



ORIGINAL ARTICLE

Effectiveness test of avocado seeds on the renal histopathology of white rats induced by isoniazid

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ABSTRACT

The kidneys are a pair of organs within the urinary system, located in the upper retroperitoneal cavity, functioning in blood filtration, reabsorption, and secretion through diffusion processes involved in blood purification and urine formation. The kidneys are highly susceptible to damage from exposure to nephrotoxic drugs, one of which is isoniazid. Avocado seeds (*Persea americana* Mill.) are known to contain antioxidant compounds with potential protective effects against renal tissue damage. This study aimed to examine the effect of avocado seed extract on the histopathological changes in the kidneys of white rats induced with isoniazid. The research employed an experimental design using 25 male rats divided into five groups: positive control (K+), negative control (K-), and three treatment groups receiving avocado seed extract at doses of 100 mg/kg body weight (P1), 200 mg/kg body weight (P2), and 400 mg/kg body weight (P3). Isoniazid induction was administered to all groups except the positive control. After a 14-day treatment period, kidney samples were collected for histopathological analysis using hematoxylin-eosin (HE) staining. The observations indicated that avocado seed extract effectively improved renal histological structure in white rats (*Rattus norvegicus*) induced with isoniazid. In the treatment group III, renal histological improvement was observed, with only 5–25% structural damage or a score of 1, compared to groups I and II, which showed 25–50% damage or a score of 2.

Keywords: avocado seed, kidney, isoniazid, histopathology

Introduction

The kidneys are fundamental organs responsible for maintaining homeostasis within the human body. They regulate fluid and electrolyte balance, acid–base equilibrium, and blood pressure, while also removing metabolic waste and producing several essential hormones. Their diverse physiological roles make them indispensable to the maintenance of systemic health. Anatomically, the kidneys are bilaterally positioned in the retroperitoneal cavity on either side of the vertebral column, protected by layers of adipose tissue and connective structures that stabilize their location in the abdominal cavity.^{1–4} Renal function encompasses ultrafiltration, electrolyte balance, maintenance of acid–base stability, erythropoiesis through erythropoietin secretion, and hormonal regulation of calcium, phosphorus, and blood pressure. On average, the kidneys filter approximately 200 liters of blood per day, producing about 2 liters of urine that facilitate the excretion of waste products and excess fluid. Disruption of these processes can result in significant physiological imbalance and indicates the onset of kidney disease or acute kidney injury (AKI).^{5–7}

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AKI is characterized by an abrupt decline in glomerular filtration rate, leading to the retention of nitrogenous waste products and disturbances in fluid, electrolyte, and acid–base homeostasis. It may be caused by ischemic events, sepsis, nephrotoxins, or obstruction of urine flow.^{8,9} In Indonesia, following the outbreak of the COVID-19 pandemic, a surge of pediatric AKI cases emerged during mid-2022. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines describe AKI as an increase in serum creatinine of at least 0.3 mg/dL within 48 hours or a 50% increase from baseline within 7 days. This condition poses a serious health threat as it can rapidly progress to chronic kidney disease (CKD) or result in mortality, particularly in children.^{10–12}

Atypical progressive acute kidney injury (AKI) is a complex, heterogeneous syndrome with significant morbidity and mortality in both critically and non-critically ill patients across all age groups, including neonates.¹³ The incidence of AKI has increased steadily worldwide, with reports suggesting 1–7% of hospitalized cases requiring medical intervention and up to 25% necessitating intensive care admission. Despite advances in critical care management, mortality rates among intensive care unit (ICU) patients with AKI remain alarmingly high, reaching 50–70%.¹⁴ Pediatric patients with AKI experience prolonged hospitalization and frequently require mechanical ventilation in pediatric intensive care units (PICUs).¹⁵

In recent years, environmental and pharmaceutical toxins have been increasingly recognized as etiological factors in AKI. One significant concern has been the contamination of medicinal syrups with diethylene glycol (DEG) and ethylene glycol (EG)—industrial solvents used in antifreeze and brake fluid production.^{16,17} In 2020, the U.S. Poison Control Center reported 6,036 EG-related cases, including 30 fatalities.¹⁸ Toxicological analyses in Indonesia detected DEG and EG residues in pediatric AKI cases, alongside calcium oxalate deposits in kidney biopsies, confirming the nephrotoxic link. These findings underscore the health risks associated with chemical contaminants in pharmaceutical products and highlight the urgent need for alternative, safer therapeutic interventions.¹⁹

To mitigate renal damage and enhance nephroprotection, researchers have increasingly explored plant-based therapies with antioxidant and anti-inflammatory properties.²⁰ One potential natural source with medicinal promise is avocado (*Persea americana* Mill.), a plant species widely cultivated in Indonesia and originating from Central and South America. The fruit contains a variety of nutrients, including minerals, phenolic compounds, carotenoids, phytosterols, proteins, and vitamins.²¹ Interestingly, the avocado seed—which is generally discarded as waste—also possesses a rich phytochemical profile and significant antioxidant potential.²² Bioactive constituents such as polyphenols, flavonoids, triterpenoids, alkaloids, and tannins have been identified in its extracts and are known to confer hepatoprotective, antimicrobial, and nephroprotective effects.²³ Studies have revealed that avocado seed extracts exhibit particularly strong antioxidant activity in their dried form. The seeds also contain 15–25% oil, characterized by poor water solubility, which limits their bioavailability. Advances in nanoparticle-based formulation techniques have facilitated the development of biodegradable delivery systems that enhance the solubility and bioavailability of these plant-derived compounds.^{24,25} Such innovation may enhance the pharmacological potential of avocado seed extract in protecting renal tissue from toxin-induced oxidative stress.

Given these considerations, further investigation into the nephroprotective properties of avocado seed extract is highly warranted. Evaluating its histopathological effects on kidney tissue—especially in models of chemically induced nephrotoxicity, such as isoniazid administration—could clarify its therapeutic value. The present study aims to assess the impact of avocado seed extract on the renal histopathology of albino rats (*Rattus norvegicus*) subjected to isoniazid-induced nephrotoxic injury, thereby contributing to the development of safe, natural, and locally available nephroprotective interventions.

Method

The present study employed an experimental quantitative design to investigate the effect of avocado seed (*Persea americana* Mill.) extract on the renal histopathological features of rats induced with isoniazid. The study was scheduled for a three-month period, from April to June 2025. The extraction process was conducted at the Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Prima Indonesia. Animal handling and treatment were carried out at the Animal Research Laboratory, Faculty of Pharmacy, Universitas Sumatra Utara. Preparation of histopathological specimens was performed at the Anatomical Pathology Laboratory, Royal Prima Hospital.

The study population consisted of male Wistar rats (*Rattus norvegicus*) aged 2–3 months, weighing 150–200 grams. The Wistar strain was selected due to its stable physiological characteristics and frequent

use in biomedical research, particularly for toxicology and drug efficacy studies. The animals were obtained from the Animal Research Laboratory, Faculty of Pharmacy, Universitas Sumatra Utara. Avocado seed samples (*Persea americana* Mill.) were collected from a local farming group in Jaba Village, Namorambe District, Deli Serdang Regency, North Sumatra Province. Botanical identification was performed at the Faculty of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatra Utara (USU) to confirm the plant species.

For the preparation of *simplicia*, collected avocado seeds were cleaned from adhering impurities, washed under running water, and soaked for 24 hours. The seeds were then oven-dried, ground into fine powder using a blender, and subjected to ethanol extraction through maceration. A total of 1,975 grams of avocado seed *simplicia* powder was immersed in 96% ethanol until fully submerged. The maceration process lasted for five days in a dark room, with occasional stirring. The macerate was filtered through filter paper and concentrated using a rotary evaporator at 40–60°C, followed by evaporation in a water bath to obtain a thick extract.

Phytochemical screening of the extract was conducted to identify its secondary metabolites. Alkaloids were tested using Dragendorff's reagent, with the formation of a brown precipitate indicating a positive result. Flavonoids were detected by adding magnesium ribbon and concentrated HCl; the appearance of red, yellow, or orange coloration confirmed their presence. Saponins were identified via the Froth test, where stable froth persisting for 30 seconds after the addition of hot water and HCl indicated a positive result. Tannins were tested using 5% ferric chloride solution, with a dark blue or greenish-black precipitate denoting positivity. Finally, triterpenoids and steroids were tested with chloroform, acetic anhydride, and H₂SO₄; the appearance of red, orange, or yellow color indicated triterpenoids, while a green color suggested steroids.

The experimental animals were healthy male Wistar rats aged 2–3 months, weighing 150–200 grams. Inclusion criteria comprised healthy male Wistar rats within the specified age and weight range, with normal appetite and no physical abnormalities. Exclusion criteria included rats that became ill during the intervention period—manifested by lethargy, severe diarrhea, or loss of appetite—in which case they were replaced with reserve animals. Prior to the experiment, all rats underwent a seven-day acclimatization period with access to standard feed and water *ad libitum*.

The research variables included the independent variable—avocado seed extract dosage (0, 100, 200, and 400 mg/kg body weight)—and the dependent variable—renal histopathological damage, encompassing degeneration, necrosis, and inflammatory cell infiltration. Controlled variables included rat species, sex, age, and body weight; environmental conditions; standard pellet feed; *ad libitum* water intake; oral administration volume; and isoniazid (INH) source.

Rats were randomly allocated into five groups, each consisting of five animals. The first group served as the normal control, receiving no treatment. The second group acted as the positive control, receiving isoniazid induction only. The three treatment groups (P1, P2, and P3) were induced with isoniazid and then administered avocado seed extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively. Isoniazid was administered at a dose of 50 mg/kg body weight. All treatments were given orally once daily for 14 days using an oral gavage needle. Upon completion of the treatment period, rat kidney samples were collected for histopathological examination to evaluate structural alterations and derive conclusions regarding the extract's effects.

Results

Table 1 presents the results of the qualitative phytochemical screening of avocado seed extract to identify the bioactive compounds present. Based on the tests conducted, the extract yielded positive results for six distinct classes of bioactive compounds. The first test was for alkaloids. Using Dragendorff's reagent, the extract showed a positive reaction indicated by the formation of a brown precipitate. To identify flavonoids, the extract was treated with a 2 cm strip of magnesium ribbon (Mg) and 1 mL of concentrated hydrochloric acid (HCl). A color change to red, yellow, or orange confirmed a positive result. The saponin test was performed by adding 1 mL of 2N HCl solution to the extract, with the presence of stable froth indicating a positive result. The tannin assay used 5% ferric chloride (FeCl₃) solution, where the appearance of a dark blue or greenish-black coloration signified positive detection.

Finally, to identify triterpenoids and steroids, the same reagent mixture—98% acetic anhydride and three drops of concentrated sulfuric acid (H₂SO₄)—was applied, following the Liebermann–Burchard test. Although both compounds used the same reagent, the positive results were distinguished by the colors

formed: the presence of triterpenoids was confirmed by red, orange, or yellow coloration, while the presence of steroids was demonstrated by a green color in the sample. Collectively, these results indicate that avocado seed extract contains a diverse range of bioactive phytochemical compounds.

Table 1. Phytochemical screening results of ethanol extract of avocado seeds

Bioactive Compound	Reagent	Avocado Seed Extract	Description
Alkaloid	Dragendorff reagent	+	Formation of brown precipitate
Flavonoid	2 cm Mg ribbon and 1 mL concentrated HCl	+	Appearance of red, yellow, or orange color
Saponin	1 mL 2N HCl	+	Formation of stable froth
Tannin	5% ferric chloride solution	+	Dark blue or greenish-black color
Triterpenoid	98% acetic anhydride and 3 drops 98% H ₂ SO ₄	+	Formation of red, orange, or yellow color
Steroid	98% acetic anhydride and 3 drops 98% H ₂ SO ₄	+	Green coloration of the sample

Note: (+) Indicates the presence of the tested compound group; (–) Indicates absence.

Table 2 outlines the scoring system used to evaluate the degree of renal damage in experimental rats based on histopathological examination under a light microscope. The assessment focused on three primary types of tissue damage: cellular degeneration, necrosis, and inflammatory cell infiltration. Each parameter was graded on a scale from 0 to 4, representing the severity and percentage of affected kidney tissue area. A score of 0 represents normal histological features with no observable tissue damage. A score of 1 indicates very mild injury, corresponding to 5–25% of tissue involvement in either degeneration, necrosis, or inflammatory infiltration.

Table 2. Histopathological scoring criteria for rat kidney damage

Score	Degeneration	Necrosis	Inflammatory Infiltration
0	None	None	None
1	5–25%	5–25%	5–25%
2	25–50%	25–50%	25–50%
3	50–75%	50–75%	50–75%
4	>75%	>75%	>75%

A score of 2 reflects mild to moderate damage (25–50% involvement), while a score of 3 indicates moderate to severe damage (50–75% involvement). The highest score, 4, denotes extensive injury affecting more than 75% of renal tissue, characterized by pronounced degeneration, extensive necrosis, and widespread inflammatory infiltration. This scoring system provides a quantitative approach to assess and compare the extent of renal damage in experimental models.

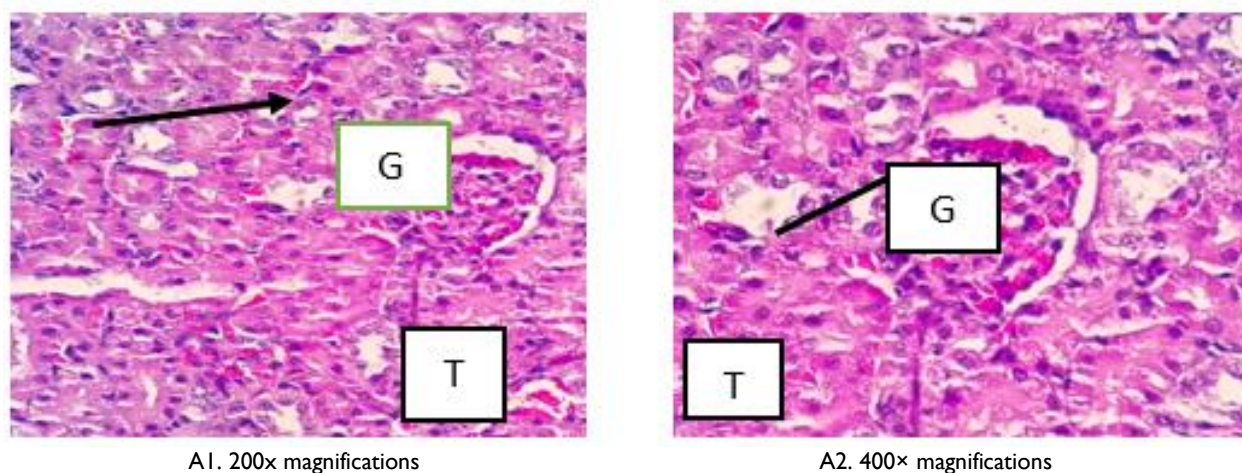


Figure 1. Histopathological examination of the rat kidneys, utilizing Hematoxylin and Eosin (H&E) staining, showed findings consistent with a damage score of 0. Figures A1 and A2 (at 200x and 400x magnification) illustrate that the tissue is free of structural alterations; both the glomerular and tubular cells appear normal.

Histopathological examination was performed using hematoxylin and eosin (H&E) staining under a light microscope at 400× magnification to observe cellular detail. The degree of structural alteration was scored as follows: score 0 for normal architecture, score 1 for minimal lesions covering 1–25% of the field, score 2 for mild to moderate lesions affecting 26–50%, score 3 for moderate to severe necrotic lesions involving 51–75%, and score 4 for extensive tissue necrosis and loss of normal architecture in 76–100% of the observed field.

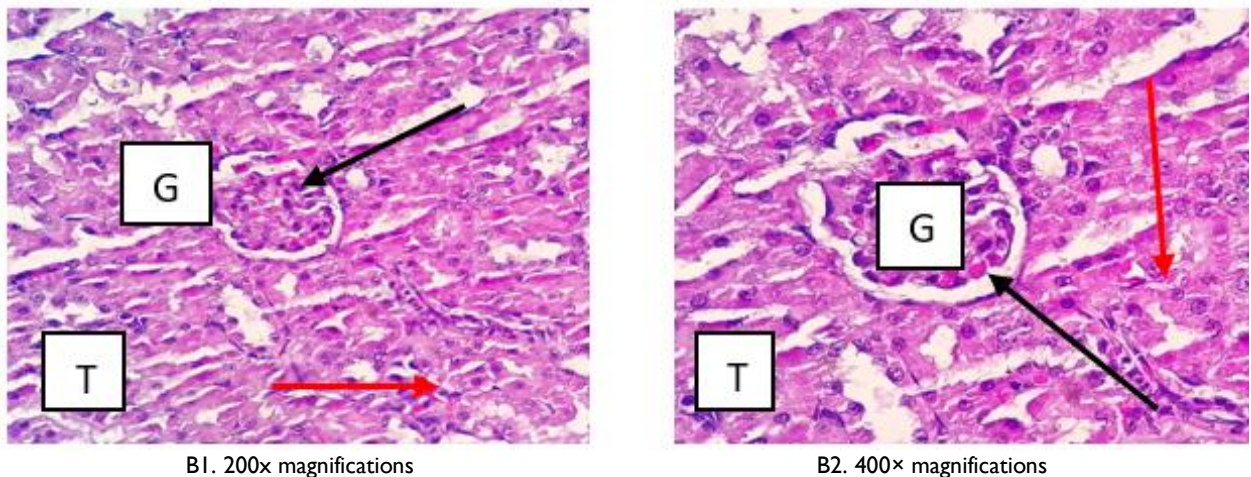


Figure 2. Histopathological analysis of rat kidneys (Hematoxylin & Eosin stain, 200x and 400x magnification) revealed a mean damage score of 2. As depicted in Figures B1 and B2, this damage level is characterized by mild, partial edema within the tubular and glomerular regions.

→ Tubular necrosis; → Glomerular necrosis

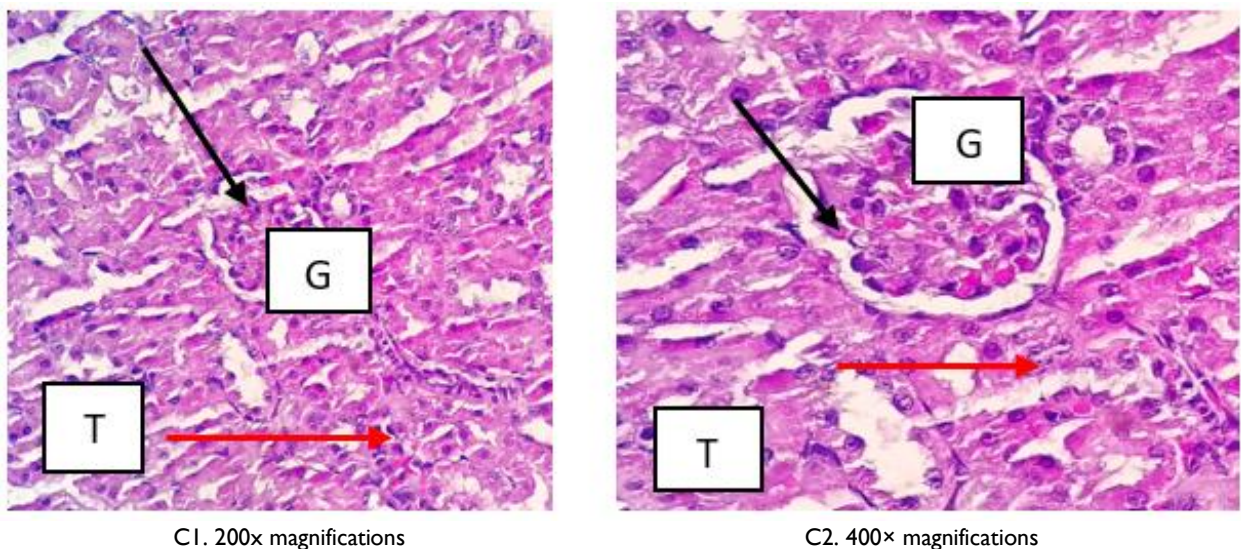


Figure 3. Histopathological analysis of the rat kidneys, utilizing Hematoxylin & Eosin (H&E) staining (200x and 400x magnification), revealed an average damage score of 2. As depicted in Figures C1 and C2, this level of renal damage is characterized by mild edema in the tubular and glomerular regions.

→ Tubular necrosis; → Glomerular necrosis

Discussion

This study aimed to investigate the protective effects of ethanolic extract of avocado seeds (*Persea americana* Mill.) on the renal histopathological profile of white rats (*Rattus norvegicus*) induced with isoniazid. The primary sample, avocado seeds, was extracted using the maceration method with 96% ethanol as the solvent. Ethanol was selected due to its non-toxic and neutral properties, good absorptive capacity, compatibility with water, and limited ability to dissolve interfering substances.

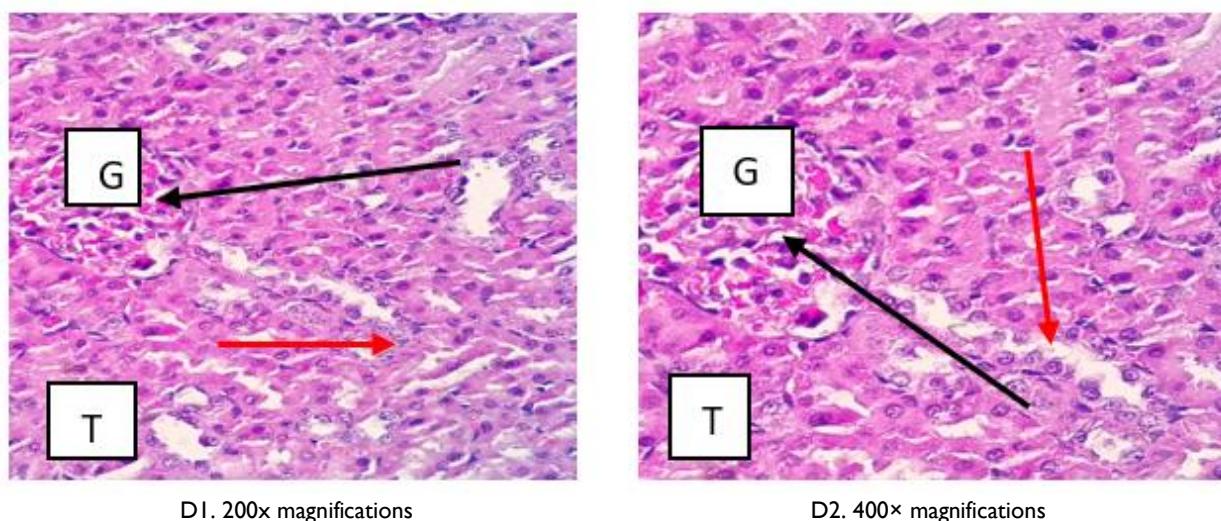


Figure 4. Histopathology of rat kidneys (Hematoxylin & Eosin stain, 200x/400x magnification) revealed a mean lesion score of 2. Figures D1 and D2, representative of this damage grade, demonstrate mild edema in the tubular and glomerular regions.

→ Tubular necrosis; → Glomerular necrosis

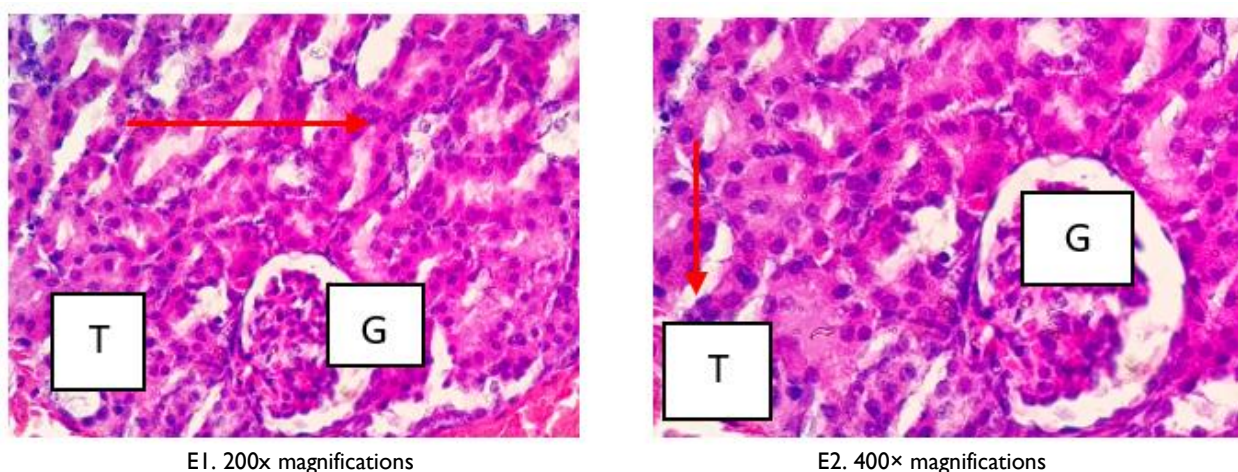


Figure 5. Histopathological analysis of rat kidney tissue, stained with Hematoxylin and Eosin (H&E) at 200x and 400x magnification, revealed a mean damage score of 1. Figures E1 and E2 show this damage is characterized by mild edema in the tubular and glomerular regions.

→ Indicates an area of tubular repair

A total of 30 white rats were randomly assigned to five experimental groups, each consisting of five animals. The first group served as the normal control, receiving neither isoniazid induction nor seed extract. The second group acted as the negative control, receiving only isoniazid induction to assess the extent of kidney damage. The remaining three groups were treatment groups that, following isoniazid induction, received avocado seed extract at doses of 100 mg, 200 mg, and 400 mg, respectively.

The treatment period lasted for 28 days. On day 29, all animals were euthanized, and kidney tissues were collected for histopathological examination. The histopathological scoring analysis revealed that the most substantial improvement and renal tissue regeneration occurred in the group treated with a 400 mg dose of avocado seed extract. These findings demonstrate that avocado seed extract possesses potential reparative effects on renal tissues damaged by isoniazid exposure.

Conclusion

The seed extract of avocado was effective in improving the structural damage of renal histology in white rats induced by isoniazid. In treatment group III, renal histological repair was observed, with structural damage ranging from 5–25% (score 1), compared to treatment groups I and II, which exhibited 25–50%

damage (score 2). Further studies are recommended using varying doses and durations of avocado seed extract administration to determine the optimal dose for ameliorating renal histological damage. Additional research should also be conducted using other animal models or clinical trials in humans to confirm the safety and efficacy of the avocado seed extract. It is also advisable to explore the underlying mechanisms of action of the extract more comprehensively, such as through biochemical analysis, enzyme activity assessment, or the evaluation of oxidative stress markers in renal tissue.

References

1. Puelles VG, Huber TB. Kidneys control inter-organ homeostasis. *Nat Rev Nephrol*. 2022 Apr 14;18(4):207–8.
2. Crambert G, Al-Qusairi L. Editorial: New advances in the renal regulation of K⁺ homeostasis in health and disease. *Front Physiol*. 2023 Oct 12;14.
3. Levassort H, Essig M. Le rein, son anatomie et ses grandes fonctions. *Soins Gériatrie*. 2024 Jan;29(165):10–20.
4. Wallace MA. Anatomy and Physiology of the Kidney. *AORN J*. 1998 Nov;68(5):799–820.
5. Obi Y, Raimann JG, Kalantar-Zadeh K, Murea M. Residual Kidney Function in Hemodialysis: Its Importance and Contribution to Improved Patient Outcomes. *Toxins (Basel)*. 2024 Jun 28;16(7):298.
6. Lawrence MG, Altenburg MK, Sanford R, Willett JD, Bleasdale B, Ballou B, et al. Permeation of macromolecules into the renal glomerular basement membrane and capture by the tubules. *Proc Natl Acad Sci*. 2017 Mar 14;114(11):2958–63.
7. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders HJ. Acute kidney injury. *Nat Rev Dis Prim*. 2021 Jul 15;7(1):52.
8. Claure R, Bouchard J. Acid-Base and Electrolyte Abnormalities during Renal Support for Acute Kidney Injury: Recognition and Management. *Blood Purif*. 2012;34(2):186–93.
9. Turgut F, Awad A, Abdel-Rahman E. Acute Kidney Injury: Medical Causes and Pathogenesis. *J Clin Med*. 2023 Jan 3;12(1):375.
10. Okusa MD, Davenport A. Reading between the (guide)lines—the KDIGO practice guideline on acute kidney injury in the individual patient. *Kidney Int*. 2014 Jan;85(1):39–48.
11. Hessey E, Perreault S, Dorais M, Roy L, Zappitelli M. Acute Kidney Injury in Critically Ill Children and Subsequent Chronic Kidney Disease. *Can J Kidney Heal Dis*. 2019 Jan 14;6.
12. Umar TP, Jain N, Aziz H. Endemic rise in cases of acute kidney injury in children in Indonesia and Gambia: what is the likely culprit and why? *Kidney Int*. 2023 Mar;103(3):444–7.
13. Gist KM, Fuhrman D, Stanski N, Menon S, Soranno DE. Subphenotypes of acute kidney injury in children. *Curr Opin Crit Care*. 2022 Dec;28(6):590–8.
14. Bazargani B, Moghtaderi M. New Biomarkers in Early Diagnosis of Acute Kidney Injury in Children. *Avicenna J Med Biotechnol*. 2022;14(4):264–9.
15. Pitabuana AR. Insidens, faktor risiko dan outcome gangguan ginjal akut (GgGA) pada anak di Unit Perawatan Intensif Anak RSUP dr. Wahidin Sudirohusodo Tahun 2020. Universitas Hasanuddin; 2020.
16. Petejova N, Martinek A, Zadrazil J, Teplan V. Acute toxic kidney injury. *Ren Fail*. 2019 Jan 25;41(1):576–94.
17. Kumar P, Rastogi S, Saini PK, Sahoo S, Raghuvanshi RS, Jadaun GPS. Minimizing the risk of ethylene glycol and diethylene glycol poisoning in medications: A regulatory and pharmacopoeial response. *Regul Toxicol Pharmacol*. 2025 Jan;155:105741.
18. Gummin DD, Mowry JB, Beuhler MC, Spyker DA, Bronstein AC, Rivers LJ, et al. 2020 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 38th Annual Report. *Clin Toxicol*. 2021 Dec 2;59(12):1282–501.
19. Eapen JV, Thomas S, Antony S, George P, Antony J. A review of the effects of pharmaceutical pollutants on humans and aquatic ecosystem. *Explor Drug Sci*. 2024 Aug 28;2:484–507.
20. Tienda-Vázquez MA, Morreeuw ZP, Sosa-Hernández JE, Cardador-Martínez A, Sabath E, Melchor-Martínez EM, et al. Nephroprotective Plants: A Review on the Use in Pre-Renal and Post-Renal Diseases. *Plants*. 2022 Mar 18;11(6):818.
21. Marra A, Manousakis V, Zervas GP, Koutis N, Finos MA, Adamantidi T, et al. Avocado and Its By-Products as Natural Sources of Valuable Anti-Inflammatory and Antioxidant Bioactives for Functional Foods and Cosmetics with Health-Promoting Properties. *Appl Sci*. 2024 Jul 9;14(14):5978.
22. Awidarta K, Rohman A, Nugroho AE. Unlocking the Therapeutic Potential of Avocado Peel Waste: A Comprehensive Review of Phytochemicals and Pharmacological Activities. *J Food Pharm Sci*. 2025 Sep 29;285–95.
23. Farhani AA, Leliqia NPE. Review: Studi Kandungan Fitokimia dan Aktivitas Antimikroba Alpukat (*Persea americana* Mill). *Pros Work dan Semin Nas Farm*. 2023;2:335–44.
24. Chotimah. Uji Total Flavonoid dan Aktivitas Antioksidan Ekstrak Daun dan Kulit Batang Dadap Serep (*Erythrina subumbrans* (Hassk) Merr.) Menggunakan Pelarut yang Berbeda. Universitas Islam Negeri Maulana Malik Ibrahim Malang; 2019.
25. Zade A, Shastri DA. Nanoparticle-Based Drug Delivery: Enhancing Bioavailability and Therapeutic Efficacy in Pharmaceutical Applications. *J Intern Med Pharmacol*. 2024 Oct 29;1(03):26–38.
26. Parisi JM, Rebok GW, Xue QL, Fried LP, Seeman TE, Tanner EK, et al. The Role of Education and Intellectual Activity on Cognition. *J Aging Res*. 2012;2012:1–9.
27. Fathinatusholihah, Destarianiy E, Efriani R, Simbolon D, Wahyuni E. Pengaruh video animasi dan e-leaflet terhadap perilaku deteksi dini stunting. *J SAGO Gizi dan Kesehat*. 2024 Aug 26;5(3A):811.
28. Yuliasih N, Sari P, Bestari A, Martini N, Sujatmiko B. Does Health Education Through Videos and E-Leaflet Have a Good Influence on Improving Students' Reproductive Health Knowledge, Attitudes, and Practices? an Intervention Study in Jatnangor, Indonesia. *Adv Med Educ Pract*. 2025 Jan;Volume 16:29–39.
29. Ihlasyandi E, Sudiyat R. Efektivitas media AVA dan leaflet dalam penyuluhan tentang TB paru terhadap peningkatan

- pengetahuan kader kesehatan di wilayah Kota Bandung. *J Ris Kesehat Poltekkes Depkes Bandung*. 2022 May 30;14(1):134–41.
30. Rosmiati, Pratikwo S, Arwani, Hartono M, Anonim T. Pengaruh pemberian edukasi kesehatan dengan media audiovisual terhadap pengetahuan keluarga dalam penanganan kejadian kejang demam pada anak. *J Lintas Keperawatan*. 2022;3(1).
 31. Meppelink CS, van Weert JC, Haven CJ, Smit EG. The Effectiveness of Health Animations in Audiences With Different Health Literacy Levels: An Experimental Study. *J Med Internet Res*. 2015 Jan 13;17(1):e11.
 32. Hasanica N, Catak A, Mujezinovic A, Begagic S, Galijasevic K, Oruc M. The Effectiveness of Leaflets and Posters as a Health Education Method. *Mater Socio Medica*. 2020;32(2):135.
 33. Saputra SNM, Isnaeni I. Efektivitas Penyuluhan Kesehatan Dengan Media Audiovisual Terhadap Peningkatan Pengetahuan Akibat Seks Bebas Pada Remaja Kelas VIII Di SMP Muhammadiyah 28 Bekasi. *Malahayati Nurs J*. 2022 Jul 1;4(7):1807–20.
 34. Ernawati A. Media Promosi Kesehatan Untuk Meningkatkan Pengetahuan Ibu Tentang Stunting. *J Litbang Media Inf Penelitian, Pengemb dan IPTEK*. 2022 Dec 25;18(2):139–52.
 35. Wilson PA, Cherenack EM, Jadwin-Cakmak L, Harper GW. Selection and Evaluation of Media for Behavioral Health Interventions Employing Critical Media Analysis. *Health Promot Pract*. 2018 Jan 19;19(1):145–56.