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Original Research

Nephroprotective effects of *Datura metel* extract in gentamicin induced mice model: biochemical and histological evidences

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Abstract: *Datura metel* is traditionally used as a remedy for renal toxicity. However, the nephroprotection has not been scientifically validated yet. To evaluate the nephroprotective like effect of methanolic extract of *D. metel* in gentamicin induced mice model, mice of either sex were divided into groups. One group received normal saline as negative control. The 2nd group received gentamicin 100mg/kg for 8 days as positive control, 3rd group received 50mg/kg silymarin as standard, while the reaming groups received 100, 200 and 300 mg/kg of MEDM and gentamicin 100mg/kg, for 8 days. The blood and urine samples were collected on 9th day, animals were then dissected and whole kidneys were removed and preserved in formalin for later histological examinations. The level of serum creatinine, blood urea nitrogen, urine creatinine and urine urea were significantly ($P<0.05$) elevated and the renal MDA level was also elevated significantly ($P<0.05$) by gentamicin in mice. After the treatment of test animals with MEDM, the elevated level of serum and urine biomarkers by gentamicin were reversed by MEDM. The nephroprotective effect was found in dose dependent manner. As the MEDM significantly protected the nephrotoxicity via its antioxidant effect. The findings of our study thus proved the scientific background for the nephroprotective effect of MEDM.

Key words: Crude methanolic extract of *D. metel*; Phytochemical screening; In vivo nephroprotective screening; Biomarkers and histopathology.

Introduction

Nephrotoxicity is caused when kidney inadequately detoxify and excrete the metabolites (1). Nephropathy is widely seen in different population of the world, irrespective of age, sex, and gender (2). Exogenous as well as endogenous toxicants are responsible for the renal toxicity. Drugs are the major contributing factors for acute nephrotoxicity. General mechanisms responsible for nephrotoxicity are rhabdomylosis, intra-glomerular hemodynamics alteration, tubular toxicity, crystal nephropathy, interstitial nephritis, thrombotic microangiopathy and inflammation (3-6). Drugs like gentamicin, acetaminophen and cisplatin induce nephrotoxicity via activation of free radicals (7, 8). Oxidative stress is the leading cause of kidney injury in gentamicin treated patients (9). Gentamicin stimulates the release of iron from mitochondria of renal cortex which leads to production of hydroxyl radical (10).

Datura metel is an annual herb, found as a wild weed as well as cultivated in tropical and temperate zones. It belongs to family solanaceae (11). *D. metel* is commonly known as thorn's apple, Indian apple or devil's trumpet which is distributed all over the world such as Nigeria, Central Asia, Bangladesh and Central America (12, 13). Similarly, it has reported that traditionally *D. metel* was remedy for renal toxicity (14, 15). IT has been reported that the crude methanolic extracts of *D. metel* has the ability to exhibit antioxidant activity due to free radical scavenging activity (16, 17). Mai *et al*., 2017, has isolated different flavonoids such as kaempferol from *D. metel* (11). Kaempferol exhibit antioxidant effect and free radical scavenging activity (18).

The ethnopharmacological evidence of the applications of *D. metel* for renal disorders accompanied with the antioxidant and free radical scavenging effects of the methanolic crude extracts of *D. metel* (16, 17) provoked us to evaluate the nephroprotective effect of *D. metel* in gentamicin induced mice model.

Materials and Methods

Plant Material

D. metel was collected from the uncultivated lands of Village Pati Khurd Takht Bhai, Pakistan in June 2018. Identification and authentication of plant specimen was done by Dr. Gul Jan, Department of Botany, AWKUM (Voucher number HDB, AWKUM-75).

Extraction and Maceration

The dried powder plant of *D. metel* (950 g) was macerated with hydroalcoholic solvent (90% methanol) at room temperature for 14 days. The hydroalcoholic soluble residues were passed through muslin cloth, dried using rotary evaporator at about 40-45 °C till the filtrate was completely dried. A solid, oily viscous crude extract of about 90 grams of was obtained.

Animals

Healthy Swiss albino mice (25-30 g) of either sex was purchased from the Veterinary Research Institute (VRI) Peshawar, Pakistan. Animals were housed in cages in well crossed ventilated animal house of the department of pharmacy, AWKUM, at a temp of (22±1 °C). All experimental measures and research were carried out according to the guidelines for laboratory animals adopted by the Ethics committee of the university.

Phytochemical Tests

Preliminary phytochemical tests for alkaloids, glycosides, flavonoids, terpenoids, tannins, saponins, and phlobatannins were performed (19).

Grouping and dosing of animals

Swiss albino mice were divided into 6 groups and were for 8 days.

1: The animals of this group were injected intraperitonially with 0.9% normal saline.

2: Gentamicine (100mg/kg/day i.p.) to the Mice of this group.

3: Animals of this group were given Silymarin (50mg/kg/day i.p.).

4: MEDM and Gentamicine (100mg/kg/day + 100mg/kg/day i.p.) to the mice of this group.

5: MEDM (200mg/kg/day) as well as Gentamicine (100mg/kg/day) to the mice of this group.

6: MEDM (300mg/kg/day) as well as Gentamicine (100mg/kg/day) to the mice of this group.

The experiment was continued for 8 days. After the collection of blood and urine samples, the animals were sacrificed at 9th day under mild chloroform anesthesia and organs (kidneys) were isolated, washed with normal saline and then conserved in 10% formalin solution for histological assessment (20, 21).

Serum creatinine and blood urea nitrogen (BUN)

The collected blood samples were then analyzed for serum creatinine, serum urea and BUN (Blood Urea Nitrogen) by using auto analyzer and different commercial diagnostic kits (22, 23).

Urine creatinine and Urine Urea

The urine samples were then analyzed for urine urea and creatinine clearance by using different available commercial diagnostic kits/ lab analysis (24).

Determination of lipid peroxides level

Malonaldehyde was measured to detect lipid peroxidation using thiobarbituric acid assay (25). MDA and thiobarbituric acid reacts and it gives red color which absorbs light at 535nm. A stock solution of hydrochloric acid 0.25 N, tricholoroacetic acid 15% w/v, and thiobarbituric acid 0.375% w/v was formed then gently heated. The mixture was cooled and centrifugated at 1000 rpm for 10 min. Flocculent layer is removed and the absorbance of sample was determined at 535nm. Extinction co-efficient of $1.56\times10^5\ M^{-1}\ cm^{-1}$ was used for MDA calculation (26).

Histological Assessment

The already preserved (10% neutral buffered formalin) kidneys were used and then embedded in paraffin for 24 h. The kidneys were incised into 4-5µm sections and deparaffinized, hydrated and stained with H&E stains. Cut sections were also stained with Periodic acid Schiff's staining. A blinded pathologist used light microscope to examine the renal sections for the detection of the extent of damages to glomeruli, tubles, interstitium as well as for capillary hemorrhage and congestion (27).

Statistical analysis

All the data are expressed as mean ± standard error mean. Oneway ANOVA was used followed by Dunnet test. $P<0.05$ were considered as statistically significant.

Results

Preliminary phytochemical tests

The phytochemical screening showed that different phytochemical groups like alkaloids, flavonoids, glycosides, tannins and phenolic compounds were present in crude MEDM while saponins, phlobatannins and terpenoids were not present (Table 1).

Biochemical analysis

Serum creatinine and blood urea nitrogen

Serum creatinine level of gentamicin treated group was found elevated highly significantly ($P<0.001$), compared against control Figure 1A. The results showed that when MEDM was administered concomitantly with gentamicin then the serum creatinine level (elevated by gentamicin) was significantly decreased by M100 ($P<0.05$), M200 very significantly ($P<0.01$) while M300 highly significantly ($P<0.001$), compared to gentamicin treated animals. The reduction in serum creatinine level was more prominent in 300mg/kg MEDM treated group. Similarly, the result of this study showed that gentamicin significantly ($P<0.001$) raised level of serum BUN thus induced kidney injury in the gentamicin treated group, compared with the control MEDM treated groups lowered the level of serum BUN level such as M100 lowered less significantly ($P<0.05$), M200 reduced serum BUN level significantly ($P<0.01$)

Table 1. Phytochemical screening of *D. metel.*

Phytochemicals	Whole plant extract
Alkaloids	+
Flavonoids	+
Glycoside	+
Saponins	–
Tannins	+
Phlobatannins	–
Terpenoids	–
Phenols	+

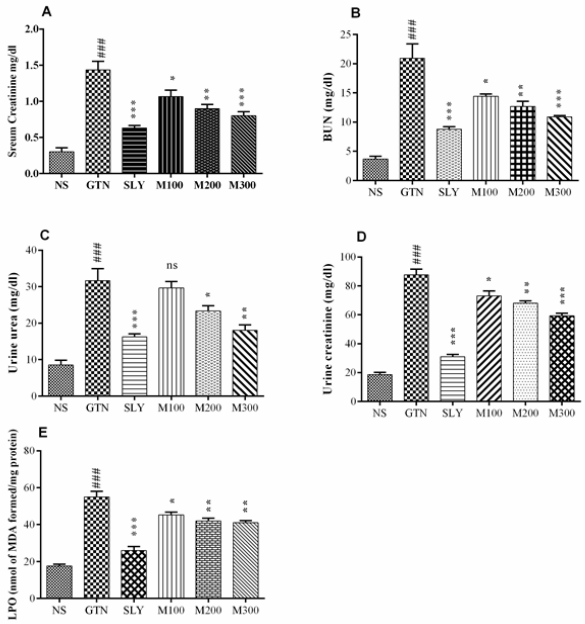

Figure 1. Effect of MEDM on Serum Creatinine level [A] Blood Urea Nitrogen [B] Urine creatinine level [C] Urine Urea level [D] renal MDA [E] level in mice. Data are expressed as mean ± SEM (*n=6)*; $^{###}P<0.001$(Compared to control group); $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (Compared to gentamicin group). Gentamicin was used as negative control while silymarin as positive control. The extract was administered in different dose to mice. MDA concentration was used as marker to detect LPO. MEDM= crude methanolic extract of *D. metel,* NS= Normal saline, GTN= gentamicin 100mg/kg, SLY= Silymarin 50mg/kg, M100= crude methanolic extract 100mg/kg + gentamicin 100mg/kg, M200= crude methanolic extract 200mg/kg + gentamicin 100mg/kg, M300= crude methanolic extract 300mg/kg + gentamicin 100mg/kg.

and M300 lowered the serum BUN highly significantly ($P<0.001$*)* when compared against gentamicin Figure 1B.

Urine Creatinine and Urine urea

The urine creatinine level lowered down after treating the test animals with different doses of MEDM Figure 1C. The urine creatinine level was decremented in M100 group significantly *(*$P<0.05$), in M200 group with gradual increase in significance *(*$P<0.01$) while in M300 group with marked increase in significance *(*$P<0.001$), compared against gentamicin group. The effect of MEDM on the urine urea level of gentamicin induced nephrotoxic animals is shown in figure 1D. The results of our investigations revealed that gentamicin group showed significant ($P<0.001$) increment in the level of urine urea when comparison were made against control hence evidenced the induction of nephrotoxicity. The M100 group did not have significant effect on lowering urine urea level, M200 group lowered urine urea level significantly ($P<0.05$) while M300 decreased urine urea level ($P<0.01$) very significantly, compared with gentamicin group.

Renal lipid peroxidation level

The effect of MEDM on the renal LPO has been shown in the figure 1E. It was observed that gentamicin has highly significantly ($P<0.001$) elevated level of renal MDA, when comparison was made against control group, which indicates that the antioxidant machinery of kidney is compromised and caused nephrotoxicity. It was noted that significant ($P<0.05$) reduction in MDA level was done in M100 group, whereas in M200 and M300 level of MDA was very significantly ($P<0.01$) decreased.

Histopathological Assessment

The effect of MEDM on the renal histopathology of test animals is shown in table 2. The histological examinations of control showed normal findings in our study (figure 2). While gentamicin group showed moderate changes in histology like presence of hyaline cast, tubular degeneration, inflammatory infiltrate and congested blood vessels (figure 3). Silymarin treated group also showed protection of kidney tissues (figure 4). After treatment with different doses of MEDM, the histological examination showed that M100 and M200 group

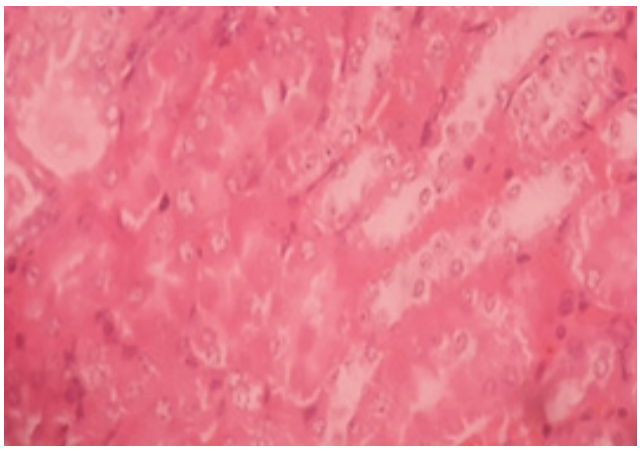

Figure 2. Photomicrograph of haematoxylin and eosin (H and E) slides of kidney of normal saline group with normal histology. The renal tubules and glomeruli are in normal shape. No histological changes were seen such as inflammatory infiltrate, congested blood vessels, tubular degeneration and hyaline cast**.**

Table 2. Effect of MEDM on kidney Histology of test animals.

Groups	Histological Parameters			
	Inflammatory Infiltrate	Tubular Degeneration	Hyalin Cast	Congested Blood vessels
Control	-	-	-	-
GTN	++	++	++	++
SLY	+	+	+	+
M100	++	++	++	++
M200	++	++	++	++
M300	+	-	+	+

Key; -: negative, +: mild, ++: moderate, +++: severe

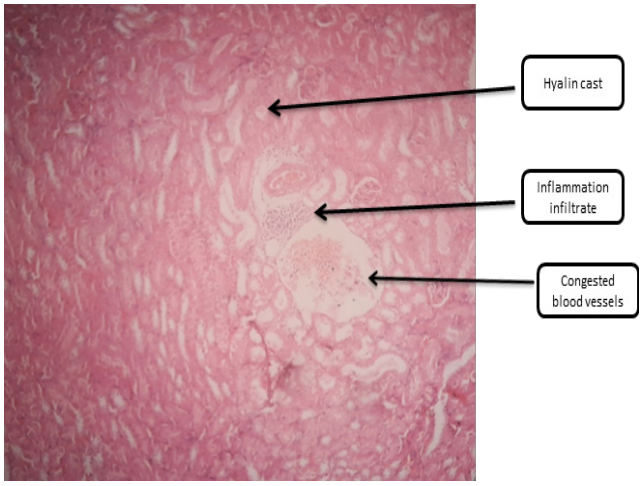

Figure 3. Photomicrograph of haematoxylin and eosin (H and E) slides of gentamicin treated kidney histology with moderate changes such as inflammatory infiltrate, congested blood vessels, tubular degeneration and hyaline cast.

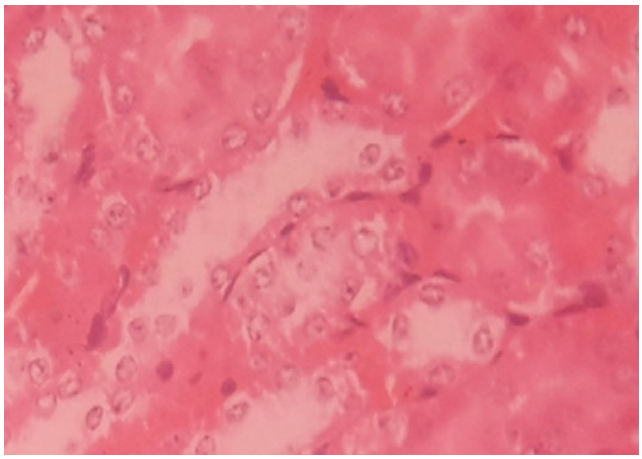

Figure 4. Photomicrograph of haematoxylin and eosin (H and E) slides of kidney of silymarin treated group with normal histology. The renal tubules and glomeruli are in normal shape. No histological changes were seen such as inflammatory infiltrate, congested blood vessels, tubular degeneration and hyaline cast.

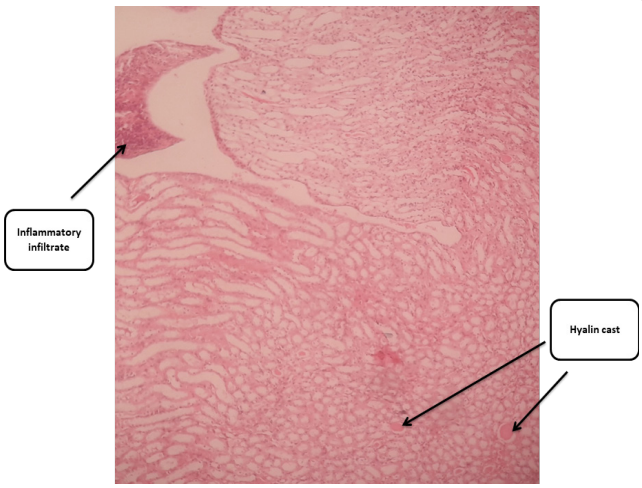

Figure 5. Photomicrograph of haematoxylin and eosin (H and E) slides of kidney histology of 100 mg/kg dose with marked changes in inflammatory infiltrate, tubular degeneration and hyaline cast.

(Figure 5 and figure 6) revealed slightly improvement in morphological changes whereas M300 treated group (Figure 7) revealed marked improvement in morphological changes such as there were minimal inflammatory infiltrate and tubular degeneration along with hyaline cast and congested blood vessels.

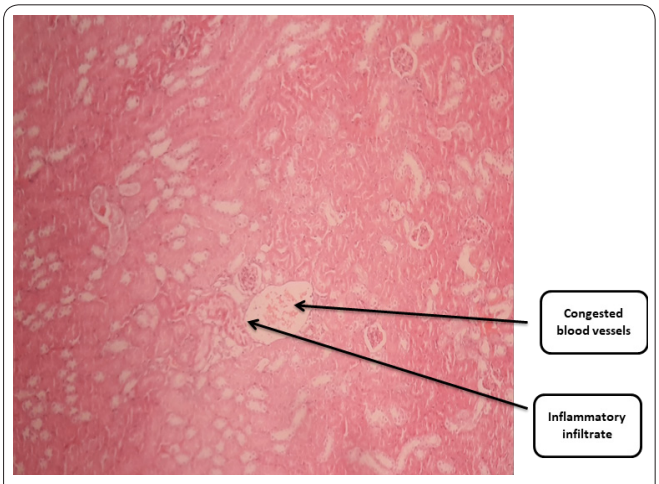

Figure 6. Photomicrograph of haematoxylin and eosin (H and E) slides of kidney histology of 200 mg/kg dose with marked inflammation infiltrate, tubular degeneration and congested blood vessels.

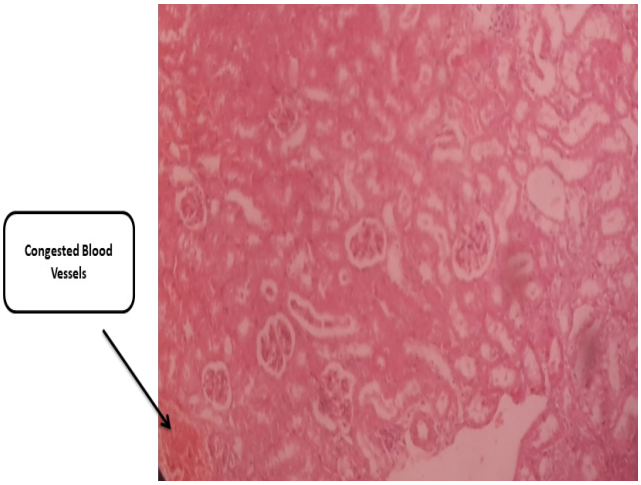

Figure 7. Photomicrograph of haematoxylin and eosin (H and E) slides of kidney histology of 300 mg/kg dose of MEDM with significant improvement in tubular degeneration, inflammation infiltrate, congested blood vessels and hyaline cast (induced by gentamicin).

Discussion

Gentamicin has been used against sever infections but its use is limited due to its side effect of causing nephrotoxicity (28). It increases the production of Reactive oxygen species (ROS) in the kidney and thus induce nephrotoxicity by elevating the serum creatinine level and serum urea level, serum BUN level, urine urea and urine creatinine level (20, 29). ROS are considered to be involved in the mechanistic pathway for tubular necrosis (30, 31). Moreover, gentamicin enhances the production of ROS which leads to inflammatory changes in kidney (32). The Nitric Oxide generation at glomerular and mesangial level (33, 34). Different researchers found that the agents that hinder the synthesis of ROS or exhibit free radical scavenging activity can protect or recover against the gentamicin induced nephrotoxicity (35-37).

Gentamicine induce nephrotoxicity which is represented by elevated levels of serum urea, BUN, serum creatinine, uric acid and necrosis of proximal tubules,

leading to renal failure (23). Serum creatinine and BUN concentrations are the biomarkers for kidney functions test. Urinary excretion of creatinine and plasma concentration of creatinine is usually constant and does not alter with urinary excretion rate, metabolic rate and dietary changes(29). Increase in serum creatinine level indicates reduction of glomerular filtration rate, accompanied with increased serum level of BUN and serum urea. The level of serum creatinine and BUN increases due to marked renal parenchyma cells injury (23, 38). Impairment of antioxidant enzymes of renal mitochondria by gentamicin supports the effect of ROS (23, 38).

In our study it has been observed from the biochemical parameters of test animals that gentamicin has markedly induced nephrotoxicity which is evident by elevated levels of serum creatinine, blood urea nitrogen, urine creatinine, urine urea and renal MDA level.

In this study, MEDM conferred nephroprotection which is supported by the ability of MEDM which significantly($P<0.05$) lowered the raised biomarkers for renal toxicity, induced by gentamicin. The nephroprotective effect of MEDM was in dose-dependent manner, it was better at higher doses (300 mg/kg) as compared to lower doses (100mg/kg and 200mg/kg).

The nephroprotective effect of MEDM might be because of the free radical scavenging and antioxidant effect of extract. Secondary metabolites such as flavonoids have been reported to have these activities. Alabri *et al*., 2014, and Ratan *et al*., 2011, found the presence of flavonoids in *D. metel* extract (16, 39). In the preliminary phytochemical tests the presence of flavonoids was also detected in MEDM (table 3). Alabri *et al*., 2014, and Hossain *et al*., 2014, has reported that the crude methanolic extracts of *D. metel* caused antioxidant activity due to free radical scavenging effects (16, 17). Mai *et al*., 2017, has isolated different flavonoids such as kaempferol from *D. metel* (11). Kaempferol exhibit antioxidant effect and free radical scavenging activity (18, 40, 41).

Histological examination demonstrated in our study that the histological findings were parallel with that of the biochemical parameters and lipid peroxidation findings. However, our histological and microscopic examination revealed that after administration of MEDM, the histological damages induced by gentamicin were markedly reversed. The reversal of renal damages was observed in dose dependent manner. The recovery of nephrotoxicity was better at 300 mg/kg dose as compared to 100 mg/kg and 200mg/kg.

Russo and his coworkers reported that withanolides possess strong antioxidant activity. Withanolides exert antioxidant effect by scavenging the free radicals which are generated by gentamicin which improves histological architecture of kidney (42). Different researchers have reported the presence of withanolides compounds in *D. metel* plant (43-45). Mai *et al*., 2017, has isolated different flavonoids such as kaempferol from *D. metel* (11). Kaempferol exhibited antioxidant effect and free radical scavenging activity (18). Similarly, Rho *et al*., 2011, reported that kaempferol and its derivative exhibit inhibitory action of NO synthesis (46).

Hence it is concluded from our research that the *D. metel* caused significant renal protective effect in in vivo studies. The effect of *D. metel* may be because of the antioxidant and nitric oxide inhibitory effect of flavonoid, kaempferol which already isolated. Our study has provided the scientific justification that *D. metel* has nephroprotective activity but till now the exact mechanism is not known for this effect. Thus, our study has paved the way for new researchers to further investigate the exact nephroprotective mechanism and identify the chemicals present in *D. metel,* responsible for nephroprotective action for clinical effects.

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