Original Article

EFFECT OF CITRULLUS LANATUS SEEDS (ETHANOLIC EXTRACT) ON NORMAL RENAL FUNCTIONS AND SERUM ELECTROLYTES OF ALBINO RATS

NeelofarYousaf Warraich,¹ Amtul Hafeez,² Wardah Siddique,³ Fouzia Parveen,⁴ Shahnaz Fatima,⁵ Nazia Rashid,⁶

ABSTRACT

Objectives: To evaluate the effect of Citrullus lanatus seeds on normal renal functions and serum electrolytes for the improvement of kidney health in order to perform its functions in normal and stressful conditions.

Methods: The study was conducted at the Pharmacology Department, KEMU, Lahore and UVAS, Lahore in 2017. It was a randomized controlled trial (RCT). A sample size of 16 healthy albino wistar rats was taken. Rats were divided into two equal groups with 8 number of rats in each group and kept separately in metabolic cages. Group A was normal control group and given normal saline once a day orally. Group B was given the ethanolic extract of Citrullus lanatus seeds I/P 600 mg/kg/day.

Renal function tests (blood urea nitrogen, serum, and urine creatinine clearance) and serum electrolytes (Ca⁺⁺, Na⁺, K⁺, Mg⁺⁺) were evaluated along with effects on urinary volume, pH and specific gravity on day zero and day 14.

Results: Urinary output markedly increased in group B taking Citrullus lanatus seeds extract in comparison to normal control Group A with significant p-value < 0.001. Urinary pH of group B was improved significantly with p-value < 0.001 in comparison to group A. Whereas, there was the insignificant difference in RFTS, serum electrolytes & urinary specific gravity of both groups at the end of the study.

Conclusion: The ethanolic extract of Citrullus lanatus seeds is helpful in maintaining kidney health to perform its functions properly.

Key Words: Citrullus Lanatus Seeds, Renal functions Tests, Serum Electrolytes

INTRODUCTION:

Kidneys are excretory and the secretory organ of the body responsible for the maintenance of the hemostatic state of the body by accomplishing the three major goals such as glomerular filtration, tubular resorption and tubular excretion¹ which are generally assessed by renal function tests. Electrolytes are minerals in body fluids that carry an electric charge and keep the heart, nerves, and muscles in proper function.

Sometimes kidneys functions get deteriorated depending upon the physical, chemical and biological circumstances that it faces. There is circumstantial evidence available now which shows rapid decline in kidney function with age, different chronic ailments and due to its exposure to toxic substances which lead to acute and chronic kidney failure. So, it is very important to maintain/ improve the health of kidneys to perform its functions in normal and stressful conditions.

As such, it is important to maintain a precise and constant balance of electrolytes to stay healthy. Our kidneys play an important role in ensuring that electrolyte levels remain invariant despite any change the body may undergo. Having excess or insufficiency of electrolytes in the body can be dangerous and in some cases fatal.

¹ Assistant Professor Pharmacology, AMDC, Lahore.

² Assistant Professor Pharmacology, IM&DC, Sialkot.

³ Assistant Professor Pharmacology, LMDC, Lahore.

⁴ Assistant Professor Pharmacology, SMDC, Lahore.

⁵ Assistant Professor Pharmacology, SMC, Narowal

⁶ Assistant Professor Pharmacology, FMJU, Lahore.

The role of herbal remedies to boost normal health conditions is always considered effective. In recent years, an increasing number of people has chosen traditional remedies and products to improve their health conditions either alone or in combination with other herbal products.²

The nephroprotective role of different naturally occurring herbs is being studied to reduce stress caused by various reasons such as Aloe vera³, Kabab chini⁴, silymarin⁵, rosemary, curcumin, propolis, ginger, black seeds, turmeric.

Citrullus lanatus fruit belongs to the Cucurbitaceae family Slight glance in past relieves the importance of Citrullus lanatus in traditional herbal medicine and remedies. It is not only used to overcome severe heat effects in the human body or quenching thirst, but its different parts such as roots, rind, pulp, and seeds are used for different purposes/treatments of different ailments because of its composition. Seeds are the most ignored part of the fruit, unknown to the fact that it possess the maximal nutrient values in comparison to the whole fruit.

Watermelon seeds are great sources of carbohydrates, protein (both essential and non-essential amino acids) and oil. These seeds comprise about 35% of protein, 50% of oil, and 5% of dietary fiber. Watermelon seed is also rich in micro- and macronutrients such as magnesium, calcium, iron, phosphorus, potassium, Watermelon seeds are a source of healthy fat (unsaturated), almost 90%, vitamins, antioxidants, minerals, proteins phytochemicals (phenols and flavonoids). According to seed analysis by Okunrobo O et al.,(2012) different minerals are present in watermelon seed in different quantities such as magnesium (11.4 mg/kg), calcium (16.8mg/kg), potassium (7.8mg/kg), sodium (5.7 mg/kg) and zinc (1.2 mg/kg).8

Seeds have magical effects as a demulcent, pectoral tonic and; hepatoprotective and hypotensive agents. Seeds are used to treat bed wetting and UTI (urinary tract infection). It is vermifuge; paralyzing tapeworms and roundworms. Their

antimicrobial action is better than that of pumpkin seed oil.¹⁰ They also possess analgesic, anti-inflammatory activity, anti-ulcerogenic and the hepatoprotective activity.¹¹

METHODS:

The fresh fruit of Citrullus lanatus was purchased from the local fruit market of Lahore. The fruit was properly cut and seeds were separated. Proper identification of seeds was done by authorized personnel from GC University, Lahore. Seeds were properly washed and dried under sunlight for 3 days and then ground into fine powder using an electric blender. The ethanolic extract of Citrullus lanatus seeds was prepared¹² and dissolved in 5 ml distilled water. The dose for individual rat i.e. 600mg/kg was calculated. Doses were administered through feeding guage no. 16. Adult healthy albino rats of male gender weighing 150-200 grams were purchased from the local market. Animals were numbered and kept in iron cages in individual groups, under good hygienic conditions, at room temperature under natural light and dark cycles so that to maintain the biological clock in the animal house of UVAS. Food and water were provided ad libitum.

Rats were divided into two experimental groups, containing eight rats in each group randomly. Group 1 was a healthy control group and 0.5 ml of distilled water was given orally once a day with a feeding tube (16 guage). Group 2 rats were given Citrullus lanatus seeds ethanolic extract in the dose of 600 mg/kg/day body weight orally with a 16 gauge feeding tube once daily. All the treatment was provided daily for 14 days. 24 hours of urine of rats was collected in a clean glass container and volume was measured in measuring glass beaker.

In a small amount of urine; its specific gravity, pH, glucose, and albumin were assessed by urine test strips of URIMED, Biotec Diagnostics UK Ltd.¹³ The results were evaluated according to the change of

colors on the strips as mentioned on the strip leaflet.

The remaining urine was not centrifuged and poured into serum cups and stored in the research freezer (-20°C) for analysis of urine creatinine later About 1-1.5 milliliters of whole blood was drawn by the cardiac puncture⁹ using disposable syringes. The collected blood sample was transferred to labeled gel tubes and allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rev/min for minutes. 14 The clear serum obtained was separated and shifted in serum cups and preserved at -20 °C till serum analysis was started. Lab tests were performed for analysis of RFTs and serum electrolytes. All the tests were performed on semiautomatic clinical chemistry analyzer, micro lab 300. Methods were followed as provided by manufacturer. Before the performance, the cuvettes and the frozen samples were warmed to desired temperature (37°C).

version 8 for statistical analysis and p-value less than 0.05 was considered significant.

All data was entered on graph pad prism

Renal function tests including blood urea

RESULTS:

nitrogen, serum, and urine creatinine and creatine clearance were statistically insignificant in normal control group A and Citrullus lanatus seeds extract group B. Similarly, electrolytes such as calcium, potassium, sodium and magnesium levels in serum of both groups were insignificant. 24 hours urinary output was significantly increased in group B in comparison to normal control group A. Urinary pH was improved in group B in comparison to group A whereas the insignificant difference was observed in the specific gravity of urine in both groups.

Table 1: comparison of RFTs among groups (n=8, mean± SEM)

(II o, Incum_ BEIII)		
RFTs	Group A	Group B
Blood Urea Nitrogen (mg/dl)	12.43 <u>+</u> 0.75	16.60 <u>+</u> 1.96
Serum Creatinine(mg/dl)	0.35 <u>+</u> 0.08	0.51 <u>+</u> 0.09

Urine Creatinine(mg/dl)	82.06 <u>+</u> 21.13	108.87 <u>+</u> 21.16
Creatinine Clearance(ml/min)	3.80 <u>+</u> 1.06	4.68 <u>+</u> 0.88

Group A: Normal control group, Group B: Citrullus lanatus seeds extract group

Table 2: Comparison of serum electrolytes among groups (n=8, mean± SEM)

······ 6 6- · ··· F · (· · · · · · · · · · · · · · · · ·			
Serum electrolytes(mm0l/l)	Group A	Group B	
Serum sodium	139.70 <u>+</u> 6.50	134.30 <u>+</u> 8.01	
Serum potassium	4.86 <u>+</u> 0.33	4.69 <u>+</u> 0.21	
Serum calcium	8.94 <u>+</u> 1.38	8.17 <u>+</u> 1.20	
Serum magnesium	1.98 <u>+</u> 0.12	1.91 <u>+</u> 0.10	

Group A: Normal control group, Group B: Citrullus lanatus seeds extract group

Table 3: Comparison of urinary parameters among groups (n=8, mean± SEM)

Urinary parameters	Group A	Group B
24hrs urinary output (ml)	23.00 <u>+</u> 0.92	31.50 <u>+</u> 3.38
Specific gravity	1.02 <u>+</u> 0.01	1.02 <u>+</u> <0.01
pН	6.50 <u>+</u> 0.37	5.65 <u>+</u> 0.17

Group A: Normal control group, Group B: Citrullus lanatus seeds exrtract group.

DISCUSSION:

Citrullus lanatus seeds are a combination of various components that are beneficial as well protective for kidneys. Electrolytes and various minerals content in the seeds are the main sources to improve functions of the kidney and deteriorate its functions in stressful conditions that may lead to acute or chronic renal failure. In our current study, we tried to evaluate the effect of Citrullus lanatus seeds in order to improve kidney health. We observed that there was no effect of Citrullus lanatus seeds extract on blood urea nitrogen when administered alone to healthy rats. This effect is in accordance with a previous study in which the effect of Citrullus lanatus seeds was evaluated in normal healthy rats. 15 But over the period of time, there was no significant difference within group A at day 0 and 14 but significant difference was present within

group B at day 0 and day 14 which shows that seeds extract improves the Blood urea nitrogen in normal healthy rats. The seeds of Citrullus lanatus neither increased nor decreased the serum creatinine and urine creatinine and creatinine clearance in normal healthy rats. No literature is found in support of the effect of Citrullus lanatus seeds on creatinine levels and creatinine clearance in normal healthy rats.

No change in serum potassium and magnesium levels of group B was noticed at day 14 in comparison to normal control group A. This effect was in accordance with a previous study in which no effect was seen in serum potassium values after using Citrullus lanatus seed extract.¹⁵

No change in serum calcium and sodium levels of group B was noticed at day 14 in comparison to normal control group A which shows that Citrullus lanatus seed extract kept the serum calcium and sodium levels within the normal range in healthy rats. Over a period of time, there was no significant difference within group an on day 0 and 14 but a significant difference was present within groups B on day 0 and day 14.

In group B diuretic effect of the Citrullus lanatus, seed extract was observed whereas urinary specific gravity and pH remained unchanged showing that seeds extract helped to maintain them in the normal range.

CONCLUSION:

According to these findings, it is concluded that the ethanolic extract of Citrullus lanatus seeds is helpful in maintaining kidney health to perform its functions properly.

ACKNOWLEDGMENTS:

I am really thankful to my supervisor Dr. Zujaja Zaheer for her kind supervision. I want to pay my gratitude to my parents and my dear husband.

REFERENCES:

1. Hall JE. Hemostasis and blood coagulation: Guyton and Hall Textbook of Medical

- Physiology. 13th ed. Philadelphia, PA: Elsevier. 2016.
- 2. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific journal of tropical biomedicine. 2013 May 1;3(5):337-52.
- 3. Virani SH, Bhatt SH, Saini MA, Saxena KK. Aloe vera attenuates gentamicininduced nephrotoxicity in wistar albino rats: histopathological and biochemical changes. Asian J Pharm Clin Res. 2016;9(1):113-7.
- 4. Ahmad QZ, Jahan N, Ahmad G. Nephroprotective effect of Kababchini (Piper cubeba) in gentamycin-induced nephrotoxicity. Saudi Journal of Kidney Diseases and Transplantation. 2012 Jul 1;23(4):773.
- Ghaznavi H, Mehrzadi S, Dormanesh B, Tabatabaei SM, Vahedi H, Hosseinzadeh A, Pazoki-Toroudi H, Rashidian A. Comparison of the protective effects of melatonin and silymarin against gentamicininduced nephrotoxicity in rats. Journal of evidence-based complementary & alternative medicine. 2016 Oct;21(4):NP49-55.
- Rahman H, Priyanka P, Lavanya P, Srilakshmi N, Kumar PR. A review on ethno botany, phytochemistry and pharmacology of Citrullus lanatus L. International Research Journal of Pharmaceutical and Applied Sciences. 2013 Apr 28;3(2):77-81.
- 7. Mehra M, Pasricha V, Gupta RK. Estimation of nutritional, phytochemical and antioxidant activity of seeds of musk melon (Cucumismelo) and water melon (Citrullus lanatus) and nutritional analysis of their respective oils. Journal of Pharmacognosy and Phytochemistry. 2015 Feb;3(6):98-102.
- Lucky OO, John UO, Kate IE, Peter OO, Jude OE. Quantitative determination, Metal analysis and Antiulcer evaluation of Methanol seeds extract of Citrullus lanatusThunb (Cucurbitaceae) in Rats. Pacific Asian Journal of **Tropical** Biomedicine. 2012 Jan 1;2(3):S1261-5.
- Kumari A, Rao J, Kumari J, Sharma N, Jain P, Dave V, Sharma S. Analgesic activity of aqueous extract of Citrullus lanatus peels.

- Advances in Pharmacology and Pharmacy. 2013 Dec;1(3):135-8.
- Aruna A, Vijayalakshmi K, Karthikeyan V. In vitro Antioxidant screening of Citrullus lanatus leaves. INTERNATIONAL JOURNAL. 2014;1:01.
- 11. Erhirhie EO, Ekene NE. Medicinal values on Citrullus lanatus (watermelon): pharmacological review. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2014;4(4):1305-12.
- 12. Padzys GS, Ondo JP, Omouendze LP. Impact of Gnetum africanum and Amaranthus rectroflexus Plant Food on Urea, Creatinine and Plasma Electrolytes. Journal of Agricultural Science. 2015 Feb 1;7(2):174.
- 13. Urine test strips of URIMED, Biotec Diagnostics UK Ltd.

- 14. Khaki A, Fathiazad F, Nouri M, Khaki AA. Effect of citrullus lanatus seeds extracts on serum total testosterone in rat. Crescent Journal of Medical and Biological Sciences. 2014:1(1):25-27
- 15. Garuba T, Sabiu S, Ambali IA, Feyijin IB. toxicological implications of acute and repeated dose administrations of hydroethanolic extract of citrullus vulgaris seeds in wistar rats. Annales of West University of Timisoara. Series of Biology. 2017;20(1):49.