Zinc Oxide Nanoparticles Ameliorates Dimethylnitrosamine Induced Renal Toxicity in Rat

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Abstract

Dimethylnitrosamine (DMN) is an established carcinogen. It is toxic to several organs viz. liver, kidney, lungs and immune system. Several drugs have been used in the past to modulate its toxicity using experimental models. Present study was designed to investigate the effect of zinc oxide nanoparticles (ZnONPs) on renal toxicity caused by DMN in laboratory rat. Since oxidative mechanisms form etiological causing its toxicity, the proposed study focuses on amelioration of oxidative stress expressed by ZnONPs, if any. Present results show that administration of ZnONPs (50 mg/kg) to DMN (2µl/100g body weight) treated rats diminuted the concentration of malonaldehyde, H_2O_2 and NO in the kidney. However, GSH concentration increased after ZnONPs treatment. Results on glutathione S-transferase, glutathione peroxidase favored an antioxidative effect of ZnONPs. These results find support from diminished oxidative DNA damage manifested by ZnONPs in DMN treated rats. Histopathological results on kidney also indicate a protective effect of ZnONPs on renal toxicity of DMN. A comparison of results between control and ZnONPs treated rats suggests that ZnONPs might be toxic to renal tissue. Nevertheless, the study suggests that ZnONPs possess strong therapeutic/antioxidative potential against DMN induced renal toxicity.

Introduction

Dimethylnitrosamine (DMN) is an established carcinogen (IARC, 1978; ATSDR, 1989). It has been reported that preferential site of biotransformation of nitroso compounds is the liver, but kidney, lungs and other organs may also participate in its degradation albeit to minor degree (Lee et al., 1964; Knecht, 1966). Magee and Barnes (1962) for the first time showed that a single dose of DMN could induce renal tumors. Later studies made to decipher the etiology of renal cancer induced by DMN attributed it to the generation of reactive oxygen species (ROS) and resulting oxidative stress (Bansal et al., 2005; Mittal et al., 2006).

Subsequently, efforts were made to protect DMN toxicity in suitable experimental models using several drugs and antioxidants. Hamza et al., (2017) showed therapeutic effects of α-lipoic acid (ALA) against DMN induced renal toxicity in rats. Rana and Kumar (2000) showed that cadmium and zinc induced metallothionein inhibits lipid peroxidation (LPO) in the kidney of DMN treated rats. Metallothionein is involved in the reduction of hydroxyl radicals (OH) and the sequestration of ROS produced under stress conditions (Ruz and Carrasco, 2013; Chasapis and Loutsidou, 2012). Furthermore, zinc plays an important role as a transcription factor and antioxidant defense system in prevention of DMN toxicity. Zinc channels create a balance between cell survival and cell death via controlling the free and intracellular zinc movements (Dhawan and Chadha, 2010). Therefore zinc has been considered as a suitable agent to prevent toxicity of several xenobiotics i.e. carbon tetrachloride (Rana and Tayal, 1981; Saldeen, 1969), ethyl alcohol (Yunice and Lindeman, 1977) and DDT (Feaster et al., 1972).

Recent advent of nanomedicine employs nanotechnology in the treatment and diagnosis of several diseases. In this context, several nanoparticles have been synthesized and tested for toxicity and health aspects (Oberdorster et al., 2007; Hulla et al., 2015). Zinc oxide nanoparticles (ZnONPs) amongst them have potent therapeutic potential since they express bioavailability, biocompatibility and high solubility. They possess the capacity to regulate cell cycle and cellular homeostasis (Vizirianakis, 2017). Recently, Food and Drug Administration (FDA) has approved zinc oxide nanoparticles for anticancer therapy (Shen et al., 2013). They

can produce selective toxicity towards cancer cells causing disequilibrium of zinc dependent protein activity (Vinderall and Mitjans, 2015). Rasmussan et. al. (2010) hypothesized that ZnONPs can kill cancer cells through the induction of oxidative stress in the cancerous cells. Thus ZnONPs emerged as nanotheranostic platform against several diseases specially those caused by oxidative stress. Nonetheless, several laboratories have published reports dealing with toxic effects of ZnONPs in specific organs and cell lines (Li et al., 2012; Fazilah, 2013; Soheili et al., 2013; Esmaeillou et al., 2013).

Therefore, there appears to be sufficient reason to examine further the antioxidative effects of ZnONPs manifested in a suitable experimental model and conditions. With this perspective, a study on the protective effects of ZnONPs against DMN induced hepatotoxicity in rat was recently made in our laboratory (Rani et al., 2018). Present study was made with an objective to assess the protective effects of ZnONPs, if any, on DMN induced renal toxicity in rat. Further, renal toxicity of ZnONPs, if any, has also been assessed.

Materials And Methods

Chemicals and reagents

Zinc oxide nanoparticles were procured from Sigma Chemical Co. Missouri (USA). According to the manufacturer, nanoparticles contained approximately 80% zinc basis, 100% purity and < 100 nm size with a surface area 15–25^2/g.

Dimethylnitrosamine, thiobarbituric acid, 5'-5'-dithiobis-2-nitrobenzoic acid, 1–2'-4' dinitrobenzene, glutathione reductase, glutathione and N-(1-Naphthyl) ethylene- diamine dihydrochloride (NEDA) were also purchased from Sigma Chemical Co. (USA). All other reagents of highest purity were obtained from High Media (Mumbai).

Characterization of zinc oxide nanoparticles

ZnONPs were characterized using a battery of methods as described earlier (Rani et al., 2018). Briefly, the size and shape of ZnONPs were observed through transmission electron microscope at Sophisticated Analytical Instrument Centre, Punjab University, Chandigarh (India). Scanning electron microscopic observations and energy dispersive X-ray analysis (EDAX) were made at Department of Physics, Choudhary Charan Singh University, Meerut (India). Analysis of size, distribution, zeta potential and XRD analysis of ZnONPs were performed at Indian Institute of Technology, Roorkee (India).

Maintenance of animals and experimental protocol

Prior approval of Institutional Animal Ethical Committee was sought to make present investigations. Experiments were conducted on male Wistar rats (150 ± 25 g), procured from the animal facility of Jamia Hamdard, Delhi. They were maintained under standard laboratory conditions (room temperature 25 ± 5°C), relative humidity (50 ± 10%) and a 12 h dark/light cycle. Each rat was individually housed in polypropylene cage, offered food pellets (Golden Feeds, Delhi) and tap water *ad libitum*.

After acclimatization to laboratory conditions for two weeks, rats were separated into four groups, each containing five rats. Rats of group A were injected DMN (2µl/100g body weight) in saline intraperitoneally (ip)

on each alternate day for 15 days as described earlier (Rani et al., 2018). Rats of group B were treated as the rats of group A and subsequently administered a pre determined NOEL of ZnONPs (50 mg/kg) on each alternate day for 30 days. Rats of group C were treated with ZnONPs only as the rats of group B. Rats of group D were injected (ip) saline (2µl/100g body weight) only on each alternate day for 45 days and treated as controls.

After 45 days, rats were starved overnight and their urine samples were collected next morning through metabolic cages. Thereafter, rats were sacrificed by light ether anesthesia. Kidneys were carefully removed and processed for the estimation of reactive species viz. malondialdehyde, nitric oxide and hydrogen peroxide. Oxidative stress was determined through standard parameters viz. reduced glutathione (GSH), glutathione S-transferase and glutathione peroxidase as described below.

Kidney function test

Creatinine in the urine samples was determined following the method of Toro and Ackerman (1975), using a commercial kit procured from M/S Span Diagnostics, Surat (Gujarat, India).

Parameters of oxidative stress

Malondialdehyde (MDA)

MDA in the kidney samples was determined using thiobarbituric acid following the method of Jordan and Schenkman, (1982). Absorbance was recorded at 532 nm using a spectrophotometer (Systronics, India). 1, 1, 3', 3', tetramethoxypropane (Sigma) was used as the standard. Protein contents in the kidney samples were determined following the method of Lowry et al. (1951). Bovine serum albumin (Sigma) was used as the standard.

Hydrogen peroxide (H_2O_2)

H_2O_2 in the kidney homogenates prepared in 0.25 M sucrose was measured employing ferrithiocyanate method as described by Thurman et al. (1972). Absorbance was recorded at 480nm using a spectrophotometer (Systronics, India).

Nitric oxide (NO)

NO in the kidney samples was estimated through Greiss reagent following the method suggested by Cortas and Wakid (1990). Absorbance was recorded at 550 nm using a spectrophotometer (Systronics, India).

Reduced glutathione (GSH)

Ellman's reagent was used to determine reduced glutathione in kidney samples (Ellman, 1959). Absorbance was recorded at 412nm using a spectrophotometer (Systronics, India).

Glutathione S-transferase

Glutathione S-transferase was assayed using 1-choloro-2', 4' dinitrobenzene (CDNB) that conjugated with glutathione. Absorbance was recorded at 340nm (Habig et al. 1972).

Glutathione peroxidase

The enzyme was assayed following the method of Paglia and Valentine (1967). Glutathione di sulphide (GSSG) produced as a result of glutathione peroxidase is reduced by an excess of glutathione reductase. Conversion of GSSG to GSH was monitored at 340nm using a spectrophotometer (Systronics, India).

Metallothionein

Metallothionein in kidney samples was analyzed following the cadmium saturation method (Onaska et al., 1978), using atomic absorption spectrophotometer (EC, Hyderabad, India).

8-hydroxy-2' -deoxyguanosine (8-OHdG)

Urine sample of each rat was collected in a sterilized vial through metabolic cage. These samples were stored at -80□c till further analysis. Competitive ELISA technique was used for the estimation of 8-OHdG (biomarker of oxidative DNA damage) using a commercial kit procured from Bioassay Technology Laboratory (China). Absorbance was recorded at 450nm using a microplate reader (EC, Hyderabad, India).

Histopathology

Small pieces of kidney were fixed in 10% neutral formaldehyde, dehydrated and embedded in paraffin. 5 µm thick sections were stained with haematoxylin and eosin and examined under a research microscope (Nikon, Japan).

Statistical analysis

Students' "t" test was employed to make intergroup comparisons amongst different groups. Differences amongst groups with p value < 0.05 were considered significant. SPSS software version 2.0 was used for inter group comparison.

Results

Characterization of ZnONPs

Shape, size, structure and electric composition of ZnONPs was determined applying standard methods. Results show that average diameter of ZnONPs was < 100nm (Fig. 1A). SEM observations showed that the nanoparticles agglomerate (Fig. 1B). Electrical composition of the ZnONPs was determined through EDAX. The XRD pattern of ZnONPs showed a hexagonal structure when compared with the standard data (JSPDS: 00-001-1136) published elsewhere (Rani et al. 2018). Zeta potential of the nanoparticles was recorded to be 18.9 mV (Fig. 2). Intensity-weighed particle size distribution of ZnONPs has been shown in Fig. 3.

Renal function

Higher concentration of creatinine showed renal impairment in DMN treated rats. Subsequent treatment of DMN treated rats with ZnONPs improved renal function. ZnONPs treated rats showed higher values for creatinine than control rats. (Table-1)

MDA, H_2O_2 and NO

Lipid peroxidation is considered to be an important mechanism involved in DMN toxicity. Malondialdehyde, the product of LPO increased in the kidney of DMN treated rats. Administration of ZnONPs to DMN treated rats diminuted malondialdehyde concentration in renal tissue. However, its concentration was higher in the kidney of ZnONPs treated rats than control rats (table-1). Higher values for NO in the renal tissue of DMN treated rats supported the results on malondialdehyde. ZnONPs therapy offered to DMN treated rats decreased NO concentration in the kidney. A comparison of NO values obtained in the kidney of ZnONPs and control rats showed higher values though insignificant in the kidney of ZnONPs treated rats (table-1). Results on hydrogen peroxide also showed higher values in the kidney of DMN treated rats. Average values of H_2O_2 in the kidney of DMN + ZnONPs treated rats were lower than DMN treated rats (table-1). Taken together, all these results suggest an antiperoxidative and antinitrosative role of ZnONPs.

GSH

In the kidney of DMN treated rats, a significant decline in GSH values was observed. GSH status improved after administration of ZnONPs to DMN treated rats. These observations also show that ZnONPs offer antioxidative protection against DMN induced renal toxicity. Treatment of rats with ZnONPs improved renal concentration of GSH. (table-1)

Glutathione S-transferase and glutathione peroxidase

Results on GSH were supported by the observations on glutathione-S-transferase. Enzyme activity decreased in the kidney of DMN treated rats. Supplementation of ZnONPs to DMN treated rats restored its activity near to control values (table-1). Glutathione peroxidase activity also decreased in the kidney of DMN treated rats. However, it increased in the kidney of DMN and ZnONPs treated rats (table-1).

Oxidative DNA damage

Present results showed greater oxidative DNA damage in the kidney of DMN treated rats as measured by 8-OHdG. ZnONPs supplementation to DMN treated rats inhibited this damage (% inhibition) to some extent. However, treatment with ZnONPs alone also induced DNA damage in kidney of rats (Fig. 4).

Metallothionein

Results on renal metallothionein (MT) concentration suggest that induction of MT decreased in the kidney of DMN treated rats. However % increase in MT was recorded in the kidney of DMN and ZnONPs treated rats. ZnONPs, alone were also found to be a potent inducer of MT in rat kidney (table 1).

Histopathology

In addition to glomerulonephritis and proximal tubular necrosis, adenocarcinoma formation in subcapsular cortex was recorded in the kidney of DMN treated rats. Epithelial degeneration was conspicuous in proximal as well as distal tubules. Nuclei of different shapes and sizes were noticed throughout cortex and medulla (Fig. 5A, B, C).

The histopathological observations in the kidney of DMN and ZnONPs treated rats indicated less severe glomerulonephritis and less pronounced tubular necrosis. Adenocarcinoma was wanting. However, neoplastic tissue formation was witnessed at few locations in the proximal cortex. Tubular epithelium was found to be intact. Nuclear changes were insignificant. (Fig. 5D, 6A).

Histopathological observations in the kidney of ZnONPs treated rats showed no nephritis. Proximal and distal tubules were well formed showing no sign of epithelial degeneration. Brush border was found to be intact. However, increased mitotic activity was noticed in distal cortex. (Fig. 6B, C)

All the pathological changes described above were wanting in the kidney of control rats. Renal tubular cortex and medulla exhibited no sign of injury. Normal nuclei in cortex as well as medulla were observed (Fig. 6D). Detailed observations are summarized in table-2.

Discussion

Present study demonstrates that DMN is equally injurious to kidney as it is to the liver and lungs. Mechanism of its toxicity has been discussed by a few workers in the past. It has been established now that dimethylnitrosamine and other nitroso compounds are preferentially metabolized in the liver, however, kidney also participates in their biodegradation. DMN is metabolized by CYP2E1 which hydroxylates one methyl group. The resulting hydroxylmethyl nitrosoamine is unstable and decomposes to formaldehyde which methylates DNA and protein or reacts with water to form methanol (Guengerich et al., 1991; Frei et al., 2001). The formation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) contributes in oxidative stress which might be one of the key factors in the induction of pathological changes, carcinogenicity, neoplastic changes and tumor formation not only in the liver but kidney and lungs also (Pradeep et al., 2007; Wills et al., 2006).

Restoration of renal function remains a challenging issue in experimental toxic renal injury. Since ZnONPs were found to be protective against DMN induced hepatic injury in rats (Rani et al., 2018), similar study on kidney was considered essential to prove the therapeutic potential of ZnONPs. Very first indication of a beneficial effect of ZnONPs against DMN toxicity was exhibited by observations on creatinine. It was elevated in the urine samples of DMN treated rats but decreased in DMN and ZnONPs treated rats. ZnONPs treatment alone also increased creatinine concentration. Elevated urinary/serum creatinine is a reliable biomarker of renal function (Bennett, 1996). It is associated with abnormal glomerular function (Bishop et al., 2005). Ali Noori et al., (2014) also reported that treatment of Balb/c mice with ZnONPs (50–300 mg/kg) increased serum creatinine concentration. They correlated it with glomerular and tubular degeneration. During present study also we found a correlation between creatinine concentration and renal morphological changes. Improved renal glomerular and tubular morphology in DMN and ZnONPs treated rats corresponded with a

decline in urine creatinine concentration. However, ZnONPs at present concentration and dose regimen exhibited moderate renal toxicity.

Several studies have documented that metabolism of DMN generates ROS in the liver of experimental animals that lead to oxidative stress (Guengerich et al., 1996.). However, very few workers have shown that ROS are responsible for its renal toxicity also (Usunomena et al., 2012). Present results confirm that DMN could induce LPO in the kidney as well. Subsequent treatment with ZnONPs inhibited the generation of ROS. Dawei et al., (2009) postulated that zinc oxide nanoparticles possess the ability to decrease malondialdehyde and increase the activity of antioxidant enzymes. Contrarily, LPO observed as malondialdehyde, increased in the kidney of ZnONPs treated rats also. Findings of other experiments conducted on the toxicity of ZnONPs have also revealed that it elevated MDA concentration in zebra fish (Zhao et al., 2013); human epidermal cells (Sharma et al., 2009) and human liver (Sharma et al., 2012).

Nitric oxides, in the kidney of DMN treated rats also showed elevated values. It declined in the kidney of DMN and ZnONPs treated rats. Earlier studies show that nitric oxide donors like $NaNO_2$ partially prevented the chronic hepatitis induced by dimethylnitrosamine (Lukivskaya et al., 2004). ZnONPs might have affected DMN induced renal toxicity by modulating NO synthase. Nitric oxide synthase inhibitors like N (ω) nitro. L- arginine (L-NNA) might attenuate the protective effects over DMN toxicity expressed by nitric oxide donors (Fukawa et al., 2017).

H_2O_2 is a major metabolic product of DMN (Pradeep et al., 2007). Elevated values were registered for H_2O_2 in the kidney of DMN treated rats. However, a decline was recorded in DMN and ZnONPs treated rats. This observation suggests that ZnONPs influence the metabolism of DMN. This influence might be at the level of CYP2E1. However, further studies are needed to confirm this presumption.

Significant increase in renal concentration of MDA, H_2O_2 and NO reciprocated with significant depression of GSH in kidney of DMN treated rats. Subsequent administration of ZnONPs to DMN treated rats restored GSH status in the kidney. ZnONPs treatment to normal rats also elevated GSH level. GSH, a non enzymatic antioxidant is known to counteract damaging effects of ROS (Rana, 1997; Rana, 2001; Dorval and Hontela, 2003). ZnONPs express antioxidant effect which may be attributed to its anti- inflammatory potential mediated by down regulation of iMOS, cyclo-oxygenase-2 and various cytokines (Nagajyothi et al., 2015). Other workers attribute the beneficial effects of ZnONPs to metallothionein (Mo et al., 2014; Jing et al., 2015). In an earlier study, Rana and Kumar (2000) showed that metallothionein protects against DMN toxicity. According to Durnam and Palmiter (1981), there appears to be strong possibility that upon release, zinc acts as a compensatory messenger of oxidative stress, stimulating a factor in the enhancer region of MT gene. Enhanced transcription of these genes could explain the elevated levels of Zn-MT in oxidant stressed cells. Genes for MT and GSH determine the protection by MT inducers (Garg and Hart, 1997).

Present results show that DMN inhibits MT in kidney as compared to its concentration in normal rat kidney. MT concentration increased in the kidney of DMN and ZnONPs treated rats. Administration of ZnONPs alone significantly increased MT concentration in renal tissue. These results suggest that ZnONPs too, are strong inducers of MT. Earlier reports show that zinc is the potential inducer of MT (Rana and Kumar, 2000; Maret,

2000). MT exchanges zinc relatively quickly in intramolecular and intermolecular reactions with other zinc/sulfur clusters despite relatively high thermodynamic stability (Maret et al., 1997).

DMN is known to affect glutathione S-transferase (GST) activity in the liver (Aniya and Anders, 1985; Armato et al., 1993; Sheweita et al., 2008). However, its effects on renal glutathione S-transferases are not known. Present investigations showed that DMN increased the expression and stimulated the activity of GST in kidney. Aniya and Anders (1985) reported that DMN administration decreased hepatic GST but increased it in serum. This elevation is accompanied by increase in serum GPT (SGPT) activity and serum bilirubin concentrations. Previous study from our laboratory has also confirmed elevation of serum transaminases in DMN treated rats (Rani et al., 2018). Treatment of rats with ZnONPs to normal rats increased GST activity in the kidney but decreased it in the kidney of DMN and ZnONPs treated rats. However, no increase in renal GSH concentration was recorded. GST and GSH play an important role in the detoxification of mutagens and carcinogens (Sheweita and Tilmisany, 2003). Further, GST can reduce covalent binding of epoxides of carcinogens like DMN (Gopalan et al., 1992).

Many workers agree that protective effects of ZnONPs against chemically induced damage in liver/ kidney are manifested through its anti oxidative potential and prevention of ROS mediated mutagenicity and carcinogenicity (Taccola et al., 2011). DMN treatment to rats affects an array of antioxidant enzymes viz: superoxide dismutase, catalase and glutathione peroxidase. Post treatment of ZnONPs to DMN treated rats increased glutathione peroxidase activity in comparison to control rats indicating its enhanced capacity to scavenge H_2O_2 and lipid hydroperoxides (Zhao et al., 2013). Morphological improvement in the kidney of DMN treated rats, manifested by ZnONPs, supported above observations. Zak et al. (1960) and Magee and Barnes (1962) confirmed that DMN could induce renal tumors in rats. Hard and Butler (1971) studied the morphogenesis of epithelial neoplasms induced in rat kidney by DMN. Riopelle and Jasmine (1969) further classified renal tumors induced by DMN. They named them as dysplastic epithelial islands. However, subsequent administration of ZnONPs abolished these tumors and suppressed other morphological lesions. Functional changes, diminished oxidative stress and improvement in antioxidative enzymes, significantly contribute to morphological repair in the kidney.

Most of the observations discussed above favour protective/ anti oxidative/ anti carcinogenic potential of ZnONPs. Several reports do describe toxicity of ZnONPs. One of the critical features of ZnONPs is their selective toxicity towards cancerous cells in comparison to normal cells (Premanathan et al., 2011). ZnONPs express cytotoxicity owing to their specific composition and surface properties. ZnONPs are chemically more active, lead to spontaneous formation of ROS at their surface and cause oxidative stress (Yang et al., 2009). Formation of ROS contributes to cellular toxicity and the release of Zn^{+} ions from the ZnONPs due to their instability in the acidic compartment of lysosomes. Yu et al., (2009) and Fukui et al. (2012) also concluded that ZnONPs toxicity arise from Zn^{2+} ions released from ZnONPs *in vitro* and *in vivo*. Wiseman et al. (2006, 2007) revealed that excess free Zn^{2+} (dissolved from ZnONPs) resulted in depletion of sulfhydryl groups in metallothionien and reduction of mitochondrial function leading to apoptotic or necrotic cell death. In principle, ZnONPs toxicity may be manifested through several mechanisms viz. oxidative stress, inhibition of anti oxidative enzymes, mitochondrial dysfunction and apoptosis. Interestingly, type of the cell system treated

with ZnONPs, the strength of oxidative stress and existing intercellular/intracellular environment are important factors that will determine ZnONPs toxicity.

Conclusion

In conclusion, present study suggests that ZnONPs possess the potential therapeutic efficiency to scavange ROS, induce GSH and dependent enzymes, stimulate metallothionein synthesis and reduce oxidative DNA damage. These mechanisms being interdependent enforce a protective action against DMN induced renal toxicity. Nevertheless, ZnONPs were found to be moderately toxic to kidney. Dose regimen is an important factor in its protective effects.

Abbreviations

DMN (dimethylnitrosamine), ZnONPs (zinc oxide nanoparticles), NEDA (N-(1-Naphthyl)ethylenediamine dihydrochloride), ip(intraperitoneally), Zn-MT (zinc metallothionein), H_2O_2 (Hydrogen peroxide), NO (nitric oxide), MDA (malondialdehyde), GSH (reduced glutathione), ROS (reactive oxygen species), CDNB (1-choloro-2’, 4’ dinitrobenzene), 8-OHdG (8-hydroxy-2' -deoxyguanosine), AD (adenocarcinoma), CO (cortex), ND (nuclear degeneration), BR (brush border), ED (epithelial damage/degeneration), GL (glomerulus), MA (mitotic activity), NPL (neoplasm), NPR (nuclear proliferation),BC (binucleated cells), EP (epithelial linning), PCT (proximal convoluted tubule), GL (glomeruli),TEM (transmission electron microscope), SEM (scanning electron microscope), XRD (X-ray diffraction), JSPDS (Joint committee on powder diffraction standards), EDAX (Energy Dispersive X- Ray)

Declarations

Conflicts of interest

The authors have no conflict of interest.

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Authors contribution

SVSR and YV conceived and designed research. YV, VR conducted experiments and analyzed data. SVSR and YV wrote the manuscript. All authors read, critically evaluated and approved the manuscript for publication. The authors declare that all data were generated in- house and that no paper mill was used.

Availability of data and materials

All data generated and analyzed during this study are included in this article.

Conflict of interest- Authors declare that they have no any conflict of interest.

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Consent to participate

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Tables

Table-1: Protective effect of ZnONPs on oxidative stress in kidney of DMN treated rat

S. N.	Parameters	DMN	DMN+ZnONPs	ZnONPs	Control
1	Renosomatic index	1.183±0.055*	1.023±0.016*	0.973±0.020*	0.916±0.029
2	Creatinine (g/l)	2.072±0.025*	1.108±0.042*	0.962±0.069*	0.498±0.057
3	MDA(n moles/mg protein)	1.006±0.032*	0.738±0.059*	0.722±0.080*	0.488±0.025
4	NO(µm/g wet kidney)	2.236±0.294*	1.046±0.012^{NS}	1.114±0.005*	1.106±0.014
5	H_2O_2(µm/g wet kidney)	1.452±0.057*	1.386±0.098*	1.074±0.023*	1.226±0.076
6	Metallothionein(µg MT/g wet kidney)	0.384±0.033*	0.548±0.076*	0.616±0.059*	0.412±0.026
7	GSH(µg/g wet kidney)	0.104±0.005*	0.124±0.011*	0.153±0.019*	0.125±0.009
8	Glutathione-S-transferase (n moles/min/mg protein)	3.386±0.552*	2.938±0.863*	2.75±0.680*	1.932±0.553
9	Glutathione peroxidase (n moles/NADPH/min/mg/protein)	8.876±5.812*	2.666±1.018*	4.654±1.584*	5.58±1.471

Results are expressed as mean± SE (n=5), where n is number of observations**. *-** denotes values significantly different from controls ($p<0.05$). NS– denotes Non-significant values.

Table-2: Summary of histopathological observations in the kidney of DMN and DMN+ZnONPs treated rat

Histopathological lesions:	DMN	DMN+ZnONPs	ZnONPs	Control
Proximal convoluted tubule				
Necrosis	++	-	-	-
Degeneration	++	-	-	-
Regeneration	-	-	-	-
Presence of cellular debris in the lumen	+	-	+	-
Hydropic cells	-	-	-	-
Lipid droplets	+	-	-	-
Interstitial cells	+	-	+	-
Tubular protuberance	+	+	+	-
Tubular proliferation	+	+	-	-
Loss of brush border	++	+	+	-
Adenocarcinoma/Neoplasia/Hyperplasia	+	+	-	-
Glomerulus				
Glomerulonephritis	+	+	+	-
Presence of leukocytes	-	+	-	-
Presence of erythrocytes	+	+	-	+
Presence of macrophages	+	+	+	-
Glomerular enlargement	+	-	-	-
Glomerular shrinkage	+	-	+	-
Presence of balloon cells	+	-	-	-
Inflammation of glomerular endothelium	-	-	-	-
Hyaline glomerulopathy	-	-	-	-
Medulla				
Necrosis	+	-	-	-
Sclerosis	-	-	-	-
pycnosis	+	-	+	-
Nuclear changes				
Binucleated cells in cortex	+	-	+	+

Binucleated cells in medulla	+	-	+	+
Intranuclear inclusions	-	-	-	-
Pycnosis	+	+	+	-
Karyolysis /karyorrhesis	+	-	+/-	-
Increase in nuclear volume	-	-	-	-
Decrease in nuclear volume	+	-	+	-
Changed nuclear outline	+	-	+	-
Prominent nuclei	+	+	-	-

+ = Present, - = Absent.

Figures

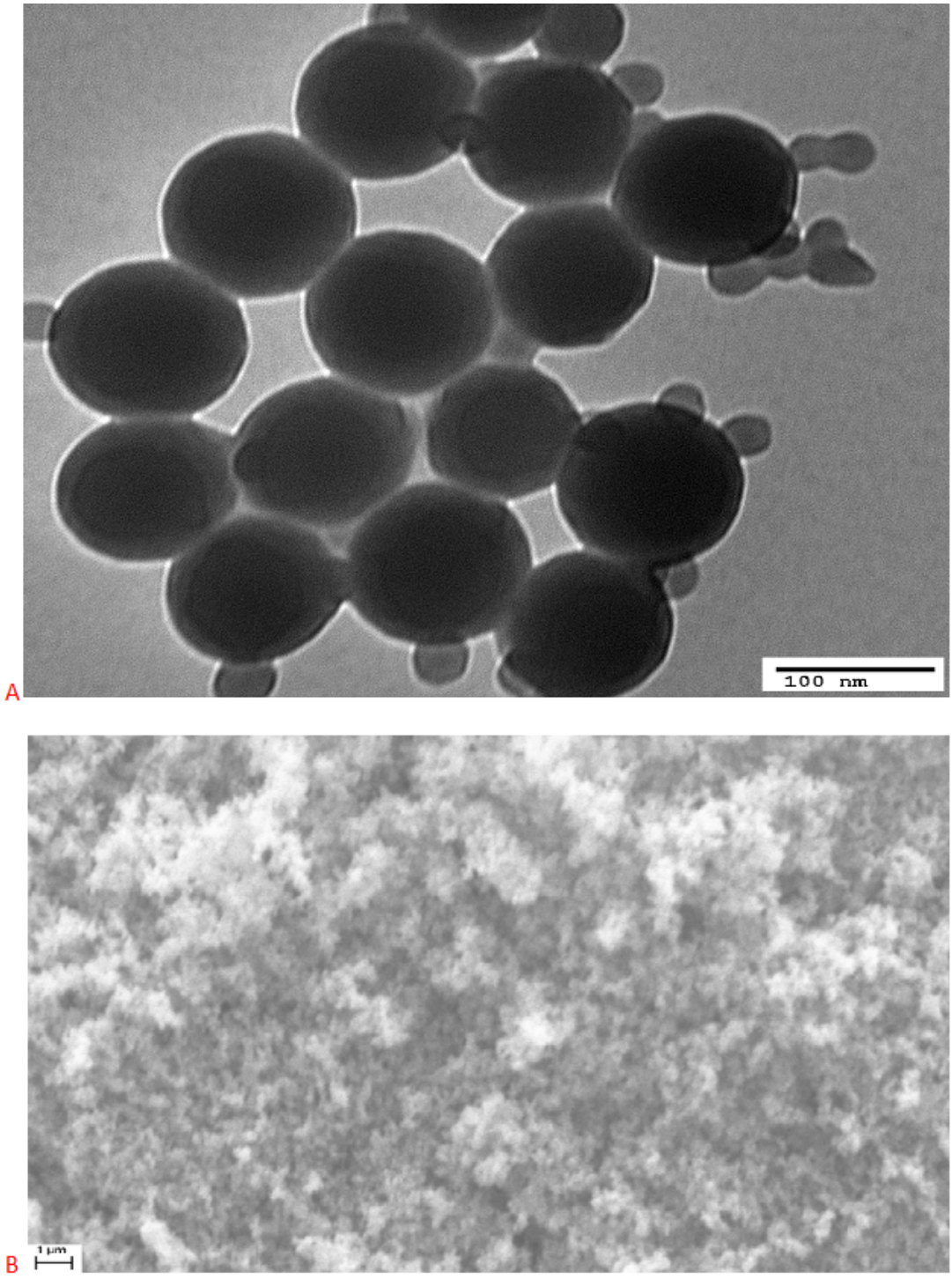

Figure 1

(A) Transmission electron microscopic image of ZnONPs, (B) Scanning Electron microscopic image of ZnONPs

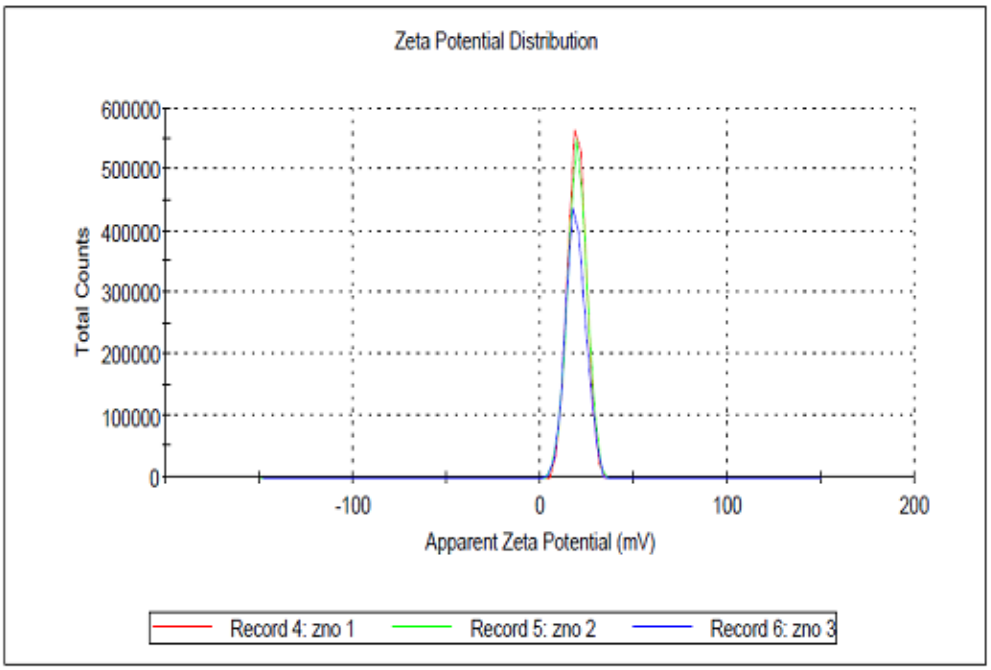

Figure 2

Zeta potential of ZnONPs

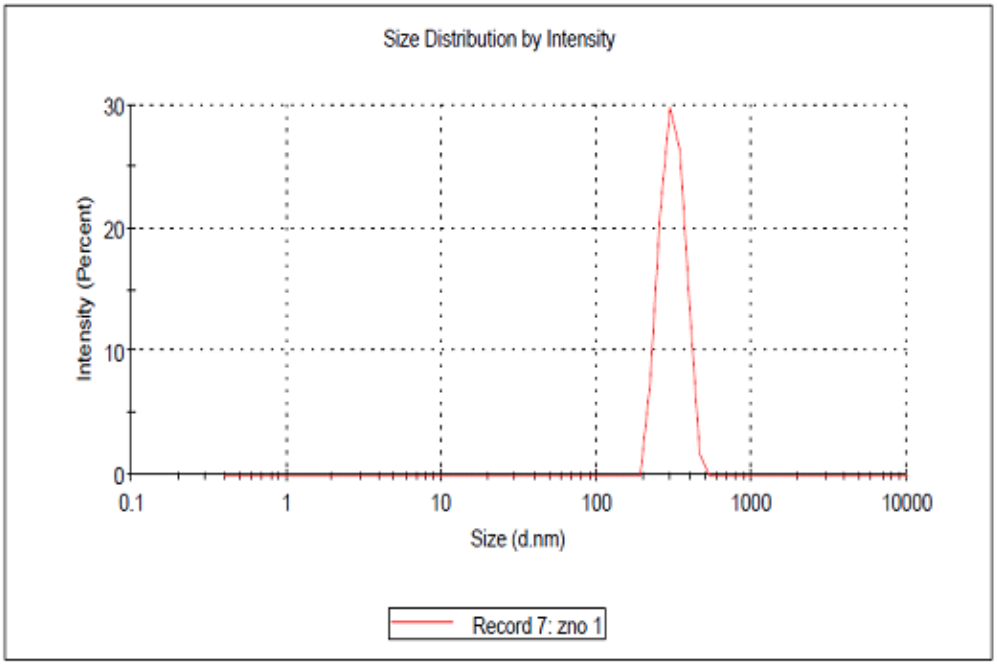

Figure 3

Size distribution (by intensity) of ZnONPs

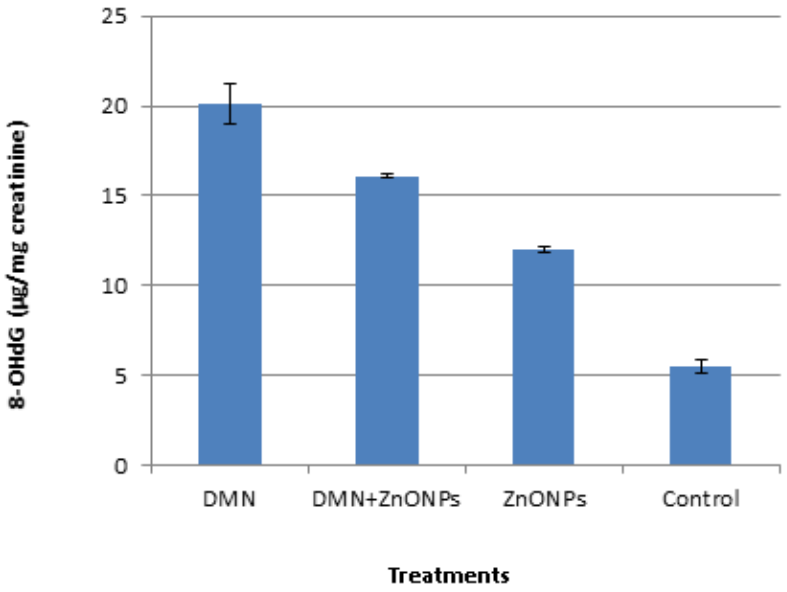

Figure 4

8OHdG in the urine samples of treated and control rats.

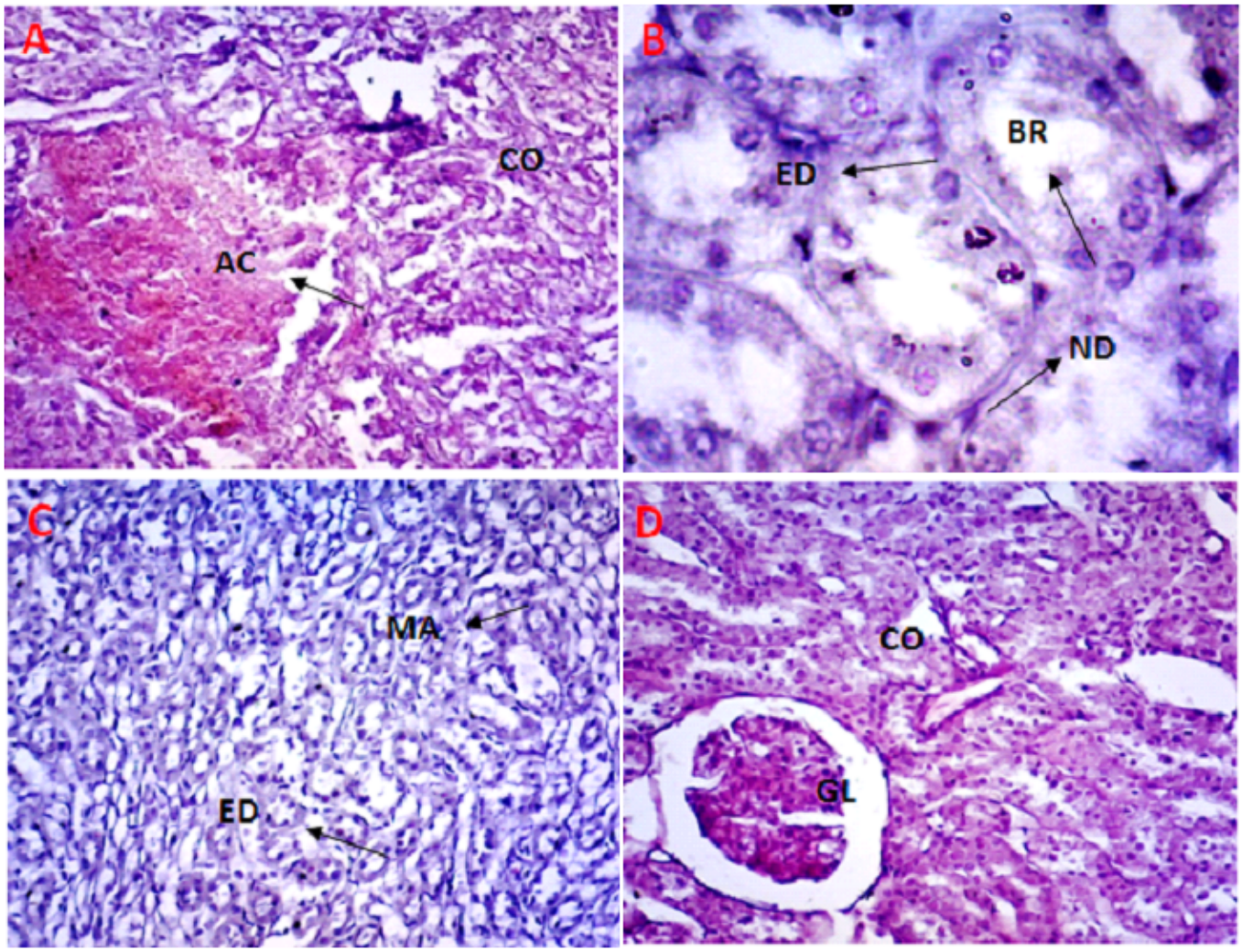

Figure 5

Histopathological observations. (A) Kidney of DMN treated rat shows the presence of adenocarcinoma (AC) in the subcapsular region of the cortex (CO). X100. (B) T.S. of a kidney of the DMN treated rat through proximal cortex shows nuclear degeneration (ND), loss of brush border (BR) and epithelial damage (ED). X400. (C) T.S. of a DMN treated rat kidney through medullary region shows epithelial degeneration (ED) and increased mitotic activity. X100. (D) The kidney of DMN+ZnONP treated rat shows well organized cortex (CO) and glomerulus (GL) Tubular necrosis is of low grade. Nuclear changes are not prominent. X100.

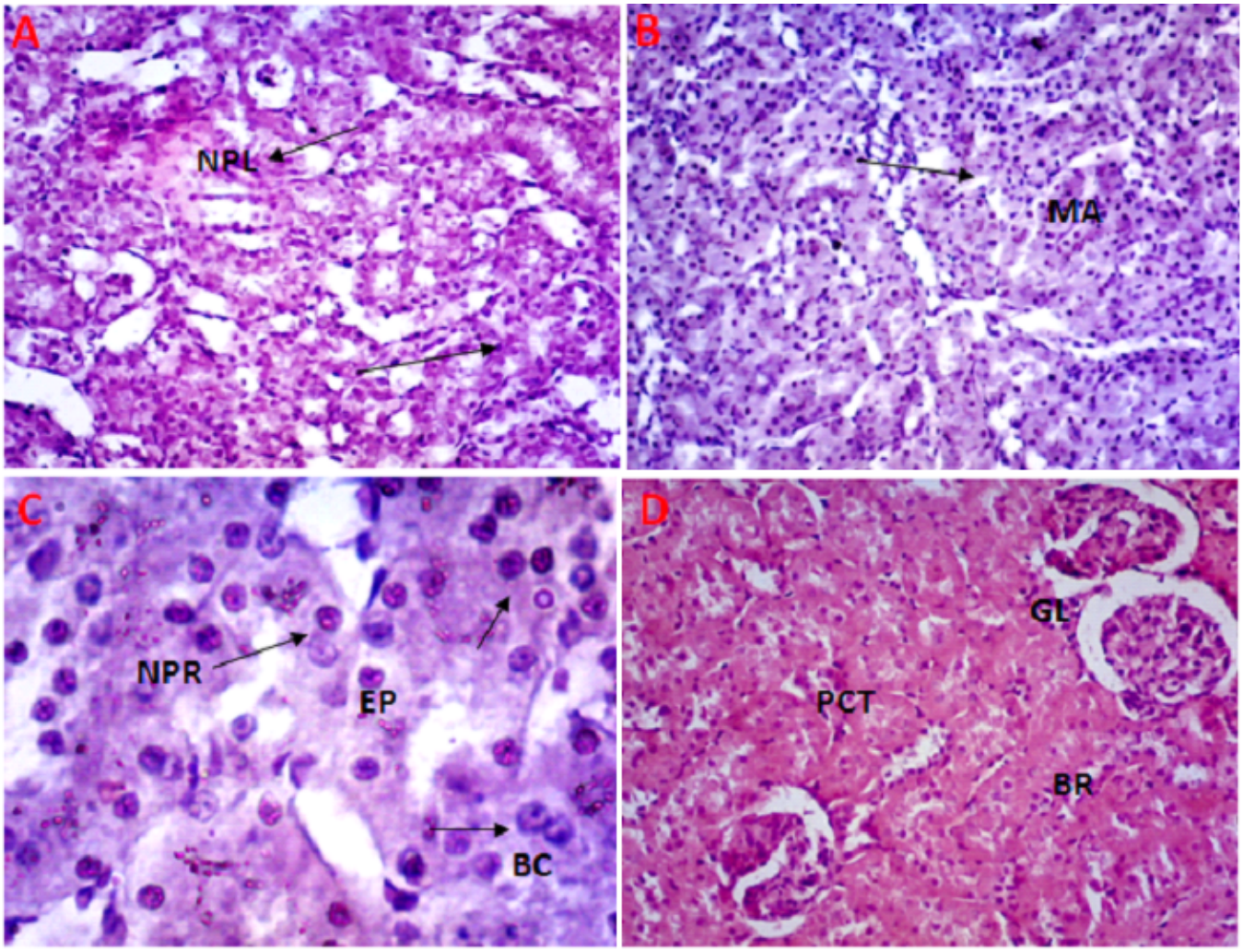

Figure 6

Hiatopathological observations. (A) Distal cortex region of DMN + ZnONP treated rat shows the presence of a neoplasm (NPL). The tubules are disorganized. Nuclear mitotic activity is high. X100. (B) The kidney of rat treated with ZnONP only shows extensive mitotic activity (MA), Tubular necrosis and epithelial damage are wanting. X100. (C) The proximal renal cortex of ZnONP treated rat shows nuclear proliferation (NPR) and binucleated cells (BC), epithelial lining (EP) remains unchanged. X400. (D) T.S. of the kidney of the control rat shows normal structure of proximal convulated tubules (PCT) and the glomeruli (GL). Nuclei are round in shape. There is no loss of brush border (BR). X100.