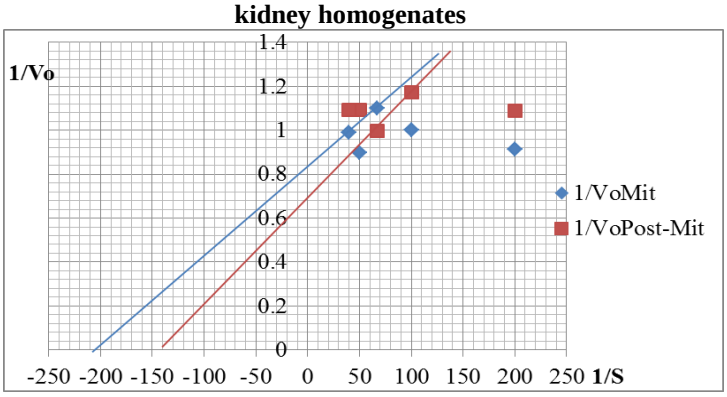


Fig 2c: Lineweaver- Burk plots of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat spleen homogenates

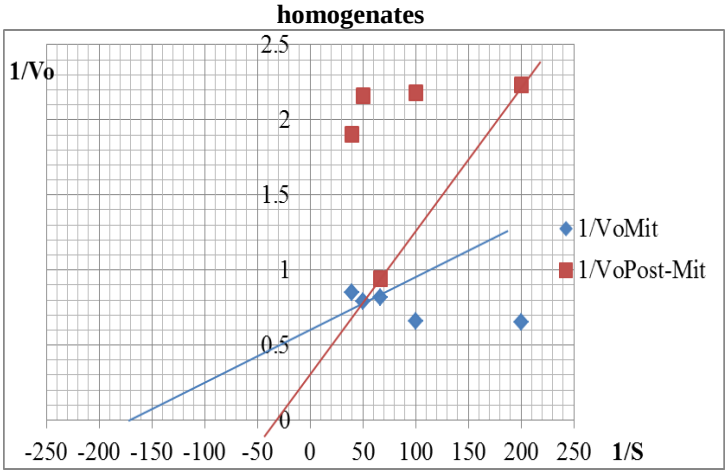


Fig 2d: Lineweaver- Burk plots of NDMA denitrosation by mitochondrial and post mitochondrial fractions of rat testes homogenates

Table 1: Values of Vmax (µM/mg protein/hr) and Km (µM) of NDMA denitrosation by mitochondrial and post-mitochondrial fractions of rat organs

Organs	Vmax (MF)	Km (MF)	Vmax (PMF)	Km (PMF)
Liver	2.00	0.005	1.11	0.013
Kidney	2.00	0.004	1.54	0.025
Spleen	1.25	0.004	1.43	0.007
Testes	1.67	0.005	3.33	0.033

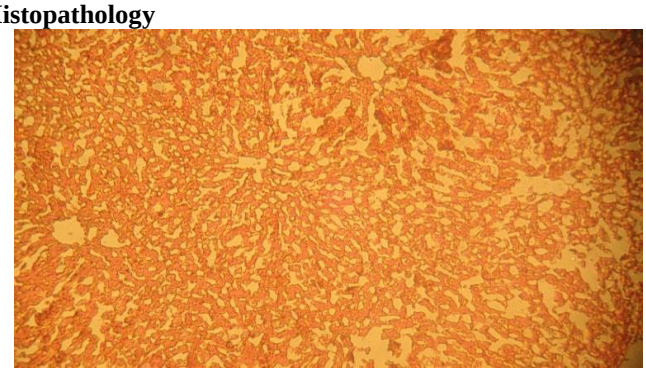
MF - Mitochondrial fraction, PMF- Post-mitochondrial fraction

Table2: Mean± SEM values of activities of ALT, AST and GGT enzymes of rat serum and liver

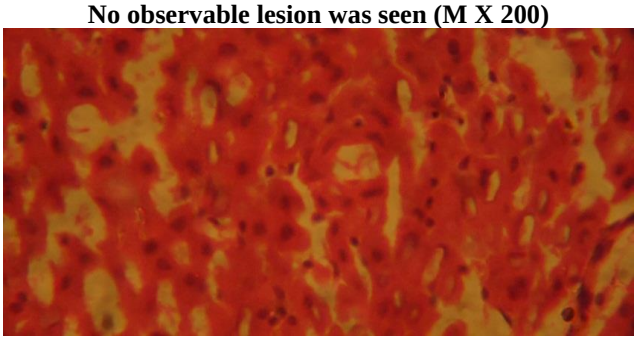
	ALT (U/L) Mean ± SEM	AST (U/L) Mean ± SEM	GGT (U/L) Mean ± SEM
Group A rats	22.51± 1.005	82.09 ± 6.995	4.948 ± 0.575
SERUM Group B rats	88.19 ± 6.361	177.10 ± 8.150	52.30 ± 6.800
P-value	0.0024	0.0012	0.0018
Group A rats	70.68 ± 4.871	86.08 ± 9.20	6.948 ± 2.104
LIVER Group B rats	110.40 ± 5.113	181.40 ± 5.20	77.00 ± 9.348
P-value	0.0013	0.048	0.004

Group A (control) – fed with normal rat pellets. Group B – fed with 2.5 mg NDMA / kg body weight. $P < 0.05$

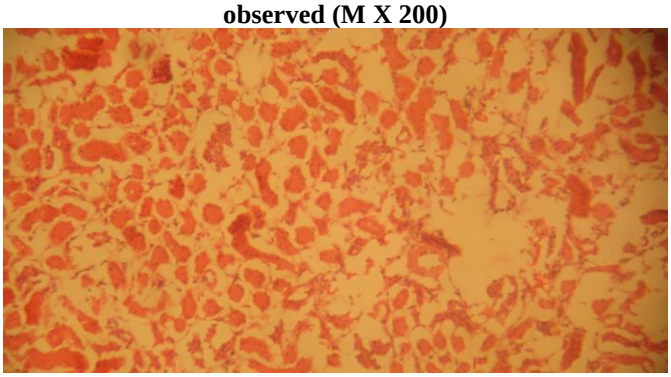
Histopathology



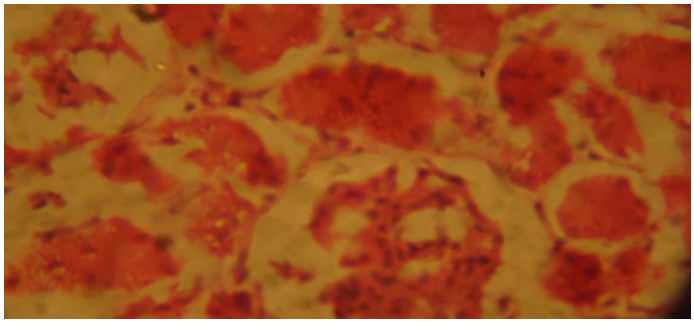
Liver (Group A-control) – Rat fed with normal rat pellet- No observable lesion was seen (M X 200)



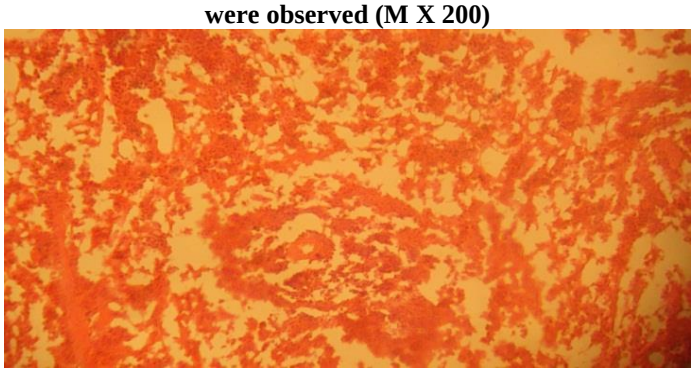
Liver (Group B-rat fed with 2.5 mg/kg bw of NDMA)- Shrunken hepatocytes with prominent sinusoids were observed (M X 200)



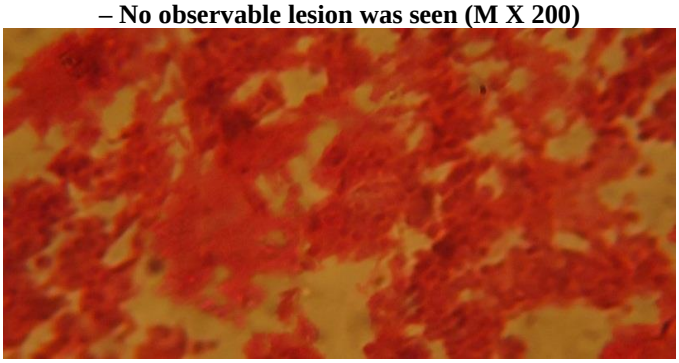
Kidney (Group A-Control)- Rat fed with normal rat pellets- No observable lesion was seen (M X 200)



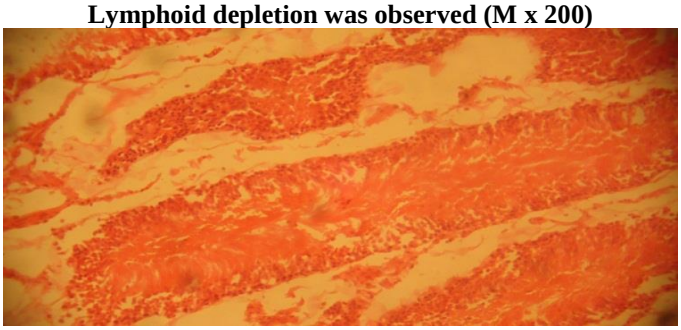
Kidney (Group B-Rat fed with 2.5 mg/kg bw of NDMA) - Mild interstitial cellular infiltration and tubular clumping were observed (M X 200)



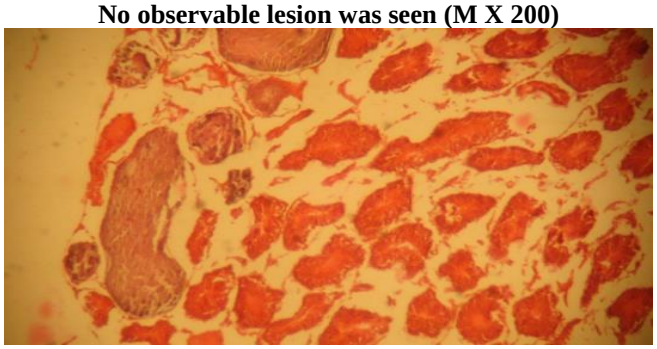
Spleen (Group A- Control) - Rat fed with normal rat pellets – No observable lesion was seen (M X 200)



Spleen (Group B-rat fed with 2.5 mg/kg bw of NDMA) - Lymphoid depletion was observed (M x 200)



Testis (Group A-Control) – Rat fed with normal rat pellets- No observable lesion was seen (M X 200)



Testis (Group B-rat fed with 2.5 mg/kg bw of NDMA) – Severe subcapsular congestion was observed (M X 200)

Discussion

Considerable evidence has been accumulated to support the degradation of Nitrosodimethylamine (NDMA) and other alkylnitrosamines by mammalian cells to form nitrite, aldehydes and alkyldiazonium compounds. The degradation has been observed to involve two major pathways, demethylation and denitrosation, requiring the activities of cytochrome p450 enzymes [24-29]. The activities of cyt p450 enzymes in the dealkylation of nitrosamines is generally believed to be an activation pathway [30], while the denitrosation through the same enzymes occurs as a detoxification process [31]. Chowdhury and co-workers [32] reported the sequential oxidation of nitrosamines to aldehydes and carboxylic acids through the activity of cyt p450, indicating processivity in the metabolism of these important toxicants found in foods and environmental specimens.

Raza *et al*.[33] observed tremendous induction of CYP2E1 protein and increase in CYP2E1- dependent NDMA metabolism in subcellular regions of different tissues. Cytochrome P450 2E1 has been found in the mitochondria [34]. In the present study, NDMA was denitrosated by the mitochondrial and post-mitochondrial fractions of liver, kidney, spleen and testes. During the course of denitrosation, concentrations of nitrite at the beginning were generally the same for the two fractions in each case (figures 1a-1d). However, there were disparities in concentrations of nitrite with time, in which the concentrations of nitrite in mitochondrial fractions were higher than those of the post-mitochondrial fractions. Figures 2a-d present the Lineweaver-Burk (double reciprocal) plots of NDMA denitrosation by mitochondrial and post-mitochondrial fractions of liver, kidney, spleen and testes, respectively. Table 1 presents the values of maximum velocity (Vmax) and Michaelis-Menten constants (Km), as calculated for the respective organs. The Vmax values in the mitochondrial fractions of liver and kidney were higher than those of the post-mitochondrial fractions of the same organs, whereas in the case of spleen and testes, the values were lower in the mitochondrial fractions than post-mitochondrial fractions. The Km values in the mitochondrial fractions were found to be lower than those in the post-mitochondrial fractions. The mitochondrial fractions of kidney and spleen were found to have the same Km value of 0.004μM, those of the liver and testes were found to have the same Km value of 0.005μM. However, the Km values in the post-mitochondrial fractions of liver, kidney, spleen and testes were observed to be 0.013, 0.025, 0.007 and 0.033 μM, respectively. The higher concentrations of nitrite in the mitochondrial fractions (fig1a-d) and the lower Km values (fig 2a-d) observed in the mitochondrial fractions could indicate greater activities of cytochrome p450 enzymes in the mitochondrial fractions than post-mitochondrial fractions in the metabolism of NDMA. Early studies of the members of CYP1 to CYP3 families involved in the biotransformation of xenobiotics have shown that these proteins are exclusively present in the endoplasmic reticulum. However, recent studies have revealed significant amounts of several members of these protein families in other cell compartments, particularly, mitochondria [35-39].The finding in the present study is in line with the work of Bhagwat *et al.* [39] who reported that the activity of NDMA-demethylase, implicating mitochondrial CYP2E1 was more than that of the corresponding microsomal activity. CYP 450 2E1 showed a preference for the mitochondria-specific electron transport proteins called Adrenoxin (Adx) and Adrenoxin reductase (Adr), and if the Adrenoxin interacting domain of CYP2E1 is modified, the activity of mitochondrial CYP2E1 in metabolizing NDMA would be effectively inhibited [36]. In vivo studies have reported the presence of full-length CYP 2E1 in rat liver mitochondria [40]. The targeting of CYP 450s to the mitochondria could be a protective mechanism of these organelles against damage due to xenobiotics, however, under pathologic conditions mitochondria could be exposed to damage by toxic metabolites produced by these proteins [41].

Table 2 shows the values of Mean ± SEM of the activities of alanine amino transferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) enzymes in the serum and liver of experimental rats. The activities of the three enzymes were statistically higher ($p < 0.05$) in both serum and liver of rats on chronic NDMA administration when compared with the controls. The elevated activities of the three enzymes in the NDMA-administered group indicated hepatic damage in this group of rats.

When NDMA is orally administered, there is rapid absorption at the upper region of the gastrointestinal tract (GIT), passing through the messentric and portal veins into the liver before entry into the general blood flow. When a large dose of NDMA is administered, nearly all of the substance is passed through the liver, unmetabolized, into the general blood circulation. However, when a small dose of the NDMA is administered, the compound is efficiently metabolized in the liver, releasing very little quantity of the unmetabolized form into the general blood circulation and finally entering the other organs [42]. The histological studies of liver, kidney, spleen and testes of experimental rats were carried out. Figure 3b shows the development of shrunken hepatocytes with prominent sinusoids in rats treated with chronic administration of NDMA (2.5mg/kg body weight) compared with the control rats (fig 3a). Sinusoids are carvenous spaces conducting blood in which hepatocytes are bathed. Chemicals could passively diffuse across the sinusoidal membrane to enter the hepatocytes[43]. The prominence of the sinusoids may indicate inflammation of the membrane of this vital hepatic blood passage as a result of the NDMA administration. High doses of NDMA, either orally or by injection, caused severe liver damage and pale hepatocytes in the centrilobular and mid-zonal areas of the organ [44], as well as the congestion of the portal vessels, periportal cellular infiltration by mononuclear cells and severe diffuse hepatic necrosis [45]. Figures 4b shows the development of interstitial cellular infiltration with tubular clumping in kidney of rats on chronic NDMA administration (2.5mg/kg body weight) compared with the controls (fig 4a). Magee and Barnes [17] found that large doses or even a single large dose of NDMA, produced kidney carcinogenesis in experimental rats. Lymphoid depletion was observed in the spleen of rats on chronic administration of NDMA (2.5mg/kg body weight) (fig 5b), the controls showed no observable lesion (fig 5a). Spleen is a major lymphoid organ that synthesizes antibodies and sequestrates antibody-coated bacteria together with antibody-coated blood cells [46]. This organ is also reservoir of monocytes [47]. The observed lymphoid depletion in the spleen of on NDMA administration, in this study, may lead to compromised immunity, due to deficiency in the synthesis of antibodies and low level of monocytes. The effect of NDMA administration on male fertility was also investigated and the testes of the experimental rats were found to show severe subcapsular congestion (fig 6b), while the controls showed no observable lesion (fig 6a). A single intraperitoneal injection of 30 or 60 mg NDMA / kg (body weight) in male rats, led to testicular damage,

which is the necrosis or degeneration of the seminiferous epithelium [48]. The toxic potential of nitrosamines has been due to their one or more of these effects; DNA damage, reactive oxygen species generation and oxidative stress [49], lipid peroxidation, adduct formation and pro-inflammatory cytokine production [50], alkylating and mutagenic potentials[51], induction of apoptosis [52], mediation of unscheduled DNA synthesis and cell death [51], induction of single-strand DNA breaks and nitric oxide production and enhancement of the xanthine oxidase activity resulting in high levels of superoxide anion, hydrogen peroxide and hydroxyl radical [53].

In conclusion, this work has shown that NDMA denitrosating enzyme in the mitochondrial fractions has greater affinity for NDMA than the same enzyme in the post-mitochondrial fractions, containing microsomal enzymes. The work also showed that NDMA at a low dose could induce toxicity in the spleen, kidney and testes of rats, in addition to the well-established information on liver toxicity by this compound.

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References

1.Ortiz de Montellano PR., De Voss I.I.: Cytochrome P450: Mechanism and Biochemistry. 3rd Ed. (Ortiz de Montellano PR. Ed) 2005, pp. 183-245, Kluwer Academic/ Plenum Publishers, New York.

2.Guengerich FP., Yun CH., Macdonald TL: J. Biol.1996, Chem. 271, 27321-27329.

3.Preussmann R. and Stewart BW: Chemical carcinogens. Vol. 2, 2nd Ed. (Searle CE. Ed), 1984, 643-828, American Chemical Society, Washington D.C.

4.Yamazaki H., Inui Y., Yun CH., Guengerich FP., Shimada T: Carcinogenesis.1992, 13, 1789-1794.

5.Crespi C.L., Penman B.W., Leakey J.A.E. Arlotto M.P., Stark A., Parkinson A., Turner T., Steimel D.T, Rudo K., Davies R.L and Langenbech R: Human cytochrome P450IIA3: CDNA sequence, role of the enzyme in the metabolic activation of promutagens. Comparison to nitrosamine activation by human cytochrome p 450IIE1. Carcinogenesis. II,1990, 1293-1300

6.Bartsch H., Hietanen E., and Malaveille C: Carcinogenic Nitrosamines: Free radical aspects of their action. Free radical. Biol. Med.1989, 7, 637-644.

7.Lewis D.F.V : Oxidative Stress: The role of cytochromes P450 in oxygen activation. J. Chem. Technol. Biotechnol.2002, 77, 1095-1100.

8.Kushida H., Fujita K., Suzuki A., Yamada M., Endo T., Nohmi T et al.: Metabolic activation of N-alkylnitrosamines in genetically engineered Salmonella typhimurium expressing CYP2EI or CYP2A6 together with human NADPH-Cytochrome P450 reductase. Carcinogenesis, 2000, 21: 1227-32.

9.Flyod R.A., Soong L.M., Stuart M.A. and Reigh D.L: Spin trapping of free radicals produced from nitrosamine carcinogens. Photochem. Photobiol.1978, 28, 857-862.

10.Grover T.A, Ramseyer J.A. and Piette L.H : Photolysis of nitrosamines and nitrosamides at neutral pH: A spin-trap study. Free radic Biol. Med.1987, 3, 27-32.

11. Hiramoto K., Ryuno Y. and Kikugawa K: Decomposition of nitrosamines and concomitant release of nitric oxide by Fenton reagent under physiological conditions. Mutat. Res.2002, 520, 103-111.

12. Wink D.A. and Desrosiers M.F: Unusual Spin-trap Chemistry for the reaction of hydroxyl radical with the carcinogen N- nitrosodimethylamine. Radiat. Phys. Chem..1991, 38, 467-472.

13.Bodgan c: Nitric oxide and the immune response Nat. Immunol. 2002,2, 907-916.

14. Nemeth E., Halasz A; Barath A; Domokos M. and Galfi P. (2007). Effect of hydrogen paroxide on interleukin- 8 synthesis and death of Caco-2 cells. Immunopharmacol. Immunotoxicol. 29, 297-310.

15. Yamamoto K., Kushima R., Kisaki O., Fujiyama Y. and Okabe H : Combined effects of hydrogen peroxide- induced oxidative stress and IL-1apha on IL-8 production in CaCo-2 cells (a human colon carcinoma cell line) and normal intestinal epithelial cells. Inflammation. 2003, 27, 123-128.

16.Hebels D.G.A.J., Briede J.J., Khampang R., Kelinjans J.C. S. and Dekok T.M.C.M: Radical mechanisms in Nitrosamine and Nitrosamide- induced whole genome gene expression modulations in CaCo-2 cells. Toxicological sciences.2010, 116 (1): 194-205.

17. Magee P.N. and Barnes JM: Induction of kidney tumors in the rat with diethylnitrosamine. J. Pathol. Bacteriol. 1962, 84: 19-31.

18. Asahina S., Friedman MA., Arnold E., Millar GN., Mishkin M., Bishop Y and Epstein SS: Acute Synergistic toxicity and hepatic necrosis following oral administration of sodium nitrite and secondary amines to mice. Cancer Reg;1971, 31:1201-1205..

19. Tomatis L: Prenatal exposure to chemical carcinogens and its effect on subsequent generations. In: Conference on perinatal carcinogenesis. Washington, DC, National cancer Institute monograph.Traditional fermentation of African oil Bean seed for Ugba production. J. Food microbiol.1977, 3: 18-24

20.Druckery H., Schildbach A., Schmahl D., Preussmann R and Ivankovic S (1963b). Quantitative analysis of the carcinogenic action of diethylnitrosamine. Arzneimittel-forsch, 13: 841-851.

21.Alexandrov VA: Blastomogenic effect of dimethylnitrosamine on pregnant rats and their offsprings. Nature (Lond.),1968, 218: 280-281.

22.Lowry OH., Rosebrough NJ., Farr AL., Randall RJ: Protein measurement with the folin-phenol reagent. J. Biol.Chem.1951, 193: 265-275.

23.Mongomery HAC. and Dymock JF. (1962). The determination of nitrite in water. Analyst,1962, 86: 4141-416.

24.Frei E., Gilberg F., Schroder N., Breuer A., Edler L. and Wiessler M. Analysis of the inhibition of N-nitrosodimethylamine activation in the liver by N-nitrodimethylamine using a new non-linear statistical method. Carcinogenesis.1999, 20: 459-464.

25.World Health Organization: N-nitrosodimethylamine. Concise international chemical assessment document,2002, 38 (CICADS 38). Wold Health organization, Geneva, Switzer-land.

26.Magee P.N and Barnes J.M : Carcinogenic nitrosocompounds. Adv. Cancer Res,1967, 10., 163-246.

27.Yang C.S., Smith T.J., Hong J.Y. and Zhou S : Kinetics and enzymes involved in the metabolism of nitrosamines. In loeppky R,N and Michejda C.J (eds). The Chemistry and Biochemistry of Nitrosamines and Related N-Nitroso compounds. ACS Symposium series 533. ACS Washington. DC,1994, pp169-178.

28.Appel K.E and Graf H : Metabolic nitrite formation from N-nitrosamines. Evidence for a cytochrome P450-dependent reaction. Carcinogenesis, 1982,3: 293-296

29.Yoo J.S.H., Guengerich F.P and Yang C.S : Metabolism of N-nitrosodialkylamines by human liver microsomes. Cancer Res.1988, 48: 1499-1504.
30.Ji C., Mirvish S.S., Nickols J., Ishizaki H., Lee M.J and Yang C.S: Formation of hydroxyl derivatives, aldehydes and nitrite from N- nitrosomethyl-n-amylamine by rat liver microsomes and purified cytochrome P450. Cancer Res.1989, 49, 5299-5304.
31.Appel K.E., Ruhl C.S and Hilderbrandt A.G : Oxidative dealkylation and reductive denitrosation of nitrosomethylamine in vitro. Chem.. Biol. Interact.,1985, 53: 69-76.
32.Chowdhury G., Calcutt M.W and Guengerich F.P: Oxidation of N-Nitroso alkylamines by human cytochrome P450 2A6 sequential oxidation aldehydes and carboxylic acids and analysis of reaction steps. Journal of Biological Chemistry, 2010, 285. 8031-8044
33. Raza H., Prabu S.K., Robin M.A., Avadhani N.G : Elevated mitochondrial cytochrome P450E1 and glutathione-s-transferase A4-4 in streptozotocin-induced diabetic rats. Tissue-specific variations and roles in oxidative stress.2004, 53: 185-194.
34. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 1997;77:517-44.
35.Bai J. and Cederbaum A.I: Overexpression of CYP2E1 in mitochondria sensitizes Hep G2 cells to the toxicity caused by depletion of glutathione. J. Biol Chem, 2006, 281:5128-5136.
36.Robin M.A., Anandatheerth-athavarada H.K., Fang J.K., Cudic M., Otvos L., Avadhani N.G: Mitochondria-targeted cytochrome P450 2E1 (P450 MT5) contains an intact N-terminus and requires mitochondrial specific electron transport proteins for activity. J.Biol.2001, chem. 276: 24680-24689.
37.Sangar M.C Bansal S. and Avadhani N.G : Bimodal targeting of microsomal cytochrome P450s to mitochondria: implications in drug metabolism and toxicity. Expert Opin Drug metab Toxicol, 2010, 6. 1231- 1251.
38.Avadhani N.G., Sangar M.C., Bansal S. and Bajpai P: Bimodal targeting of cytochrome P450s to endoplasmic reticulum and mitochondria: the concept of chimeric signals. FEBS J.2011, 278: 4218-4229.
39.Bhagwat S.V., Boyd M.R and Ravindranath V: Multiple forms of cytochrome P450 and associated monooxygenase activities in human brain mitochondria. Biochem Pharmacol, 2000, 59: 573-582.
40.Bansal S., Liu C.P., Sepuri N.B., Anadatheerth-athavarada H.K., Selvaraj V., Hoek J., Milne G.L., Guengerich F.P and Avadhani N.G: Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol- mediated oxidative stress. J. Biol Chem, 2010, 285: 24609-24619.
41.Sangar M.C., Anandatheerth-athavarada H.K., Tang W., Prabu S.K., Martin M.V., Dostalek M., Guengerich F.P. and Avadhani N.G : Human liver mitochondrial cyt P450 2D6-individual variations and implications in drug metabolism. FEBS J.2006,276:3440-3453.
42.Gomoz D : The absorption and metabolism in rats of small oral closes of dimethyl-nitrosamine. Implication for the possible hazard of NDMA in human food. Biochem. J. 1977, 164:497-500.
43.Hodgson E: A textbook of modern Toxicology. 3rd edition.2004, A John Wiley and sons.Inc. publication, Hoboken, New Jersey.
44. Barnes, J. M., and Magee, P. N: Some Toxic Properties of Dimethylnitrosamine. Brit. J.Ind. Med.,1954, 11: 167-174.
45.Usunomena U., Sunday J.J., Spencer N., Esosa U.S., Kingsley O. and Maduagwu E.N: Toxicity evaluation of the liver and in vitro metabolisms in wistar rats on exposure to N-Nitrosamine precursors. British Journal of Pharmacology and Toxicology,2011, 2 (3): 138-142
46.Loscalzo J., Fauci A.S., Braunwald E., Dennis L.K., Hauser S.L. and Longo D.L: Harrison's Principles of internal medicine. McGraw-Hill Medical.2008, ISBN 9780071466332
47.Swirski F.K., Narendorf M., Etzrodt M., Wildgruber M., Cortez-Retamozo V., Panizzi P., Figueiredo J.L., Kohler R.H., Chudnovskiv A., Waterman P: Identification of splenic reservoir monocytes and their development to inflammatory sites. J. Sci.2009, 325: 612-616.
48. Hard, G.C. and Butler, W.H: Toxicity of dimethylnitrosamine for the rat testis. J. Pathol., 1970,102:201-207.
49.Swann PF and Magee PN (1968) Nitrosamine-induced carcinogenesis. The alklylation of nucleic acids of the rat by N-methyl-N-nitrosourea, dimethylnitrosamine, dimethyl sulphate and methyl methanesulphonate. Biochem J.1968, 110 (1):39–47.
50.Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP, Wink DA:A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann N Y Acad Sci.2002, 962:195–206.
51. Bolzan AD and Bianchi MS. (2002). Genotoxicity of streptozotocin. Mutat Res.2002, 512(2-3):121–134.Chemistry, Vol3 (Mariano PS. Ed). 191-241, JAI Press, Greenwich,CT.
52.Murata M, Takahashi A, Saito I, Kawanishi S: Site-specific DNA methylation and apoptosis: induction by diabetogenic Streptozotocin. Biochem Pharmacol.1999, 57 (8):881–887.
53.Nukatsuka M, Sakurai H, Yoshimura Y, Nishida M, Kawada J. Enhancement by streptozotocin of O2- radical generation by the xanthine oxidase system of pancreatic beta-cells. FEBS Lett.1988, 239(2):295–298.