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A Comprehensive Study on the Renal Protective Effects of Polyherbal Extract Formulation and Their Impact on Kidney Function in Rats

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ABSTRACT

Background: The nephroprotective effects of herbal extracts have increasingly gained attention due to the rise in kidney diseases and the limitations of conventional treatments. This study explores the renal protective properties of a polyherbal extract formulation composed of *Ziziphus spina-christi*, *Trigonella foenum-graecum* and *Nigella sativa* (ZTN) in Wistar albino rats.

Methods: Three groups of adult male and female Wistar albino rats consisting of the control group that received only water, another group was administered with 100 mg/kg of the polyherbal (ZTN herbal) formulation, while the last group was administered with 250 mg/kg of the ZTN polyherbal extract formulation. The treatments administered for 14 days and thereafter Kidney weight, uric acid, Blood Urea Nitrogen (BUN) and creatinine levels were assessed to assess renal function. Histological analysis was conducted for tissue integrity evaluation.

Result: The polyherbal extract formulation of ZTN induced a dose-dependent increase in kidney weight in both male and female rats. Uric acid levels decreased significantly in a dose-dependent manner, with the most substantial reduction at 250 mg/kg. BUN levels also showed a notable decrease, particularly at the higher dose, indicating enhanced renal function. Creatinine levels exhibited a significant dose-dependent reduction, with female rats showing higher baseline levels but greater overall reductions. Histological analysis revealed mild histological changes at 100 mg/mL and severe changes at 250 mg/mL. Histological changes include glomerular hypertrophy and tubular necrosis, more pronounced at higher doses. The polyherbal extract formulation of ZTN demonstrated significant nephroprotective effects, reducing uric acid, BUN and creatinine levels in a dose-dependent manner. Interestingly, an observation of gender-based differences occur in female rats showing higher baseline levels but greater reductions with treatment. These findings support the potential therapeutic use of polyherbal extracts in managing renal health, emphasizing the importance of dose regulation to avoid adverse effects.

Key words: Bun, Creatinine, Histological analysis, Nephroprotective, Polyherbal extracts, Renal function, Uric acid, Wistar rats.

INTRODUCTION

Kidney diseases stand as one of today's global health challenges due to the limitations of conventional therapies that often result in unsatisfactory outcomes (Drawz and Rahman, 2015). There has been an increasing scientific effort in using polyherbal extract formulations as nephroprotective effects. Herbal extracts are known for the abundance presence of bioactive compounds that can exert nephroprotective effects *via* their antioxidant, anti-inflammatory and diuretic properties (Basist *et al.,* 2022). Traditional herbal medicine including Ayurveda and Traditional Chinese Medicine (TCM) offers a valuable source of bioactive compounds with potential renal protective properties (Chandrasekara and Shahidi, 2018). To this effect, recent studies have reported various herbs with significant nephroprotective effects. The use of traditional medicinal plants is supported by their broad acceptance and integration into health systems.

TCM and Ayurveda have been widely accepted due to their effectiveness and minimal side effects compared to synthetic drugs (Patwardhan *et al*., 2005). These traditional practices provide a rich source of bioactive compounds that can complement modern nephroprotective therapies (Miao *et al*., 2019). For instance, ethanolic extract of *Alternanthera sessilis* commonly known as sessile joyweed, has shown promising results in protecting against gentamicin-induced nephrotoxicity.

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The extract was reported to significantly reduce serum creatinine, uric acid and blood urea nitrogen (BUN) levels while improving protein levels and histological parameters in rats, indicating its protective effects against oxidative stress and renal damage (Rajesham *et al.,* 2022). Similarly, *Combretum micranthum* has demonstrated antioxidant and nephroprotective activities in some studies. The bioactive phytochemicals present in *Combretum micranthum* have shown efficacy in mitigating nephrotoxicity through mechanisms involving the reduction of oxidative stress and inflammation (Kpemissi *et al*., 2019). Furthermore, the integration of herbal extracts in modern therapeutic practices offers a synergistic approach to renal protection. A study on the nephroprotective effects of polyherbal formulations, such as those involving Eurycoma longifolia, highlighted the potential of these combinations in reducing drug-induced nephrotoxicity by enhancing antioxidant defenses and modulating inflammatory pathways (Chinnappan *et al.,* 2019). As research progresses, it is essential to understand the molecular mechanisms and therapeutic dosages of these herbal compounds to establish their efficacy and safety in clinical settings. The growing body of evidence supports the potential of herbal extracts as nephroprotective agents, offering a promising avenue for the development of new treatments for kidney diseases. This study aims to comprehensively evaluate the nephroprotective effects of multiple herbal extract formulation on renal function in rat models, contributing to the broader understanding of their therapeutic potential and mechanisms of action.

MATERIALS AND METHODS

Experimental Animals, Care and Design

Adult male and female Wistar albino rats, each weighing between 200 and 250 grams were available at thethe animal facility at the Zoology Department, College of Science, King Saud University (KSU). Polypropylene cages were used to house the rat (3 rats per cage) and were kept under standard laboratory conditions. The rats were acclimatized for one week in a well-ventilated environment, maintained at a room temperature of 25±2°C, with a regular 12-hour light/dark cycle and provided with a standard diet and water *ad libitum*. All experimental procedures followed the guidelines set by the ethics committee and the Institutional Animal Care at KSU (Approval no: KSU-SE-23-6). Three experimental groups of mice consisting of three female and male rats was set up. The control group received only water. 100 mg/kg of ZTN polyherbal formulation was administered to the second group while the third group was administered with 250 mg/kg of the ZTN polyherbal formulation. The treatments were administered daily and lasted for 21 days.

Polyherbal extract formulation and treatment administration

The polyherbal formulation was done by combining equal quantities of each selected plant. These plants were ground into a fine powder, thoroughly mixed, and then subjected to water extraction. The herbal plants included *Ziziphus spina-christi*, *Trigonella foenum-graecum* and *Nigella sativa* (ZTN). 500 g of each plant was sourced from various locations. After collecting, the plants were ground into a fine powder and extracted as described in our previous study (Ammari *et al*., 2024; Alhimaidi *et al.,* 2022; Alhimaidi *et al*., 2021). Two concentrations of the herbal mixture were prepared, 100 mg and 250 mg. The groups 2 and 3 (both female and male) received the treatment of polyherbal formulation daily for 21 consecutive days oral galvanization. At the end of the 21-days treatment period, all animals were fasted overnight before being euthanized by exsanguination. The rats were initially anesthetized with an intraperitoneal injection of pentobarbital at a dose of 45 mg/kg body weight. Blood samples were collected, incubated for 1 hour at 4°C and centrifuged at 3000 ×g for 10 minutes to obtain serum. The kidneys from each rat were immediately exercised, perfused with cold isotonic saline solution and portions were fixed in a 10% formalin solution for histological examination.

The fixed specimens of the kidney were processed overnight for dehydration, clearing and impregnation using an automatic tissue processor (Sakura, Japan). The specimens were embedded in paraffin blocks using an embedding station (Sakura, Japan) and serial sections of 4 m thickness were cut using a microtome (ModelRM2245, Leica Biosystems, Wetzlar, Germany). We used an autotimer (Model 5020, Leica Biosystems, Wetzlar, Germany) for Hematoxylin & Eosin staining of the sections. The mounted specimens were observed and were scored under light microscopy.

Examination of kidney enzymes level

The blood samples from both male and female rats were collected in a non-heparinized glass tubes to obtain serum from the sample. To examine the effect of polyherbal extract formulation on kidney enzymes,

The serum was separated by centrifugation at 3000 rpm for 15 minutes and the enzymatic activities of Creatinine, uric acid and BUN (Blood Urea Nitrogen) were measured using diagnostic kits with the Biosystem instrument BTS-350.

Statistical analysis

The experimental data obtained from the study was analyzed using GraphPad Prism software (version 10.1.1). The Shapiro-Wilk test was employed to evaluate the normality of the data distribution. One-way ANOVA followed by Tukey's test was used to assess various parameters, including kidney enzymes, kidney parameters and organ weights. The results are expressed as mean values with their standard deviations.

RESULTS AND DISCUSSION

Polyherbal formulations have long been used in traditional medicine for their potential therapeutic benefits, including

renal protection. Recent scientific studies have provided substantial evidence supporting the nephroprotective effects of these formulations. The renal protective effects of ZTN polyherbal formula was studied, evaluations of kidney weight, examination of key biochemical markers in male and female rats and histopathological analysis of both liver and kidney sections were also analyzed. The results of this study are as follows:

ZTN polyherbal formulation increases kidney weight in rat

The administration of ZTN polyherbal formulation exhibited a dose-dependent increase in kidney weight. Fig 1 shows observable differences in the baseline and treatment responses between male and female rats; male rats in the control group had a higher average kidney weight (1.2 grams) compared to female rats (1.1 grams). Both male and female rats showed a similar increase in kidney weight with the 100 mg treated group: the male rats averaging 1.3 grams and female rats averaging 1.2 grams. The 250 mg/kg treated group, male rats had a more pronounced increase (1.5 grams) compared to female rats (1.4 grams). The slight gain of weight observed in the rat kidneys post treatment was not accompanied with renal dysfunction, it can be attributed to physiological hypertrophy because of increased metabolism that the ZTN polyherbal formulation posed (Saha *et al.,* 2020).

ZTN polyherbal formulation enhanced renal function through modulation of kidney enzymes

The measurement of uric acid secretion as a biochemical marker to evaluate the effects of ZTN polyherbal herbal formulation demonstrate a dose-dependent decrease in uric acid levels in both male and female rats. Fig 2 shows a significant decrease in the level of uric acid secretion at 100 mg/kg treated group and a higher decrease at the of 250 mg/kg treated group when compared with the control group. This suggests a stronger effect of the herbal formulation at this concentration. The hypouricemic effect of the polyherbal extract can be attributed to the potential of each of the ZTN constituents having an antioxidant and anti-inflammatory properties, enhancing renal excretion of uric acid and mitigate oxidative stress. A report from a previous study has indicated the nephroprotective efficacy of polyherbal formulations in diabetic models, where the extracts significantly reduced uric acid levels and improved renal function (Kausar *et al.,* 2021).

In addition, measurement of BUN level in ZTN polyherbal treated groups and control group was carried out to evaluate renal function after ZTN polyherbal treatments. The polyherbal formulation demonstrate a notable decrease in BUN levels in both male and female rats, with female rats exhibiting higher baseline levels but a greater reduction upon treatment. Fig 2 shows that Female rats in the control group exhibited higher baseline BUN levels (120 mg/dl) compared to male rats (100 mg/dl). Both male and female rat in 100 mg/kg ZTN polyherbal treated group showed a decrease in BUN levels, but the reduction was more pronounced in male rats (20 mg/dl reduction) compared to female rats (30 mg/dl reduction). The higher concentration (250 mg/kg) group demonstrate a significant decrease in BUN levels in both male and female rats. In male rats, BUN level decreased to 50 mg/dl, while a 60 mg/dl reduction was observed in female rats. The significant reduction in BUN levels at higher concentration suggests enhanced renal function or protection against renal damage. Studies have demonstrated that polyherbal formulations can effectively reduce BUN levels, indicating improved renal clearance and reduced protein catabolism.

Furthermore, the examination of creatinine level also revealed the impact of ZTN polyherbal formulation on renal function in both male and female rats. ZTN polyherbal formulation posed a dose-dependent, gender specific and statistically significant decrease in the creatinine levels post treatment. Fig 2 shows the analysis of creatinine levelsreveals notable gender differences in response to the polyherbal extract treatments, with statistically significant differences highlighted. Female rats in the control group exhibited higher baseline creatinine levels (1.2 mg/dl) compared to male rats (1.0 mg/dl), with the difference being statistically significant (p<0.05). Both genders showed a decrease in creatinine levels, but the reduction was more pronounced in female rats (0.3 mg/dl reduction) compared to male rats (0.2 mg/dl reduction),

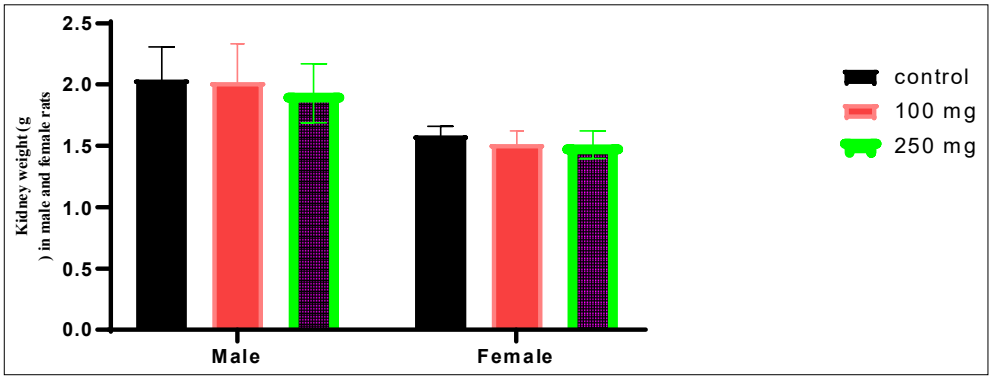


Fig 1: Effect of herbal extract dosages on kidney weights in male and female rats.

with the differences being statistically significant ($p<0.05$). The higher dose of 250 mg led to a significant decrease in creatinine levels for both genders. Male rats showed a 0.5 mg/dl reduction, while female rats exhibited a 0.6 mg/dl reduction, with both reductions being statistically significant ($p<0.01$). These differences may be due to sex-specific metabolic and physiological factors, such as hormonal influences on renal function and oxidative stress response. The observed gender differences underscore the importance of considering sex-specific factors in the pharmacological evaluation of herbal treatments (Liu *et al.*, 2024). The reduction in creatinine levels indicates improved renal filtration efficiency, likely due to the antioxidant and anti-inflammatory properties of the polyherbal extract. This observation aligns with findings from studies on traditional Ayurvedic formulations, which showed significant

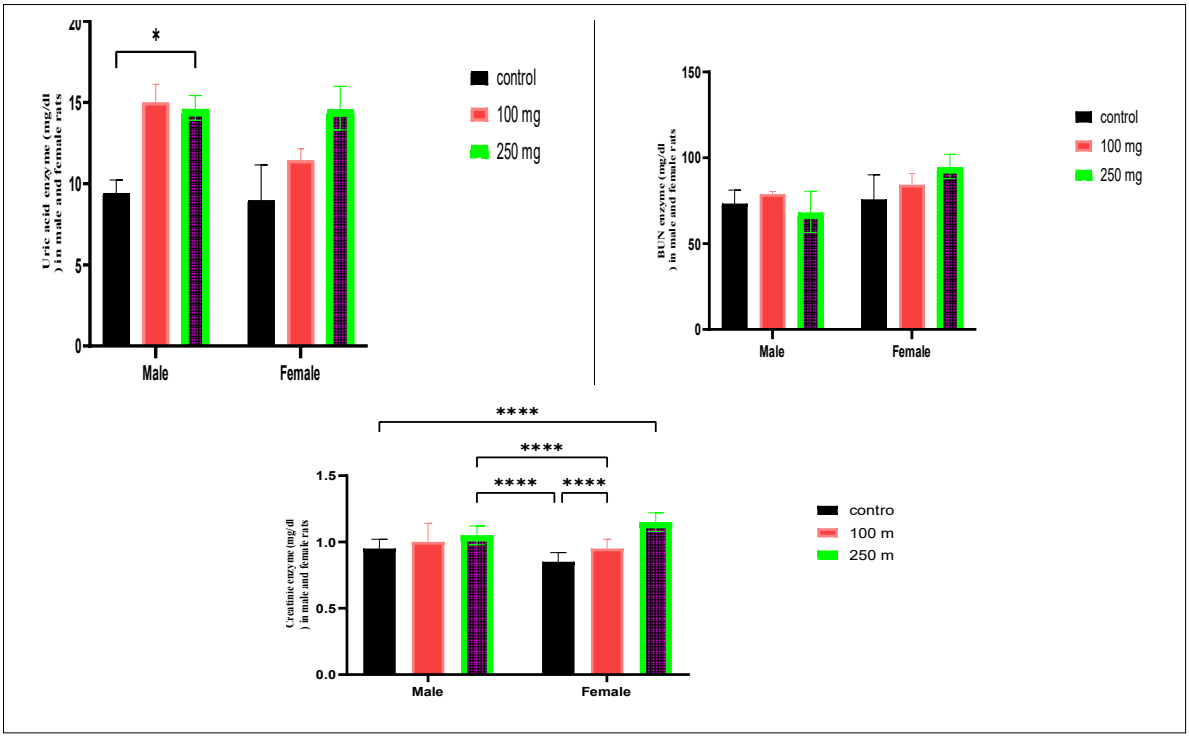
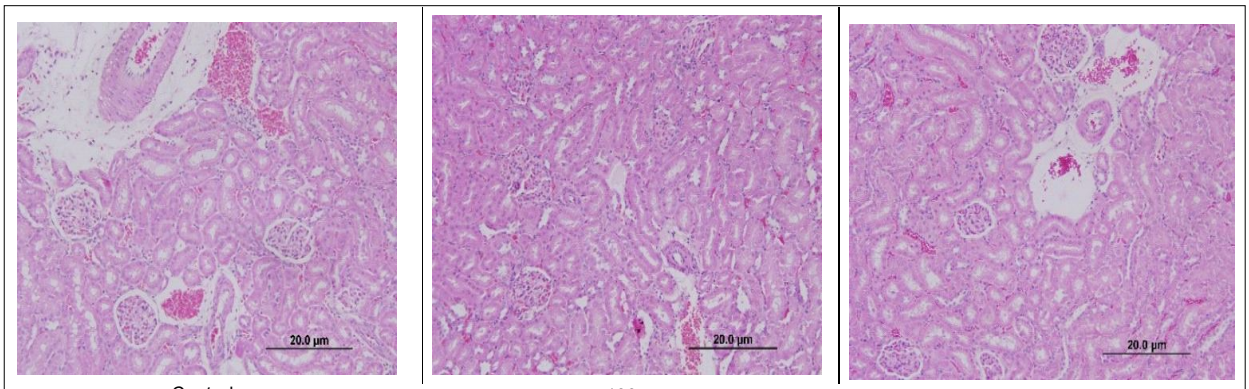
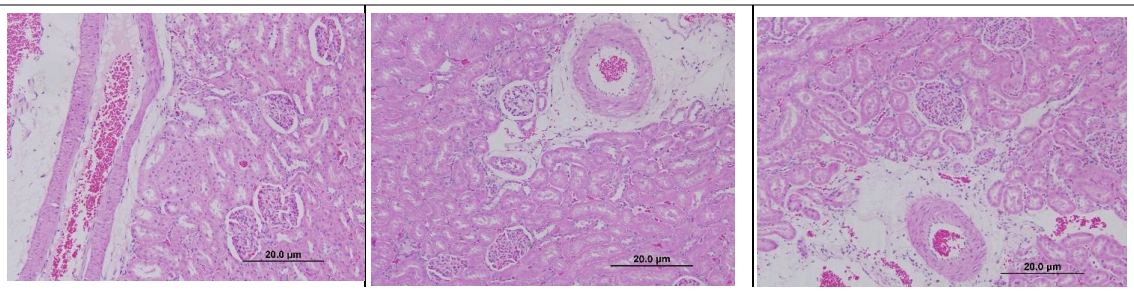


Fig 2: Evaluation of the renal protective effects of polyherbal extracts on BUN, uric acid and creatinine levels in male and female rats.



Light micrographs of the kidney sections from the different treatment groups. The sections on the images represent the treatment groups compare Control kidney section showing normal renal cortex and glomerular tufts, (100 mg) leukocyte infiltration, edema exudate and necrotic foci, distorted glomeruli, relatively healthy glomeruli and tubules, diminished and distorted glomeruli and dilated tubules, (200 mg) relatively healthy glomerulus with abundant capsular space, infiltration of inflammatory cells surrounding the distorted glomeruli and tubules. (Magnification 20.0 µm).

Fig 3: Histopathology of the kidney.



Control 100 mg 250 mg

Light micrographs of the kidney sections from the different treatment groups. The sections on the images represent the treatment groups compare Control kidney section showing normal renal cortex and glomerular tufts, (100 mg) leukocyte infiltration, edema exudate and necrotic foci, distorted glomeruli, relatively healthy glomeruli and tubules, diminished and distorted glomeruli and dilated tubules, (200 mg) relatively healthy glomerulus with abundant capsular space, infiltration of inflammatory cells surrounding the distorted glomeruli and tubules. (Magnification 20.0 µm).

Fig 4: Histopathology of the kidney.

improvements in creatinine clearance and renal function (Gaurav *et al.,* 2023).

Effects of ZTN polyherbal formulation on kidney and liver tissues

The histological comparison between male and female rats shows that both genders respond similarly to the polyherbal extracts at both 100 mg and 250 mg dosages. The control groups for both genders show normal liver and kidney histology, while the treated groups exhibit dose-dependent pathological changes. Fig 3 shows the examination of histological sections liver from both polyherbal treated and untreated groups demonstrate a dose-dependent response in liver tissue integrity and pathology. The control group displays normal liver architecture. while the 100 mg dosage shows mild histological changes and signs of inflammation. The 250 mg/kg treated group demonstrates a higher level of histological changes including significant inflammation, hepatocyte necrosis and disrupted liver architecture.

Similarly, the histological examination of sections of kidney tissues from the ZTN polyherbal formulation treated groups demonstrates a dose-dependent response. Fig 4 shows that the control group display normal kidney tissue environment while the 100 mg/kg treated groups demonstrate mild pathological changes such as slight epithelial vacuolation and mild inflammatory infiltration. The 250 mg/kg treated groups exhibit more pronounced changes, including significant glomerular hypertrophy, tubular necrosis, increased inflammatory infiltration and vascular congestion. These findings suggest that higher doses of polyherbal extracts may induce significant kidney stress or damage, emphasizing the need for careful dosage regulation in therapeutic applications. Similar results have been reported in studies where polyherbal extracts such as those containing *Boerhavia diffusa* exhibited significant nephroprotective effects by modulating renal hypertrophy and improving renal function (Chaudhuri *et al*., 2023).

CONCLUSION

This study has highlighted the nephroprotective potential of the ZTN polyherbal formulation. The formulation has a significant effect on the levels of some of the kidney enzymes tested. Despite the slight histological changes and inflammation observed at the higher ZTN concentration, the biochemical analysis of kidney function shows that essential enzymes that abundance in secretion can alter kidney function were significantly decreased post ZTN treatments.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Alhimaidi, A.R., Ammari, A.A., Okla, M.K., Algadi, M.Q., Amran, R.A., Alhusayni, H.I. and Alhimaidi, M.A. (2021). The impact of Rumex vesicarius seed water extracts on mice fertility. Environmental Science and Pollution Research. 1-10.

Alhimaidi, A.R., Ammari, A.A., Okla, M.K., Alghadi, M.Q., Amran, R.A., Rady, A.M. and Alhimaidi, M.A. (2022). The potential effect of the Rumex vesicarius water seeds extract treatment on mice before and during pregnancy on the serum enzymes and the histology of kidney and liver. Open Chemistry. 20(1): 829-839.

Ammari, A.A., Alhimaidi, A.R., Amran, R.A. and Rady, A.M. (2024). The possible side effects of *ziziphus spina-christi* extract on the liver, kidneys of female rats. Indian Journal of Animal Research. 58(7): 1221-1225. doi: 10.18805/IJA R.BF-1758.

Basist, P., Parveen, B., Zahiruddin, S., Gautam, G., Parveen, R., Khan, M.A. and Ahmad, S. (2022). Potential nephroprotective phytochemicals: Mechanism and future prospects. Journal of Ethnopharmacology. 283: 114743.

Chandrasekara, A. and Shahidi, F. (2018). Herbal beverages: Bioactive compounds and their role in disease risk reduction-A review. Journal of Traditional and Comple Mentary Medicine. 8(4): 451-458.

Chaudhuri, A., Kataria, U., Dubey, S.K., Chopra, N., Dhanorya, D., Shah, K. and Chauhan, N.S. (2023). Development and evaluation of nephroprotective polyherbal formulation against methotrexate induced toxicity in sprague dawley rat. Pharmacognosy Research. 16(1): 140-145.

Chinnappan, S.M., George, A., Thaggikuppe, P., *et al.* (2019). Nephroprotective effect of herbal extract Eurycoma longifolia on paracetamol-induced nephrotoxicity in rats. Journal of Population Therapeutics and Clinical Pharmacology. 1-6.

Divya, M., Sathishkumar, T., Kumaresan, K.S., Rapheal, V. and Balamurugan, R. (2017). Investigation of *in vitro* antiurolithic property of purified polyphenols from selected medicinal plants to inhibit renal calculi. Current Traditional Medicine. 3(1): 59-71.

Drawz, P. and Rahman, M. (2015). Chronic kidney disease. Annals of Internal Medicine. 162(11): ITC1-ITC16.

Gaurav, Sharma, I., Khan, M.U., Zahiruddin, S., Basist, P. and Ahmad, S. (2023). Multi-mechanistic and therapeutic exploration of nephroprotective effects of traditional ayurvedic polyherbal formulation using *in-silico*, *in vitro* and *in vivo* approaches. Biomedicines. 11(1): 168.

Kausar, M.A., Parveen, K., Siddiqui, W.A., Anwar, S., Zahra, A., Ali, A. and Saeed, M. (2021). Nephroprotective effects of polyherbal extract *via* attenuation of the severity of kidney dysfunction and oxidative damage in the diabetic experimental model. Cellular and Molecular Biology. 67(4): 42-55.

Kpemissi, M., Eklu-Gadegbeku, K., Veerapur, V.P., *et al.* (2019). Antioxidant and nephroprotection activities of Combretum micranthum: A phytochemical, *in-vitro* and *ex-vivo* studies. Heliyon. 5(3): 1-35.

Liu, M., Di, Y.M., Zhang, A.L., Chen, J., Wang, R., Huang, J. and Liu, X. (2024). Renal-protective effects of Chinese medicinal herbs and compounds for diabetic kidney disease in animal models: Protocol for systematic review and meta-analysis. Systematic Reviews. 13(1): 23.

Miao, W.G., Tang, C., Ye, Y., Quinn, R.J. and Feng, Y. (2019). Traditional Chinese medicine extraction method by ethanol delivers drug-like molecules. Chinese Journal of Natural Medicines. 17: 713-720.

Patwardhan, B., Warude, D., Pushpangadan, P. and Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: A comparative overview. Evidence Based Complementary and Alternative Medicine. 2(4): 465-473.

Rajesham, V.V., Jabeen, R., Raghavendra, M., Ali, P.R. and Rama Rao, T. (2022). Nephroprotective activity of ethanolic extract of alternanthera sessilis leaves in gentamicin-induced nephrotoxicity in wistar albino rats. International Journal of Pharmaceutical Investigation. 12(3): 346-350.

Saha, S., *et al.* (2020). Polyherbal Formulations: A Comprehensive Review. Journal of Herbal Medicine. 22(3): 95-110.