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Nephroprotective effect of *Opuntia ficus indica* purple variety fruit juice in rats induced to renal damage by gentamicin

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ABSTRACT

Introduction: Chronic Kidney Disease (CKD) has become a silent epidemic affecting about 10% of the world's population, the discovery of new protective functional foods is important to hinder the development of this disease.

Objective: Evaluate the nephroprotective effect of fruit juice of *Opuntia ficus indica* purple variety in rats induced by renal damage by gentamicin.

Methods: 32 male Holtzman rats were involved in the experiment; we used the fruit juice of *Opuntia ficus indica* purple variety to test its nephroprotective properties. The rats were randomized into four groups (n = 8). Which were treated orally for 27 days with the following: Group I and II-1 ml / kg of physiological serum; Group III-20 ml / kg of *Opuntia ficus indica* juice (OJ); Group IV-40 ml / kg of *OJ*. From day 21, Group II to IV received intramuscular gentamicin at a dose of 50 mg/kg for 7 days.

Results: The administration of 40 ml/kg of *Opuntia ficus indica* evidenced a statistically significant decrease of serum creatinine, urea, uric acid and urine proteins, histologically, we observed a conserved structure at the level of the glomerulus, capsule of Bowman and a slight desquamation of the level of the proximal and distal tubules.

Conclusion: The juice of *Opuntia ficus indica* purple variety at a dose of 40 ml / kg exerted a nephroprotective effect in the renal tissue with the improvement of the biochemical parameters and the histological study.

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KEYWORDS

Medicinal plant; *Opuntia ficus indica*; Gentamycin; Prickly Pear; Nephroprotection.

ABBREVIATIONS

CKD: Chronic kidney disease.

AKI: Acute kidney injury.

OJ: Opuntia juice.

OSS: Oral saline solution.

ISS: Intramuscular saline injection.

AA: Ascorbic acid.

NAG: N-Acetyl-/β-glucosaminidase.

NF-κβ: Nuclear factor kappa beta.

TNF-α: Tumoral necrosis factor alpha.

INTRODUCTION

Chronic kidney disease (CKD) has become a silent epidemic affecting more than 10% of the global population. This prevalence, similar to that of other chronic diseases, presents a significant challenge for public health due to the high treatment costs in the terminal stages of the disease, the increased frequency of complications, and the higher risk of cardiovascular diseases[1].

CKD and its principal risk factors, such as diabetes and hypertension[2], have shown a sustained increase in prevalence and incidence, becoming critical public health issues both locally and globally[3]. Many of these risk factors have a nutritional origin, closely related to excessive consumption of sugars, fats, cholesterol, alcohol, sodium, and a limited

intake of fruits and vegetables, compounded by sedentary lifestyles[4].

Other diseases, such as acute kidney injury (AKI), significantly increase the hazard of developing CKD (5–7). One of the contributing factors to AKI is antibiotics, which are estimated to cause 60% of AKI cases in hospitalized patients[8].

Diet plays a fundamental role in the treatment of CKD, not only as a protective measure in its various stages but also to avoid the dangers of overweight, which can further complicate the progression of the disease. The kidneys, with their multiple functions in maintaining body homeostasis, assume a vital role in this context, regulating the excretion of metabolic waste products, fluid balance, electrolyte composition, and blood pressure[9].

The discovery of functional foods with nephroprotective properties holds great importance as a preventive measure in public health. Previous studies have found evidence that *Opuntia ficus* indica extract exerts nephroprotective properties; however, its effect at lower doses in more common forms of consumption, such as juices, in the prevention of antibiotic-induced kidney damage has not been studied. The aim of this study is to determine the nephroprotective effect of *Opuntia ficus indica* juice in rats with gentamicin-induced kidney damage.

METHODS

The study has a pure experimental design, with a control group and post-test. The acquisition of the purple variety of *Opuntia ficus indica* fruit was carried out at the Wholesale Fruit Market located in the San Luis district, sourced from the Ayacucho region. The fruits were selected intact, fresh, without bruises or browning, then packed in cloth bags and boxes for transport to the laboratory. The Opuntia juice (OJ) was obtained daily, after disinfecting and peeling to extract the edible part of the fruit (mesocarp) and using an extractor Oster® to obtain the fruit juice. The juice was placed in an amber bottle for protection from light.

The sample size calculation was based on a previous study[10]. We used the necessary sample size to observe a 12.1% decrease in creatinine levels between the experimental group and the control group after treatment, considering a 15% increase for possible skewness and a 20% attrition rate. Using the KISS approach, we estimated a sample size of eight rats per group.

Evaluation of the nephroprotective effect: Male Holtzman *Rattus norvegicus* rats, three months old, weighing between 200-220 g, were used. They were acquired from the National Agrarian University - La Molina (Lima, Peru) and acclimated for one week at an average temperature of 22 ºC, with alternating 12-hour light and dark cycles, balanced diet, and water ad libitum.

The Welwood technique with modifications was applied to induce nephrotoxicity[11]. The animals were randomly divided into four groups (n=8), receiving the following treatments via orogastric administration for 27 days:

- Group I: physiological saline (0.9% NaCl) 10 mL/kg
- Group II: physiological saline (0.9% NaCl) 10 mL/kg
- Group III: Opuntia juice 20 mL/kg
- Group IV: Opuntia juice 40 mL/kg

From day 21 to day 27, groups II-IV received 50 mg/kg of gentamicin - Gentacar® (G) via intramuscular injection, while group I received 50 mg/kg of 0.9% NaCl via the same route. At the end of the experimental treatment (day 27), the rats fasted for 24 hours with water ad libitum and were placed in individual metabolic cages for urine collection over 24 hours. They were then anesthetized with diethyl ether vapor for blood extraction by cardiac puncture and subsequently given a lethal dose of pentobarbital. Cervical dislocation was then performed to proceed with bilateral nephrectomy, where both kidneys were removed from the abdominal cavity, washed with 0.9% NaCl, and preserved in 10% buffered formaldehyde solution for histological study.

The urine volume was measured using a graduated cylinder, then centrifuged at 2000 rpm for 5 minutes. The determination of protein was done using the Lowry method, a colorimetric technique based on the reaction of urinary proteins with Na2CO3, NaOH, CuSO4, tartrate, and the Folin-Ciocalteu reagent, measuring the color intensity at 580 nm[12].

Serum uric acid levels were determined using the Searcy method with Tietz modifications, employing reagents such as 4-Aminoantipyrine, 3-5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS), uricase, and peroxidase. The final reaction was read at 505 nm[13,14].

Serum urea was determined by the Faucet and Scott method, where urea is enzymatically decomposed in an alkaline environment, forming a green complex measured at 600 nm[15].

Serum creatinine quantification was evaluated through a reaction with alkaline picrate, forming a chromogen measured at 510 nm[16]. All the reagents used for uric acid, urea and creatinine quantification were obtained from Wiener Lab.

The histopathological analysis of kidney tissue was conducted at the Institute of Pathology of the National University of San Marcos located at the National Archbishop Loayza Hospital. The analysis of the histological sections was performed by Dr. José Ernesto Raez Gonzales, a pathologist at the Institute. Indicators considered for evaluation included aspects of histological structure, luminal spaces, glomeruli, Bowman's capsule, and renal tubules.

Statistical analysis: For indicators that met the assumptions of normality of residuals and homoscedasticity, we used

mean ± standard deviation. For log-normal residuals with homoscedasticity, we used the geometric mean with 95% confidence intervals. Statistical analysis was conducted using R 4.2.2. To determine the distribution of the residuals, we applied the Q-Q plot and Shapiro-Wilk normality test. The ANOVA test was used to compare the means of the groups, and the Dunnett test was employed as a post hoc test to evaluate intergroup differences. A value of $p<0.05$ was considered to indicate a statistically significant difference.

Ethical approval: This study was approved by the Ethics and Research Committee of the Professional School of Nutrition at the National University of San Marcos (RD N°0034-D-FM-2015). Ethical standards and procedures were followed in accordance with Peruvian Law N° 30407, the Animal Protection and Welfare Law[17].

RESULTS

The administration of 50 mg/kg of gentamicin in Group II resulted in a notable increase in creatinine, uric acid, and urea levels compared to Group I (Table 1). The OJ at 20 mL/kg (Group III) did not show a significant impact on creatinine and urea levels compared to Group II (Table 1). However, uric acid (Table 1), protein levels and 24-hour protein excretion were significantly reduced ($p<0.01$) (Table 2). In contrast, when OJ was administered at 40 mL/kg (Group IV), there was a significant decrease in creatinine, uric acid, urea, protein levels, and 24-hour protein excretion compared to Group II ($p<0.01$) (Table 1 and 2).

Histopathology findings showed preserved nephron structure in OJ+G groups **(Figure 1C-D)** compared with gentamicin-only group **(Figure 1B)**, showing glomeruli, Bowman's space and capsule in preserved state similar to control group **(Figure 1A)**.

Table 2. Protein excretion in urine samples according to treatment group

Treatment groups	Protein §	24 h protein excretion *
	(mg/mL)	(mg/dL)
Group I OSS + ISS	8.25 (6.33–10.77) [a]	103.95 ± 54.35[a]
Group II SS + G	13.38 (11.54–15.51)	291.13 ± 66.08
Group III OJ 20 mL/kg + G	9.28 (8.08–10.66) [a]	169.38 ± 34.22[a]
Group IV OJ 40 mL/kg + G	9.34 (8.51–10.26) [a]	95.93 ± 20.13[a]

OSS: Oral saline solution (NaCl 0.9%), ISS: Intramuscular saline solution, OJ: Opuntia juice, G: Gentamicin.
§ Geometric mean (95% Confidence intervals).
* Mean ± standard deviation.
(a) $p<0.01$ compared to group II – ANOVA, Post hoc Dunnet test.

DISCUSSION

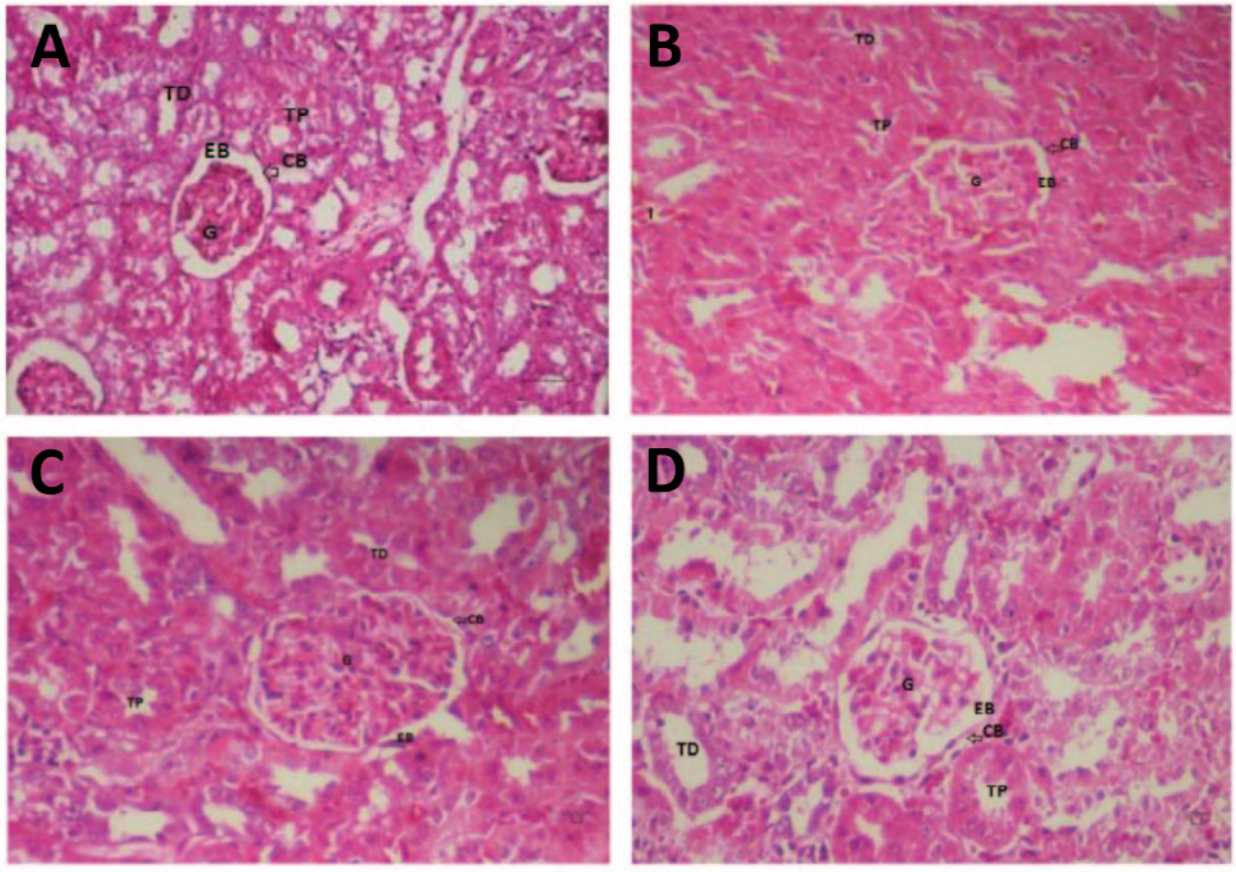
Our results indicate that a natural formulation of *Opuntia* fruit (juice) exhibits nephroprotective properties by preserving the cytoarchitecture of the nephron and reducing markers of kidney damage, including creatinine, uric acid, urea, and protein excretion.

The results can be explained considering the effects of the phytochemicals found in *Opuntia*, this include quercetin, isorhamnetin, kaempferol and luteolin, vitamins such as ascorbic acid (AA) and tocopherols are also found in high quantities in OJ. Quercetin has shown to be an inhibitor of both 15-lipoxygenase-1[18] and cyclooxygenase activity[19], which are mediators in renal ischemia mechanism of injury[20,21].

Table 1. Biochemical analysis of blood samples according to treatment group

Treatment groups	Creatinine *	Uric acid *	Urea §
	(mg/dL)	(mg/dL)	(mg/dL)
Group I OSS + ISS	11.9 ± 0.9 [a]	0.77 ± 0.10 [a]	32.6 (29.6–36.0) [a]
Group II SS + G	13.7 ± 0.9	1.11 ± 0.15	56.3 (51.0–62.2)
Group III OJ 20 mL/kg + G	12.9 ± 1.0	0.83 ± 0.15 [a]	53.4 (45.1–63.2)
Group IV OJ 40 mL/kg + G	10.8 ± 0.9 [a]	0.37 ± 0.13 [a]	33.3 (29.1–38.2) [a]

OSS: Oral saline solution (NaCl 0.9%), ISS: Intramuscular saline solution, OJ: Opuntia juice, G: Gentamicin.
§ Geometric mean (95% Confidence intervals).
* Mean ± standard deviation.
(a) $p<0.01$ compared to group II – ANOVA, Post hoc Dunnet.



(A) Group I shows glomeruli (G) in normal conditions, Bowman's capsule (CB) preserved, distal tubules (TD) and proximal tubules (TP) without desquamation. **(B)** Group II shows congested glomeruli (G), damaged Bowman's capsule (CB), reduced Bowman's space (EB), proximal tubules (TP) and distal tubules (TD) with pronounced desquamation, luminal space obstruction and infiltration (I). **(C)** Group III shows glomerular congestion (G), reduction of Bowman's space (EB); proximal tubules (TP) and distal tubules (TD) with mild desquamation. **(D)** Group IV: Glomeruli (G) in normal state, normal Bowman's space (EB) without thickening of its capsule (CB) and proximal tubules (TP) and distal tubules (TD) with slight desquamation. **H-E. 20X.**

Figure 1. Photomicrographs of kidney tissue

Mechanistic evidence has shown that AA is an effective inhibitor of N-Acetyl-/β-glucosaminidase (NAG)[22], enzyme associated with tubular damage[23]. Both quercetin and AA have shown to be noticeable scavengers of reactive oxygen species (ROS)[24,25], thereby reducing oxidative stress, one of the most important components in AKI mechanisms[26]. Luteolin has also exerted protective properties in AKI by inhibition of caspase-3, downregulation of NF-κβ and TNF-α[27].

Previous findings focused on the plant extract, not the fruit, have also reported the nephroprotective effect of *Opuntia ficus indica*[10,28], Saad *et al* (2017) used a lyophilized extract at doses of 100 mg/kg on rats for 60 days before lithium-induced kidney toxicity. The authors reported only minor damages in kidney structures; serum urea and creatinine were significantly minor compared to control group[10].

Similar results were evidenced in Hfaiedh *et al* (2018) where 100 mg/kg of *Opuntia* for 40 days before sodium dichromate-induced kidney toxicity prevented tissue necrosis and biochemical alteration. Our findings are aligned with those results, showing that lower doses of *Opuntia* (40 mL/kg) can also exert protective properties in less time (27 days)[28].

The strengths of our study include the use of a common dietary formulation to assess nephroprotection, we also included histopathological findings to assess the robustness of our findings. The limitation of our study include not measuring kidney tissue antioxidant activity, having both enzymatic and non-enzymatic antioxidant profiles would have provided a more comprehensive view of nephroprotection.

CONCLUSION

The juice of *Opuntia ficus indica* purple variety at a dose of 40 ml / kg exerted a nephroprotective effect in the renal tissue with the improvement of the biochemical parameters and the histological study.

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