ISSN: 0975-5160

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2021; 11(4); 07-17

Original Research Article

Experimental Evaluation of Nephroprotective Effect of *Punica granatum* Peel Extract aganist Gentamicin Induced Nephrotoxicity in Albino Rats

Shakira Fathima Syeda[1], Mohammed Mohsin[2], Dr. T.Sunitha[3], Dr. J. Margaret Viola[4], Dr. G. Jyothsna[5]

[1,3,4,5] Department of Pharmacology, Government Medical College, Ensanpally, Siddipet, Telangana, India.

[2]Department of Pharmacology, Deccan Medical College, Hyderabad, Telangana, India.

Received: 07-05-2021 / Revised: 23-05-2021 / Accepted: 25-06-2021
Corresponding author: Dr. G. Jyothsna
Conflict of interest: Nil

Abstract

Nephrotoxicity is a disease characterised by derangement in carbohydrate, protein and fat metabolism which is caused by the complete or relative insufficiency of insulin secretions. Long-term complications from high blood sugar can develop heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor circulation of limbs leading to amputations. **AIM:** The aim of this study was to investigate the potential Nephroprotective activity of *Punica granatum* peel extract in Gentamicin induced Nephrotoxicity. Rats were divided into 6 groups, **Group I** Normal control group received distilled water 5ml/kg body weight orally daily, **Group II** were treated with Gentamicin 100mg/kg body weight induced nephrotoxicity administered i.p daily for 8 days. **Group III** Gentamicin 100mg/kg administered i.p for 8 days and *Punica granatum* extract 100 mg/kg administered orally daily for 10 days. **Group IV** Gentamicin 100mg/kg administered i.p. for 8 days and *Punica granatum* extract 200 mg/kg administered orally daily for 10 days & **Group V** treated with *Punica granatum* peel extract 200mg/kg alone for 8 days administered respectively. *Punica granatum* extract had shown Significant in protecting the gentamicin- induced renal failure in rats. The result of this study demonstrates the potentiality of *Punica granatum* peel extract as a source of a Nephroprotective activity. Observations were recorded and results were analyzed by one way analysis of variance (ANOVA). P<0.001was considered highly significant.

Key Words: Nephroprotective, Gentamicin, *Punica granatum* peel extract.

Introduction

Normal renal function is essential for appropriate excretion of metabolic waste, maintenance of fluid homeostasis, regulation of electrolyte status, and hormonal balance.

However, the kidneys are continuously exposed to multiple insults, including toxins, alterations in blood supply, and exposure to oxidative stresses, and thus must adapt to sustain life-supporting functions. This is accomplished via multiple molecular and biochemical mechanisms, mediated by several cell types in and out the kidney. It is common for stresses to overwhelm these naturally protective processes, resulting in the damage of the kidney.

Nephrotoxicity was defined in 1991 by the World Health Organization as a renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals[1]. As per WHO report, approximately 2.62 million people received dialysis worldwide, and this number may well double by the year 2030[2]. Much of the expenditure, morbidity and mortality previously attributed to diabetes and hypertension are attributable to kidney disease and its complications [3,4].

Drug induced nephrotoxicity is a serious adverse reaction observed in clinical practice that can limit the use of effective therapeutic agents[5] and can have detrimental effects on a patient's overall health and well-being. Nowadays, patients have multiple diseases, multiple medications and undergo multiple diagnostic and therapeutic procedures which cause potential harm to the kidneys.

Gentamicin is an antibiotic of aminoglycoside group, used for the treatment of dissimilar bacterial infections. 90% of administrated gentamicin is excreted unchanged in the proximal renal tubule that leads to a necrosis at a higher dose[6]. Chronic and high dose of gentamicin initiates *in vitro* and *in vivo* free radical productions and induction of oxidative stress. Gentamicin elicits mitochondrial superoxide anions that cause the generation of hydroxyl radicals[7]. By the 1950s, kidney disease was clearly recognized as a common complication of diabetes, with as many as 50% of patients with diabetes of more than 20 years having this complication.

Over the past decade, pomegranate (*Punica granatum*) is titled as a wonder fruit because of its voluminous pharmacological properties. In 1830, *Punica granatum* fruit was first recognized in United States Pharmacopeia; the Philadelphia edition introduced the rind of the fruit was introduced. There are significant efforts and progress made in establishing the pharmacological mechanisms of peel (pericarp or rind) and the individual constituents responsible for them. However, the medicinal properties of Punica granatum as a fruit peel have very scantily studies. This review provides an insight on the phytochemical components that contribute too antihyperglycemic[8], Nephroprotective[9], antihyperlipidemic[10] effect, and numerous other effects of wonderful, economic, and eco-friendly pomegranate peel extract [11]

Its antioxidant agents reveal a renoprotective effect in gentamicin-induced nephrotoxicity. Pomegranate peel contains 50% of bioactive constituents including flavonoids, phenolics, proanthocyanidin, and ellagitannins with different minerals. Pomegranate edible part contains 10% seeds and 40% arils, arils mainly enclose anthocyanins, while the seeds contain mainly anthocyanin and glucosides. The most important phytochemical in the pomegranate juice is an ellagitannin which also named punicalagin which inhibits peroxidation through scavenging of free radicals[12]. The current study is focused on achieving nephroprotective activity from a natural source with no or negligible side effects so that it can be used in patients with cardiac, hormonal or renal compromise with no fear of deleterious effects[13] & hence the present study was planned.

Materials and Methods

Collection and authentication of plant material

Punica granatum (Pomegranate) were collected from the local area of Karimnagar (Telangana) in the month of January and authenticated by Dr. Srinivas Garu, Professor & HOD, Department of Botany, SRR Degree & PG College of Sciences, Karimnagar, Telangana, India.

Extraction of *Punica granatum* material

Freshly collected Pomegranate fruits were cleaned with water to remove adhering dust & peel was separated & shade dried. The dried samples were powdered in grinder to a coarse powder. The powder was successively extracted with 50% Ethanol using Soxhlet apparatus. The Ethanolic extract of *Punica granatum* peel was filtered to remove the peel particles. The residue was reextracted with the same solvent. The extracts were pooled and concentrated under vaccum at 45°C and stored in an airtight container for subsequent analysis[14].

Prior to the actual experiment, pilot studies were done to determine the:

1. Dose of Gentamicin injected
2. Effective dose of *Punica granatum* peel extract.

1. Dose of Gentamicin[15].

To induce a stable Nephrotoxicity state in rats, the dose of Gentamicin suggested in literature is a single intraperitoneal injection of 100 mg/kg body weight. All the animals developed Nephrotoxicity with no mortatlity.

2. Effective dose of *Punica granatum* peel extract

As the dose of *Punica granatum* peel extract was not known, the effective dose in which it shows nephroprotective activity at 50% of Gentamicin induced nephrotoxicity rats (ED50) was determined. The animals were administered orally with logarithmically graded dose increments of *Punica granatum* peel extract. Pairs of rats were given 10, 50, 100, 500, 1000 and 5000 mg/kg body weight of the test drug. A quantifiable Nephroprotective effect was observed from 50 mg/kg onwards. Before proceeding with the study, the animals were tested for acute toxic effects of the test drug.

Acute Toxicity study

Seven groups of rats, two in each group were taken. Extract of *Punica granatum* peel was administered in doses of 10, 50, 100, 500, 1000, 5000 and 10000 mg/kg body weight orally and the animals were observed for toxic effects and mortality. No severe adverse effects or mortality was observed in any of the groups except for diminished activity and the animals tolerated the drug well.

Animals: Adult Albino rats of either sex weighing between 150 to 250 gms were used in this study, laboratory bred albino rats were obtained from M/S Sainath Agencies, Hyderabad and placed in individual cages in central animal house of Department of Pharmacology, Chalmeda Anand Rao Institute of Medical sciences, Bommakal, Karimnagar, Telangana, the animals were stabilized for 1 week under standard conditions at temperature 25±1°C, 60±5% relative humidity and 12 hrs dark light cycles. They had been given free accesses to standard pellet diet and water ad libitum. The study was conducted according to the ethical norms approved by the IAEC, and was carried out in accordance with the recommendations of CPCSEA. Ref:

(CAIMS/Academics/IAEC/PhD/1/2014/CPCSEA).

Experimental design

Animals were randomly divided into 5 groups with 6 animals in each which were divided as Follows **Group I** (Normal control) received distilled water 5ml/kg body weight p.o, **GroupII**

Gentamicin 100mg/kg body weight administered i.p daily for 8 days. **Group III & Group**

IV received *Punica granatum* peel extract 100 mg/kg & 200mg/kg administered orally daily

For 10 days simultaneously Gentamicin 100mg/kg i.p for 8 days. **Group V** received *Punica granatum* peel extract 200mg/kg alone for 8 days.

Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of biochemical parameter like serum creatinine and blood urea.

Histopathological examination:

At the end of the experimental period, the rats were sacrificed by cervical dislocation and kidneys were dissected out, washed with saline, fixed with 10% neutral buffered formalin, dehydrated through graded alcohol series, cleared, embedded in paraffin and cut into 5mm sections. Deparaffinized sections were stained with heamatoxylin and eosin (H&E) stain for microscopic examination. The slides were examined by light microscopy under 400x magnification for microscopic alterations of pathological significance.

Renal function parameters

Serum was used for the estimation of Creatinine and Blood urea nitrogen (BUN). The estimation of the above mentioned parameters was carried out using biochemical kits i.e Blood urea was estimated by GLDH-kinetic method and serum creatinine was measured by Jaffes method.

Renal function parameters: A marked increase in serum creatinine levels & BUN in gentamicin treated group was noted in animals compared to control. Peel extract of *Punica granatum* at all doses (100mg/kg & 200mg/kg) significantly decreases the levels of Serum Creatinine and Blood urea levels.

A significant decreased concentration levels of Serum Creatinine and BUN is noted in the Group V (200 mg/kg) animals respectively. When compared to control group rats and gentamicin induced treated group animals. *Punica granatum* peel extract at the dose of 200 mg/kg significantly decreased the concentration levels of Creatinine & BUN in serum respectively.

Statistical analysis

All the values were expressed as Mean ± S.D., standard error of mean and percentage reduction were calculated. The test of significance was done by using ANOVA.

Results

Group I: The animals in this group received distilled water 5ml/kg body weight orally for 8 days. Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of the biochemical parameter like serum creatinine and blood urea levels were estimated, and the kidneys were dissected out for microscopic examination which shown normal kidney architecture. Biochemical parameters are shown in Table, Histopathology section were shown in fig I and the corresponding graphical representation is shown in Graph-I & II

Group II: The nephrotoxicity control group animals induced with Gentamicin 100mg/kg body weight, orally for 8 days. Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of the biochemical parameters like serum creatinine and blood urea levels were increased, and the kidneys were dissected out for microscopic examination showing with diffuse glomerular congestion, inflammatory cell infiltration, hemorrhage and necrosis of kidney. Biochemical parameters are shown in Table, Histopathology section were shown in fig II and the corresponding graphical representation is shown in Graph-I & II.

Group III: The nephrotoxic animals i.e., induced with Gentamicin 100mg/kg for 8 days in these group animals were treated with *Punica granatum* peel extract at a dose of 100mg/kg body weight orally for 10 days. Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of the biochemical parameters, there is reduction in serum creatinine and blood urea levels, and the kidneys which were dissected out for microscopic examination showed a few areas of glomerular congestion and reduced inflammatory cells, the results of biochemical parameters are shown in Table, Histopathology section were shown in fig III and the corresponding graphical representation is shown in Graph-I &II respectively.

Group IV: The nephrotoxicity animals i.e., induced with Gentamicin 100mg/kg for 8 days in this group were treated with *Punica granatum* peel extract at a dose of 200mg/kg body weight orally for 10 days. Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of the biochemical parameters, there is reduction in serum creatinine and blood urea levels, and the kidneys which were dissected out for microscopic examination showing almost normal structure with mild vacuolation of the kidney, and the results of biochemical parameters are shown in Table-IV, Histopathology section were shown in fig IV and the corresponding graphical representation is shown in Graph-I & II respectively.

Group V: The animals in this group were treated with *Punica granatum* peel extract at a dose of 200mg/kg body weight orally for 8 days to compare a difference with the normal group of animals. Twelve hour after the last treatment, the blood samples from all the rats were collected separately from orbital sinus for the estimation of the biochemical parameters, there is reduction in serum creatinine and blood urea levels, and the kidneys which were dissected out for microscopic examination showed clear and similar kidney architecture, and the results of biochemical parameters are shown in Table-V, Histopathology section were shown in fig V and the corresponding graphical representation is shown in Graph-I & II respectively.

Biochemical parameter

There is increase in the mean values of both serum creatinine and blood urea in gentamicin treated group (4.25±0.187, 185.33±13.45) as compared to control group (0.65±0.05, 21.83±2.31).

The decrease of elevated serum creatinine and blood urea level in gentamicin + *Punica granatum* peel extract group IV (1.01±0.11, 26.5±2.42) was statistically highly significant ($P < 0.001$) when compared to gentamicin group (4.25±0.187, 185.33±13.45).

The increase in serum creatinine and blood urea level with gentamicin indicates severe toxicity.

When *Punica granatum* peel extract (200mg/kg) was coadministered with gentamicin, the serum creatinine and blood urea levels were remained in normal limits indicating nephroprotective effect.

Whereas for group V only *Punica granatum* peel extract was administered in normal animals, the serum creatinine and blood urea levels (0.53±0.081, 13.66±2.16) remained in normal range when it compared with group I, group II, group III and group IV. Satistically it is highly significant when compared with all the groups.

Histopathological Studies

The histopathology of Group I animals sections of the kidney revealed normal architecture of glomerulus, tubules with loop of henle showing normal kidney. Whereas, the Group II animals kidney section revealed

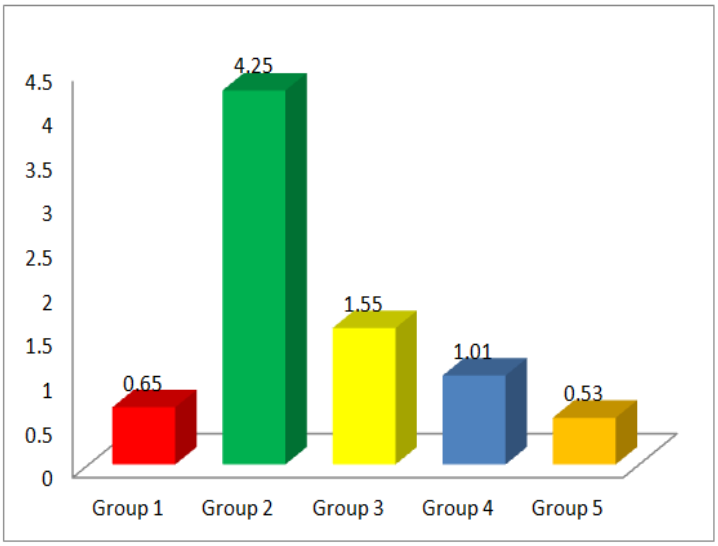
diffuse glomerular congestion, necrosis of tubular epithelial cells that have become detached (from their basement membranes) and been sloughed into the tubular lumens, whereas others are swollen, vacuolated. Intense inflammatory cellular infiltration was seen with extensive hemorrhages - nephrotoxic kidney. Group III animal's kidney section Sections of the kidneys reveals focal glomerular congestion mild reduction of inflammatory cellular infiltration and hemorrhages when compared to gentamicin group, which is suggestive of protective effect of *Punica granatum* peel in gentamicin induced nephrotoxicity. Groups IV Section of the kidney revealed improvement in the histological structure compared to group II and group III. There was improved glomerular and tubular structure of the renal tissue with mild haemorrhages and vacuolations suggestive of protective action of *Punica granatum* peel in gentamicin induced nephrotoxicity and Group V animals kidney section revealeds glomerulus, tubules with loop of henle suggestive as almost as normal structure.

Table 1: Mean Serum Creatinine and Blood urea levels of all the groups

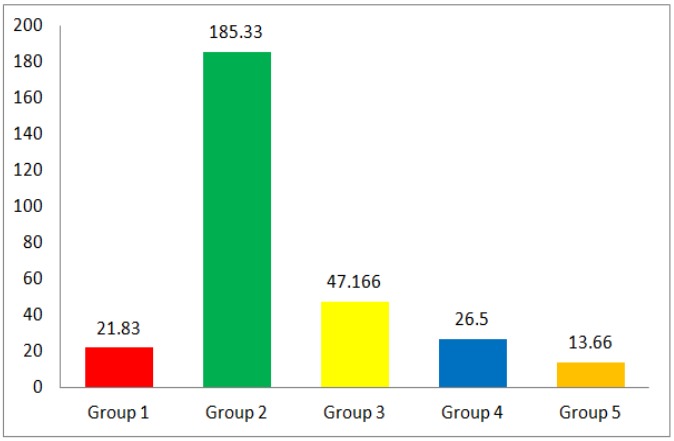
Group	Parameters	Serum Creatinine	Blood Urea
Normal Control (Normal Saline 5ml/kg)	Mean ±SD	0.65±0.05	21.83±2.31
	SEM	0.022	0.945
Gentamicin Induced (100 mg/kg)	Mean ± SD	4.25±0.187	185.33±13.45
	SEM	0.076	5.493
Test -1 (Gentamicin 100mg/kg + *Punica granatum* 100 mg/kg)	Mean ± SD	1.55±0.27*	47.166±4.44*
	SEM	0.111	1.815
Test-2 (Gentamicin 100mg/kg + *Punica granatum* 200mg/kg)	Mean ± SD	1.01±0.11**	26.5±2.42**
	SEM	0.047	0.99
Test-3 (*Punica granatum* 200mg/kg)	Mean ± SD	0.53±0.081**	13.66±2.16**
	SEM	0.033	0.881

*P value<0.001, **P value<0.0001

All the values are expressed as Mean ± SEM.



Graph 1: Serum Creatinine



Graph 2: Blood Urea

Histopathology of Rat Kidney

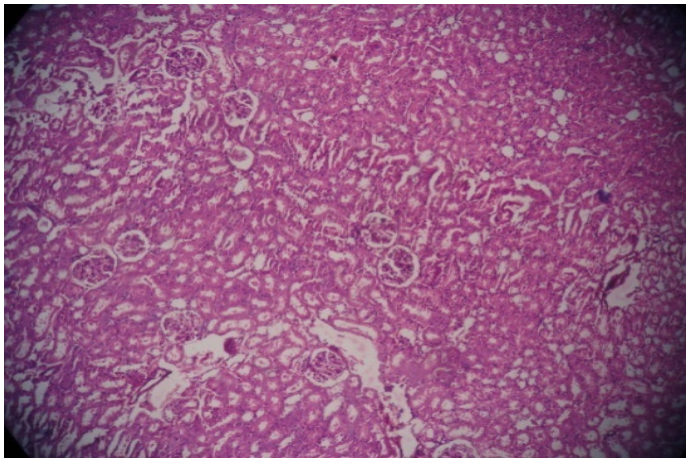


Fig I: Group I (control group) showing normal kidney architecture.

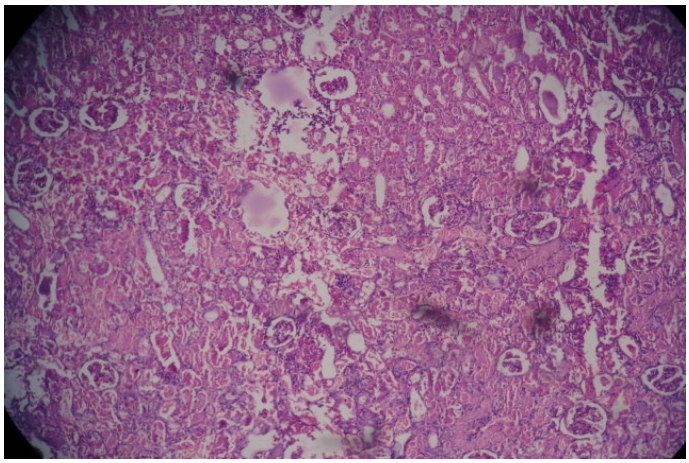
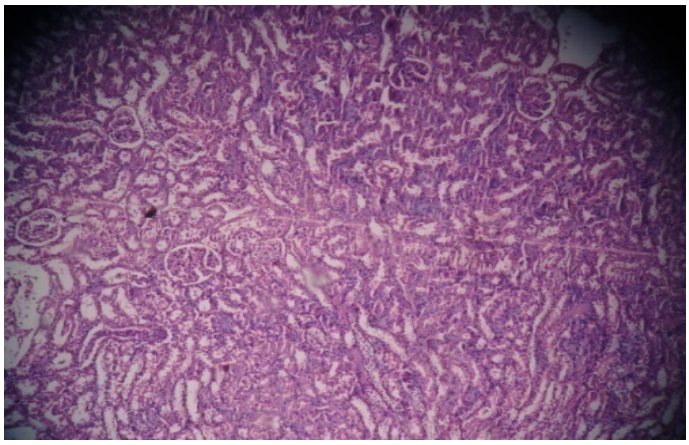


Fig II: Group II (Gentamicin 100mg/kg/day i.p for 8 days) showing diffuse glomerular congestion, inflammatory cell infiltration, hemorrhage and necrosis of kidney.



FigIII: Group III (Gentamicin 100mg/kg/dayi.p for 8 days + *Punica granatum* peel extract 100mg/kg/day orally for 10 days) showing a few areas of glomerular congestion and reduced inflammatory cells.

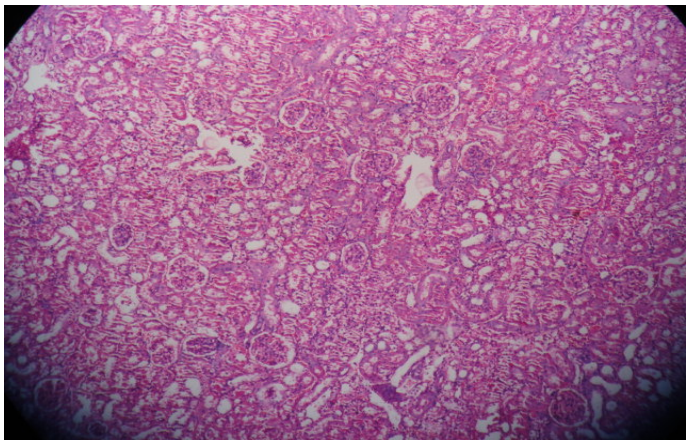


Fig IV: Group IV (Gentamicin 100 mg/kg/day i.p for 8 days + *Punica granatum* peel extract 200mg/kg/day orally for 10 days) showing almost normal structure with mild vacuolation of the kidney architecture .

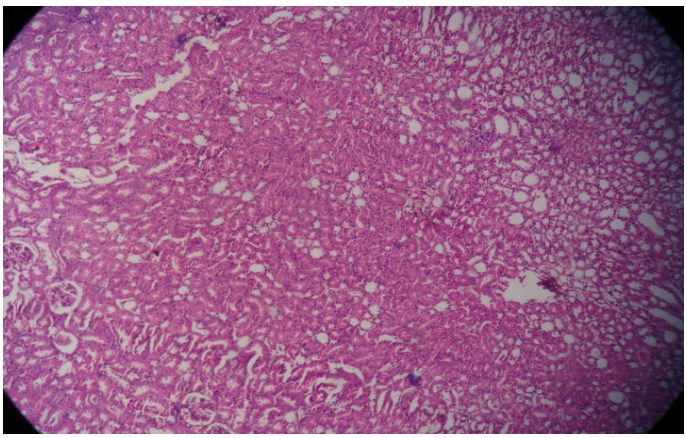


Fig V: Group V (only *Punica granatum* peel extract 200mg/kg/day orally for 8 days) showing clear and similar to normal kidney.

Discussion

This study aimed to evaluate the protective effect of the *Punica granatum* peel extract against gentamicin-induced nephrotoxicity in rats. Gentamicin administered rats had encountered acute kidney dysfunction an evidenced by significant elevation of serum creatinine & BUN with multiple histological damages. Treatment with the *Punica granatum* peel extract at the dose level of 100 mg/kg body weight and 200 mg/kg body weight for 8 days significantly lowers the level of serum creatinine & BUN when compared with the toxic group.

The statistical significance of the nephroprotective activity of *Punica granatum* peel extract treated group & Group V only Punica granatum extract was found almost equal as both groups gained the same level of significance against the toxic group in most of the parameters including serum creatinine & BUN. Hence, the review of the study is concluded that the herbal drug possesses nephroprotective activity and it has been proven by different animal models, which gives many links to develop the future trials.

A relationship between oxidative stress and nephrotoxicity are well demonstrated in many experimental models[16]. Several animal and clinical studies suggested that antioxidants have protective effect in Gentamicin induced nephrotoxicity[17]. Estimation of serum creatinine, blood urea and histopathological changes after drug therapy is evidence of its nephrotoxicity. Several mechanisms are involved in nephrotoxicity, one of them is due to production of reactive oxygen species (ROS) [18] and antioxidants have got beneficial effects.

Gentamicin is common antibiotic used in the treatment of severe infections specially caused by gram –ve organisms. It is used alone or in combination with other antibiotics. Nephrotoxicity, side effect of gentamicin was seen in 5% - 25% of patients receiving gentamicin for more than 3-5 days [19].

Evidence suggests that gentamicin is actively concentrated in renal cortex and proximal tubular cells leading to renal injury by forming reactive oxygen species (ROS)[20,21,22].

Agents with antioxidant action have protective effect on the gentamicin induced nephrotoxicity [23,24].

Punica granatum peel extract belongs to Punicaceae family exhibit antioxidant property by its ability to scavenge free radicals and inhibit lipid peroxidation[25].

In the previous study by[26], where nephrotoxicity was induced by giving gentamicin 100mg/kg/day i.p. for 8 days and similar dose and duration was taken in our study and the duration of ethanolic peel extract of *Punica granatum* was given for 10 days as evident from our experimental work.

In the present study nephrotoxicity induced by gentamicin in albino rats, group II animals have been demonstrated by significant increase in serum creatinine, blood urea and histopathologic changes-showing diffuse glomerular congestion, inflammatory cell infiltration, hemorrhage and necrosis of kidney (Fig II).

Gentamicin induced group III (*Punica granatum* peel extract100mg/kg) and group IV (*Punica granatum* peel extract 200mg/kg) animals, showed a significantly reduction in biochemical parameters and histopathologic changes have been significantly reduced by co-administration of *Punica granatum* peel extract. Group V was administered with high dose of *Punica granatum* peel extract i.e 200mg/kg body weight which has shown more significant value when it compared with normal control group animals.

The findings of present study correlates with the study conducted by Naidu MU et al[27]

which demonstrated the beneficial effect of ginkobiloba extract on gentamicin induced nephrotoxicity in rats due to its antioxidant properties.

Thus the data of this study suggests that *Punica granatum* peel extract was able to produce considerable alleviation from the nephrotoxic action of gentamicin in the albino rats. Further studies may be needed for its clinical use.

Conclusion and Summary

Nephrotoxicity is a globally prevalent chronic debilitating illness. The goals in management of nephrotoxicity are to alleviate various symptoms and signs, and to prevent or reduce the acute and chronic complications. There were range of number of drug agents available for the treatment of nephrotoxicity but they are costly and have many adverse effects which warrant the continued research for newer drugs.

approval: The study was approved by the Institutional Ethics Committe

References:

1. Robert F.M, Herber.Nephrotoxicity.In: John H. Duffus, Howard G. J. Worth, Fundamental Toxicology. Cambridge: The Royal Society of Chemistry, 2006, pg 198.
2. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet. 2015 May 16, 385(9981),1975–82.
3. Murray CJ, Barber RM, Foreman KJ, Abbasoglu Ozgoren A, Abd-Allah F, Abera SF, et al.; GBD 2013 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. Lancet. 2015 Nov 28, 386(10009), 2145–91.
4. Tonelli M, Muntner P, Lloyd A, Manns BJ, Klarenbach S, Pannu N, et al.; Alberta Kidney Disease Network. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. Lancet. 2012 Sep 1, 380(9844), 807–14.
5. Izzedine et al. Diabet Med, 2005, Dec, 22(12), 1761-1762.
6. Virani SH et al, Aloe vera attenuates gentamicin-induced nephrotoxicity in Wistar albino rats, Histo-pathological and biochemical changes. Asian J Pharm Clin Res 2016, Vol 9:113-7.
7. Geetha K, Ramarao N, Sindhu B, Rao VU. Nephroprotective, nephrocurative activity of Mimosa pudica root against gentamicin induced nephrotoxicity. Int J Pharm Pharm Sci 2015, Vol7, 173-7.
8. Sushil kumar middha et al A review on antihyperglycemic and antihepatoprotective activity of Eco-friendly *Punica granatum* peel waste (Evidence-Based Complementary and Alternative Medicine, Vol 2013)
9. Nidhal AK Mohammed Ali et al., Nephro-Protective Effect of *Punica granatum* peel in Gentamicin-Induced Nephrotoxicity in Rats. Medical Journal of Babylon. Vol. 9-No. 1-2012, page no 220-228.
10. Esmaillzadeh A, Tahbaz F, Gaieni I, Alavi-Majd H, and Azadbakht L. Cholesterol- lowering effect of concentrated pomegranate juice consumption in type II diabetic patients with hyperlipidemia. International Journal Vitamin Nutritional Resources. 2006, 76(3), 147-51D100-111.
11. Sushil kumar middha et al A review on antihyperglycemic and antihepatoprotective activity of Eco-friendly *Punica granatum* peel waste

(Evidence-Based Complementary and Alternative Medicine, Vol 2013)
12. Hamza RZ, Abdel-Azez AM, Hussien NA. Evaluation of the antioxidant potential for different extracts of Al-taif pomegranate (*Punica granatum* L.) induced by atrazine and malathion pesticides in liver of male albino mice. Int J Pharm Sci 2015, 7:89-94.
13. Gupta YK, Briyal S. Animal models of cerebral ischemia for evaluation of drugs. Indian J PhysiolPharmacol 2004, 48, 379-94.
14. M.N. GHOSH., Fundamentals of Experimental Pharmacology, 6th Edition.
15. Mehul V. Makwana1, Nilesh M. Pandya1, Dharmesh N. Darji, Sarav A. Desai, Bhaskar VH4, Assessment of nephroprotective potential of *Sida cordifolia* Linn. In, experimental animals, Scholars Research Library Der Pharmacia Lettre, 2012, 4 (1):175-180.
16. Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management Mahaboob Khan sulaiman Diabetology & Metabolic Syndrome,2019, 11:7
17. Shifow AA, Kumar KV, Naidu MUR, Ratnakar KS. Melatonin, a pineal hormone with antioxidant property, protects against gentamicin-induced nephrotoxicity in rats. Nephron. 2000, 85(2):167–174.
18. Naidu MU, Shifow AA, Kumar KV, Ratnaker KS. Ginkgobiloba extract ameliorates gentamicin-induced nephrotoxocity in rats. Phytomedicine 2000; 7: 191-97.
19. Maarten Naesens,Dirk R. J. Kuypers, and Minnie Sarwal,Calcineurin Inhibitor Nephrotoxicity, Clin J Am Soc Nephrol 2009, 4: 481–508
20. John R, HerzenbergAM. Renal toxicity of therapeutic drugs. J ClinPathol 2009, 62:505–515.
21. Perazella MA. Renal vulnerability to drug toxicity.Clin J Am SocNephrol 2009, 4: 1275–1283
22. Pannu N, Nadim MK. An overview of drug-induced acute kidney injury. Crit Care Med 2008, 36: S216–S223.
23. Bertram G. Katzung , Basic and clinical pharmacology. 12thEdition,New Delhi : Tata McGraw Hill Education Private Limited, 2012 : 811.
24. Badreldin H. Ali et al.Basic & Clinical Pharmacology & Toxicology, 109: 225–232.
25. Gurpreet kauraur, G. Jabbar, Z. Athar, M. Alam, M.Sarwar Alam. *Punica granatum (*Pomegranate*)* flower extract possesses potent antioxidant activity and abrogates. Fe-NTA induced hepatotoxicity in mice. Food and Chemical Toxicology. 2006;44(7): 984-993.
26. Nidhal AK Mohammed Ali et al., Nephro-Protective Effect of *Punica granatum* peel in Gentamicin-Induced Nephrotoxicity in Rats. Medical Journal of Babylon. Vol. 9-No. 1-2012, page no 220-228.
27. Naidu MU, Shifow AA, Kumar KV, Ratnaker KS. Ginkgobiloba extract ameliorates gentamicin-induced nephrotoxocity in rats. Phytomedicine 2000, 7, 191-97.