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An In-Vivo Study on Nephroprotective Effect of Rotula aquatica Lour. (*Pashanabheda*) against Cisplatin Induced Nephrotoxicity in Swiss Albino Mice

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ABSTRACT

Rotula aquatica Lour. (Pashanabheda) is a very known drug in treatment of urinary retention, dysuria, renal calculi and diseases of *mutravaha srotas in* Ayurveda; which come under the group of diseases of different organs of the renal system. Being one of the vital organs, kidneys are at high risk of getting diseased, so in need to find out effective drug in renal disorders; this is an attempt to investigate the nephroprotective effect of *Rotula aquatica Lour.* against cisplatin-induced nephrotoxicity in Swiss Albino mice. Five groups of mice were formed, each containing six mice. First group was normal control (without treatment and without Inj. cisplatin), second group was cisplatin control (Inj. cisplatin given without drug treatment), in third, fourth and fifth groups; *Rotula aquatica Lour.* was given in three different doses i.e. 25 mg, 12.5 mg, 50 mg, respectively, along with Inj. cisplatin. In comparison to the normal control group, cisplatin control group showed increased levels of Serum Urea, Serum Creatinine, Serum BUN and decreased levels of antioxidant enzymes – glutathione, glutathione peroxidase, catalase and superoxide dismutase. Histopathological examination of kidney tissue from cisplatin control group showed signs of nephrotoxicity, i.e. hyalinized glomeruli, glomeruli atrophy, widening of Bowman's capsule, etc., compared to normal control group. On the other hand, groups treated with *Rotula aquatica Lour.* alleviated all the parameters, e.g. biochemical, antioxidant enzymes and histopathological changes. In conclusion, nephroprotective effect of *Rotula aquatica Lour.* was observed in Swiss Albino mice. The observations were noted, analysed statistically, results were discussed and concluded.

Key Words *Pashanabheda, Rotula aquatica Lour., Nephrotoxicity, Nephroprotective*

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INTRODUCTION The Ayurvedic science of medicine has been propounded with the sole objective of maintaining a disease-free society. Ayurveda is based on drugs derived mainly from the plant kingdom. Drug therapy based on medicinal plants also forms the major aspect of therapeutics. And because of the promising effectiveness of these drugs with seldom any side

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effects, research on the medicinal plants has undergone a phenomenal growth during the last few decades. Detailed experimental, clinical and phytochemical investigations of these plants can lead to the development of effective plant drugs for dreadful diseases. Ayurveda offers a challenging area for research on combating these diseases and developing targeted herbal drugs for their cure. Diseases like *murtaghata* (urinary retention), *mutrakruccrah* (dysuria) and *mutravaha srotas vikara* (diseases of mutravaha srotas) etc. have very well relevance with the diseases of the kidneys[1] and other organs of the renal system, which are described in the modern system of medicine. Kidneys[2] are the main organs in the renal system, which regulate homeostasis inside the human body through the excretion of waste products formed during metabolism. Kidneys[3] are the most complex organs both anatomically and functionally. Any toxic insult to the kidney can immediately and invariably affect all its functions, and so as the whole body. Its damage is frequently observed under toxic conditions caused by different types of compounds and chemicals acting through a variety of biological mechanisms. As the man has been exposed constantly to the polluted atmosphere and toxic environment by circumstances and occupation, the kidneys are at high risk of getting diseased due to overload and ultimately lead to renal failure, which is a fatal condition. All the above factors hint at the need to find out the remedy which is effective and safe for the management of the nephrotoxicity. In Ayurveda, our *Acharyas* - *Charaka*, *Sushruta*, and *Vagbhata* have told *mutravirechaneeya*, *mutravirajaniya*, *vellantaradi gana dravyas* etc. for the treatment of *murtaghata*, *mutrakruccrah* and *mutravaha srotas vikara*. *Punarnava*, *Gokshura*, *Varuna*, *Pashanabheda* etc. were in common use to treat such ailments as classical Ayurvedic medicines. Out of that, *Pashanabheda* is one of the very effective drugs used to cure *murtaghata*[4], *mutrashmari*[5], *mutrakruccrah*[6] etc. The plant used as *Pashanabheda* in South India, especially in Kerala is *Rotula aquatica Lour*. Hence, this is an attempt to conduct an *in-vivo* study on Nephroprotective effect of *Pashanabheda* i.e. *Rotula aquatica Lour*. against cisplatin induced nephrotoxicity[7] in Swiss Albino mice.

MATERIAL AND METHODS

Collection of drug (i.e. *Rotula aquatica Lour*.)

Plants of *Rotula aquatica Lour*. were procured from the rocky riverbeds i.e. natural habitat of Kerala, India. It was authenticated by Dr. P. Jayasree, MD, *Dravyaguna Vigyana*, Professor, Department of *Dravyaguna Vigyana*, Government Ayurveda college, Thiruvananthapuram, Kerala, India. It was also pharmacognostically identified by macroscopic, microscopic evaluation, and also purity of the drug was observed by preliminary phytochemical evaluation.

Preparation of *churna* (i.e. powder of drug)

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The useful part of *Rotula aquatica Lour*. i.e. roots were properly cleaned by pure water and dried in the shade. The drug was finely ground into a powder of 120 mesh size.

Dose of the *churna* (i.e. powder of the drug)

The dose of *churna* for an adult human being is12 g, considering this; the effective dose of the drug for rats was calculated using the formula given below:

Animal dose = Human dose × 0.018 for 200 g of animal[8]

e.g. for *churna*: Human dose is 12 g;

then the dose of *churna* for a 200 g rat is = 12 × 0.018 = 0.216g / 200g of rat.

So, the dose of *churna* for 25 g of mice is 25 mg.

The dose of the test drug suspension was prepared by adding 0.2 ml of distilled water in 25 mg of *churna* of drug (i.e. *Rotula aquatica Lour.)* and administered to the animals according to their body weight with the help of an oral feeding cannula attached to a 1 ml syringe

Selection of animals

Properly healthy Swiss Albino mice of any sex weighing between 20-25g were procured from a small animal breeding station in Mannuthy, Kerala, India. The animals were kept in standard laboratory conditions of a light (day) and dark (night) cycle of 7 am to 7 pm, at 25 ºC ± 2 ºC temperature and relative humidity of 30-60 % in well-ventilated cages. Animals were fed with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water ad libitum. The animals were acclimatised at laboratory hygienic conditions for 7 days before starting the experiment. All the animal experiments were done as per the instructions prescribed by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India, and executed through the Institutional Animal Ethical Committee of the Research Centre. The Nephroprotective effect of *Rotula aquatica Lour*. in mice was studied at Amala Cancer Research Institute, Thrissur, Kerala, India.

Method of inducing Nephrotoxicity

The Inj. Cisplatin is widely used to induce the Nephrotoxicity to assess Nephroprotective effect in experimental studies. Hence the same was used for this study. A single dose of Inj. Cisplatin was given intraperitoneally in a dose of 16 mg/kg body wt. to induce Nephrotoxicity[9] in Swiss albino mice.

Procedure

A total of 30 Swiss albino mice of any sex weighing between 20-25 g were chosen in this study. These mice were kept under observation for 7 days, and then five groups were made; so that each group contains 6 mice.

Group 1 was a Normal Control group in which neither Inj. Cisplatin was injected, nor the drug was administered. Group 2 was considered as a Cisplatin Control group in which a single injection of Inj. Cisplatin was injected intraperitoneally into the mice in a dose of 16 mg/kg body wt. and no drug was given to them. The groups 3, 4 and 5 were meant for the drug i.e. *Rotula aquatica Lour*. To group 3, the drug was administered in the effective dose, i.e. 25 mg

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orally through oral feeding cannula for 5 days and on the next day i.e. 6th day Inj. Cisplatin was injected intraperitoneally in a dose of 16 mg/kg body weight as a single dose, and also the drug was administered for next 3 days, i.e. 72 hours. To group 4, the drug was administered as half the effective dose, i.e. 12.5 mg for 5 days and on the next day i.e. 6th day, Inj. Cisplatin was injected and the drug treatment was continued for the next 3 days. To group 5, the drug was administered as double the effective dose, i.e. 50 mg for 5 days and Inj. Cisplatin was injected on the 6th day, and the drug treatment was continued for the next 3 days.

Table 1 Grouping of animals

No.	Groups
Group 1.	Normal control
Group 2.	Cisplatin control
Group 3.	Inj. Cisplatin + 25 mg churna of *Rotula aquatica Lour.*
Group 4.	Inj. Cisplatin + 12.5 mg churna of *Rotula aquatica Lour.*
Group 5.	Inj. Cisplatin + 50 mg churna of *Rotula aquatica Lour.*

The body weight of all the mice was noted on the 1st day of the experimental study and at the end of the study.

Group 1: Normal Control group - these mice were sacrificed after 8 days

Group 2: Cisplatin Control group - these mice were sacrificed after 3 days, i.e. 72 hours after the injection of Inj. Cisplatin single dose.

Group 3, 4, 5: The drug was administered to the mice of these groups for 5 days and then Inj. Cisplatin was injected as a single dose on the 6th day, and the test drug was continuously administered for the next 3 days i.e. 72 hours. At the end of 72 hours, all these mice were sacrificed, and blood was collected by heart puncture and stored properly in containers. Also, kidneys were separated and kept in proper containers containing 10% formaldehyde solution for histopathological examination and in -20^{0}C freezer for the assay of antioxidant enzymes related to kidney tissue. Nephrotoxicity assessment and nephroprotective efficacy of the drug were evaluated with the help of three parameters: morphological parameters, biochemical investigations, and histopathological changes.

Morphological parameters include -

Change in body weight.

Biochemical investigations include –

Serum Creatinine

Serum Urea

Serum Blood Urea Nitrogen (BUN)

Antioxidant enzymes -

• SOD-Superoxide dismutase enzyme

• Catalase enzyme

• GSH – Glutathione

• Gpx – Glutathione peroxidase enzyme

Histopathological studies include –

a) Atrophied glomeruli due to necrosis of vessels

b) Widened Bowman's capsule

c) Hyalinised glomeruli

d) Morphological structure of the renal tubules: proximal convoluted tubules, distal convoluted tubules.

The changes seen in these three parameters in group 3, group 4, and group 5 were compared with the normal control and cisplatin control

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groups. The efficacy of three different doses within groups 3, 4, and 5 was also compared and statistically evaluated.

Statistical analysis

The observations and results of nephroprotective study are given below. To do the analysis of observed parameters following statistical methods were used: Descriptive statistics: Mean (± SD) was reported for summarizing the collected data. One-way ANOVA was carried out to compare different groups to each parameter. TukeyKramer Multiple comparison test: If One-Way ANOVA showed significance, then this test was applied for comparing any two groups for each parameter. If the p-value was less than 0.05 then it was considered as statistically significant.

RESULTS AND DISCUSSION

Observation of Physical activities

Group 1 i.e. Normal control: In this group all the mice were healthy with normal physical activities.

Group 2 i.e. Cisplatin control: In this group all the mice experienced notable weight reduction, less food consumption and lesser activities.

Group 3, 4, 5**:** All the mice of these groups experienced a gradual increase in the consumption of food, body weight and activities as compared to the group 2 i.e. Cisplatin control group after its injection intraperitoneally.

Morphological parameters

Group 1 - which was the Normal control group, experienced an average of 0.9 g of increase in body weight and of Group 2 - which was the Cisplatin control group, experienced an average of 6.12 g of decrease in body weight, which is the difference of body weight on the 6th day before induction of nephrotoxicity by Inj. Cisplatin and body weight on the ninth day before sacrifice. In case of Group 3, Group 4, Group 5, which were given 25 mg, 12.5 mg, 50 mg *churna* of the drug i.e. *Rotula aquatica Lour.*; showed 0.44, 0.36, 0.62 g of increase in body weight, respectively. Table no. 2 shows the comparison of the groups by One-Way Analysis of Variance (ANOVA) by Tukey Multiple Comparisons Test. Comparison of Group 1 vs. Group 2 showed p-value which was less than 0.001 and was highly significant. And the same was true in the case of treatment groups from groups 3, 4, and 5 vs. Group 2, where P< 0.001, which was highly significant.

Table 2 Efficacy of *Rotula aquatica Lour.* treatment on body weight in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Group 1.	0.9	0.09	P< 0.001
Group 2.	-6.12	1.05	
Group 3.	0.44	0.12	P< 0.001
Group 4.	0.36	0.14	P< 0.001
Group 5.	0.62	0.19	P< 0.001

Biochemical investigations

In table no. 3, 4, and 5, the mean and standard deviation values of serum creatinine, serum urea, and serum BUN are given (mg/dl) in the second and third columns, respectively. Compared to the values in group 1 i.e. normal control, group 2, i.e. cisplatin control showed much increased values of serum creatinine, serum urea and serum BUN. In the treatment groups i.e. from group 3 to group 5, the values reverted to the normal. In the groups 3, 4 and 5; serum creatinine, serum urea and serum BUN values showed a gradual decline

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concerning the dose of the drug i.e. 25 mg, 12.5 mg and 50 mg *churna* of the drug received; by which it can be interpreted that the efficacy of drug is in dose dependent manner. Comparison of group 1 vs. group 2 shows a p-value which is less than 0.001 and is highly significant. And the same is true in case of treatment groups from 3rd to 5th group vs. group 2, where P< 0.001, which is highly significant.

Table 3 Efficacy of *Rotula aquatica Lour.* treatment on serum creatinine in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Group 1.	0.95	0.158	P< 0.001
Group 2.	3.15	0.316	
Group 3.	1.288	0.295	P< 0.001
Group 4.	1.663	0.278	P< 0.001
Group 5.	1.143	0.183	P< 0.001

Table 4 Efficacy of *Rotula aquatica Lour.* treatment on serum urea in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p-value
Group 1.	39.57	5.599	P< 0.001
Group 2.	151.0	6.864	
Group 3.	49.16	6.163	P< 0.001
Group 4.	63.94	8.670	P< 0.001
Group 5.	40.81	5.033	P< 0.001

Table 5 Efficacy of *Rotula aquatica Lour.* treatment on serum BUN in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	–value
Group 1.	18.477	2.615	< 0.001
Group 2.	70.517	3.205	
Group 3.	22.96	2.881	< 0.001
Group 4.	29.859	4.048	< 0.001
Group 5.	19.057	2.350	< 0.001

Efficacy of *Rotula aquatica Lour.* treatment on antioxidant enzymes in cisplatin-induced nephrotoxicity in mice: In the previous biochemical investigations, it has been seen that for all the three parameters – serum creatinine, serum urea, and serum BUN- the groups 3, 4, and 5; receiving 25 mg, 12.5 mg, and 50 mg *churna* of the *Rotula aquatica Lour.* respectively, demonstrated dose-dependent effectiveness. This shows that group 5 exhibited maximum efficacy. To evaluate the role of antioxidant enzymes in alleviating toxicity induced by Inj. Cisplatin, group 5, which received 50 mg *churna* of the drug, was selected.

The second column in table no. 6 presents the mean values of superoxide dismutase enzyme in U/mg protein; in table no. 7, presents the mean values of catalase enzyme in k/mg protein; in table no. 8, presents the mean values of glutathione in nmol/mg protein; in table no. 9, presents the mean values of glutathione peroxidase enzyme in nmol/mg protein; and in the third column, the standard deviation values are shown. In group 1, the value of the superoxide dismutase enzyme was 2.48, which declined in the cisplatin control group to 1.40. In group 5, the values reverted to normal, i.e., 2.59.

In group 1, the value of the catalase enzyme was 11.016, which declined in group 2 to 6.238. In group 5, the values reverted to normal, i.e., 10.990.

In group 1, the value of glutathione was 15.82, which declined in group 2 to 8.70. In group 5, the values again reverted to normal, i.e. 17.40.

In group 1, the value of the glutathione peroxidase enzyme was 17.775, which declined in group 2 to 8.198. In group 5, the values again reverted to normal, i.e. 18.947.

The tables show the comparison of the groups by One-Way Analysis of Variance (ANOVA) by the Tukey Multiple Comparisons Test. A comparison of group 1 vs. group 2 shows a p-value which is less than 0.001; it shows that it is highly significant. Also, in the case of group 5 vs. group

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2, the p-value is less than 0.001, which shows that it is highly significant.

Table 6 Efficacy of *Rotula aquatica Lour.* treatment on the Superoxide dismutase enzyme in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Normal control (Group 1)	2.48	0.749	P< 0.001
Cisplatin control (Group 2)	1.40	0.064	
Group 5	2.59	0.258	P< 0.001

Table 7 Efficacy of *Rotula aquatica Lour.* treatment on Catalase enzyme in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Normal control (Group 1)	11.016	1.350	P< 0.001
Cisplatin control (Group 2)	6.238	0.515	
Group 5	10.990	1.034	P< 0.001

Table 8 Efficacy of *Rotula aquatica Lour.* treatment on Glutathione enzyme in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Normal control (Group 1)	15.82	2.250	P< 0.001
Cisplatin control (Group 2)	8.70	1.298	
Group 5	17.40	2.750	P< 0.001

Table 9 Efficacy of *Rotula aquatica Lour.* treatment on Glutathione peroxidase enzyme in cisplatin-induced nephrotoxicity in mice

Groups	Mean	S.D.	p–value
Normal control (Group 1)	17.775	1.773	P< 0.001
Cisplatin control (Group 2)	8.198	0.553	
Group 5	18.947	5.434	P< 0.001

Histopathological evaluation

In group 1, the colour of the kidneys was uniformly dark red, which indicates proper vascularisation within the kidney tissues. In group 2, the kidneys were pale in colour compared to those of group 1. In group 3 and group 5, the kidneys showed a gradual increase in red colour, respectively, with the dose of the drug they received. To do the histopathological studies, 5 different blocks of renal tissues were taken from various sites of the kidney. Paraffin sections were done and stained with haematoxylin-eosin. In group 1, kidneys of mice showed normal renal tubules, normal glomeruli with normal Bowman's capsule, and interstitial tissues were normal with minimal oedema. In group 2, kidneys of mice showed atropy of many glomeruli , some were hyalinized, widening of Bowman's capsules, oedematous interstitial tissue with scattered inflammatory cells and the lining cells of renal tubules were larger. In group 3 histopathological study showed normal glomeruli and some with widening of Bowmen's capsule, normal renal tubules; in some interstitial tissue, collection of lymphocytes and plasma cells were seen. In group 5, histopathological study of the kidneys of mice showed normal glomeruli with normal Bowman's capsule, normal renal tubules, the lining cells of renal tubules in some parts showed increased intracytoplasmic granules and also scattered inflammatory cells were observed in the interstitial tissue.

In group 2, damage to the kidneys has been produced, i.e. nephrotoxicity. In the results of group 3, and group 5, it was observed that there was qualitative improvement in the normality of the nephrons in the kidneys, so this effect of the *Rotula aquatica Lour.* can be attributed to its nephroprotective effect against cisplatin-induced nephrotoxicity. The nephroprotective efficacy of *Rotula aquatica Lour.* was evaluated by changes in morphological parameters, biochemical investigations and histopathological evaluation. Observation of results of all these parameters

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infers the Nephroprotective effect of the *Rotula aquatica Lour.* in the Swiss Albino mice.

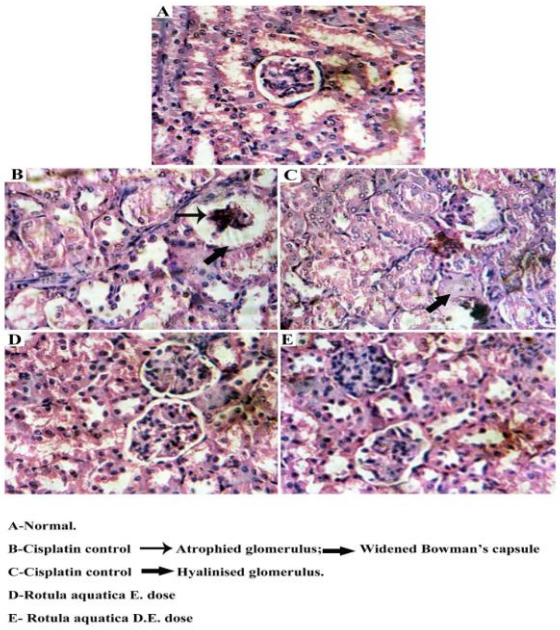

Figure 1 Histopathology of the Kidney - Comparison of the changes in the kidney tissues
A. Group 1- normal control

B. Group 2- cisplatin control -atrophied glomerulus, widened Bowman's capsule

C. Group 2- cisplatin control – hyalinized glomerulus

D. Group 3 - Inj. Cisplatin + 25 mg churna of *Rotula aquatica Lour.*

E. Group 5 - Inj. Cisplatin + 50 mg churna of *Rotula aquatica Lour.*

Mode of action of *Rotula aquatica Lour.* (*Pashanabheda*): An Ayurveda science view

There are number of causative factors (*Hetu*) involved in the production of diseases like *mutrakruccrah* (dysuria) and *mutraghata* (urinary retention). Out of those causative factors (*hetu*); '*tikshnah aushadha sevana*'[10] (i.e. consumption of sharp, potent or extreme pungent medicine) is involved in the vitiation of the *pitta* (*dosha* responsible for regulating body temperature and metabolic activities), which causes it to increase (*pittavriddhi*) abnormally; *tikshnah* (sharpness) *guna* is dominantly comprised of *agni mahabhuta* and so it is *daha* and *pakakara*[11] (causes burning and inflammation). Its overuse results in the destruction of *dhatus* (major structural components of the body), causing weakness. Due to these characteristics of *tikshnah guna*, normal functioning of the kidneys gets affected, and it results in the manifestation of *mutraghata* (urinary retention). As a result of *mutraghata*, the *kleda* (moistness) in the body gets increased, which is normally excreted through the urine according to Ayurveda. The *Pashanabheda* has *kashaya* (astringent taste), *tikta rasa* (bitter taste), *sheeta virya* (cold potency), *katu* (pungent) *vipaka* and *laghu* (light), *snigdha guna* (unctuous property). It has *mutrajanana* (diuretic) action as it is included in *mutravirechaneeya mahakashaya*[12] and *ashmarihara* (anti-urolithic) *prabhava*. Because of its *Kashaya*[13] and *tikta rasa* that are *Pittashamaka rasas*, and its *sheeta virya*, which also has *pittashamaka* property; it alleviates *Pittavriddhi* and normalises pitta. So, the effects of *Pittavridhhi* were mitigated by the use of *Rotula aquatica Lour.* because of its *Pittashamana* property. Again, the *sheeta virya* (cold potency) of *Pashanbheda* possesses *mutrajanana*[14] action, so the *kleda* that was accumulated inside the bodily systems got its way through the urine due to its proper formation. In addition, the *sheeta virya*[15] possesses properties like *daha hara* (decreases burning), *jeevanam* (gives life) and *rakta* (blood tissue) - *pitta prasadanam* (quality enhancing); it

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provides protection to the *dhatus* from damage. It protects the *dhatus*, supports normal functioning of *mutra* (urine) formation and maintains normal functions of the *mutravaha srotasa*, thus maintaining homeostasis and keeping body physiology normal.

Mode of action of *Rotula aquatica Lour.* (*Pashanabheda*): Modern science view

Nephrotoxicity is the potential of certain substances to cause damage to the kidneys, leading to impaired kidney function or kidney failure; such substances are called as nephrotoxins. Nephrons, the functional unit of the kidneys are the main site of damage in case of cisplatin induced nephrotoxicity. Cisplatin is mainly responsible for the production of free radicals within the kidney tissue which increases the oxidative stress which further results in the damage to the nephrons – proximal convoluted tubule, glomerulus, Bowman's capsule and distal convoluted tubules. So, the proper functioning of the kidneys got hampered and as a result Serum Creatinine, Serum Urea and Serum BUN got increased. And the levels of antioxidant enzymes e.g. superoxide dismutase, catalase, glutathione, glutathione peroxidase etc. were decreased which normally gives protection to the kidney tissue by neutralizing free radicals and so as a result of all this damage done to the kidneys, the urine formation also gets hampered. So, in the treatment part, the drug should have an anabolic effect on the antioxidant defense mechanism within the kidney tissue which improves the functioning of antioxidant enzymes against free radicals resulting in the neutralization of free radicals. So, the damage done to kidney tissue can be reversed. The drug which shows diuretic action can help the kidneys for improvement in urine formation. *Pashanbheda* i.e. *Rotula aquatica Lour.* improves the antioxidant defense system within the kidney tissue which further protects the kidneys from damage. *Rotula aquatica Lour.* improves different antioxidant enzymes – superoxide dismutase, catalase, glutathione and glutathione peroxidase within the nephrons (the main functional unit of kidneys) and help improve its normal functioning. Allantoin – one of the phytochemical constituents of *Rotula aquatica Lour.* that shows diuretic action helps kidneys to eliminate excretory products through the urine. The diuretic action of the drug primarily supported by its anabolic effect on the antioxidant defense system results in the protection of the kidney tissue against the damage induced by Inj. Cisplatin. In this way, it combats with the free radicals and protects the kidneys from the damage induced by Inj. Cisplatin to the kidney tissue results in an improvement in the normal functioning of the kidneys. In this way, the drug *Pashanbheda* i.e. *Rotula aquatica Lour.* gives protection to the kidneys, i.e. the nephroprotective effect.

CONCLUSION

Rotula aquatica Lour. i.e., *Pashanbheda* is an effective drug for treating *mutraghata*,

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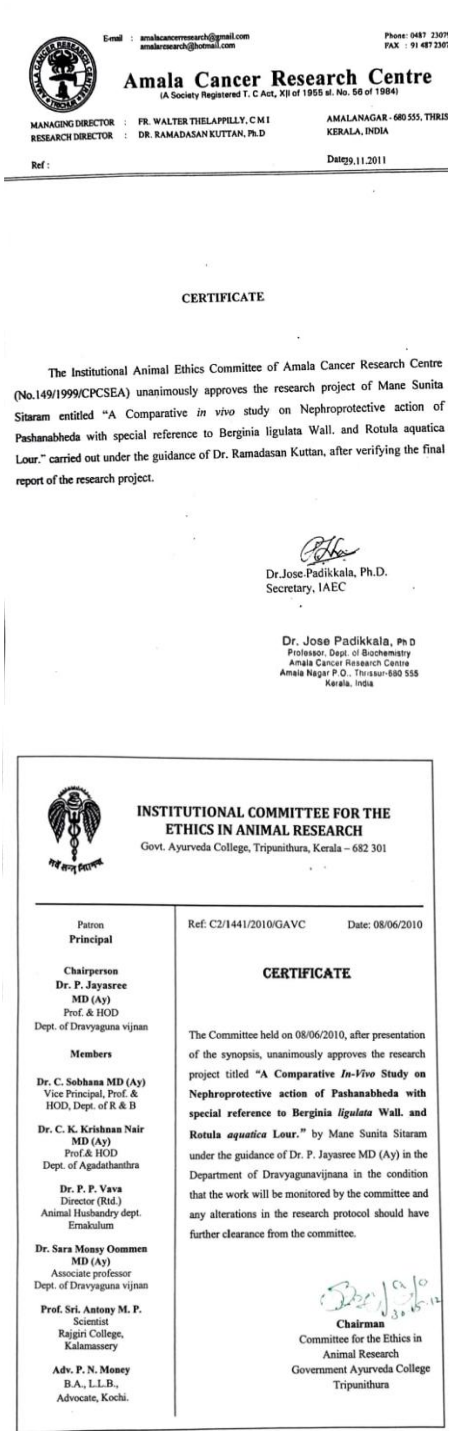
mutrakruccrah, and *mutravaha srotasa* diseases as it is nephroprotective. Further experimental studies on larger samples may be carried out to confirm the results of this study. A clinical trial is required to establish the effects in various kidney diseases.

ETHICAL STATEMENT

All animal handling procedures in this study were approved by the institutional committee for ethics in animal research [Ref: C2/1441/2010/GAVC], Government Ayurveda College, Thripunithura, Kerala and the Institutional Animal Ethics Committee of Amala Cancer Research Centre [No: 149/1999/CPCSEA], Trissur, Kerala, India.



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Date 29.11.2011

CERTIFICATE

The Institutional Animal Ethics Committee of Amala Cancer Research Centre (No.149/1999/CPCSEA) unanimously approves the research project of Mane Sunita Sitaram entitled "A Comparative *in vivo* study on Nephroprotective action of Pashanabheda with special reference to Berginia ligulata Wall. and Rotula aquatica Lour." carried out under the guidance of Dr. Ramadasan Kuttan, after verifying the final report of the research project.

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Ref: C2/1441/2010/GAVC Date: 08/06/2010

CERTIFICATE

The Committee held on 08/06/2010, after presentation of the synopsis, unanimously approves the research project titled **"A Comparative *In-Vivo* Study on Nephroprotective action of Pashanabheda with special reference to Berginia *ligulata* Wall. and Rotula *aquatica* Lour."** by Mane Sunita Sitaram under the guidance of Dr. P. Jayasree MD (Ay) in the Department of Dravyagunavijnana in the condition that the work will be monitored by the committee and any alterations in the research protocol should have further clearance from the committee.

Chairman
Committee for the Ethics in Animal Research
Government Ayurveda College
Tripunithura

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CONFLICT OF INTEREST

No conflict of interest.

RESEARCH ARTICLE

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