IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2021 Vol.:20, Issue:2

Nephroprotective Effect of Tukhm-E-Khurfa (*Portulaca oleracea* L.) and Tukhm-E-Kahu (*Lactuca sativa* L.) against Cisplatin Induced Nephrotoxicity in Experimental Animals

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Abul Faiz[1]*, Zaibunissa Begum[2]

[1] PG Scholar, Dept.of Ilmul Advia (Pharmacology), Govt. Nizamia Tibbi College, Charminar, Hyderabad, India.*

[2] Associate professor & HOD, Dept.of Ilmul Advia (Pharmacology), Govt. Nizamia Tibbi College, Charminar, Hyderabad, India.

Submitted: 05 December 2020
Revised: 26 December 2020
Accepted: 16 January 2021

Keywords: Nephrotoxicity, Cisplatin, Nephroprotective, Kurfa, Kahu, Unani Medicine

ABSTRACT

Background: In nephrotoxicity, toxic metabolites retain in the body leading to complications like edema and ascites, etc. Tukhm-e Khurfa and Kahu (Seeds of *Portulaca oleracea* and *Lactuca sativa*) were used as nephroprotective (Muhafiz-e-Kuliyah) drug in Unani system of medicine. **Objectives:** To study the nephroprotective effect of ethanolic extract of Tukhm-e-Khurfa and Tukhm-e-Kahu (EETKH & EETK) against cisplatin induced nephrotoxicity in rats. **Methods:** All the animals of negative control group were administered cisplatin 5 mg/kg b.w. I.P. on the first day and same day extract of tukhm-e-khurfa and tukhm-e-kahu (55 mg/kg b.w. and 75 mg/kg b.w. respectively) in test group A and B as single drug. Whereas, in group fifth and sixth combination of both the extract was started orally (70 mg/kg b.w. and 130 mg/kg b.w. respectively). On 8th day, cisplatin 5 mg/kg b.w. was injected i.p. in the animals of test groups then again test drugs were given orally for next 7 days. At the end of study (on 15thday) blood was collected for the estimation of serum creatinine and BUN. Histopathological examination of kidney was also conducted. **Result:** Test group treated with test extracts showed statistically significant ($P<0.05$, $P<0.01$) reduction in the elevated serum creatinine and blood urea nitrogen. Histopathological study of kidney showed almost normal tissue structures in comparison to negative control where severe structural disintegration was observed. **Conclusion:** The study demonstrated that EETKH and EETK as single as well as in combination possesses a potential nephroprotective activity.

INTRODUCTION

Kidneys are the main excretory organs of the body responsible to excrete the waste, undesirable and toxic substances out of the body. It is a predominant organ involved in the regulation of extracellular fluid volume, control of electrolyte and acid base balance and regulates the composition and volume of the blood and removes the water from the blood in the form of urine. They excrete selected amount of various wastes, assume a role in erythropoiesis by forming renal erythropoietin factor, and help to control blood pH, regulate the blood pressure by secreting renin, which activate the renin angiotensin pathway and participate in the activation of vitamin D.

For many years, various nephroprotective interventions have been used to curtail the side effects but the protective effects are mostly partial, suggesting the need for alternative approaches *(P. Kalra et al 2018.).* Unani system of medicine possesses very effective and safe nephroprotective drugs, which are useful in renal disorders since long time. For example, in recent years, drugs like kasni (*Chicorum intybus*), Khar-e-khask (*Tribulus terristeris*), Kaknaj (*Physalis alkekengi*), *Kabab Chini (Pipeber cubeba),* Habb-al-Qilt (*Dolichos biflorous*) are reported to be Muqawwi-e-Gurda (Renal Tonic/Nephroprotective), anti-inflammatory and diuretic by these studies *(Alam et al 2016, Roohi Zaman et al 2017).* These drugs were shown to produce beneficial effect in kidney disease without producing any major or subtle toxicity on other systems of the body. Among all these drugs *Portulaca oleracea* and *Lactuca sativa* are commonly known as 'Common Purslane and Garden Lettuce' in English. They have antioxidant, diuretic and anti-inflammatory property (*Gholam reza karimi et al 2010; Rafael LLorach et al 2004; Younus et al 2019*). The present study was undertaken to evaluate the ethanolic extract of Tukhm-e-Khurfa and Kahu as a potential nephroprotective that reduce cisplatin induced nephrotoxicity.

MATERIALS AND METHODS

Experimental animals

The study was carried out on Albino Wistar rats, each weighing 150-200 gm. The experimental protocol was approved by Institutional Animal Ethics Committee of Govt. Nizamia Tibbi College, Charminar, Hyderabad. Reg. No.1070/GO/Re/S/07/CPCSEA, dated 24.03.2018. All experimental procedures and animal care are in accordance to CPCSEA guidelines for care and use of Animals in scientific research. The animals were housed in

clean polypropylene cages with temperature (25 ± 2 C) and relative humidity of (60 ± 5%) under a 12 h light/dark cycle. Animals were fed with normal rat chow and water *ad libitum* throughout the study period. The animals were purchased from VAB Bio Sciences, CPCSEA No.282/PO/RcBt/S/2000/CPCSEA, #7-12 Medipally Village, Narapally, Ghatkesar Mandal, Medchal District, Hyderabad-500039.

Test Drugs and Chemicals

Tukhme-e-Khurfa and Tukhm-e-Kahu (seeds of *Portulaca oleracea* & *Lactuca sativa*) have been taken from the department of Ilmul Advia (Pharmacology), Govt. Nizamia Tibbi College, Hyderabad. Crude drugs were authenticated by Dr. Mohd Kashif Hussain (Botanist) of Survey of Medicinal Plants Unit (SMPU) of NRIUMSD, Hyderabad vide Voucher specimen No.SMPU/CRI-Hyd 14107 for *P. olerace* and SMPU-Hyd 14108 for *L. sativa*. Cisplatin (CISTERO) was procured from KIMS BIBI Healthcare Pvt. Ltd. Malakpet, Hyderabad, TS, India.

Extraction of the test drug

Tukhme-e-Khurfa and Tukhm-e-Kahu (Seeds of *Portulaca olerace* and *Lactuca sativa)* were cleaned and pulverized with the help of electric grinder. The 100 gm powder of each drug was used for ethanolic extraction separately using Soxhlet apparatus. Extracts were concentrated on waterbath at 80 °C until it becomes semisolid in nature. Dried extracts were weighed for yield percentage and it was found to be10 % in *P. oleracea* and 11% in *L. sativa* respectively. It was labeled and kept in airtight container in refrigerator for further use.

Dosage of the test drug

The doses of the test drugs for albino wistar rats were calculated accordingly using the human dose mentioned in Unani literature multiplied by the conversion factor of 6 (*Shannon Reagan-Shaw et al 2008, Nair & Jacob et al 2016*), proposed by Shannon Reagan-Shaw *et al* and Nair and Jacob *et al*, is based on their body surface area). The dose of the test drug "khurfa" in Unani literatures has been described to be 3-7 gm and dose of Kahu is mentioned as 6-10 gm but the dose for animal was calculated taking the human dose as 5.5 gm and 8 gm respectively (Fixed after toxic study), ethanolic extract of both test drugs were calculated in this way at same formula, was found to be 55mg/kg body weight and 75mg/kg body weight at which the drug was studied.

Acute toxicity

The acute toxicity study was performed by using Organization for Economic Cooperation and Development (OECD) guidelines 423. Female Albino Wistar rats were used for the study. The dose of 2000mg/kg, P.O. of both extracts administered in the acute toxicity study. The animals were observed continually for 2hrs for gross behavioral changes and intermittently once every 2hrs and finally at 24 and 72 hours to note any signs of toxicity including death. After this observation, no sign of toxicity or death was recorded.

Experimental design

Nephroprotective activity of EETKH and EETK seeds as single drug as well as in combination were used. Nephrotoxicity was induced in Albino Wistar rats following the method of Alam *et al.*, (2019) with slight modification. Animals were divided randomly in to six groups with six animals in each group.

GROUP I served as normal control. Normal saline (3ml/kg/day i.p) single dose and rat chow and water orally once daily for 14 days.

GROUP II served as negative control/Cisplatin control. Cisplatin (5mg/kg) was injected i.p. to all rats of this group as a single dose on 8th day.

Group III: Animals in this group received extract of Tukhm-e-Khurfa (EETKH) in a dose of 55 mg/kg .orally, once daily for 7 days. On 8th day cisplatin in the dose of 5 mg/kg was injected as single dose i.p. Test drug was given again for 7 days p.o and this group was considered as test group A.

Group IV: Animals in this group received extract of Tukhme-e-Kahu (EETK) in a dose of 75 mg/kg orally, once daily for 7 days. On 8th day cisplatin in the dose of 5 mg/kg was injected as single dose ip, and test drug was given again for 7 days p.o. This group was considered as test group B.

Group V: Animals in this group received extract of combined test drug in a dose of 70 mg/kg orally, once daily for 7 days. On 8th day cisplatin in the dose of 5 mg/kg was injected as single dose intraperitoneally, and test drug was given again for 7 days p.o. This was considered as test group combined low dose (CLD).

Group VI: Animals in this group received extract of combined test drug in a dose of 130 mg/kg orally, once daily for 7 days. On 8th day cisplatin in the dose of 5 mg/kg was injected as single dose intraperitoneally and test drug was given again for 7 days p.o. This was considered as test group Combined High dose (CHD).

At the end of experiment (on 15th day) after 1 hr of oral administration of test drugs all the animals were anaesthetized (Pentobarbitone sodium 60mg/kg; i.p.) blood was collected by cardiac puncture for biochemical assay. Rats were sacrificed and both kidneys were removed and sent for histopathological study.

Estimation of biochemical parameters

Serum creatinine and blood urea nitrogen, blood was collected in labeled test tubes and sent to the veterinary lab Diagnopet, Hyderabad for analysis.

Histopathological Studies

Kidneys of animals were dissected out washed with normal saline, and preserved in 10% formalin for the purpose of histopathological examination and sent to the Veterinary pathology lab (veterinary lab Diagnopet, Hyderabad).

Statistical Analysis

Results were expressed as Mean±SEM. The different values determined were compared with each other and comparison was made using One-way ANOVA with Tukey's Multiple Comparison test. The difference of mean was considered significant at $p< 0.05$.

RESULT

Effect of ethanolic extract of Tukhm-e-Khurfa and Tukhm-e-Kahu on biochemical parameters

The levels of serum creatinine and blood urea nitrogen levels were significantly ($p<0.001$) increased in negative control group. Treated with ethanolic extract of *Tukhm-e-Khurfa* (EETKH) reduced the elevated levels of serum creatinine and blood urea nitrogen as compared to negative control group ($P< 0.001$). Whereas, animals treated with ethanolic extract of Tukhm-e-Kahu (EETK) (test group B) have no significant reduction in serum parameters. Combines extract in low dose treated group showed significant ($P< 0.05$ & $P< 0.01$) reduction in elevated levels of serum creatinine and blood urea nitrogen in comparison

to negative control. High dose combination of test extract reduced the elevated levels of serum creatinine and blood urea nitrogen significantly (P< 0.001).

Effect of ethanolic extract of Tukhm-e-Khurfa and Tukhm-e-Kahu on kidney tissues

Histopathological evaluation of cisplatin treated kidneys has revealed severe damage consisting of tubular necrosis extensive loss of tubular epithelial cells and tubular dilation as compared to normal control. Appearance of nearly normal and no inflammation of tubular cells were observed in rat kidneys treated with extract of tukhm-e-khurfa single and in combination with tukhm-e-kahu. Extract of Tukhm-e-Khurfa and Tukhm-e-Kahu group showed normal morphology of cortex and proximal tubule (Fig 1).

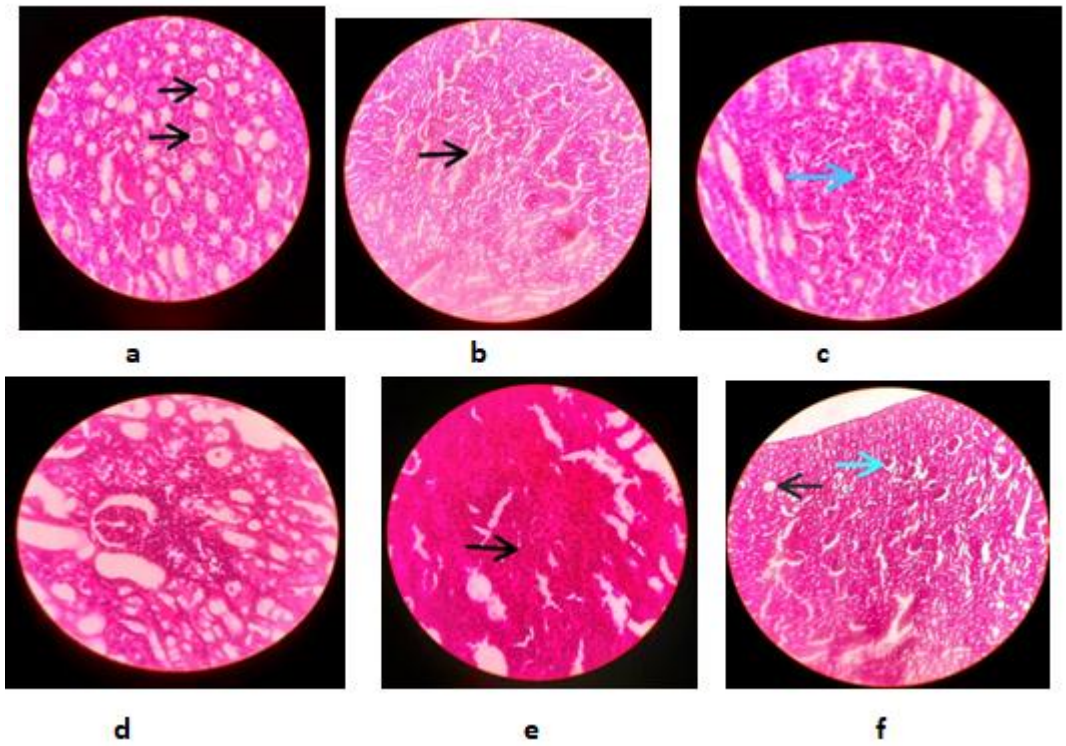

Figure No. 1: (1) Plain control group (2) Negative Control (3) Test group A (4) Test group B (5) Combined extract low dose group (6) Combined extract high dose group

Table No. 1: Effect of ethanolic extract of Tukhm-e-Khurfa and Tukhm-e-Kahu on renal function against cisplatin induced nephrotoxicity

Groups	Serum Creatinine mg/dl	Blood Urea Nitrogen mg/dl
Plain Control	0.65±0.04282	21.42±1.037
Negative Control	2.517±0.09458^{***a}	62.27±1.126^{***a}
Test group A	0.6167±0.04773^{***b}	19.77±0.6815^{***b}
Test group B	2.233±0.1726	46.17±5.889
Combined extract low dose group	2.017±0.1778^{*c}	46.17±5.889^{**d}
Combined extract high dose group	0.55±0.02236^{***e}	18.35±0.1648^{***e}

One-way ANOVA followed by tukey's multiple comparison tests. Data expressed in mean±SEM. N=6 animals in each group.

* P<0.05, ** P<0.01, *** P<0.001

a- Plain control vs Negative control

b- Negative control vs Test group A

c- Negative control vs Combined low dose (CLD)

d- Negative control vs Combined low dose (CLD)

e- Negative control vs Combined high dose (CHD)

DISCUSSION

Numerous processes are involved in cisplatin-induced nephrotoxicity such as inflammation, production of reactive oxygen species (ROS), cell apoptosis and mitochondrial dysfunctioning (*Kilic et al.* 2013*; Kotins et al.* 2014). This study demonstrates that *P. oleracea* and *L. sativa* possess nephroprotective effect against cisplatin-induced nephrotoxicity. It was observed that cisplatin induced severe renal injury, which was evidenced by the elevated serum creatinine and blood urea nitrogen (BUN) levels (Table 1) in group of animals treated with cisplatin only. Elevation of serum creatinine and BUN has been considered as one of the most important manifestation of severe tubular injury of kidney (*Ali et al.*, 2001: *Bennit et al.*, 1982; *Gilmao et al.*, 1992) especially if the elevation in both is

proportional (*Braum e, al, 1975*). Cisplatin has been reported to produce nephrotoxicity (*Devi MA DMNBPK et al*. 2016). It is an indication of severe tubular cell damage that was further confirmed by the histopathological studies where the features of acute tubular necrosis were observed (Figure 1).

Treatment with test extract the two serum markers i.e. urea and creatinine significantly ($p< 0.001$) reduced in test group A and combined high dose group when compared with negative control group. Whereas, in combined low dose group showed significant decrease in serum urea ($p<0.05$) and creatinine ($p<0.01$) level in comparison to negative control. The histopathological analysis of kidney tissue also showed tremendous recovery as the features suggested only mild to moderate degree of tubular necrosis in treated groups in comparison to negative control. Thus, the test drug was found to exhibit nephroprotective activity.

There are several evidences that diuretic drugs of Unani system of medicine help to prevent the kidney from nephrotoxicity (Wasim Ahmad *et al 2011* and Ghufran Ahmad *et al 2013).* Several studies have suggested that oxidative stress is the major reason for nephrotoxicity caused by lipid peroxidation and free radicals. Free radicals formation is one of the mechanisms of nephrotoxicity induced by cisplatin and antioxidants have protective effects against renal toxicity induced by this drug (*Gholam reza Karimi et al, 2010*). It has been reported that quercetin has nephroprotective activity (*Devipriya S, et al* 1999). Lettuce is a rich source of carotene and vitamin C. It is also a fair source of vitamin E **(**Nicolle *et al.* 2004**)**. *P. oleracrea* and *L. sativa* having chemical constituents like Flavonoids (Quercetin), omega-3, ascorbic acid, β-carotene and glutathione which have anti-oxidant activity. And these plants have also diuretic property **(***Golam reza karimi et al 2010, Md. Kamal Uddin et al 2014, Database on Midicinal plant used in Ayurveda vol-3***).** Previous studies on *Aegle marmelos* (Safarjal Hindi), *Rosa damascena* (Gul-e-Surkh) *and Cichorium intybus* (Kasni) (*Kore K. J et al, 2011*) have a potent antioxidant and renal protective activity and preclude oxidative damage inflicted to the kidney due to presence of quercetin (*Zaid Ahmad Q et al 2016*) These drugs were shown to produce beneficial effect in kidney disease without producing any major side effect on other systems of the body.

CONCLUSION

The findings of the present study suggests that the Ethanolic extract of Tukhm-e-Khurfa single as well as in combination protect against cisplatin induced nephrotoxicity and may be considered as a potentially useful candidate in nephrotoxicity. It also validated the claim of

Unani physicians that Tukhm-e-khurfa and Tukhm-e-Kahu which can be used as nephroprotective agent in renal diseases. Extensive and multidimensional further research is needed to elucidate the exact mechanism of nephroprotective action of these plants extract.

ACKNOWLEDGEMENT

The authors are thankful to principal and administration of Govt. Nizamia Tibbi College, Charminar, Hyderabad, for providing facilities to carry out this research work.

REFERENCES

1. Aurther GC Text book of medical physiology 8th edition WB, Saunders company, hartcourt publisher international company Singapore 2000, pp 364-379
2. Tortora .J.F, Nicholas P.A. 1990, "Principles of Anatomy and Physiology,6th Edn,Harper and Raw publishers, New York,273-353
3. Kalra P, Karwasra R, Gupta YK, Ray SB, Singh S. Terminalia chebula supplementation attenuates cisplatin-induced nephrotoxicity in Wistar rats through modulation of apoptotic pathway. Natural Product Research. 2018 Jan 17;33(11):1641-5.
4. Md Alam A, Quamri MA, Siddiqui MA, Hai U, Sofi G. Nephroprotective effect and unani medicine: A review. J Nephrol Ther. 2016;6 (236):2161-0959.
5. Zaman R, Alam A, Jafri MA, Sofi G, Ahmad G. Nephroprotective effect of Beekh Kasni (Roots of Cichorium intybus) in the form of methanolic and aqueous extract in Gentamycin induced rat models. Journal of Pharmacognosy and Phytochemistry. 2017;6(3):337-41.
6. Karim MS, Ashraf N, Kalam A, Jahan N, Jafri MA, Ahmad G. Effects of Biskhapra (Trianthema portulacastrum Linn.) leaves extract in adriamycin induced nephrotic syndrome. International Journal of Green Pharmacy (IJGP). 2011;5(4).
7. Baitar I. Al-Jam-e-ul-Mufradat-Al-Adviah-Wal-Aghziya (Urdu translation). CCRUM publication, New Delhi. 1999. 257-58,122,123
8. Hubal BI. Kitab Al-Mukhtarat Fit Tibb (Urdu Translation by CCRUM). Vol II. New Dehli: Ministry of Health & Family Welfare. 2007:84-5,282-3
9. Sina I. Al Qanoon Fit Tibb. Urdu Trans: Kantoori GH.) part I Vol-II, New Delhi: Idarae Kitabus Shifa. 2010: 60,223
10. Momin HM. Tohfa al Momineen. Nawal kishore, Lucknow. YNM 53.
11. Azam, M.K. Muheet Azam Persian,part I Vol-IV and part I Vol-II, Matba Nizami, Kanpur,1895; 22-3, 184-6
12. Ghani M.N., Khazainul Advia, Idara kitab al shifa, New Delhi, 55,108,240-43,458-59,667-68,1012-13
13. YBU Ibne Ali, Kitabul Motamad fil advia Mufrada, Dar al Kutub Alarabia Alkubra Misr,1907. 29.,126-27
14. Karimi G, Khoei A, Omidi A, Kalantari M, Babaei J, Taghiabadi E, Razavi BM. Protective effect of aqueous and ethanolic extracts of Portulaca oleracea against cisplatin induced nephrotoxicity. Iranian journal of basic medical sciences. 2010;13(2):31-5.
15. Llorach R, Tomás-Barberán FA, Ferreres F. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. Journal of Agricultural and Food Chemistry. 2004 Aug 11;52(16):5109-16.
16. Younus I, Ismail H, Rizvi CB, Dilshad E, Saba K, Mirza B, Tahir M. Antioxidant, analgesic and anti-inflammatory activities of in vitro and field-grown Iceberg lettuce extracts. Journal of Pharmacy & Pharmacognosy Research. 2019;7(5):343-55.
17. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. The FASEB journal. 2008 Mar;22(3):659-61.
18. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. Journal of basic and clinical pharmacy. 2016 Mar;7(2):27.

19. Afzal M, Khan NA, Ghufran A, Iqbal A, Inamuddin M. Diuretic and nephroprotective effect of Jawarish Zarooni Sada—a polyherbal unani formulation. Journal of ethnopharmacology. 2004 Apr 1;91(2-3):219-23.
20. Shameem A, Khan NA, Ahmed G. Effect of Banadequl buzoor in some renal disorders. Hamdard Medicus XL. II (4), Hamdard Foundation, Karachi, Pakistan. 1999:31-6.
21. Shirwaikar A, Issac D, Malini S. Effect of Aerva lanata on cisplatin and gentamicin models of acute renal failure. Journal of ethnopharmacology. 2004 Jan 1;90(1):81-6.
22. Khatoon R, Aslam M, Chaudhary SS. Nephroprotection in Unani Medicine through Herbal Medicine & their research scope: A Review. Journal of Drug Delivery and Therapeutics. 2019 Aug 15;9(4-s):687-95.
23. Zaid Ahmad Q. Herbs as Nephroprotective Agent: An Over View with Reference to Unani System of Medicine. Austin Renal Dis. 2016;1(1):1001.
24. Kilic U, Kilic E, Tuzcu Z, Tuzcu M, Ozercan IH, Yilmaz O, Sahin F, Sahin K. 2013. Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. Nutr Metab. 10:01–08.
25. Kotins MS, Patel P, Menon SN, Sane RT. 2014. Renoprotective effect of Hemidesmus indicus, a herbal drug used in gentamicin-induced renal toxicity. Nephrol. 9:142–147.
26. Ali, B.H., Ben Ismail, T.H., Basheer, A.A., 2001. Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity: influence of gonadectomy and hormonal replacement therapy. Indian Journal of Pharmacology 33, 369–373.
27. Bennit, W.M., Parker, R.A., Elliot, W.C., Gilbert, D., Houghton, D., 1982. Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity. Journal of Infectious diseases 145, 370–374
28. Gilman, G.A., Rall, T.W., Nies, A.S., Taylor, P., 1992.The Pharmacological Basis of Therapeutics, vol. 2, 8th ed. McGraw-Hill, Singapore, pp. 1106–1109.
29. Devi MA DMNBPK. Evaluation of nephroprotective activity of ethanolic extract of Annona reticulata in gentamicin and cisplatin induced nephrotoxicity in rats. Journal of Pharmaceutical Sciences and Research.. 2016; 8(9): p. 995.
30. Wasim A, Khan NA, Ghufran A, Shamshad A. Study of Carthamus tinctorius Linn for diuretic and nephroprotective effect in albino rats. Unani Medicus. 2011:76-82.
31. Gufran A, Wasim A, Khan NA, Shamshad A. Evaluation of Ethanolic Extract of Habb-e-Kaknaj (Physalisalkekenji Linn. Fruit) in Rat. Hippocratic Journal of Unani Medicine. 2013;8(3):1-9.
32. Devi PS, Shyamala DC. Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney. Indian journal of pharmacology. 1999 Oct 1;31(6):422.
33. Nicolle C, Cardinault N, Gueux E, Jaffrelo L, Rock E, Mazur A, Amouroux P, Rémésy C. Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clinical Nutrition. 2004 Aug 1;23(4):605-14.
34. Uddin M, Juraimi AS, Hossain MS, Un A, Ali M, Rahman MM. Purslane weed (Portulaca oleracea): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. The Scientific World Journal. 2014 Oct;2014.
35. Database on Midicinal plant used in Ayurveda vol-3 Sharma PC, Yelne MB, Dennis TJ, Joshi A.
36. Kore KJ, Jadhav PJ, Shete RV, Shetty SC. Diuretic activity of Tectona grandis leaves aqueous extract in wistar rats. International Journal of Pharmaceutical Research and Development. 2011;3:141-146.