

## Effect of *Mulaka (Raphanus sativus Linn.) panchanga* and *Mulaka kshara* against cisplatin-induced nephrotoxicity

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Ayurveda has given prime importance to *Mutravaha srotas* (urinary system) and *Srotogata vikaras* (urinary disorders). Acharyas of Ayurveda has described many aspects of *Mutravaha srotasa* (urinary system) and described diseases pertaining to it. *Mulaka (Raphanus sativus Linn.)* belongs to family Brassicaceae, traditionally being used in kidney disorders. *Mulaka* was firstly introduced in *Charak Samhita* and used for urinary system related problems. Hence, present study was planned with an aim to prove the traditional claims on Ayurvedic formulations of *Mulaka* i.e., powder of *Mulaka panchanga* and *Mulaka kshara*. *Mulaka panchanga* (450 and 900 mg/kg) and *Mulaka kshara* (90 and 180 mg/kg) were evaluated for nephroprotective activity by using cisplatin (5 mg/kg, ip) –induced nephrotoxicity in albino rats. Cisplatin increases the levels of blood urea, uric acid and serum creatinine which suggest the kidney damage in rats. Cisplatin-induced the hepatotoxicity was confirmed by increase in the level of serum transaminases, alkaline phosphatase and bilirubin with increase in the level of serum cholesterol and triglyceride. Both formulations reversed the serum biochemical parameters and urine creatinine levels with an increase in the urine volume. Further, powder of *Mulaka panchanga* and *Mulaka kshara*, attenuated the level of free radicals with concomitantly increase in antioxidant parameters in kidney tissue homogenate and thus, protect the kidney cytoarchitecture in histopathological study. The result of present study indicates that *Mulaka panchanga* and *Mulaka kshara* both having nephroprotective and antioxidant properties in dose dependent manner in cisplatin-induced nephrotoxicity in rats.

**Keywords:** Cisplatin, *Mulaka*, *Mulaka kshara*, *Mulaka panchanga*, Nephrotoxicity

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Renal disorders have always remained a major area of concern for health. These days, use of analgesic, Nonsteroidal anti-inflammatory drugs (NSAID), antidepressant, antibiotic and chemotherapy agents carry adverse drug effects on kidney. Kidneys are frequently exposed to these drugs and/or toxic

them which belongs to family Brassicaceae and traditionally used in kidney disorders. The important Ayurvedic formulations of *Mulaka*, widely used in clinical practice for urinary disorders are *Mulaka panchanga* and *Mulaka kshara* mentioned in classics. Further, Ayurvedic Formulary of India mentioned *Mutrakreccha* (dysuria), *asmari* (calculus), *gulma* (abdominal lump) and *vatavikara* (disorder due to *vata dosa*). The dose of *Mulaka kshara* is 1g per day<sup>3</sup>.

toxicity results in serious clinical syndromes<sup>1</sup>. *Charaka Samhita* contains several sections on urologic ailments. Acharyas of Ayurveda has described many aspects of *Mutravaha srotasa* and described many diseases pertaining to *Mutravaha srotasa* (urinary system) like *Mutraghata* (urinary obstruction), *Mutrakricchra* (dysuria) and *Mutrasmari* (urinary calculus). Many herbs have been proven to be effectual as nephroprotective agents<sup>2</sup> while, many more are claimed in the texts. *Mulaka (Raphanus sativus Linn.)* one of

Chemotherapy has been recognized to be efficacious in cancer treatment. Those agents usually demolish the physiological homeostasis in various organs during treatment of cancer. Physiological side effects can occur that are induced in non tumor cells mostly by radical formation and oxidant injury<sup>4</sup>. Cisplatin is one of the most potent chemotherapeutic antitumor drugs which is inorganic platinum compound with a broad spectrum antineoplastic

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activity against various types of tumors<sup>5</sup>. The chief dose limiting side effect of cisplatin is cumulative nephrotoxicity in man and in animals. Cisplatin is causing nephrotoxicity by recruiting oxidative stress, inflammation and cell death pathways<sup>6</sup>.

Various medicinal plants that possessing nephroprotective activity may attenuate the kidney toxicity when co-administrated along with different nephrotoxic agents. In this background, it was thought worthwhile to evaluate the drugs which could be useful as adjuvant as nephroprotective agent. Further, rationale behind the concept on traditional uses of *Mulaka* (*Raphanus sativus* Linn.) has not well established through systematic pharmacological studies. Therefore, present study was planned to evaluate the *Mulaka panchanga* powder and *Mulaka kshara* for nephroprotective activity against cisplatin-induced nephrotoxicity in wistar albino rats.

## Materials and Methods

### Drugs and chemicals

*Mulaka* (*Raphanus sativus* Linn.) was cultivated at organic farm "Ram Vadi" of Anandabava Aashram, Jamnagar, Gujarat, India. The fresh matured samples of *Mulaka* was identified by Botanical Survey of India, Howrah with specimen No. VS 02 dated 30 March 2015 and verified by the name of *Raphnus sativus* Linn. Family: Brassicaceae. Two different dosage forms, *Mulaka panchanga* powder and *Mulaka kshara* were taken for experimental study. *Kshara* was prepared with modified reference to Ayurvedic Formulary of India. *Mulaka panchanga* leaned with water, cut into small pieces and allowed to dry. After complete drying, the *Mulaka panchanga* was subjected for ash preparation. Obtained ash was used for *Kshara jala* preparation by addition of water in 6:1 ratio and kept the solution overnight. Next day, the supernant was collected by filtering through muslin cloth and collected solution was evaporated completely to obtain *Mulaka kshara*. All other chemicals used were of analytical grade.

### Animals

Wistar albino rats of either sex weighing between 200±20 g were procured from the Animal house attached to the Pharmacology laboratory, IPGT&RA, Gujarat Ayurved University. The animals were maintained as per standard husbandry conditions in terms of temperature (22±3°C), relative humidity (50–60%) and 12 h light and dark cycle. The selected animals were kept under acclimatization for

7 days before experiment started. Animals were fed with standard rat pellet feed and drinking water was given *ad libitum*. The experiments were carried out after approval of Institutional Animal Ethics Committee (IAEC/15/2013/43) in conformity with the guidelines of the CPCSEA, New Delhi.

### Dose calculation

Dose of the drug was fixed by extrapolating the human dose to laboratory animals on the basis of body surface area ratio as per the table of Paget and Barnes (1964)<sup>7</sup> as given in Table 1.

### Experimental design

Wistar albino rats of either sex weighing between 200±20 g were divided into six groups, each consists of six rats (n=6). Group (I) kept as normal control group received distilled water (10 mL/kg, po), Group (II) was kept as cisplatin control (CG), received distilled water (10 mL/kg, po). Group (III) and (IV) treated with *Mulaka kshara*, at oral dose levels of 90 and 180 mg/kg (MKL and MKH) respectively. Group (V) and (VI) treated with *Mulaka panchanga* powder, at oral dose levels of 450 and 900 mg/kg (MPL and MPH) respectively.

The test drugs and distilled water to respective groups were administered for 10 days. On the 5<sup>th</sup> day of experiment, cisplatin was injected intraperitoneally at the dose of 5 mg/kg to all groups (II to VI) of animals except to normal control group (I). On 10<sup>th</sup> day, all rats were hydrated (2 mL/100 g) and kept under metabolic cages for urine collection for 24 h. The animals were weighed again on 11<sup>th</sup> day, blood was collected from retro orbital plexus under light ether anesthesia for estimation of serum biochemical parameters. Thereafter, animals were sacrificed and important organs were dissected out, cleaned off and wet weight was noted down. Kidney sample was transferred to 10% buffered formalin solution and sent for histopathological study while, other kidney collected for tissue homogenate parameters.

Serum biochemical parameters were carried out using fully automated biochemical random access analyzer (BS-200, Lilac Medicare Pvt. Ltd.,

Table 1 — Animal dose of test drugs

Drugs	Human dose (g/day)	Animal dose (mg/kg)
<i>Mulak panchangapowder</i>	5 g/day	450 mg/kg
<i>Mulak kshara</i>	1 g/day	90 mg/kg

Mumbai). The parameters were blood sugar, serum cholesterol, triglycerides, HDL-cholesterol, SGPT, SGOT, blood urea, uric acid, total protein, calcium, albumin, globulin, total bilirubin, direct bilirubin, creatinine, and alkaline phosphatase.

The kidneys were dissected out immediately, chilled and perfused with ice-cold saline. After washing with ice-cold saline, the kidneys were patted dry and weighed, and divided into fragments. One fragment was used to prepare homogenate (2.5% w/v) in normal saline. The homogenates were centrifuged at  $4000 \times g$  for 10 minutes at  $4^{\circ}C$  and the supernatants were used for the total protein<sup>8</sup>, lipid peroxidase<sup>9</sup>, catalase<sup>10</sup>, nitric oxide<sup>11</sup>, lactase dehydrogenase<sup>12</sup>, superoxide dismutase<sup>13</sup> and alkaline phosphatase<sup>14</sup>. Second fragment homogenized (5% w/v) in 3% metaphosphoric acid solution for total glutathione<sup>15</sup> and glutathione peroxidase<sup>16</sup>. For histopathological study, kidney was carefully dissected, cleaned of extraneous tissue and was transferred to 10% buffered formalin solution for fixation for histopathological studies.

### Statistical analysis

The data are expressed as mean±standard error of mean for six rats per experimental group. One way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple t-test and Students t-test for unpaired data as applicable to determine significant difference between groups at  $p < 0.05$ .

### Result and Discussion

Cisplatin (cis-diamminedichloroplatinum II,) is an inorganic complex formed by an atom of platinum

surrounded by chloride and ammonia atoms in cis- position of a horizontal plane. It is a major anti- neoplastic drug for the treatment of various forms of cancers, with cisplatin therapy the major organs accumulating platinum are the kidneys, the liver and the spleen. In the kidney, cisplatin causing nephrotoxicity is there by recruiting oxidative stress, inflammation, and cell death pathways<sup>17</sup>. The exact mechanisms of cisplatin-induced nephrotoxicity are still not fully understood. However, lipid peroxidation and free radical generation in the tubular cells have been suggested to be responsible for the nephrotoxicity<sup>18</sup>.

Cisplatin treatment resulted in significant reduction in body weight, significant increase in relative weight of kidney and liver in comparison to control group. Prior treatment with drugs resulted in protection against the cisplatin-induced deleterious effects on general health and relative weight of kidney and liver. *Mulaka kshara* at both dose levels produced significant decrease while, *Mulaka panchanga* powder produced non-significant decrease in relative weight of kidney in comparison to cisplatin control group (Table 2). Cisplatin-induced weight loss, already reported by previous authors, may be due to gastrointestinal toxicity or by lessened ingestion of food<sup>19</sup>.

The plasma concentrations of urea and creatinine determine renal function and are thus, biomarkers for kidney disease. In present study, the increased levels of blood urea, uric acid and serum creatinine and urine creatinine due to administration of cisplatin in rats signify the severe kidney damage in rats<sup>20</sup>. *Mulaka kshara* at both the dose levels produced significant decrease while, *Mulaka panchanga* produced non-significant decrease in serum creatinine

Table 2 — Effect of test drugs on the body weight and relative weight of organs in cisplatin-induced kidney damage in rats

Parameters	NC	CC	MKL	MKH	MPL	MPH
Initial body weight (g)	210.00±5.34	197.00±5.79	204.00±4.03	204.66±6.86	197.33±7.58	211.00±4.05
Final Body weight (g)	207.00±5.90	179.33±7.11 <sup>@#</sup>	173.33±6.48 <sup>@</sup>	170.00±7.60 <sup>@</sup>	177.33±9.08 <sup>**</sup>	187.66±7.80
Heart (g/100g body weight)	0.308±0.007	0.296±0.011	0.354±0.016 <sup>*</sup>	0.334±0.013	0.322±0.018	0.276±0.019
Liver (g/100g body weight)	2.873±0.113	3.305±0.105 <sup>###</sup>	3.236±0.102	3.163±0.237	2.888±0.126 <sup>*</sup>	3.121±0.052
Kidney (g/100g body weight)	0.694±0.009	0.897±0.023 <sup>####</sup>	0.792±0.035 <sup>*</sup>	0.805±0.031 <sup>*</sup>	0.855±0.055	0.875±0.031
	01.42↓	09.13↓	15.03↓	16.93↓	10.13↓	11.05↓

Data: Mean±SEM; \* $p < 0.05$ , \*\* $p < 0.01$ , \* $p < 0.001$  when compared with initial body weight (Paired 't' test); # $p < 0.02$ , ## $p < 0.05$ , #### $p < 0.001$  when compared with normal control group (Unpaired t-test).

and blood urea level in comparison to cisplatin control group. However, drugs did not affect the serum uric acid and urine creatinine level in comparison to cisplatin control group (Table 3).

Cisplatin administration in rats non-significantly decreases the blood glucose level and significantly increased the serum cholesterol and triglyceride level in comparison with control group. The results suggest the deteriorating role of cisplatin on carbohydrates and lipid metabolism due to its liver toxicity in rats. *Mulaka panchanga* powder at both dose levels significantly revert the blood glucose level while, triglyceride level was significantly decreases by *Mulaka kshara* in doses dependent manner. HDL-cholesterol level was significantly increase by *Mulaka kshara* at both dose levels and powder at higher dose level. Previous report also suggests that, *Raphanus sativus* can decrease the plasma cholesterol, triglyceride and phospholipids in normal rats. Consequently in the present study, the significant decrease in albumin may be evidence on cisplatin-induced nephrotoxicity. The significant alleviation in albumin level due to prior administration of test drugs also provides the evidence of protective role of *Mulaka* to alleviate nephrotoxicity (Table 3).

Oxidative stress is one of the key mechanisms by which cisplatin perpetuate renal tissue injury<sup>21</sup>. Therefore, present study investigated the various endogenous markers of oxidative stress and anti-oxidant parameters in kidney tissue homogenate.

Treatment of rats with cisplatin results in a significant increase in the activity of calcium-independent nitric oxide synthase in kidney and liver leading to enhanced nitric oxide formation<sup>22</sup>. Stress to the epithelial cells caused an increase in immune detectable inducible NO synthase (iNOS). Nitric oxide is known to react with the superoxide radical, forming peroxynitrite, an even more potent oxidizing agent. Peroxynitrite can react directly with sulfhydryl residues in the cell membrane leading to lipid peroxidation or with DNA resulting in cytotoxicity<sup>23</sup>. Administration of cisplatin in rats produced non-significant decrease in nitric oxide level in comparison to normal control group which may be due to decrease in cNOS. Test drugs at both dose levels significantly revert the nitric oxide level in compared to CC group.

Cisplatin-induced cardiotoxicity could be a secondary event following cisplatin-induced lipid peroxidation of cardiac membranes with the consequent increase in the leakage of LDH from cardiac myocytes<sup>24</sup>. Serum alkaline phosphatase and transaminases are cytoplasmic in location and are released in to circulation after cellular damage. Serum alkaline phosphatase increased while, decrease in kidney homogenate suggests the damage to kidney tissue. Administration of cisplatin in rats produced non-significant increase in LDH. All groups of test drugs treated reversed both the parameters but, significant result observed for LDH by MKH, MPL,

Table 3 — Effect of test drugs on serum biochemical parameters in cisplatin-induced kidney damage in rats

Parameters	NC	CC	MKL	MKH	MPL	MPH
Glucose (mg/dl)	63.16±4.078	52.16±1.66 <sup>#</sup>	55.83±4.04	48.83±4.34	66.00±4.27*	66.83±3.33 <sup>@</sup>
Cholesterol (mg/dl)	42.16±1.53	45.50±2.06	56.33±6.32	66.00±3.74 <sup>@</sup>	49.66±2.29	56.33±3.40*
Triglycerides (mg/dl)	53.80±13.39	105.8±15.75 <sup>#</sup>	63.40±3.72*	53.40±3.75**	68.20±8.11	75.00±8.69
HDL-Chol. (mg/dl)	31.33±1.20	32.50±1.52	39.50±1.94**	42.00±6.95 <sup>@</sup>	34.16±2.86	39.66±0.91 <sup>@</sup>
SGPT (IU/dl)	54.66±3.49	52.16±11.16	57.16±9.76	56.66±6.10	67.83±6.26	63.16±3.76
SGOT (IU/dl)	156.83±8.15	117.33±30.97	171.50±18.56	158.00±16.88	172.66±12.18	174.83±10.39
ALP (IU/dl)	117.33±14.76	168.00±28.13	107.16±35.79	65.83±6.48 <sup>@</sup>	147.50±38.02	96.50±8.717
Urea (mg/dl)	38.83±6.18	61.66±19.43	50.80±10.80	38.40±4.36	68.67±12.17	49.67±11.46
Creatinine (mg/dl)	0.91±0.038	2.84±0.225 <sup>#</sup>	1.28±0.358 <sup>@</sup>	1.29±0.139 <sup>@@</sup>	2.00±0.318	2.44±0.559
Total protein (g/dl)	6.40±0.191	6.35±0.411	6.90±0.349	6.85±0.198	6.58±0.168	6.91 ±0.373
Albumin (g/dl)	3.93±0.08	3.90±0.16	3.96±0.14	3.95±0.08	3.96±0.13	3.96±0.11
Globulin (g/dl)	2.46±0.143	2.45±0.305	2.93±0.220	2.90±0.183	2.61±0.122	2.95±0.382
T. Bilirubin (mg/dl)	0.683±0.087	0.767±0.136	0.800±0.129	0.567±0.084	0.650±0.131	0.733±0.095
D. Bilirubin (mg/dl)	0.200±0.026	0.350±0.152	0.233±0.042	0.167±0.021	0.183±0.031	0.233±0.042
Uric acid (mg/dl)	1.03±0.042	1.20±0.234	1.45±0.171	1.30±0.139	1.21±0.149	1.03±0.152
Calcium (mg/dl)	10.41±0.172	10.06±0.105	9.58±0.210	10.0±0.191	9.73±0.242	9.98±0.306

Data: Mean±SEM; <sup>#</sup>p<0.05 when compared with normal control group; \*p<0.05, \*\*p<0.02, <sup>@</sup>p<0.01, <sup>@@</sup>p<0.001 when compared with CC group (Unpaired t-test).

MPH when compared to cisplatin control group suggesting that drug may have potential protective effect against cisplatin-induced cardiac and kidney damage (Table 4).

Catalase is an enzymatic antioxidant and helps in neutralizing the toxic effect of H<sub>2</sub>O<sub>2</sub>. Hydrogen peroxide is not reactive enough to cause a chain of lipid peroxidation reactions, but its combination with super oxide radical produces hydroxyl radical that is highly reactive and thus initiates lipid oxidation reactions<sup>25</sup>. *Mulaka kshara* and *panchanga* increases the catalase, but significant result observed in MPH group in comparison to cisplatin control group. Treatment with *Mulaka kshara* and *Panchanga* significantly ameliorated renal tissue injury by significantly decreasing lipid peroxidation. *Mulaka* formulations inhibit lipid peroxidation and provide protection by strengthening the antioxidants like glutathione and catalase level in kidney<sup>26</sup>.

GSH protects the biomolecules from oxidative tissue damage by scavenging ROS<sup>27</sup>. Decreased GSH levels in the renal tissues further perpetuates cisplatin-induced renal damage. The depletion of GSH seems to be a prime factor that permits lipid peroxidation<sup>28</sup>. Glutathione is one of the essential compounds for regulation of variety of cell functions. It has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized

glutathione (GS-SG) and other disulfides. Glutathione S-transferase (GST) and Glutathione peroxidase (GSH-Px) are GSH-dependent antioxidant enzymes<sup>29</sup>. Many of the earlier experimental studies demonstrated the depletion of renal GSH concentration and antioxidant activities in cisplatin-induced renal damage<sup>30,31</sup>. Therefore, administration of exogenous GSH or its esters effectively protected cisplatin-mediated renal toxicity<sup>32</sup>. In present study, administration of cisplatin in rats produced significant decrease in the total glutathione and non-significantly decreases in glutathione peroxidase suggest the deterioration

of defense mechanism. Administration of *Mulaka panchanga* powder produced significant increase in glutathione while, *Mulaka kshara* produced non-significant increase in glutathione and significant increase in GPx content in kidney homogenate in comparison to cisplatin control group (Table 4).

Cisplatin-induced renal damage is associated with increased renal vascular resistance and histologic damage to proximal tubular cells<sup>33</sup>. In this present study, histopathological examination cisplatin showed, fatty degenerative and cell infiltration due to inflammation, loss of cytoarchitecture indicate nephrotoxicity-induced in rats by cisplatin in comparison to normal control group. Treatment with *Mulaka panchanga* attenuates changes like, mild fatty and edematous changes. At higher dose, almost

Table 4 — Effect of test drugs on tissue biochemical parameters in cisplatin-induced kidney damage in rats

Parameters	NC	CC	MKL	MKH	MPL	MPH
Total Protein (mg/g tissue)	49.66±1.58	45.82±1.48	46.33±1.12	45.44±1.24	50.75±4.71	45.44±2.09
Total Glutathione (n mol/gm)	1158.33±93.51	933.06±32.23 <sup>#</sup>	1037.35±45.06	973.38±37.84	1201.44±69.37**	1172.23±77.78*
GPx (nmole of GSH utilized/min)	1.086±0.071	0.971±0.033	1.996±0.278**	2.039±0.224 <sup>@</sup>	2.431±0.395**	2.310±0.282 <sup>@</sup>
Catalase (Unit/mg protein)	3.70±0.181	3.77±0.23	4.09±0.190	4.44±0.204	4.25±0.330	4.61±0.210**
LPO (n moles of MDA formed /g tissue)	21.76±2.56	43.54±8.55 <sup>#</sup>	20.32±4.52*	13.96±1.52**	24.15±7.02	15.44±2.01*
Nitric oxide (μ mole/g tissue)	3.73±0.24	3.33±0.830	4.81±0.354	4.17±0.448	3.75±0.603	3.52±0.290
LDH (μ mole pyruvate liberated/ gm tissue/min.)	34.69±0.94	37.84±1.44	30.96±4.04	29.45±2.79*	18.48±0.91 <sup>@</sup>	22.54±3.37**
ALP (μmole of phenol/mg protein /min.)	2.09±0.157	1.08±0.142 <sup>##</sup>	1.37±0.121	1.75±0.182*	1.35±0.173	1.63±0.160*

Data: Mean±SEM; <sup>#</sup>p<0.05, <sup>##</sup>p<0.01 when compared with normal control group; \*p<0.05, \*\*p<0.01, <sup>@</sup>p<0.01 when compared with CC group (Unpaired t-test).

normal cytoarchitecture in *Mulaka panchanga* powder administered group. However, in *kshara* treated group mild to moderate fatty degenerative and edematous changes and nearly normal cytoarchitecture showed in comparison to cisplatin control group (Fig. 1 & 2). The findings of serum

biochemical and tissue parameters are well supported by histopathological study in which, the kidney of treated rats clearly indicate *Mulaka panchanga* and *kshara* at both doses level have protective effects kidney cytoarchitecture from damaged by cisplatin.

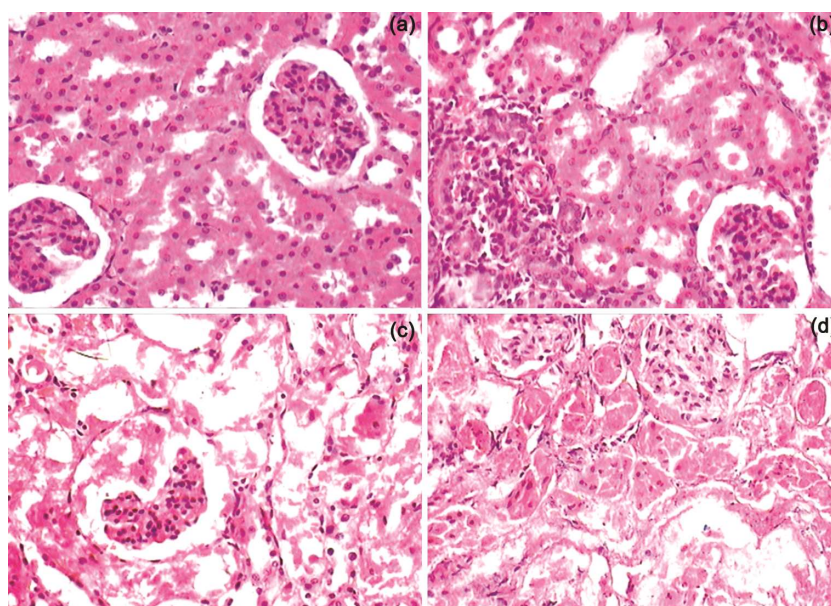


Fig. 1 — Photomicrographs of representative sections of Kidney taken at x 400 magnification. (a) Normal cytoarchitecture (Normal Control group) (b) Fatty degenerative and cell infiltration due to inflammation (Cisplatin control group) (c) Severe fatty degenerative and inflammatory changes, loss of cytoarchitecture (Cisplatin Control group) (d) Severe fatty degenerative and inflammatory changes, loss of cytoarchitecture (Cisplatin Control group)

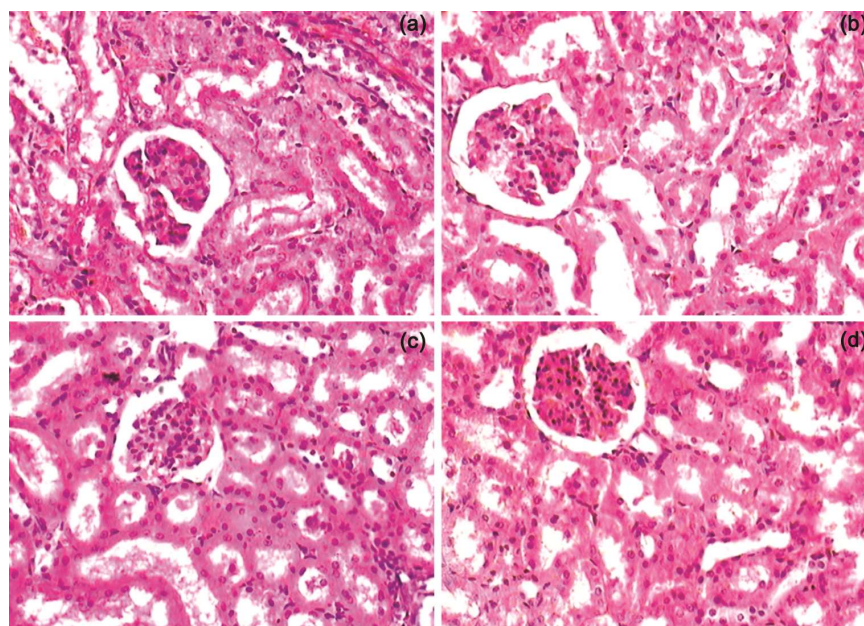


Fig. 2 — Photomicrographs of sections of Kidney taken at x 400 magnification. (a) Moderate fatty degenerative and inflammatory changes (MKL group) (b) Almost normal cytoarchitecture (MKH group) (c) Mild fatty changes, inflammatory cells and oedema in tubules (MPL) (d) Mild fatty and oedematous changes in tubules (MPH)

## Conclusion

From the present study it is concluded that, *Mulaka panchanga* and *Mulaka kshara* both having nephroprotective and antioxidant properties in dose dependent manner in cisplatin– induced nephrotoxicity in rats. Flavonoids present in all parts of *Mulaka (Raphanus sativus* Linn.) in varied quantity and are known to modulate the activities with various biomolecules. Carotenoids, kaempferol and quercetin are well known free radical scavengers present in *Mulaka*. Hence, the possible mechanism of *Mulaka panchanga* powder exerts nephroprotection could be attributed to its free radical scavenging property and its diuretic activity.

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