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Nephroprotective Effect of Methanolic Extract of *Lantana Camara* L. against Acetaminophen and Cisplatin-Induced Kidney Injury

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ABSTRACT

Lantana camara L. (Verbenaceae) is a perennial shrub, brought to India some 80 years ago from South America, which has become exotic and spread to different regions of the country. All the parts of this plant are traditionally used for several ailments such as antiseptic, antitumoural and antimicrobial. The current investigation was undertaken to explore the protective effect of methanol extract of *Lantana camara* L. *(MELC)* against acetaminophen and Cisplatin induced acute renal toxicity in rats. In each model of nephrotoxicities, thirty adult male Wistar rats were evenly divided into 5 groups. Groups I and II served as untreated and model controls, respectively while groups III–V were the treatment groups which were pretreated with 200, 400 mg/kg/day of *MELC* and group V was pretreated with Vit-E 1 hr before each dose of the nephrotoxicants (acetaminophen and Cisplatin) for 14 days (acetaminophen induced model) and 5days (Cisplatin induced model). On the 15th day (in acetaminophen) and 6th day (in Cisplatin), blood samples for serum urea, total protein and creatinine while the rat kidneys for histology were obtained under inhaled diethyl ether anesthesia. In the acetaminophen nephrotoxic rats, 200 and 400 mg/kg/day significantly ($p < 0.05$, $p < 0.01$,$p < 0.001$) attenuated elevations in the serum creatinine, total protein and blood urea nitrogen levels in dose related fashion, as well as, attenuation of acetaminophen induced tubulonephrosis. Similar effects were also recorded in the Cisplatin model of acute renal injury. In the near future, *MELC* could constitute a lead to discovery of a novel drug for the treatment of drug-induced nephrotoxicity.

Keywords: *Lantana camara* L. methanolic extract, acetaminophen and Cisplatin induced nephrotoxicities, Protective activities.

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INTRODUCTION

Drugs are a common source of acute kidney injury. Compared with 30 years ago, the average patient today is older, has more comorbidities, and is exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function. Drugs shown to cause nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Therefore, successful prevention requires knowledge of pathogenic mechanisms of renal injury, patient-related risk factors, drug-related risk factors, and preemptive measures, coupled with vigilance and early intervention. Some patient-related risk factors for drug-induced nephrotoxicity are age older than 60 years, underlying renal insufficiency (e.g., glomerular filtration rate of less than 60 mL per minute per 1.73 m2), volume depletion, diabetes, heart failure, and sepsis. General preventive measures include using alternative non-nephrotoxic drugs whenever possible; correcting risk factors, if possible; assessing baseline renal function before initiation of therapy, followed by adjusting the dosage; monitoring renal function and vital signs during therapy; and avoiding nephrotoxic drug combinations.[1-3]

Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication (nephrotoxins are chemicals displaying nephrotoxicity) on the kidneys. A number of antibiotics including the penicillins, cephalosporins, tetracyclines, as well as aminoglycosides and sulfonamides, are potential nephrotoxins. Aminoglycoside nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate (GFR) [4]. Approximately 8% to 26% of patients who receive aminoglycosides for more than 7-10 days (Australian Medicines Handbook) develop mild renal impairment which is almost always reversible. It usually presents as gradually worsening non oliguric renal failure. Severe acute tubular necrosis may occur rarely.

Acetaminophen (APAP), also known as paracetamol, is a widely used nonprescription analgesic–antipyretic agent. The drug is safe at therapeutic doses; however, acute overdose is fairly common and can lead to potentially fatal hepatic and renal damage in humans and experimental animals [5]. Although nephrotoxicity is less common than hepatotoxicity in APAP overdose, acute renal failure can occur even in the absence of liver injury [6-7]. Current evidence suggests that oxidative stress with increased generation of reactive oxygen species, depletion of reduced glutathione (GSH) and lipid peroxidation play a crucial role in the development of

APAP-induced hepatic and renal damage [8-9]. Indeed, several antioxidants were proved effective in protecting the kidneys against the deleterious effect of APAP overdose [10-11]. Heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme catabolism, is important for regulating the adaptive protection of tissues against oxidative stress and inflammation [12-13]. Hemin, the HO-1 inducer, was proved successful in protecting different organs against oxidative and inflammatory injuries in various experimental models [14-17]. Cisplatin (CP) remains the drug of choice in many platinum based chemotherapy regimens. It acts by damaging DNA owing to platination to form covalent platinum DNA adducts [18]. DNA damage elicits a series of signal transduction cascades, involving chromatin remodeling, which eventually lead to DNA repair, cell cycle arrest or apoptosis [19-22]. The therapeutic effects of Cisplatin are dose dependent but the chief limit to its promising efficacy as an antineoplastic drug is its Nephrotoxicity [23-24]. Use of other means to prevent Nephrotoxicity e.g. hydration protocols [25], antioxidant supplementation[26] have proven to be partially successful due to the repeated dosing schedules of CP. Therapeutic interventions aimed at ameliorating renal damage require the use of combination therapies. This indicts the involvement of reactive oxygen species (ROS) generation as a mediator in inflicting nephrotoxic insult in CP therapy. Interaction of Cisplatin with CYP2E1 results in the generation of reactive oxygen metabolites that causes renal injury and initiates apoptosis.

L. camara typically occurs where there is a moderate to high summer rainfall and well-drained sloping sites. Most variants have a preference for fertile organic soils, but some or all can survive on siliceous sands and sandstone-derived soils where these are of moderate depth and other conditions, especially year-round moisture, are suitable. It is a native to tropical regions and exists as dozens of strains and varieties that are highly variable in appearance. Lantana has significant adverse effects on biodiversity. It typically forms dense thickets, suppressing native vegetation and seedlings through shading, nutrient competition, smothering and allelopathy (i.e., chemically suppresses the germination and/or growth of other plant species). All the parts of this plant are traditionally used for several ailments. Leaves of the plant are antiseptic, antitumoural, and antimicrobial[27] whereas, roots are used in the treatment of malaria, rheumatism, and skin rashes[28.] Phytochemically the plant has been reported to contain Flavonoids. *Lantana* plant has been reported to possess a number of medicinal properties[29-30]. Some metabolites isolated from their leaves possess antitumor activity, antithrombin activity, anti-inflammatory, antinociceptive and antipyretic activity [31-35].

However, no work is reported related to nephroprotective effect of *Lantana camara* L. Therefore, the present study was designed to investigate the nephroprotective effect of the

methanolic extract of *Lantana camara* L. on acetaminophen and Cisplatin induced nephrotoxicity in Wistar rats.

MATERIAL AND METHODS

Collection of plant materials

Different parts (flower, leaf, stem, and root) of *L. camara* were collected from Jalpaiguri, W.B.-(India) and was identified at the Department of Botany, The City College, Jiwaji University, Gwalior, (M.P.) and sample specimen no. F/Herb/2010/3408 was submitted. Plant parts were separately shade dried and finely powdered using a blender.

Preparation of *Lantana camara* L. extract.

The dry methanolic extract of *Lantana camara* (MELC) was taken 200mg and 400mg in electronic balance and dissolve in distilled water and 200mg/kg body weight & 400mg/body weight was administered as different dose treatment.

Treatment of animals

Healthy male and female rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioural veterinary examination from Institutional Animal house (955/A/06/CPCSEA). The weight range was fall within ± 20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee.

Sixty young adult male Wistar rats, weighting 120–150 g were obtained from the Institutional Animal House of Gupta College of Technological Sciences. The rats were housed in polyethylene cages in the Animal House. The rats were housed in polyethylene cages, allowed one week of acclimatization, and maintained on standard rat chow and standard laboratory conditions throughout the experiment.

Experimental induction of acetaminophen and acetaminophen nephrotoxicity and their treatments with the extract

The present animal study experiment was conducted in two experimental models of drug-induced nephrotoxicity. In each of the models, the rats were systematically randomized into 5 groups of 6 rats per group such that the differences in the average weights between and within groups do not exceed ±20% of the average weights of all the rats. Twelve to fourteen hours before each of the experiment began, the rats were fasted of feed but distilled water was made available *ad libitum*.

In the acetaminophen model of the experiment, groups I and II rats, that served as untreated and model controls, respectively, were orally administered 10mL/kg/day of normal saline while groups III–IV rats were pretreated with single daily oral doses of 200 and 400 mg/kg of *MELC* while group V treated with Vit-E 1 h before the intraperitoneal injection of 200mg/kg/day of acetaminophen, for 14 days. For group I rats, in place of single daily intraperitoneal dose of 200mg/kg of acetaminophen, 10 mL/kg/day of normal saline was administered, for 14 days. In the Cisplatin model, five days study was conducted where single dose of Cisplatin (7.5 mg/kg body wt) was injected intraperitoneally on the second day in group II-V, group I treated with normal saline and here group V was used as standard control of Cisplatin.[36]

On days 0 and 14 (in acetaminophen treated) and 6 (in Cisplatin treated) of the experiment, the rat weights were measured, respectively, with Mettler weighing balance. The absolute and change in weights in reference to the initial weight per group were calculated.

Biochemical assays

Prior to termination of the experiment on the 14th day (in acetaminophen treated) and 5th days(in Cisplatin treated), the rats were fasted overnight. On the 15th day and 6th day, the fasted rats were sacrificed under diethyl ether anesthesia and blood samples for serum creatinine, total protein and urea were collected into plain sample bottles. Blood samples were obtained by cardiac puncture with 21G needle mounted on 5mL syringe. The animals were analyzed according to standard methods for effect of the extract on various biochemical parameters of rats such as Serum creatinine (CREST BIOSYSTEMS. A Division of Coral Clinical Systems) by Alkaline Picrate method, Total Protein Kit. by Biuret Method (CREST BIOSYSTEMS and blood urea (Span Diagnostics Ltd., Surat, India) by DAM method as well as some ions like sodium, potassium, chloride and uric acid(Table 4 and Table 8).

Histopathological studies of rat kidneys

Kidneys of sacrificed animals were identified and carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4–5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X).

Statistical analysis

Results are expressed as the mean value ± standard error of mean (S.E.M.). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant

difference between control and experimental groups was assessed by student's t-test. A probability level of less than 5 %($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Effect of 14 days of oral administration of 200-400mg/kg/day Of *MELC* on the average body and its kidney weight of acetaminophen nephrotoxic rats

Table 1 and Table 2 shows effect of single, daily 200-400mg /kg/day of oral *MELC* on the average weight in acetaminophen (200mg/kg) nephrotoxic rats treated for 14 days. As indicated in the table, repeated intraperitoneal injection with acetaminophen induced significant ($p < 0.05$) progressive weight loss in the treated rats. However, weight loss was significantly ($p < 0.01$, $p < 0.001$) enhanced by *MELC* treatment in dose related fashion and the other hand, kidney weight also changes significantly (* $P < 0.05$. ** $P < 0.01$ ***$P<0.001$) by acetaminophen treatment while recovery was seen by *MELC* treatment.

Table 1: Effect of 200mg/kg/day intraperitoneal acetaminophen and graded oral doses of *MELC* on the average body weight

Group	Average body weight (g) on	
	Day 0	Day 15
I	149.82 ± 10.19	159.83 ± 8.23
II	150.33 ± 7.58	138.67 ± 7.87*
III	151.83 ± 10.26	142.83 ± 8.82*
IV	150.33 ± 7.53	146.67 ± 7.63*
V	153.00 ± 12.00	150.50 ± 4.89**

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows: * $P < 0.05$. ** $P < 0.01$. I = control group, II = acetaminophen only treated group, III = acetaminophen (200mg/kg b.w.) + 200mg/kg.b.wt. *MELC*, IV = acetaminophen (200mg/kg b.w.) + 400mg/kg.b.wt. *MELC*, V = acetaminophen (200mg/kg b.w.) + 250mg/kg.b.wt. Vitamin-E.

Table 2: Effect of 200mg/kg/day intraperitoneal acetaminophen and graded oral doses of *MELC* on the average kidney weight

Dose group	Wet Weight (g; mean ± S.D.; n=6) Kidney
ControlD_0 (0g/kg)	1.8 ± 0.05
Control D_{14} (0g/kg)	1.9 ± 0.13
Acetaminophen (D_{14}).	1.2 ± 0.03***
Acetaminophen + 200mg/kg b.w.(D_{14}).	1.4 ± 0.55
Acetaminophen + 400mg/kg b.w.(D_{14})	1.6± 0.09***
Acetaminophen +Vitamin-E.(D_{14}).	1.8±0.25***

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows: * $P < 0.05$. ** $P < 0.01$ ***$P<0.001$.

Effect of 5 days of oral administration of 200–400mg/kg/day of *MELC* on the average body and its kidney weight of Cisplatin nephrotoxic rats

Table 5 and Table 6 shows effect of single, daily 200–400mg/kg/day of oral *MELC* on the average weight of Cisplatin nephrotoxic rats treated for 5 days. As shown in the table also, daily intraperitoneally injection with 200 mg/kg cisplatin for 5 days induced significant ($p < 0.05$) progressive weight loss in group II rats. Equally, weight loss was significantly ($p < 0.01$, $p < 0.001$) enhanced by the extract in dose related fashion and on the other hand, kidney weight also changes significantly (* P < 0.05. ** P < 0.01 ***P<0.001) by Cisplatin dose while recovery was seen by *MELC* treatment.

Table 3: Effect of 7.5 mg/kg/day intraperitoneal Cisplatin and graded oral doses of *MELC* on the average body weight

Group	Average body weight (g) on	
	Day 0	Day 6
I	138.17 ± 7.39	145.17 ± 7.81
II	132.00 ± 9.17	119.33 ± 7.34*
III	133.83 ± 12.09	122.00 ± 7.64*
IV	136.17 ± 9.70	126.83 ± 5.74*
V	135.50 ± 7.99	134.24 ± 5.57***

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01. ***P < 0.001. I = control group, II = Cisplatin only treated group, III = Cisplatin (7.5mg/kg b.w.) + 200mg/kg.b.wt. *MELC*, IV = Cisplatin (7.5mg/kg b.w.) + 400mg/kg.b.wt. *MELC*, V = Cisplatin (7.5mg/kg b.w.) + 250mg/kg.b.wt. Vitamin-E.

Table 4: Effect of 7.5mg/kg/day intraperitoneal cisplatin and graded oral doses of *MELC* on the average kidney weight

Dose group	Wet Weight (g; mean ± S.D.; n=6) Kidney
ControlD_0 (0g/kg)	1.6 ± 0.05
Control D_6 (0g/kg)	1.7 ± 0.13
Cisplatin (D_6).	1.24 ± 0.03**
Cisplatin + 200mg/kg b.w. (D_6).	1.32± 0.55***
Cisplatin + 400mg/kg b.w.(D_6)	1.45 ± 0.59
Cisplatin +Vitamin-E. (D_6).	1.67 ±0.25**

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01. ***P<0.001.

Effect of graded oral doses of *MELC* on blood urea nitrogen, total protein and serum creatinine concentrations in acetaminophen-nephrotoxic rats for 14 days

Table 3. also shows that single daily intraperitoneal administration of 200mg/kg/day of acetaminophen for 14 days induced significant ($p < 0.05$) rise in serum creatinine, total protein and blood urea levels in the acetaminophen treated rats (group II rats). However, elevations in the serum creatinine, total protein and blood urea were significantly ($p< 0.01$, $p < 0.001$)

attenuated by *MELC* pretreatments, in dose related fashion and no treatment related effect on uric acid and ions (Figure 1-3). However, the levels of uric acid as well as some ions like sodium, potassium and chloride were not affected by the administration of the root extract. (Table 5 and 6).

Histopathological examination of sections from rat kidney treated with Acetaminophen show severe and generalized tubular epithelial cell necrosis associated with diffuse tubular lumina. Kidney sections from rats pretreated with 200, 400-mg/kg body weight doses of *MELC* before Acetaminophen administration showed generalized degeneration and necrosis of tubular epithelial cells; diffuse moderate degeneration and necrosis of tubular epithelial cells; and generalized and severe degeneration and coagulative necrosis of tubular epithelial cells respectively with dilated hypocellular tubules and proteinaceous material in tubular lumina in all treatment groups. The 400 mg/ kg dose of *MELC* provided the best histological protection against Acetaminophen renal tubular damage. The Vitamin-E & Acetaminophen treated kidney shown histological protection against Acetaminophen renal tubular damage which was used as standard nephroprotective for comparison with the test component. **(Figure 4a-e)** [37]

Table 5: Effect of 200mg/kg/day intraperitoneal acetaminophen and graded oral *MELC* on serum creatinine, total protein and blood urea in treated rats for 14 days

Parameters	Groups				
	Control	Acetaminophen	Acetaminophen +200mg Extract	Acetaminophen +400mg extract	Acetaminophen Vitamin E
Creatinine	0.85±0.09	4.33 ±0.27**	3.66 ±0.15**	2.2 ±0.10**	1.2 ±0.12**
Total Protein	3.60±0.12	5.67 ±0.06**	5.21 ±0.06*	4.3 ±0.12	3.2 ±0.15
Blood Urea	16.34±1.26	40.03±3.05**	30.36±2.25**	20.94 ±1.23*	17.92±1.45**

The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

Table 6: Effect of *MELC* on some kidney function parameters of rat on acetaminophen treatment.

Groups	Dose	Na+(mmol/L)	K+(mmol/L)	CL(mmol/L)	HCO-3	Uric acid
I	Control	152.40±2.24	3.92±0.27	83.60±0.61	33.10±0.77	0.31±0.01
I I	Acetaminophen	138.75±2.65	2.95±0.17	87.00±0.54	34.55±1.02	0.49±0.03
I I I	Acetaminophen + 200mg extract	144.25±1.49	3.21±0.10	84.50±2.69	33.50±0.49	0.33±0.02
IV	Acetaminophen + 400mg extract	146.00±2.13	3.71±0.14	85.20±1.58	33.20±0.46	0.30±0.03
V	Acetaminophen+ 250mg Vit-E	150.33±1.84	3.91±0.25	84.33±0.56	33.00±0.36	0.31±0.04

Not significant when compared with control P>0.05. Data are expressed as mean ± SEM (n= 6)

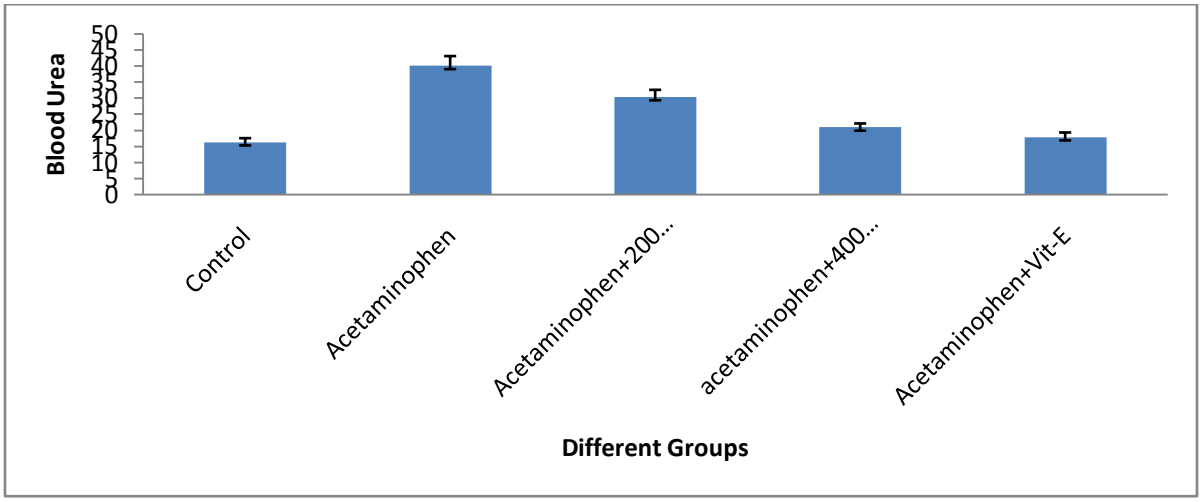


Figure1: Effect of *MELC* extract on blood urea concentrations in rats treated with acetaminophen.

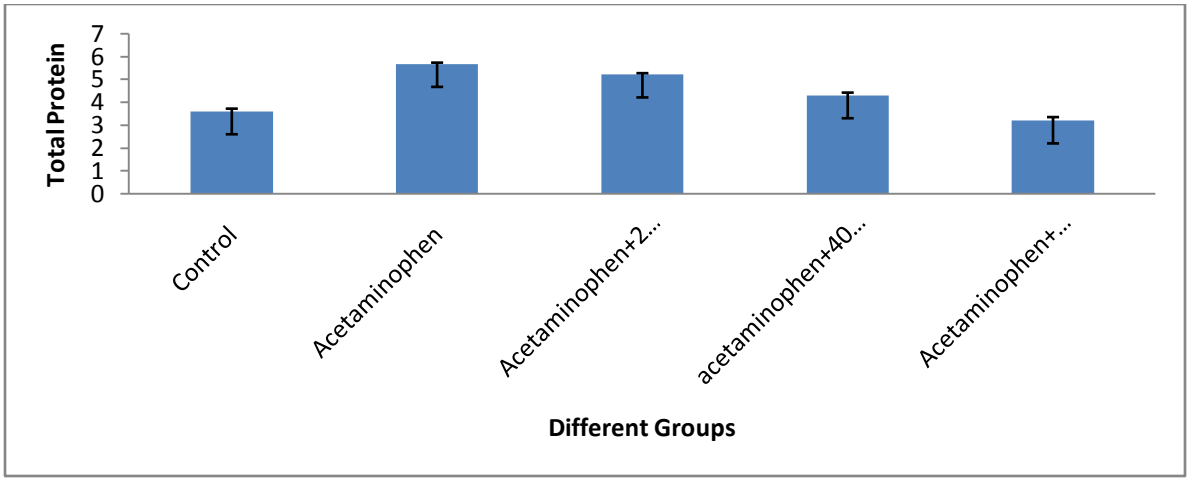


Figure 2: Effect of *MELC* extract on total protein concentrations in rats treated with acetaminophen.

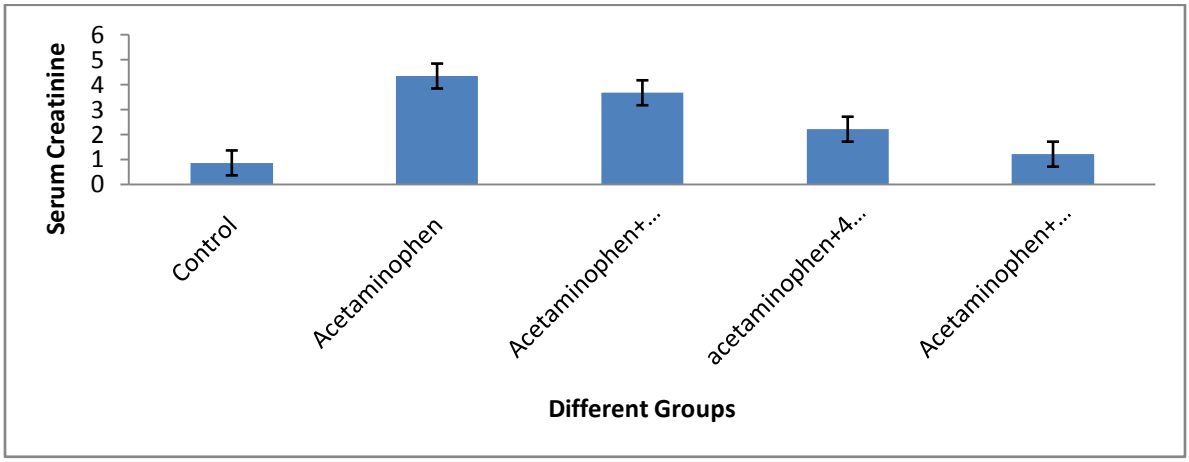
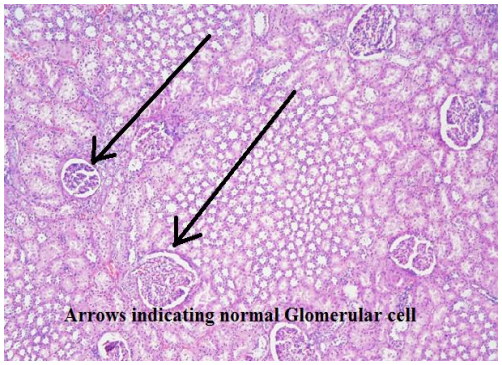
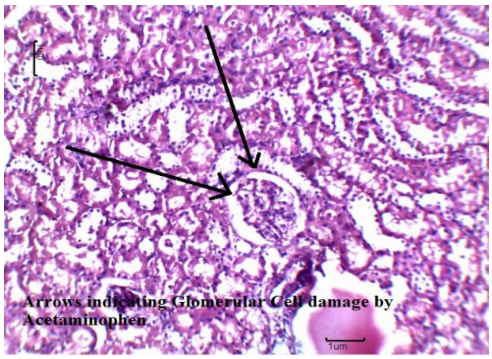


Figure 3: Effect of *MELC* extract on serum creatinine concentrations in rats treated with acetaminophen.

(Magnification-40x, Haematoxylin and eosin stain)



(a)



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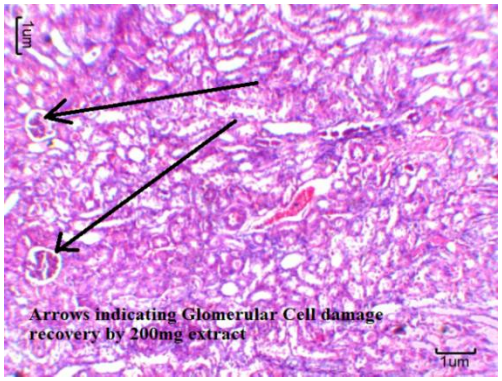

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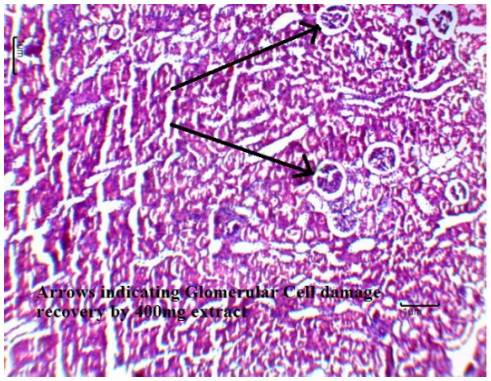

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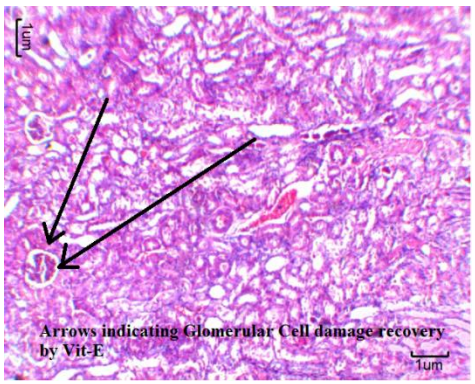


(e)

Figure. 4(a-e). Effects of the *MELC* on Kidney histomorphology in rat. Figure4a: control. Figure4b: treatment with 200mg/kg b.w. acetaminophen 7 day p.o. Figure 4c: treatment with 200mg/kg b.w. acetaminophen and 200mg/kg b.w. *MELC* 7 day p.o. Figure4d: treatment with 200mg/kg b.w. acetaminophen and 400mg/kg b.w. *MELC* 7 day p.o Figure4e: treatment with 200mg/kg b.w. acetaminophen and 250mg/kg b.w. vitamin-E 7 day p.o (40X)

Effect of graded oral doses of *MELC* on blood urea nitrogen, total protein and serum creatinine concentrations in Cisplatin-nephrotoxic rats for 5 days

Table 7 shows the effects of varying oral doses of *MELC* on the circulating serum creatinine, total protein and blood urea concentrations in Cisplatin treated rats. As shown in the table, single daily intraperitoneal administration of 7.5 mg/kg Cisplatin for 5 days was associated with significant ($p < 0.05$) elevations in the circulating levels of serum creatinine, total protein and blood urea in the group II rats (Table 7). However, the significant elevations in the serum concentrations of these measured parameters were significantly ($p < 0.01$, $p < 0.001$) attenuated by the oral doses of *AB*, [36] also in dose dependent manner and no treatment related effect on uric acid and ions. However, the levels of uric acid as well as some ions like sodium, potassium and chloride were not affected by the administration of the extract (Table 8)(Figure 5,6 and 7).

Table 7: Effect of 7.5mg/kg/day intraperitoneal cisplatin and graded oral *MELC* on serum creatinine, total protein and blood urea in treated rats for 5 days

Parameters	Groups				
	Control	Cisplatin	Cisplatin+100mg Extract	Cisplatin+200mg extract	Cisplatin + Vitamin- E
Creatinine	5.38 ±0.13	8.83 ±0.12**	7.01 ±0.11**	6.43 ±0.14**	5.22 ±0.15
Total Protein	8.71 ±0.11	10.02±0.19**	9.08 ±0.13**	8.26 ±0.12*	8.36 ±0.01**
Blood Urea	108.22±2.22	153.22±3.5**	134.60 ±2.11**	120.04 ±3.56	110.24±2.43

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

Table 8: Effect of *MELC* on some kidney function parameters of rat on Cisplatin treatment.

Groups	Dose	Na+(mmol/L)	K+(mmol/L)	CL(mmol/L)	HCO-3	Uric acid
I	Control	160.40±2.24	4.70±0.27	81.60±0.61	25.20±0.77	0.43±0.02
I I	Cisplatin	149.75±2.65	3.53±0.17	86.00±0.54	26.55±1.02	0.41±0.02
I I I	Cisplatin + 100mg extract	156.25±1.49	3.46±0.10	84.50±2.69	25.40±0.49	0.54±0.02
IV	Cisplatin + 200mg extract	159.00±2.13	4.43±0.14	86.20±1.58	25.50±0.46	0.32±0.02
V	Cisplatin + 250mg Vit-E	165.33±1.84	4.51±0.25	85.31±0.56	25.00±0.36	0.42±0.03

Not significant when compared with control P>0.05. Data are expressed as mean ± SEM (n = 6).

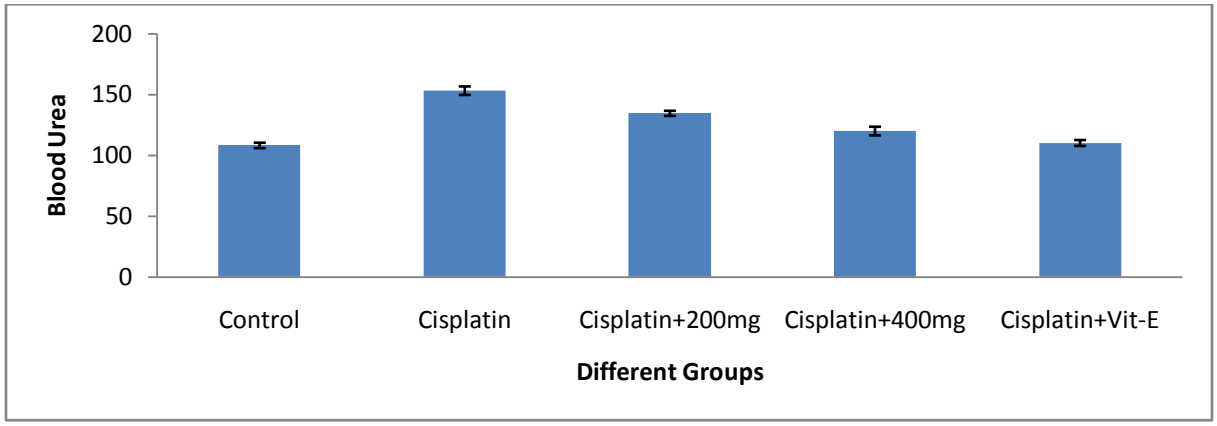


Figure 5: Effect of *MELC* extract on blood urea concentrations in rats treated with cisplatin.

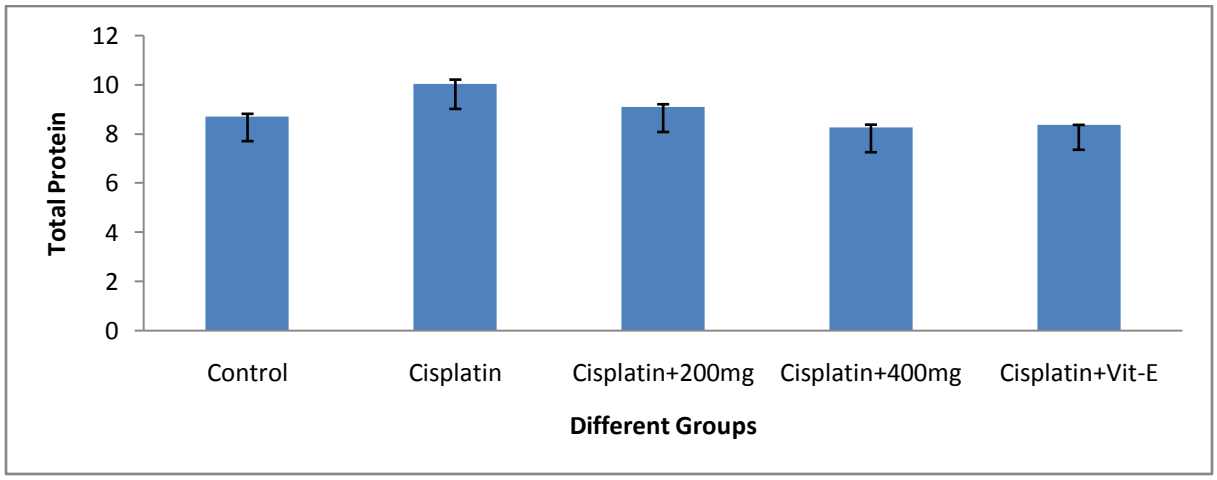


Figure 6: Effect of *MELC* extract on total protein concentrations in rats treated with Cisplatin.

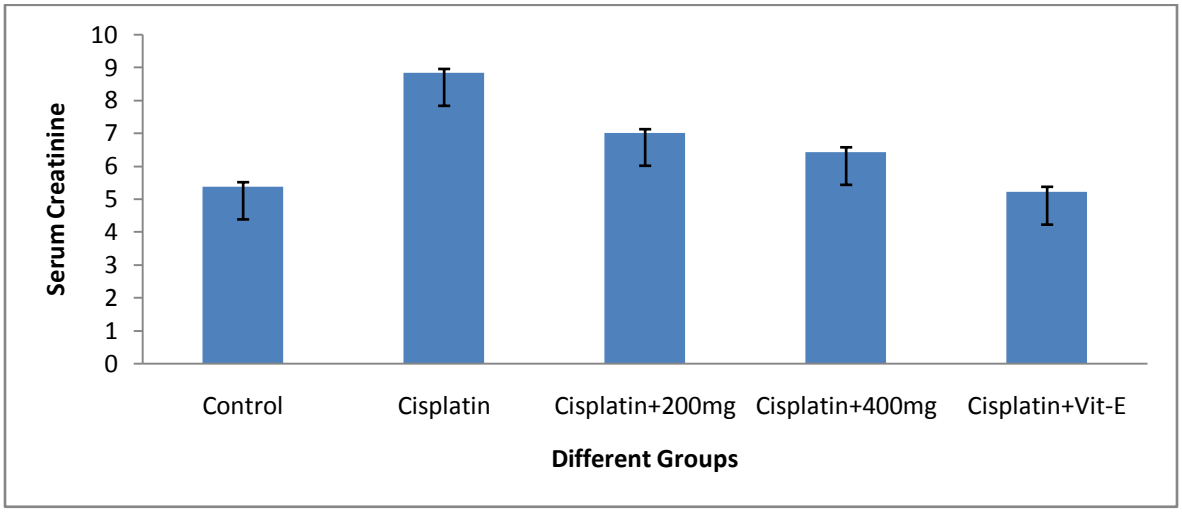
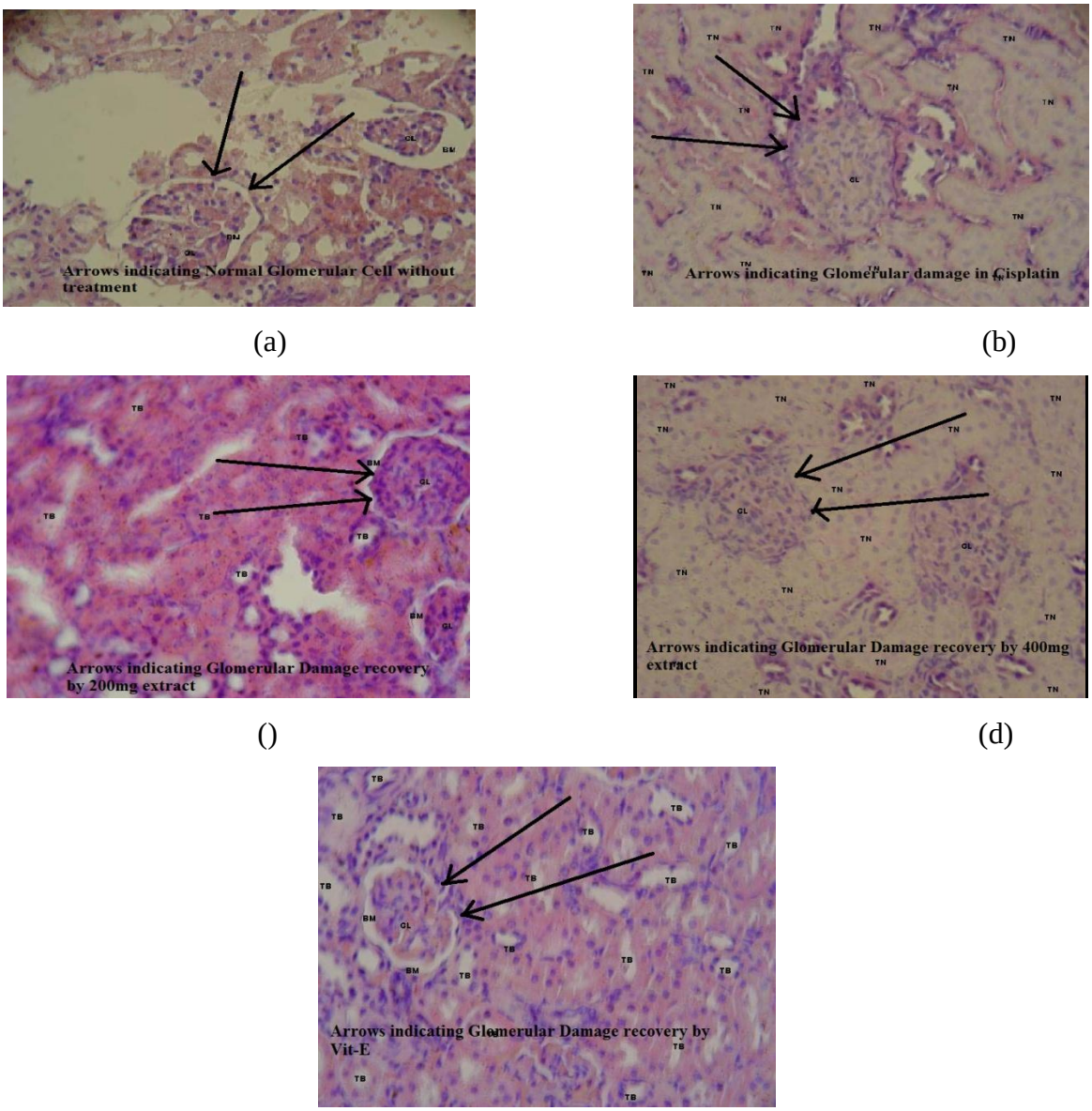


Figure 7: Effect of *MELC* extract on serum creatinine concentrations in rats treated with Cisplatin.

(Magnification-40x, Haematoxylin and eosin stain)



(a) (b)

() (d)

(e)

Figure. 8(a-e). Effects of the *MELC* on Kidney histomorphology in rat. Figure8a: control. Figure8b: treatment with 7.5mg/kg b.w. Cisplatin 5 day p.o. Figure 8c: treatment with 7.5mg/kg b.w. Cisplatin 5 day p.o and 200mg/kg b.w. *MELC* 5 day p.o. Figure8d:treatment with 7.5mg/kg b.w. Cisplatin 5 day p.o and 400mg/kg b.w. *MELC* 5 day p.o Figure8e:treatment with 7.5mg/kg b.w. Cisplatin 5 day p.o and 250mg/kg b.w. vitamin-E 5 day p.o (40X).

Histopathology Photomicrographs of kidney sections from various treatment groups are shown here. Histopathological examination of sections from rat kidney treated with Cisplatin show severe and generalized tubular epithelial cell necrosis associated with diffuse tubular Lumina

(hyalinized casts). Kidney sections from rats pretreated with 200, 400-mg/kg body weight doses of *MELC* before Cisplatin administration showed generalized degeneration and necrosis of tubular epithelial cells; diffuse moderate degeneration and necrosis of tubular epithelial cells; and generalized and severe degeneration and coagulative necrosis of tubular epithelial cells respectively with dilated hypocellular tubules and proteinaceous material in tubular Lumina in all treatment groups. The 400 mg/ kg dose of *MELC* provided the best histological protection against Cisplatin renal tubular damage. The Vitamin-E & cisplatin treated kidney shown histological protection against Cisplatin renal tubular damage which was used as standard nephroprotective for comparison with the test component (Figure 8a-e).

Acetaminophen (popularly called Paracetamol, brand names Tylenol® in US and Panadol® in UK) is an effective, well-tolerated, household, over-the-counter analgesic and antipyretic alternative to aspirin. Its ingestion in large doses or chronic use is commonly associated with hepatotoxicity and nephrotoxicity in humans and animals [38]. The clinical usage of cisplatin is limited due to its renal toxicity. Establishing the gene expression patterns and understanding the mechanism of cisplatin-induced toxicity should allow earlier identification of clinically relevant toxicological findings in compound screening, and aid in the development of therapeutics to reduce nephrotoxicity. Thus, acetaminophen- and acetaminophen-induced nephrotoxicies are well established experimental models of drug-induced renal injury [39-40]. Many animal experiments have demonstrated overwhelmingly, the positive correlation between oxidative stress and Nephrotoxicity [41].

Acetaminophen nephrotoxicity results from the toxic effects of its highly reactive intermediate metabolite, *N*-acetyl-*para*-amino-benzoquinoneimine (NAPQI), which arylates proteins (specifically selenium-binding protein and glutamine synthetase) in the S3 segment of the proximal tubule, initiating cell death of renal tubular cells[42-43].These drug-induced nephrotoxicities are often associated with marked elevations in blood urea nitrogen, serum creatinine and acute tubular necrosis [44]. Thus, biochemical parameters such as blood urea, serum creatinine, creatinine clearance, enzyme urea and urinary excretion of β_2-microglobulin have been used to investigate drug-induced nephrotoxicity in animals and man [45]. Cisplatin is known to accumulate in mitochondria of renal epithelial cells [46] and induces ROS primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH [47] and also causes the peroxidation of membrane lipids [48]. Cisplatin covalently binds to DNA bases and disrupts DNA functions. The cytotoxic action of the drug is often thought to be associated with its ability to bind DNA to form cisplatin–DNA adducts [49]. Cisplatin induced

oxidative stress can activate some protein kinases (MAPKs) c-Jun N-terminal kinase (JNK) and p38 which sensitize the injured cell to apoptosis. In the present study, drug-induced nephrotoxicities were established by single daily intraperitoneal injection of the nephrotoxins, acetaminophen and Cisplatin, for 14 days and 5days respectively. These toxicities were characterized by marked ($p < 0.05$) elevations in the circulating levels of blood urea nitrogen and serum creatinine and histological features of tubulonephritis in the model control (group II) rats when compared to untreated (group I) rats. However, these changes were attenuated by pretreatments with single, daily, graded oral doses of *MELC*, in dose related fashion. *MELC* at the oral doses of 400mg/kg/day significantly ($p < 0.01$ and $p < 0.001$) lowered acute elevations in the serum concentrations of blood urea nitrogen and serum creatinine, maintaining their values within the normal range when compared to the 200mg/kg/day of *MELC* treated rats and model control rats. Apart from the direct nephrotoxic effect of acetaminophen and Cisplatin in group II rats, the acute elevations in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolonged anorexia associated with acetaminophen and Cisplatin nephrotoxicities.

Also significant are the histopathological findings of the present study which were in accord with the histological lesions recorded for acetaminophen- and cisplatin-induced renal lesions [(50-51)]. However, our present histological findings of the effects of the *MELC* on the renal architecture were at variance with that of. The observed variance could be attributed to differences in the doses of administered extract, animal models and duration of drug exposure. Beside these, several independent independent animal [51] and human studies have reported and confirmed the high safety profile of the plant extracts, which seem to be in accord with our findings. However, more studies would be needed in this area. In addition, previous phytochemical studies of *MELC* have reported the isolation and structural determination of Flavonoids. Taking into account that flavonoids is present in the *MELC* to be responsible for the nephroprotective activity. It is also possible for the extract to be replenishing renal glutathione storage. Thus, the present study seems in accord with the earlier report. The weight losing effects of plant extracts rich in saponin and tannins are well documented in literature. The results of the investigation reveal that the *MELC* possessed significant protective effect against both Cisplatin and acetaminophen induced Nephrotoxicity and the effect was found to be in a dose dependent manner. The present study demonstrates that Cisplatin and acetaminophen induce renal injury as evident from the elevated serum urea, total protein and creatinine levels and also from the histopathological features of

acute renal failure. Treatment with *MELC* restored the elevated serum urea and creatinine level, indicating its significant nephroprotective effect.

CONCLUSION

In conclusion, the results of the present studies indicate that the aqueous extract of *MELC* possesses profound nephroprotective activity. The experimental results also reveal that the nephroprotective activity of the extract is comparable to that of vitamin E. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. In the near future, *MELC* could constitute a lead to discovering a novel drug which will be useful in treatment of drug-induced Nephrotoxicity.

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