IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Review Article ISSN: 2320-5091 Impact Factor: 6.719

EXPERIMENTAL MODELS AND METHODS FOR NEPHROPROTECTIVE ACTIVITY STUDIES– AN OVERVIEW

C Padmakiran

Corresponding Author: kiranasdm@gmail.com

https://doi.org/10.46607/iamj06p9032025

(Published Online: March 2025)

Open Access

Article Received: 11/02/2024 - **Peer Reviewed:** 28/02/2025 - **Accepted for Publication:** 13/03/2025.

Check for updates

ABSTRACT

Evidence for the safety of any new drug through pre-clinical trial is mandatory before human trials. Toxicity studies are among pre-clinical trials that ensure the drug has no acute/sub-acute or chronic toxic effect on any organ/tissue/system. On the other hand, if a drug or combination is to be used to treat any organ damage, it must be tested for its organ protective activity in animals. Nephroprotective activity studies provide evidence that the trial drug has a protective/curative effect on the kidney; hence, it can be used as a treatment for renal diseases. In practice, Wister albino rats are used as an animal model where the drug is tested for its nephroprotective activity against gentamycin-induced acute toxicity and cisplatin-induced chronic toxicity. If the test drug shows better results in serum markers and histopathological tests, it is said to have nephroprotective activity.

Keywords: *Pre-clinical trial, toxicity studies, Nephroprotective activity, acute toxicity, chronic toxicity*

INTRODUCTION

Kidneys are more susceptible to poisoning than any other organ in the body because of the variety and huge number of poisons that are delivered to them. By 2040, it is anticipated that kidney diseases will rank fifth in terms of causes of mortality.[1] Nephrotoxicity means damage to the kidneys by either any exogenous or endogenous toxins. The severity of toxicity may range from reversible structural/functional damage to irreversible structural/functional loss, or it may be micro damage to that of system failure. The increased levels of BUN and creatinine biomarkers in the blood occur when the kidneys are damaged by around 30% - 59%, accompanied by anaemia and lethargy. These biomarkers may not always give the

exact idea about the extent of damage in the kidney. Even though the kidney can regenerate, with a cell damage rate of up to 50%, it will be difficult to restore its physiological functions normally. Hence, early detection of the extent of kidney damage is essential in clinical practice.

In recent years, unhealthy food and lifestyle and irrational use of medicines have increased the chances of renal damage. Hence, any food or medicine must be tested for its nephrotoxicity effect before marketing.

Before the trial on humans, medicines are tested for their effect on the kidneys in 2 ways. The first is the nephron toxicity effect, and the second is the nephroprotective effect. As irrational use of medicine is one of the causes of nephrotoxicity, any new drug or combination must be tested for its nephrotoxicity activity (as a part of general toxicity studies) before the clinical trial. The second test is a nephroprotective activity, where the medicines used to treat acute or chronic, structural or functional kidney damage are tested. Hence, the model for nephroprotective activity is essential and apt for the increasing incidence of the disease.

Nephrotoxicity studies and Nephroprotective activity studies:

Nephrotoxicity studies evaluate the toxic effect of any drug or combination on the kidney. These studies also give an idea regarding the dosage of a drug or combination that is toxic to the kidneys. They also show whether the drug/combination causes acute or chronic damage. Conversely, nephroprotective activity studies are planned to evaluate the protective effect of any drug/combination on the kidney against toxicity.

There is an urgent need for safe and effective nephroprotective drugs which may rectify the damage to the kidney at different levels and in various ways. Hence, any drug/combination said to have a protective effect on the renal system through literature, or cell line studies can be tested for nephroprotective activity. The CPCSEA has guidelines for different toxicity studies.[2] The model selected for testing nephroprotective activity is using rats.

Models for the study:

Nephroprotective activity study: There are two different models available for nephroprotective activity against acute toxicity and nephroprotective activity against chronic toxicity3. The purpose of this type of study is to check whether the trial drug has any nephroprotective activity against acute toxicity and chronic toxicity. Usually, any test drug is tried for both these types of toxicity studies.

Prerequisite for acute and chronic toxicity study:

(i)Animal ethics committee clearance: It is mandatory to get clearance from the study center's animal ethics committee before the study's initiation. The ethics committee permits this based on the evidence in the literature and the initial cell line study report.

(ii)Preparation of test sample: Any medicine, either a single drug or a combination of drugs, which is mentioned to have nephroprotective effects in the literature or proven in an In-Vitro trial, may be selected as a trial drug. The medicine may be tested in this model, whether fresh or dry, in fresh juice, decoction, powder, or paste form. The trial drug prepared in different forms, like ointment, syrup, granules etc, may also be tested. The trial drug administered in different routes of drug administration, like oral, rectal, transdermal, vaginal, and so on, may be tested in this model. If the active extract of the trial drug is to be tested, then the ethanol extract may be prepared for the study.

(iii) Selection of the animal model: For nephroprotective activity, the standard animal model is Wister albino rats [4].

- **Inclusion criteria**: A Wistar strain of albino rats of either gender, with a body weight range from 200 to 300 gms, may be selected. They are exposed to natural day and night cycles and kept at ambient temperature and humidity during the study. These animals are fed normal food and water as per the guidelines.
- **Exclusion criteria**: Pregnant rats, diseased rats, and rats under other trials are excluded.

(iv)Dose calculation: The human dose is used to calculate the animal dose using Young's formula [5].

For any formulation and any route of drug administration, the rat dose is calculated using this formula.

a)Procedure for nephroprotective activity against chronic toxicity:

For this study, a total of 24 rats were included. Usually, the study is done with a comparison of 4 groups, and each group must have a minimum of 6 rats.

The selected 24 rats will be divided into four groups (6 rats each).

- **Group I—Normal control group: The rats in this group will be administered simple tap water and a** normal diet throughout the study.
- **Group II—Standard group: To induce nephrotoxicity6, the rats will be injected with Gentamicin 80 mg./kg body weight intraperitoneally once daily for 8 days. The renal function tests in this group of rats will be considered the** standard value in the study.
- **Group III—Trail/Test group: The rats will be injected with Gentamicin 80 mg./kg body weight intraperitoneally once daily for 8 days. The test drug in the calculated dose and route will be given for 15 days.** The medicine in any form and any route of drug administration is tested here.
- **Group IV:** The rats will be injected with 80 mg./kg body weight Intra peritoneal, once daily for 8 days. Once the toxicity features are set, the test drug in the calculated dose and route will be given for 8 or 15 days.

On the 16th day, the rats from all the groups will be tested based on the following assessment parameters.

- **For blood investigation**: 2 ml blood will be drawn from intra orbital sinus using microhematocrit on the 8th day/16th day and used to estimate serum parameters. Commonly selected parameters are Blood urea nitrogen, Serum creatinine, Alkaline phosphatase, Serum bilirubin, Serum uric acid and Cystatin C.
- **Biochemical parameters in the kidney homogenate are** Lipid peroxidation, Glutathione content, Catalase activity, kidney injury molecule 1 (Kim-1), Cystatin C and neutrophil gelatinase-associated lipocalin (NGAL).
- **Urine** tests like urinary protient(UP) and urine micro albumin (mALB)
- **Ponderal changes** based on Body weight / Heart weight / Kidney weight are noted
- **For histopathological studies:** On the 16th day, the animal will be weighed and sacrificed by cervical dislocation. The kidney will be dissected, weighed, and preserved for histopathological studies.
- Group II values/ histopathological changes are considered standard values for the test group. The values/ histopathological changes obtained in the I and III groups are compared with Group II values.

Some other drugs which may be used to induce different acute toxicity are [7]

- Glycerol-induced renal failure - closely mimics the rhabdomyolysis, ischemia-reperfusion-induced ARF simulate the hemodynamic changes-induced changes in renal functioning
- Drugs such as gentamicin, cisplatin, NSAID - induced ARF mimic renal failure due to the clinical administration of respective drugs
- Uranium, potassium dichromate-induced ARF mimics the occupational hazard.
- S-(1,2-dichlorovinyl)-L cysteine-induced ARF simulates contaminated water-induced renal dysfunction
- Sepsis-induced ARF mimics the infection-induced renal failure

b) Procedure for nephroprotective activity against acute toxicity:

- **Group I -Normal control group:** In this study, the rats in the first group were treated similarly to those in the acute toxicity study.
- **Group II- Standard group** rats are injected with Inj CISPLATIN (40 mg./kg body weight. Intra peritoneally on the first day in a single dose. [8] The rats are left as they are with normal food and water for the remaining 15 days.
- **Group III—Trail/Test group rats will be injected with Inj CISPLATIN (40 mg./kg body weight) intraperitoneally on the first day in a**

single dose. The test drug, in the calculated dose and route, will be given for 8 days.

On the 8th day, the rats will be treated and tested as in the chronic toxicity study.

How do we conclude the study?

After the evaluation of different tests,

- If the blood urea and serum creatinine values are reduced in the trial group, it can be concluded that the medicine has a nephroprotective effect compared to the standard.
- The test group's reduction in test values, such as urine albumin, indicates better glomerular protective activity of the test drug.
- The microglobulin reduction, reduced kidney injury molecule 1 (Kim-1), and reduced cystatin C show better protective effects over the proximal tubule.
- Increased EGFR shows better action over the distal tube and collecting ducts.
- In histopathology of the kidney, if the normal structure of the kidney is preserved /slightly altered than standard, that indicates the nephroprotective effect of the test drug. The gentamycin is said to cause erosion of endothelial cells and necrosis of Bowman's capsule and proximal tubular cells. Inflammatory cells are often seen around damaged nephrons. If no changes/mild damage is seen in the test group, then the test drug is said to be nephroprotective

Cost: The approximate cost for each type of study may be approximately INR 40,000/ (3-group study with 18 rats)the cost increases based on the number of groups added to the survey. The charges for biochemical tests and urine tests are additional to the study cost. The cost of histopathological changes is approximately INR 10,000/.

CONCLUSION

The benefit of nephroprotective studies is if the drug is proven to be effective in protecting the kidney or in reversing the structural or functional ability of the kidney, then those drugs can be prescribed (after clinical trial) to patients of mild to moderate renal damage so that we can prevent the organ failure or establish the normal physiology. These medicines can also be used to postpone/arrest the progress of the disease. It can also be prescribed to patients in whom renal damage is more likely a secondary/complication to any ongoing illness or who cannot avoid the causative factors for renal damage. Hence, there is an urgent need for such a set of medications that have protective effects on different levels of the kidney, which are tested using these nephroprotective activity models. These nephroprotective activity models play an essential role in providing evidence about the safety of the trial drug before the clinical trial.

REFERENCES

1. Manar A. Gamaan, Heba S. Zaky, Hebatalla I. Ahmed, Gentamicin –induced nephrotoxicity: A Mechanistic approach, Azhar International journal of pharmaceutical and medical sciences2023; Vol3 (2):11-19
2. http://cpcsea.nic.in/, Ministry of Environment, Forest and Climate Change, Government of India, Compendium of CPCSEA 2018, pages 1-202
3. M N Ghosh, fundamentals of experimental pharmacology, Hilton & company, Kolkata, 3rd edition, 2005, pp 268, p-190-197
4. Bikash Medhi, Ajay Prakash, Practical manual of experimental and clinical pharmacology, Jaypee brothers' medical publishers (P) ltd, New Delhi, 2010 Ed, Pp 374, P -9
5. Bikash Medhi, Ajay Prakash, Practical manual of experimental and clinical pharmacology, Jaypee brothers' medical publishers (P) ltd, New Delhi, 2010 Ed, Pp 374, P -24
6. Premkumar Govindappa et al., Effect of Withenia somnifera on gentamycin-induced renal lesions in rats, Brazilian Journal of Pharmacognosy, 29(2019) 234-240
7. Amrit Pal Singh et al., Animal models of acute renal failure, Pharmacological reports, Vol 64, Issue 1, Jan-Feb 2012, 31-44
8. Rasha E. Mostafa*, Dalia O. Saleh, Dina F. Mansour, Journal of Applied Pharmaceutical Science Vol. 8(04), April 2018, pp 043-050

Source of Support: Nil

Conflict of Interest: None Declared

How to cite this URL: C Padmakiran: Experimental models and methods for nephroprotective activity studies– an overview. International Ayurvedic Medical Journal {online} 2025 {cited March 2025}