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Enumerating Nephroprotective Potency of Ethanolic root extract of *Operculina turpethum* against N-Nitrosodimethylamine incited renal Carcinogenesis in Swiss Albino Mice

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Abstract:
N-nitrosodimethylamine (NDMA) is an important carcinogen frequently present in human environment and food chain. Nitrosamines such as NDMA produce oxidative stress due to generation of reactive oxygen species and alter the antioxidant defense system in the tissues. Chronic kidney disease due to a number of factors is a common and serious problem that adversely affects human health, limits longevity and increases costs to health-care systems worldwide. Oxidative stress is prevalent in kidney diseases and is considered to be an important causative mechanism. It develops from an imbalance between free radical production often increased through dysfunctional mitochondria formed with increasing age, inflammation, and reduced anti-oxidant defenses. The present study investigates the influence of ethanolic extract of *Operculina turpethum* roots on the kidney of NDMA intoxicated mice. The nephrotoxicity and therapeutic effect of the plant was assessed by the analysis of kidney marker enzymes, antioxidant enzymes and kidney histopathological studies. NDMA exposure produced detrimental effects on the redox status of the kidney indicated by a significant decline in the levels of protein and antioxidants such as superoxide dismutase, catalase, and glutathione and increased cholesterol, AST, ALT, ALP and lipid peroxidation. *Operculina turpethum* manifested chemopreventive effects by significantly restoring the enzymatic levels and reducing the nephrotoxicity in mice. Histological examination of kidney revealed patho-physiological changes in NDMA treated mice and improved plant treated renal histopathology.

Keywords: Antioxidant, Carcinogenesis, Nephrotoxic, N-nitrosodimethylamine, *Operculina turpethum*

Introduction

Cancer is one of the leading causes of death in the world and its incidence is still increasing, particularly in developing countries. It is the second leading cause of death in developed countries, and is among the three leading causes of death for adults in developing countries with the main organs affected as kidney, liver and lungs [(1)]. Chemopreventive agents may act by multiple pathways to block carcinogenesis. Environmental factors, of either biologic or chemical origin, may act as initiators, promoters, or both of carcinogenesis [(2)]. Chemical carcinogens such as N-nitrosodimethylamine (NDMA), 7, 12-dimethylbenz(*a*) anthracene, benz(a)pyrene, 4-nitroquinoline- 1-oxide, and N-nitroso-N-methylurea are commonly employed to initiate and promote neoplastic transformation in experimental animals [(3)]. Among the diverse pathways, modulation of carcinogen-induced genotoxicity, inhibition of carcinogen activation by altering the activities of phase I and II detoxification enzymes, and scavenging excess reactive oxygen species (ROS) by improving antioxidant defense systems have assumed significance [(4)]. Mechanistic data on renal carcinogenesis in rodent kidney have been largely contributed from experimental studies using classical genotoxic carcinogens from established animal models of renal cancer induction.

N-Nitrosodimethylamine (NDMA) is a member of a family of extremely potent carcinogens, the *N*-nitrosamines (Figure 1).

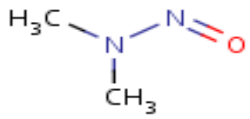

Figure 1: Structure of N-Nitrosodimethylamine

The potent nephrotoxic effect of NDMA, include proximal tubule dysfunction, perturbation of mitochondrial structure, development of nuclear inclusion bodies, interstitial fibrosis, tubular atrophy and renal failure [5]. NDMA produces a variety of health hazards in humans and animals due to its ability to induce severe alterations in various organs and tissues including the nervous system [6]. Kidneys are considered to be one of the main target organs for chronic toxicity. Redox disturbances are known to negatively impact the body system through ROS generation which destructs proteins, lipids and DNA by oxidation. As there is no reliable nephro protective drug in modern medicine, now a day attention has been drawn towards medicinal recipes for kidney ailments [7].

Medicinal plants rich in antioxidants and bioactive phytochemicals have received growing attention over the past few years as potential chemopreventive agents [8]. Nutritional factors have been implicated in the pathogenesis as well as prevention of carcinogenic process, together with metabolic and genetic factors. Antioxidants are the natural defense mechanism existing in our system and these are capable of scavenging the deleterious free radicals. A number of dietary antioxidant compounds have been shown to influence the membrane characteristics such as fluidity, stability and susceptibility to membrane oxidative damage [9]. Any natural or synthetic compound with antioxidant properties might contribute towards the total protection of the oxidative damage.

The plant *Operculina turpethum*, which is commonly known as *trivit,* has been used as a folkloric medicine in many countries to treat liver disorders, constipation, jaundice, rheumatism, chronic gout, piles and tumors, obesity and many other diseases [10]. The plant contains various secondary metabolites including saponins, flavonoids, phenolics, a glycosidic resin, which has the insoluble glycoside turpethein and two ether soluble glycosides. *Operculina turpethum* when consumed may add to the antioxygenic potential and hence may prove useful in protection against oxidative stress caused by a large number of xenobiotics including carcinogens. In view of the above considerations, the aim of this study was designed to evaluate the nephroprotective efficacy of *Operculina turpethum* by NDMA induced renal carcinogenesis in the kidney of mice.

MATERIAL AND METHODS

Chemicals: TBA (Thiobarbituric acid), TCA(Trichloroactetic acid), HCl, pyrogallol, H_2O_2, triton-x, BSA, copper sulphate, ascorbic acid, thiourea etc. All chemicals used in the study were of analytical reagent grade and were purchased from reliable firms (SRL (India), MERCK, RANBAXY, HIMEDIA). NDMA was purchased from SIGMA.

Animal care and Monitoring:

Healthy male Swiss albino mice (*Mus musculus*) (4-6 months old, weighing 25-30 g) were procured from C.C.S. Haryana Agricultural University (Hisar, India). They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). Animals lead free access to standard food

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pellet diet (Hindustan Lever Limited: metal contents in parts per million dry weight: Cu 10.0, Zn 45.0, Mn 55.0, Co 5.0, Fe 75.0) and drinking water *ad libitum* throughout the study.

Plant Material:

Operculina turpethum was collected from Pharmacological garden of CCSHAU Hisar, Haryana, India in the month of November 2011.The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra Rohtak, Haryana, India.

Preparation of Ethanolic extract:

The freshly collected *Operculina turpethum* roots were dried in shade and coarse powder was extracted. Dried powdered material was placed in the Soxhlet thimble with 80% ethanol in 500 ml flat bottom flask. Further refluxed for 18 h at 80^0 C for two days. Collected solvent was cooled and poured in a glass plate. The marc was dried in hot air oven below 50^0C for 48 h and kept in dissector for 2 days [11]. The yield of the extract was 12.5% w/w of powdered plant material for further exploration. Collected dried extract was stored at 5^0C in air tight containers.

Ethical Clearance:

The animal experiments were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee approved experimental design performed in this study for the use of Swiss Albino mice as an animal model for the study.

Treatment Regime:

The male Swiss Albino mice *(mus musculus)* weighing 25-30g were randomly selected from laboratory stocks and were placed into various groups.

Group 1 - Control

Group 2 - NDMA treated (10 mg/kg body weight)

Group 3 - NDMA + OTE (300mg/kg body weight)

Group 4 - NDMA+ OTE (400mg/kg body weight)

Group 5 - OTE (300 mg/kg body weight)

Group 6 - OTE(400mg/kg body weight)

Group 7 - NDMA + Standard antioxidant (BHA1%)

Group 8 - BHA (1%)

The doses of the plant extract, NDMA and standard antioxidant were decided on the basis of previously published reports [12]. NDMA was given on three consecutive days of each week for three successive weeks along with the plant extract simultaneously.

BIOCHEMICAL ASSAYS

After 21 days of duration the mice were fasted overnight and then sacrificed under light ether anesthesia. Kidneys were dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight noted and then stored at -80°C for various biochemical assays, and histological studies.

Preparation of kidney homogenate

Kidney tissue was minced and homogenized (10% w/v) in ice-cold 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15-20 min at 48°C twice to get the enzyme fraction. The supernatant was used for biochemical assays [13].

LPO (Lipid peroxidation)

LPO was estimated colorimetrically by measuring malondialdehyde (MDA) formation as described by Nwanjo and Ojiako [14]. In brief, 0.1 ml of homogenate was treated with 2 ml of a 1:1:1 ratio of TBA-TCA-HCl (TBA 0.37%, TCA 15%, HCl 0.25 N) and placed in water bath at 65°C for 15 min, cooled, and centrifuged at 5,000 rpm for 10 min at room temperature. The optical density of the clear supernatant was measured at 535 nm

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against a reference blank. The MDA formed was calculated by using the molar extinction coefficient of thiobarbituric acid reactants (TBARS; 1.56 x105 l/mole cm-1). The product of LPO was expressed as nmol of MDA formed per g of tissue.

Superoxide dismutase (SOD)

Hepatic SOD activity was assayed according to the method of Marklund and Marklund [15]. For the control, 0.1 ml of 20 mM pyrogallol solution was added to 2.9 ml of Tris buffer and mixed, and reading was taken at 420 nm after 1.5 and 3.5 mins. The absorbance difference for 2 min was recorded and the concentration of pyrogallol was adjusted in such a way that the rate in change of absorbance per 2 min was approximately 0.020-0.023 optical density units. Kidney sample (200 ml) was treated with 10 ml of 25% triton X-100 and kept at 48°C for 30 min. To 2.8 ml of Tris buffer, 0.1 ml of treated sample was added and mixed, and the reaction was started by adding 0.1 ml of adjusted pyrogallol solution (as for control). Reading was taken at 420 nm after 1.5 and 3.5 mins and the difference in absorbance was recorded. The enzyme activity was expressed as U/ml of liver extract and 1 U of enzyme is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50%.

Catalase (CAT)

Catalase (CAT) activity was estimated following the method of Aebi [16]. Kidney sample (100 ml) was treated with ethanol (10 ml) and placed on an ice bath for 30 min. To this, 10 ml of 25% triton X-100 was added and again kept for 30 min on ice. To 200 ml phosphate buffer (0.1 M), 50 ml of treated kidney sample and 250 ml of 0.066 M H_2O_2 (prepared in 0.1 M phosphate buffer, pH 7.0) were added in a cuvette. The decrease in optical density was measured at 240 nm for 60s. The molar extinction coefficient of 43.6 cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/min/mg protein.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [17]. In brief, 0.2 ml of kidney fraction and 0.5 ml of substrate solution (for AST: aspartate and 2-ketoglutarate; for ALT: alanine and 2-ketoglutarate) were incubated at 37°C for 60 min for AST and 30 min for ALT. After incubation, 0.5 ml of DNPH solution was added to arrest the reaction, which was kept for 20 min at room temperature. To this, 1 ml of 0.4 N NaOH was added and absorbance was read at 510 nm. Activities were expressed as U/L.

Reduced Glutathione(GSH)

Reduced glutathione (GSH) level was determined by the method of Ellman modified by Jollow et al.,[18]. Sulphosalicylic acid (0.5 ml 10%)was added to mixture of 0.4 ml homogenate and 0.6 ml of distilled water as protein precipitant. Supernatant (0.5 ml) was mixed with the reaction mixture of 4.5 ml of 0.5M Tris-buffer and 0.5 ml of 10mM DTNB and the absorbance was measured immediately at 412 nm .The GSH contents were calculated using GSH as standard and expressed as mM/g tissue.

Total Protein

Protein content was determined by the method of Lowry et al., [19] with bovine serum albumin as a standard.

Total Cholesterol

Cholesterol was determined by the method of Zak [20] with cholesterol as a standard.

Alkaline phosphatase (ALP)

Activity of alkaline phosphatase (ALP) were determined according to the protocol described

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in a laboratory practical manual [21]. Substrate solution (3 ml) was incubated at 37°C for 15 min and then 0.5 ml kidney homogenate was added. It was mixed well and immediately 0.05 ml of the mixture was removed and mixed with 9.5 ml of 0.085 N NaOH. This corresponded to zero time assay (blank). The remaining solution (substrate-enzyme) was incubated for 15 min at 37°C and then 0.5 ml was drawn and mixed with 9.5 ml of 0.085 N NaOH. Absorbance was measured at 405 nm against the reference blank. Specific activities were expressed as µmoles of p-nitrophenol formed per min per g tissue.

Histopathological studies

The kidneys were excised and fixed in buffered 10% formalin at room temperature for 72 h. It was then thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol , cleared and embedded in soft paraffin. Tissue sections of about 6mm were cut, stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of nephrotoxicity [22]. Results of the histopathological studies are shown in the (Figures 4-7).

Statistical analysis

Data are expressed as the mean±SEM. The data was analyzed by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (S.P.S.S. 11).

Results

Effect of *Operculina turpethum* on lipid peroxidation:

The level of TBARS as an index of lipid peroxidation, a degradative process of membranous lipids, in kidney tissue of NDMA treated mice was significantly (p<0.01) elevated (103.15±0.01) when compared to control animals (82.57±0.11). The remarkable increase of lipid peroxides in kidney tissue during NDMA administration indicates the formation of reactive oxygen species (ROS), which plays a major role in cell injury and nephrotoxicity. Lipid peroxidation level was restored towards its normal value by treatment with the *Operculina turpethum* extract on NDMA induced toxicity (Figure 2).

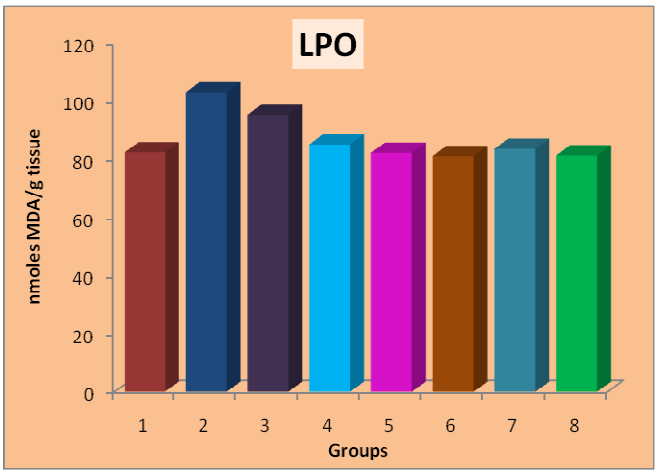

Figure 2: Effect of ethanolic extract of *Operculina turpethum* on the lipid peroxidation in different groups of mice.

Effect of *Operculina turpethum* on marker enzymes:

Biochemical parameters in the control and various experimental groups are depicted in Table1.Administration of intraperitoneal NDMA to mice caused kidney damage as indicated by a significant increase in enzymes AST, ALT, ALP activity compared to control mice. Elevated levels of these enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in kidney. The treatment of animals with the plant extract significantly (p<0.01) recovered the normal range of enzymes.

Table 1: Effect of ethanolic extract of *Operculina turpethum* (OTE) on the levels of marker enzymes AST, ALT and ALP against NDMA induced nephrotoxicity.

GROUPS	AST(U/L)	ALT(U/L)	ALP(µmoles/min/g)
Control	48.234±0.030#	34.981±0.194#	56.146±0.012#
NDMA (10 mg/kg)	65.194±0.023*	47.186±0.018*	73.143±0.008*
NDMA+OTE (300mg/kg)	61.192±0.007#	40.305±0.006#NS	69.752±0.004#
NDMA+OTE (400mg/kg)	53.213±0.001#	37.136±0.002#	61.841±0.012#
OTE(300 mg/kg)	47.219±0.003*	34.359±0.076*	56.766±0.009*
OTE(400mg/kg)	47.556±0.004*	34.671±0.071*	56.130±0.006*
NDMA+BHA (1%)	50.296±0.013#	35.154±0.010#	63.130±0.005NS
BHA (1%)	47.701±0.019*	34.108±0.003*	55.153±0.009*

Values are expressed as mean ± S.E.M for six mice in each group. *p< 0.01 vs. control, NS=Non significant and #p<0.01 vs. NDMA treated group.

Effect of *Operculina turpethum* on antioxidant enzymes:

On liver SOD level:

Effect of NDMA alone and co-treatment with *Operculina turpethum* on SOD activity has been shown in Table 2. SOD activity in NDMA treated kidney tissue (3.302±0.015) was reduced markedly than the control group (8.336±0.003) and co-treatment with *Operculina turpethum* at a dose of 400 mg/kg body weight to the NDMA treated mice significantly (p<0.01) recovered that SOD depletion.

On liver CAT level:

CAT activity in the NDMA treated group showed marked reduction compared to normal group (3.139±0.009 in NDMA treated group vs 7.876±0.044 in control group). Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effect. As shown in Table 2, co-treatment with *Operculina turpethum* at a dose of 400 mg/kg body weight, to the NDMA treated mice significantly (p<0.01) restored the CAT activity.

On total protein and total cholesterol level:

NDMA enhanced the levels of cholesterol in mice (37.164±0.003) and depleted the protein level (56.164±0.011) which were significantly recovered as (31.487±0.027) and (69.243±0.007**)** with the simultaneous dosing of the extract at 400 mg/kg body weight to the NDMA treated mice as shown in Table 2.

Table 2: Effect of ethanolic extract of *Operculina turpethum* on the levels of antioxidant enzymes SOD, CAT, total protein, total cholesterol against NDMA induced nephrotoxicity.

GROUPS	SOD(U/ml)	CAT (nanomoles/min/mg)	CHOLESTEROL (mg/g)	PROTEIN (mg/g)
Control	8.336±0.003#	7.876±0.044#	28.153±0.015#	76.159±0.020#
NDMA (10 mg/kg)	3.302±0.015*	3.139±0.009*	37.164±0.003*	56.164±0.011*
NDMA+OTE (300mg/kg)	5.148±0.007#NS	5.137±0.005#	35.262±0.019#	62.492±0.174#
NDMA+OTE (400mg/kg)	6.777±0.022#	6.151±0.018#	31.487±0.027#	69.243±0.007#
OTE (300 mg/kg)	8.139±0.009*	7.208±0.007*	27.282±0.005*	75.986±0.018*
OTE (400mg/kg)	8.069±0.006*	7.187±0.015*	26.153±0.016*	76.142±0.010*
NDMA+BHA (1%)	7.665±0.019#	6.476±0.180#	29.129±0.002*NS	72.370±0.187#
BHA (1%)	8.112±0.006*	7.144±0.008*	28.160±0.028*	76.318±0.162*

Values are expressed as mean ± S.E.M for six mice in each group. *p < 0.01 vs. control,NS=Non significant and #p<0.01 vs. NDMA treated group.

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Effect of *Operculina turpethum* on GSH level.

GSH level as measured from the kidney tissue of all the experimental groups has been shown in Figure 3. NDMA administration caused massive reduction in GSH level (2.140±0.011 vs. 4.729±0.004 in normal mice). Administration with the ethanolic extract at a dose of 400 mg/kg body weight simultaneously with NDMA significantly ($p<0.01$) elevated that reduction in animals.

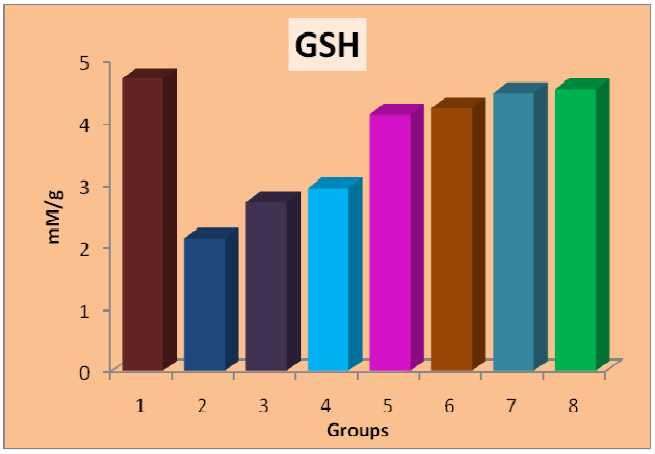

Figure 3: Effect of ethanolic extract of *Operculina turpethum* on the GSH levels in different groups of mice.

KIDNEY HISTOPATHOLOGY

Renal histopathological studies provided supportive evidence for biochemical analysis. Histopathological examination of tissues under light microscope was done to observe the effect of NDMA on the structural integrity of the cells of kidney tissues. The histopathology reports of various groups are shown in Figure 4. The histoarchitecture of control group (a) animals was showing well developed proximal and distal convoluted tubules and normal glomerulus and Bowman's capsule .While the animal treated with NDMA (b) showing degenerated glomerulus with distorted proximal and distal convoluted tubules. Animals treated simultaneously with plant extract (400 mg/kg b.wt.) showing well rejuvenated proximal and distal convoluted tubules with glomerulus (c). After treatment with BHA (d) the change in the kidney tissue was observed including the regeneration of the normal histoarchitecture.

Control group showed normal glomerular and tubular histology of kidney. The tubules were largely intact without the presence of any mononuclear infiltrates in the interstitium and blood vessels were also unremarkable. The histopathology of tissue sections suggest that the treated group had encountered vast histological damages as evidenced by the glomerular and tubular congestion with blood vessel congestion, epithelial cell desquamation, and presence of tubular cast. Inflammatory cells were also seen in kidney section from the NDMA treated group. The plant treated animals showed almost normal glomerular and tubular arrangements with minimal blood vessel congestion, epithelial cell desquamation, and presence of tubular cast with very few inflammatory cells. In the kidney sections of the mice intoxicated with NDMA and simultaneously treated with the extract, the normal cellular architecture was retained as compared to NDMA treated, thereby confirming the protective effect of the extract. In accordance with these results, it may be hypothesized that alkaloids, saponins and flavonoids, glycosides which are present in extract, could be considered responsible for the nephroprotective activity.

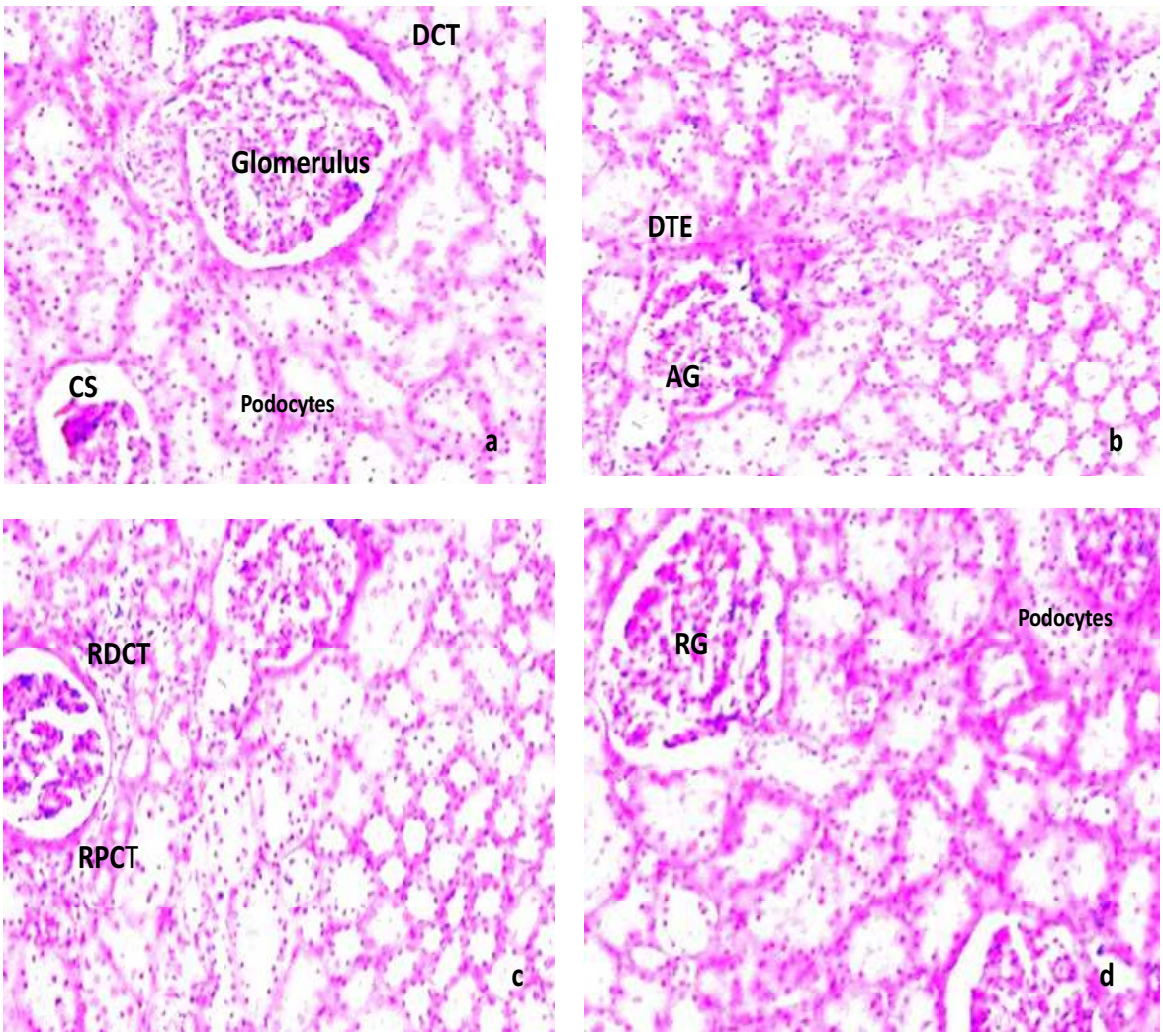

Figure 4: (a) Transverse section of kidney showing normal architecture with intact glomerulus proximal, Capsular space (CS) and distal convoluted tubules (DCT). **(b)** Transverse section of the kidney of mice treated with NDMA showing degenerated glomerulus with distorted proximal and distal convoluted tubules, atrophied glomerulus (AG) and Degenerated tubular epithelium(DTE). **(c)** The kidney showing rejuvenated proximal and distal convoluted tubules (RPCT& RDCT) and glomerulus in plant (400 mg/kg b.wt.) treated animals. **(d)** The kidney tissue of animals treated with NDMA+BHA (1%) showing regenerated glomerulus (RG) and the regeneration of normal histoarchitecture.

The decrease in the damage demonstrated by the extract as well as decrease in the infiltration of the inflammatory cells in the kidney lobules is indicative of therapeutic efficacy of *Operculina turpethum*.

Discussion

Renal failure is a common clinical syndrome. It is defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted [23]. One of the key functions of the kidneys is to filter waste products that build up in the blood. Renal failure determines that waste products are not removed completely or sufficiently. This can occur quickly (acute renal failure, or acute kidney injury) often as the result of toxins or mechanical trauma. More often, however, the development of renal failure is gradual and insidious, with resultant chronic kidney disease [24]. During the pathogenesis of kidney diseases, perturbations in cellular oxidant

handling influence downstream cell signalling and, in the kidney, promote renal cell apoptosis, senescence, decreased regenerative ability of cells and fibrosis [25].

Chemoprevention has evolved as a promising and valuable strategy to inhibit, suppress or control the incidence of carcinogenesis by using specific natural and synthetic agents [26]. The presence of nitroso compounds and their precursors in human environment together with the possibility of their endogenous formation in human body have led to suggestions of their potential involvement in human cancers [27,28].Nitroso compounds such as N nitrosodimethylamine (NDMA) have been suggested to cause oxidative stress and cellular injury due to involvement of free radicals [29,30]. ROS react with cellular components and certainly play an important role in the initiation and promotion of cells to neoplastic growth [31]. Oxidative stress may be defined as a disturbance in regular cellular and molecular function caused by an imbalance between production of reactive species and the natural antioxidant ability of our cells [32]. Lipid peroxidation plays an important role in carcinogenesis [33] and may lead to the formation of several toxic products, such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDE) and 4 hydroxynonenal. These products can attack cellular targets including DNA, thereby inducing mutagenecity and carcinogenicity [34].

Free radical scavenging enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect the biological systems from oxidative stress. Catalase, a haem-containing redox enzyme of about 250 kDa, catalyses conversion of hydrogen peroxide to water and oxygen, and is a powerful tool in removing cellular toxicity [35]. Studies have shown SOD and CAT levels were decreased in conditions such as oxidative stress. Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effects induced by oxidative stress and occurs readily in the tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids [36]. We found that levels SOD, CAT, LPO and that of kidney marker enzymes (AST, ALT and ALP) were recovered significantly by treatment with the plant extract to the normal values.

Glutathione is a tripeptide metabolic regulator and a putative indicator of health. Its main function is detoxification of endogenous metabolic peroxides through glutathione pathway and also the exogenous substances such as NDMA [37]. In the present study, the GSH level was found to decline in NDMA treated animals which was further recovered by the dosing of plant. The observed change in these enzymes showed that *Operculina turpethum* preserves the structural integrity of the tissues from the toxic effect of NDMA.

The main histological finding of this study was that NDMA treated animals showed acute tubular necrosis and glomerular widening and was responsible for dilatation of proximal and distal tubules in the cortex. The treatment with plant recovered the kidney architecture induced by NDMA. However, it is important to mention that higher dose of *Operculina turpethum* extract was more effective against the architectural damage induced by NDMA nephrotoxicity. In conclusion, mechanism of the protective action of *Operculina turpethum* is due to its free radical scavenging activity and great affinity to overcome the harmful effects caused by NDMA. However more studies are required before the

clinical application of *Operculina turpethum* as an anticancer drug.

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