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Study the Toxic Effect of N-nitroso dimethylamine on Testes and Kidney: Oxidative Stress Damage through Histopathological Analysis

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Abstract
Because the lack of sufficient studies about the effect of NDMA on kidney and testes tissues in addition to the focusing on liver toxicity research, thus this study is performed to evaluate such damage. Forty-five male rats were used in the study as follow; GI: control group, GII: treated with (3mg/kg NDMA) and GIII: treated with (1.5 mg/kg NDMA) intra-peritonially 3 times a week for 3 months. After completion of the study periods, blood sample were collected for biochemical analysis, tissue sample (kidney and testes) also taken for histopathological study. Result reveals a significant *(P<0.05)* increase in serum level of MDA and BUN in groups treated with NDMA in comparison to control. However, there is a significant *(P<0.05)* decrease in serum CAT and GSH in NDMA treated groups when compared to control. Histopathological study of kidney show area of atrophy of glomeruli, vacuolation and tubular epithelial degeneration and necrosis. On the other hands, testes presented with distortion of seminiferous tubules, vacuolation and necrosis of germinal layers along with suppression of spermatogenesis and absence of sperm in NDMA when compared to control groups. In conclusion, NDMA produce oxidative stress; which adversely affect both renal and testicular tissue along with their architecture distortion and impaired function.
Keywords: NDMA; MDA; BUN; Oxidative stress; Histopathology.

Introduction
N-Nitrosamine is a notorious group of chemical substances known for their carcinogenic effect and widespread prevalence in the environment. They arefound in the atmosphere, diet, beverages, pesticides, pharmaceutical and tobacco products (Beard and Swager, 2021). Among these, N-nitrosodimethylamine (NDMA) also referred to as dimethyl-nitrosamine, which is notable for its volatile nature (George *et al*., 2020). The hepatotoxicity of NDMA were firstly described following liver cirrhosis evolution in two cases among industrial workers in the UK, by Barnes and Magee (Sheweita *et al*., 2017).
NDMA is a vigorous carcinogenic agent that induces cancer in various tissues, including the liver, stomach, kidneys, bladder and lung. This carcinogenicity arises from metabolism of NDMA into numerous toxic substances such as methyl-carbocation, diazonium, formaldehyde and methanol (Gao *et al*., 2018), rather than from the initial compound itself (Wang *et al*., 2015). Among these, the methyl-diazonium ion acts as a powerful alkylating agent, causing protein and DNA methylation of hepatocytes. Different doses of NDMA administration associated with hepatic tissue degeneration, necrosis and liver fibrosis (Lim *et al*., 2016).
Researches highlighted that tertiary amine, quaternary amines which are key functional groups in polymers of water treatment and pharmaceutical medication that can serve as sources of NDMA production. This occurs when a reaction occur between chlorine and secondary amines result from polymer degradation (Liu *et al*., 2014; Lv *et al*., 2015). Additionally, NDMA production has been detected in water swimming pool due to anthropogenic substances that released from swimmers such as lotions, urine and sweat. These materials contribute secondary amines, which can subsequently form NDMA (Mustapha *et al*., 2021). Other sources of NDMA exposure include cigarette smoke, pesticides used in agriculture, hospitals and industrial materials such as rubber, dyes and tires (Kim *et al*., 2019). NDMA commonly induces tumors in the liver and lungs, with incidence rates ranging

from 50% to 100%. Other tissues, such as the testes and kidneys, may also develop tumors (Takahashi *et al*., 2000).

Finally, due to the lack of sufficient studies about the effect of NDMA on kidney and testes tissues in addition to the focusing on liver toxicity research, it has become necessary to investigate toxicity of NDMA in renal and testicular tissue.

Material and Methods

Animals

Forty-five male rats, adult with body weight ranging (195-205 gm) were used in this study. All animals maintained for 2 weeks in plastic cage for adaptation in the animal house of Veterinary Medicine College/ Baghdad University according to the local ethical committee, under No (P.G/2198).

Experimental design

The study consist from three groups divided randomly and including, negative control (GI), 2nd group (GII) treated with (3mg/kg NDMA) I.P, 3 times weekly for 3 months (Sultan and Al-Kaisie, 2025). 3rd group (GIII) treated with (1.5 mg/kg NDMA) I.P, 3 times weekly for 3 months (Al-Sabaawy and Al-Kaisie, 2021).

When study period is completed, the animals were sacrificed, blood was assembled for biochemical assay, oxidative stress analysis along with tissue sample for histopathology.

Estimation of oxidative stress

Oxidative stress has been analyzed with measurement of malon-dialdehyde (MDA) serum level using (BT LAB Rat malondialdehyde kit, China), catalase activity (CAT) using (BT LAB Rat Catalase kit, China) and reduced glutathione (GSH) using (BT LAB Rat Glutathione kit, China) by microplate reader 800 TS, BIOTEK (Aghetaa *et al*., 2023; Khalaf and Salih, 2023; ABD ALSAADA and AL-KAISEI, 2024).

Kidney biochemical evaluation

SEAMATY-120V fully automated, veterinary chemistry analyzer was used to measure serum level of urea according to (Adeleke and Adaramoye, 2017).

Histopathology examination

Kidney and testes tissues were preserved in 10% formalin and histopathological assessment with H&E stain done according to (Alkaisie *et al*., 2021; Abdulla and Al-Okaily, 2022; Abbas and Jawad, 2023).

Statistical analysis

The data recorded as Mean ± Standard Deviation (M±SD) and analyzed with SPSS 22.0 version. When (P<0.05), the differences among groups is regarded significant.

Result

1- Oxidative stress measurement

The analysis of oxidative stress represented through measurement of serum MDA, CAT and GSH as in table below;

Table: effect of NDMA on serum MDA, CAT and GSH.

arameters	**DA**	**AT**	**SH**
roup	**ean ± SD**	**ean ± SD**	**ean ± SD**
1, Control	46 ± 0.42 **b**	1.1 ± 10.1 **a**	7.2 ± 16.8 **a**
2, (3mg/kg)	17 ± 0.39 **a**	6.6 ± 6.67 **b**	3.0 ± 3.72 **b**
3, (1.5mg/kg)	00 ± 0.18 **a**	1.6 ± 10.3 **ab**	9.0 ± 22.4 **a**
SD	**433**	**1.33**	**).08**

First: There is a significant (P<0.05) increase in serum level of MDA in 2nd and 3rd group (3.17 and 3.00) respectively, in comparison to control group, with no significant differences between them.

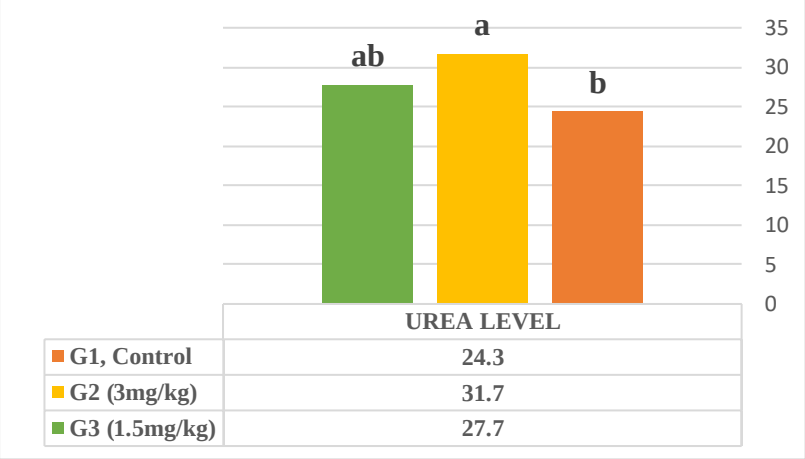
Second: CAT result shows a significant (P<0.05) decrease in serum level of the 2nd group (16.6) when compared to control (31.1) with no significant changes between 2nd and 3rd groups.

Finally: There is a significant (P<0.05) decrease in serum level of GSH in the 2nd group (48.0) when compared to control and 3rd group (87.2 and 69.0) respectively, however the later groups did not show any significant changes between them.

2- BUN analysis

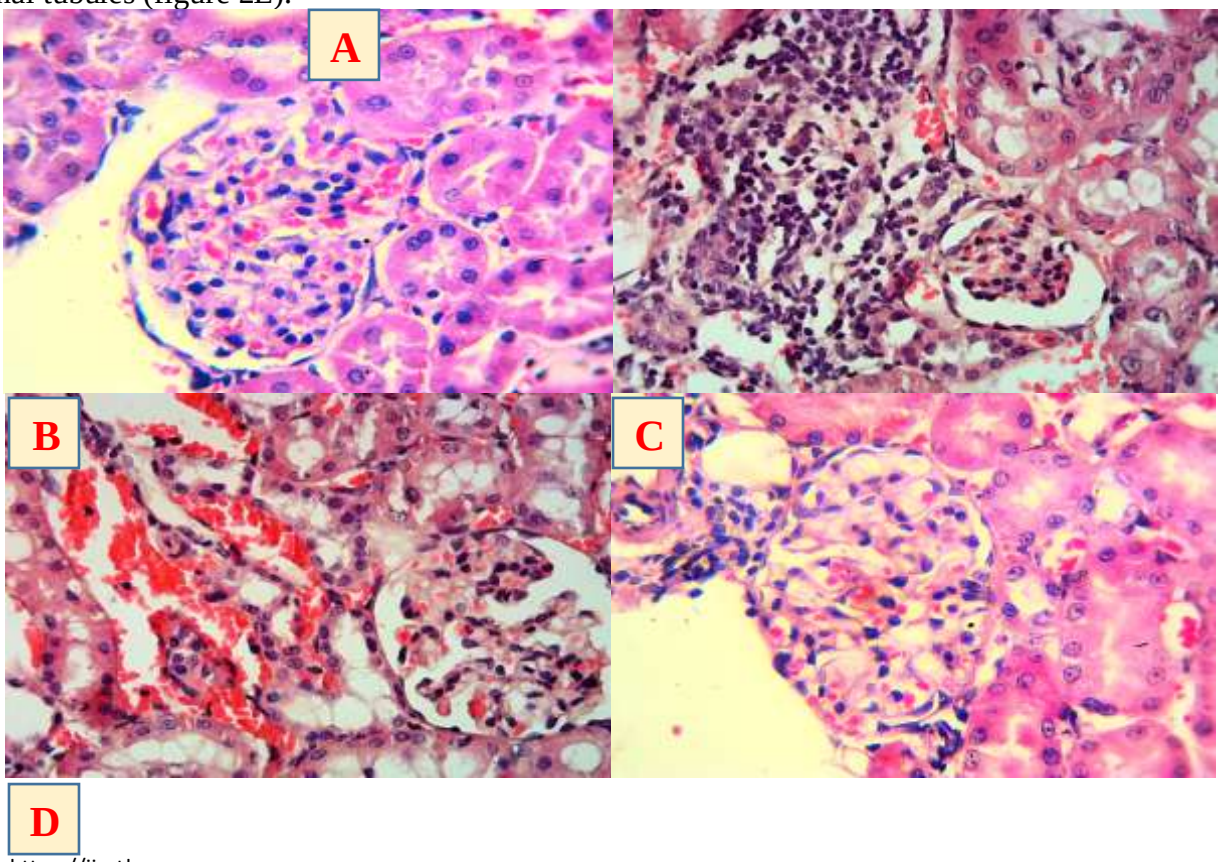
There is a significant (P<0.05) increase in serum urea level in the 2nd group (31.7) as compared to the control (24.3). However, there is no significant differences between 2nd and 3rd group as in figure1.

Figure (1): Effect of NDMA on BUN level.



3- Histopathological evaluation

Kidney histopathology showed normal renal tissue consisting from glomeruli and renal tubules (figure 2A). Renal tissue of the 2nd group treated with NDMA, reveals atrophy of glomeruli along with thicken basement membrane, vacuolation of tubular epithelium and tubular necrosis with heavy infiltration of inflammatory cells (figure 2B). Also noticed dilation of bowman capsule and extensive hemorrhage in the interstitial tissue (figure 2C). However, the 3rd group presented with acute tubular hydrobic degeneration and vacuolation of glomerular epithelium (figure 2D). Furthermore, heavy accumulation of chronic inflammatory cells with area of tubular damage and hemorrhage among renal tubules (figure 2E).



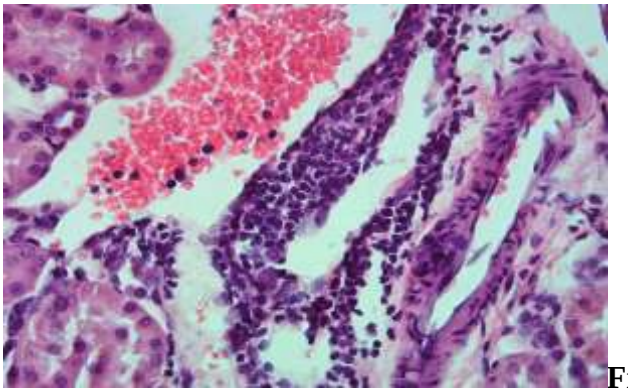
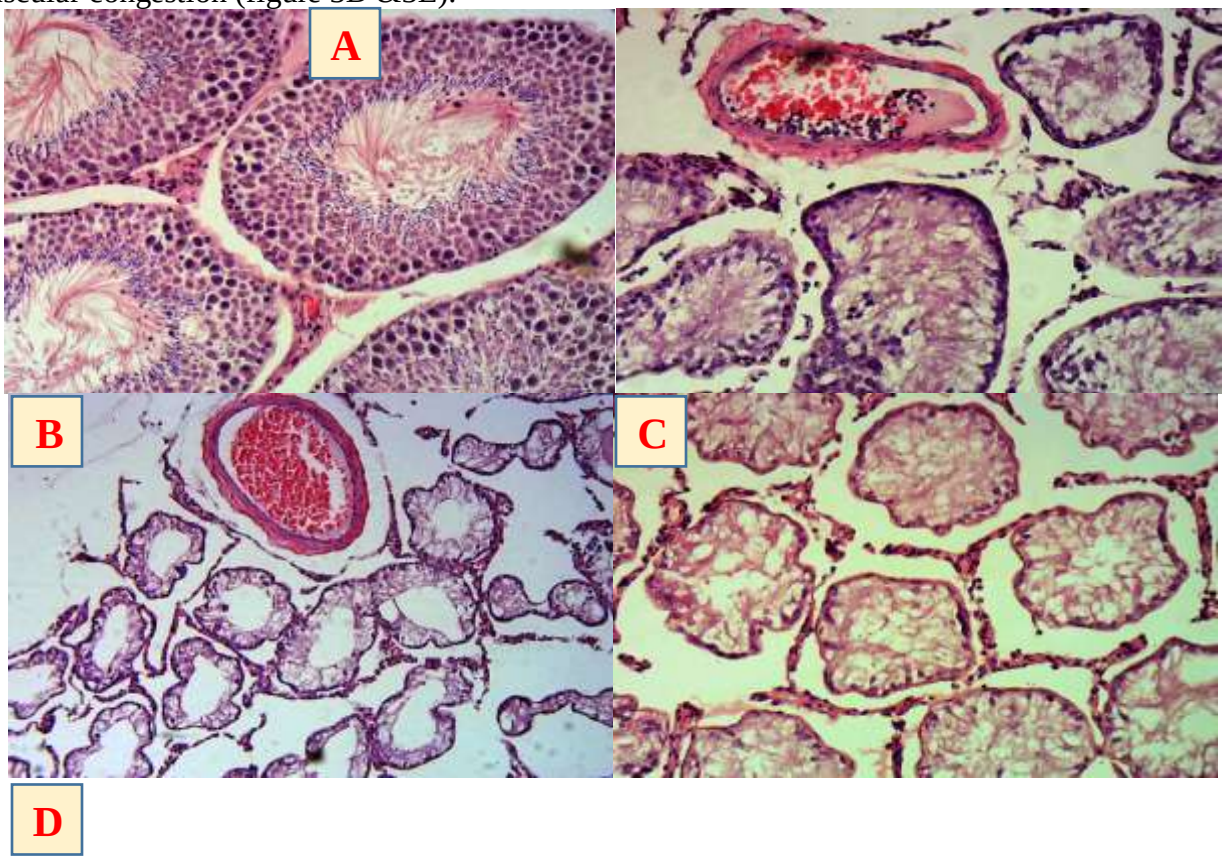


Figure (2): (A) Normal renal glomeruli and tubules of control group. (B) Kidney of 2nd group show glomerular atrophy, vacuolar degeneration of tubular epithelium, tubular necrosis and heavy infiltration of inflammatory cells. (C) Kidney of 2nd group show areas of tubular epithelium swelling and hemorrhage in renal parenchyma. (D) Kidney of 3rd group presented with cellular swelling of renal tubular and glomerular epithelium. (E) Kidney of 3rd group, noticed area of tubular necrosis with heavy infiltration mononuclear cells and interstitial hemorrhage. H&E stain.

Testes of control group presented with normal seminiferous tubules filled with normal sperm and spermatogenesis (figure 3A). Testes of 2nd group showed irregular seminiferous tubules, degeneration and necrosis of germinal epithelium with dead sperm and vascular congestion and inflammatory cell pavementing (figure 3B). Moreover, atrophy of seminiferous tubule, degeneration of interstitial tissue and absence of spermatogenesis (figure 3C). On the other hand, the 3rd group show foamy cytoplasmic appearance, vacuolated and necrotic seminiferous tubules associated with absence of sperm, impaired spermatogenesis, mononuclear cells infiltration in testicular interstitium and vascular congestion (figure 3D &3E).



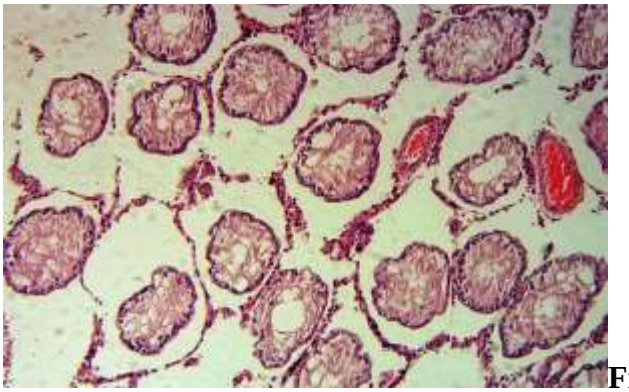


Figure (3): (A) Testes with normal architecture of control group. (B) Testes of 2nd group show vacuolation, necrosis of seminiferous tubular tissue with dead sperm and vascular congestion. (C) Testes 2nd group show seminiferous tubule atrophy, absence of germinal epithelium and spermatogenesis. (D) Testes of 3rd group reveals foamy cytoplasmic structure of seminiferous tubules, vacuolation of germinal epithelium and infiltrated with mononuclear cells in the interstitial tissue. (E) Testes of 3rd group shows tissue destruction and degeneration of seminiferous tubules with empty sperm and vascular congestion.

Discussion

One of the most important characteristic of NDMA is production of oxidative stress either through increase generation of reactive oxygen species or interference with antioxidant enzyme activity, thus generation of oxidative stress and initiating the pathogenesis of their toxic metabolites (Asejeje *et al*., 2023).

Our result reveals a significant increase in serum level of MDA along with a significant decrease in level of serum CAT and GSH in the 2nd and 3rd groups treated with NDMA (Choi *et al*., 2016; Asejeje *et al*., 2023). This elevation of these oxidative stress biomarkers result from the disturbance in the homeostasis between ROS generation and the potential effect of serum antioxidant enzyme; such imbalance associated with distortion of several tissue membrane integrity because of their fatty acid composition. This result is in similarity with (Pan *et al*., 2015; Usunomena and Okolie, 2020), they reported that membrane lipid peroxidation accompanied with elevation of MDA byproduct.

Several research explained the reduction in antioxidant enzymatic activity with NDMA administration, related to the suppression of nuclear factor erythroid 2- related factor 2 (Nrf-2) transcription factor; plays a pivotal role in activating the antioxidant defense system (Hamza *et al*., 2017; El-Shenawy *et al*., 2017).

NDMA found to affect renal tissue and we found that groups treated with nitrosamine suffered from elevation of serum urea level. This result is in line with (Adeleke and Adaramoye, 2017), they reported that serum urea and creatinine increase significantly with NDMA administration. The renal adverse effect obtained possibly associated to the excretion ability of kidney to eliminate toxic metabolite of NDMA; either through affecting glomerular filtration or renal tubular epithelium thus elevation of serum urea concentration. Kidney damage with NDMA result from excessive generation of ROS that react with unsaturated fatty acid membrane and ultimately ending with lipid peroxidation and elevation of renal biomarkers as a sign of kidney toxicity (Jalili *et al*., 2019).

Histopathologically renal tissue experienced damaging effect either on glomeruli, such as atrophy or tubular epithelium vacuolation, degeneration and even tubular cell necrosis; this damage also associated extensive hemorrhage and heavy infiltration of mononuclear cell which reflect the degree of damage experienced by renal tissue. This result is matched with (Ashtiani *et al*., 2015; Adeleke and Adaramoye, 2021), they stated that NDMA cause extensive glomerular damage and renal tubule necrosis with occlusion of tubules with proteinaceous materials resulting from diminished ability of nephron to excrete waste products; further deterioration of kidney function (Verma *et al*., 2017; Somade *et al*., 2021).

Reproductive function is adversely altered with NDMA administration through spermatogenesis suppression and seminiferous tubule damage. Our result reveals irregular seminiferous tubule with

vacuolation of germinal layer and tubular necrosis, such damage is usually related to NDMA. This result in agreement with (Leisegang *et al*., 2017; Salahshoor *et al*., 2022), they reported that NDMA use associated with distortion of cyto-architecture of seminiferous tubules, a decrease in the height and diameter of germinal epithelial layer and impaired spermatogenesis or presence of abnormal sperm. These changes may be related to the reduction in GSH level; a primary factor required for normal sperm development (Kopalli *et al*., 2015). Moreover, interference of ROS with DNA synthesis of sperm, thus disrupting germ cell division (Salahshoor *et al*., 2019). Therefore, studies mentioned that NDMA caused abnormal sperm morphology, motility and sperm count; confirm testicular damage especially seminiferous tubules (Nakung *et al*., 2018; Somade *et al*., 2021).

Conclusion

According to the present finding, NDMA exert oxidative stress condition through generation of excess ROS and impaired antioxidant enzyme activity. Therefore, NDMA produce reproductive and nephrotoxic effect with damaging of histological architecture of both organs.

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