PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci1625.com. Email: managingeditorpjms1625@gmail.com.

CANNABIS SATIVA EXACERBATES KIDNEY TOXICITY IN MALE WISTAR RATS: A DOSE DEPENDENT STUDY

Running title: Toxic effect of *cannabis sativa* on kidney in male Wistar rats.

OLUWASOLA A.

Department of Physiology, Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Osun State, Nigeria

Corresponding author: dr.amuda19@gmail.com

CANNABIS SATIVA EXACERBATES KIDNEY TOXICITY IN MALE WISTAR RATS: A DOSE DEPENDENT STUDY

Running title: Toxic effect of *cannabis sativa* on kidney in male Wistar rats.

OLUWASOLA A.

Department of Physiology, Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Osun State, Nigeria

Corresponding author: dr.amuda19@gmail.com

ABSTRACT

The kidneys play a critical role in removing waste and excess water from the blood, as well as maintaining the normal body's chemical balance. Damage to the kidneys can pose serious health risks. *Cannabis sativa* (CS) is one of the most abused substances worldwide and has been associated with adverse health effects. This study investigated the effects of CS on kidney function in male Wistar rats. Twenty male rats were randomly assigned into four groups of five animals each. Groups 1, 2, 3, and 4 received orally 1.0 ml of distilled water, CS (2 mg/kg), CS (4 mg/kg), and CS (6 mg/kg), respectively, for three weeks with free access to food and water. Kidney function parameters, including urea, creatinine, induced protein (IP), albumin, sodium (Na), and potassium (K), were measured using standard laboratory methods. CS administration significantly ($p<0.05$) increased urea and creatinine levels in the high-dose (4 and 6 mg/kg) treated groups compared with the control and low-dose (2 mg/kg) treated groups. Conversely, induced-protein (IP), albumin, sodium, and potassium levels were significantly ($p<0.05$) decreased in the high-dose treated groups. Additionally, the group treated with 6 mg/kg CS revealed more toxic effects in all the parameters when compared to 4 mg/kg CS treated group.

The effects of CS on kidney function were dose dependent. This study suggests that high-dose CS consumption may impair renal function. Therefore, withdrawal from CS consumption may be recommended to prevent its deleterious effects on kidney health.

Keywords: *Cannabis sativa*, Kidney toxicity, Low dose, High-dose, dose dependent

Submitted: June 2026, Accepted July 2026

INTRODUCTION

Cannabis (C) is a genus of flowering plants in the family *Cannabaceae*. Three species are commonly recognized: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. Some researchers consider *C. ruderalis* as part of *C. sativa*, and all three species may be treated as subspecies of a single *C. sativa* species [1]. Cannabis is widely used as an illicit substance worldwide [2], and its consumption has been associated with adverse effects on human and animal health. Numerous studies have documented the negative consequences of CS consumption or smoking on various physiological systems [3-15].

The kidneys are paired organs located in the posterior abdominal wall. Their primary functions include the elimination of toxic metabolites and waste products from the blood, as well as the regulation of fluid and electrolyte balance in the body [16]. Previous research has reported both beneficial and deleterious effects of CS on kidney function in humans and animals [17-19]. However, these studies did not examine the potential impact of different doses of CS on kidney toxicity.

This study aimed to address this gap by investigating the effects of CS on kidney function in male Wistar rats, with a focus on dose-dependent effects. Specific kidney parameters including urea, creatinine, induced protein (IP), albumin, sodium, potassium, and total protein were evaluated, as they provide critical information about renal function.

The objectives of this study were: to investigate the effects of CS on kidney toxicity in male Wistar rats; and to explore dose-dependent effects of CS on kidney toxicity. The study was guided by the following hypotheses:

Null hypothesis (H_0): CS does not exacerbate kidney toxicity in male Wistar rats.

Alternative hypothesis (H_1): CS exacerbates kidney toxicity in male Wistar rats.

MATERIALS AND METHODS

Sample collection

Cannabis sativa (CS) leaves were donated by the National Drug Law Enforcement Agency (NDLEA), Nigeria, for research purpose only.

Extraction of *Cannabis sativa* leaves

Extraction of *Cannabis sativa* (CS) was done with Soxhlet apparatus by soaking 300 grams of CS in 98% ethanol for 48 hours. It was filtered and the filtrate was poured into a round-bottom conical flask fixed with a rotary evaporator.

The filtrate was evaporated and cooled. The dried yield of the extract was 24g (weight of the extract obtained after drying) [7].

Experimental animals

Twenty male rats with average mean weight (160g ± 1.98) that were used for this research were obtained from Temilade Animal Venture, Ogbomoso, Oyo State. They were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. The experimental protocol was approved by Ethical Committee of University of Ilesa, Ilesa, Osun State, Nigeria with approval number (UNILESA/ERC/2025/021).

Experimental protocol

After 2 weeks of acclimatisation, the animals were separately and randomly assigned into four groups of five animals each.

The rats in groups 1, 2, 3, and 4 were administered orally (in the morning) 1.0mL of distilled water (control), 2.0mg/kg body weight (bw) of CS, 4.0mg/kg bw of CS and 6.0mg/kg bw of CS respectively, once daily via oral gavage between 8:00 am and 10:00 am for twenty-one (21) days. The animals had access to food and water ad-libitum and were sacrificed after day 21.

Preparation of serum samples

The rats were sacrificed under ketamine anesthesia. The blood was collected by cardiac puncture into properly labeled sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×*g* for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England).

Each of the serum was collected into plain bottle with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Determination of albumin and IP

Albumin and IP activity were determined spectrophotometrically (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA) following the kit manufacturer's procedures (product code BXC0243; Fortress Diagnostics, UK).

Determination of Urea

Urea was determined by urease method [20]:

Urea was hydrolyzed by urease to produce ammonia and carbon(iv)oxide. The ammonia released reacted with a phenol-hypochloride reagent in an alkaline medium to form blue-coloured indophenols dye. The intensity of the blue colour was measured spectrophotometrically and this was directly proportional to the urea concentration in the sample.

Determination of creatinine

In an alkaline medium, creatinine reacts with picric acid to form an orange-red colored complex

(creatinine-picrate complex). The intensity of the color produced is directly proportional to the creatinine concentration in the sample [21].

Determination of Sodium

The method is based on the reaction of sodium with a selective Chromogen producing a chromophore whose absorbance is directly proportional to sodium concentration in the sample [22].

Determination of Potassium

Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of turbidity produced is directly proportional to the concentration of potassium in the sample [23].

Statistical analysis

Results were expressed as the mean ± standard error of mean. Data was analyzed using a two-way Analysis of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS

The results obtained are presented in Table 1. CS administration significantly ($p<0.05$) increased urea and creatinine levels in the high-dose groups (4 and 6 mg/kg) compared with the control and low-dose treated groups (2 mg/kg). Conversely, a significant ($p<0.05$) decrease in induced-protein (IP), albumin, sodium, and potassium levels was observed in the high-dose treated groups relative to the control and low-dose treated groups. Furthermore, the group treated with 6 mg/kg CS revealed more toxic effects in all the parameters when compared to 4 mg/kg CS treated group.

Table 1: Kidney parameters of male rats for control and treated (2.0 mg/kg bw CS, 4.0 mg/kg bw CS, and 6.0 mg/kg bw CS) groups.

Groups	Urea (U/dl)	Creatinine (µmol/L)	Induced protein	Albumin (g/dl)	Sodium ion (Na^+) (mmol/l)	Potassium ion (K^+) (mmol/l)
Control	24±0.94	74.34±2.56	5.00±1.02	19.20±2.21	85.00±0.49	8.00±3.11
2 mg/kg CS	28±0.82	79.45±1.21	5.00±o.42	18.80±1.67	82.00±1.42	7.40±1.96
4 mg/kg CS	33±0.75#	87.20±0.98#	4.00±0.71#	12.60±0.86#	65.00±1.53#	4.20±1.71#
6 mg/kg CS	42.8±0.32#µ	93.67±1.06#µ	3.20±0.92#µ	10.80±1.74#µ	50.00±1.37#µ	3.20±1.90#µ

Values are expressed as mean ± SEM; #P< 0.05 vs control and 2.0 mg/kg bw CS groups; µP< 0.05 vs 4.0 mg/kg bw CS group.

DISCUSSION

The use of cannabis may have significant implications for kidney health. Research suggests that heavy cannabis use is associated with an increased risk of chronic kidney disease (CKD) [24], a condition characterized by the gradual loss of kidney function over time [24-26]. Cannabis can influence renal blood flow dynamics, potentially altering kidney function [26]. Δ9-Tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, can affect blood vessel dilation and constriction, thereby impacting renal blood flow and filtration [26]. Cannabis use has also been linked to changes in urinary parameters, including increased urine volume and frequency, which may reflect its effects on hydration status and renal function regulation [27].

In the kidney, cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2) play key roles in regulating renal blood flow, glomerular filtration rate (GFR), glomerular permeability, interstitial collagen production, and tubular function [28]. Our study showed that urea and creatinine levels increased in a dose-dependent manner, with no observable changes in the low-dose group. This aligns with previous studies [29-30], which reported elevated urea and creatinine levels in rats treated with CS. The potential mechanism underlying this effect may involve alterations in renal blood flow and GFR [29], thereby impairing the kidney's ability to filter and excrete urea and creatinine, which are waste products of protein and muscle metabolism [31].

These findings contrast with those of [32], who reported decreased biliary urea and creatinine in rats. The discrepancy may be attributed to differences in sample type, as their study measured bile rather than blood. Additionally, the observed reductions in induced-protein (IP), albumin, sodium, potassium, and total protein in the high-dose groups suggest abnormal loss of these substances from the kidney into urine rather than retention in the blood. This effect may be mediated by CS activation of CB_1 and CB_2 receptors in the kidney and renal vasculature, leading to excretion and filtration of these parameters [33]. These results are consistent with prior reports of [34] who revealed that CS-induced nephrotoxicity.

Our study has some limitations. First, it is unclear whether prolonged administration of low-dose CS (2.0 mg/kg body weight) would produce effects similar to those observed with higher doses (4.0 and 6.0 mg/kg body weight). Second, it is unknown whether administration at different times of the day (e.g. afternoon or night) would yield results comparable to the morning treatments used in this study.

CONCLUSION

This study concluded that *Cannabis sativa* (CS) may impair kidney function by activating cannabinoid receptors, which in turn may alter renal blood flow and key kidney parameters, including urea, creatinine, induced-protein, albumin, sodium, and potassium. The effects of CS on kidney toxicity were found to be dose dependent. Therefore, withdrawal from CS consumption may be recommended due to its potential deleterious effects on renal health. Further research is warranted to explore strategies for preventing CS-induced kidney toxicity, particularly at high doses, in the context of both medicinal and recreational use.

REFERENCES

1. Wiggans GR, Cole JB, Hubbard SM. Genomic selection in dairy cattle: the USDA experience. Annual review of animal biosciences. Vol. 5 (1), March 2017: 309-27.
2. Chouvy PA. Cannabis cultivation in the world: heritages, trends and challenges. EchoGéo. July 2019, 13(48).
3. Oluwasola A. Melatonin ameliorates the effects of Cannabis sativa-induced oxidative stress in Wistar rats: A dose-dependent and gender-specific study. Journal of Cannabis Research. Vol. 8(27), January 2026: 2-7
4. Oluwasola A, Ayoola OE, GarbaS aa'du. A comparative study on the protective effect of Ascorbic acid (Vitamin C) on Cannabis sativa-induced oxidative stress in male and female Wistar rats. Pacific Journal of Medical Sciences. Vol. 27(2), March 2026: 1-11.
5. Ayoola OE, Oluwasola A, Garba S. Cannabis sativa exacerbates inflammatory responses in male and female wistar rats. Pacific Journal of Medical Sciences. Vol. 25(2), December 2025: 50-55.
6. Oluwasola A, Lukman J, Comfort AM. Effect of Cannabis sativa on Haematological Parameters in Male and Female Wistar Rats. Asian Pacific Journal of Medical Toxicology. Vol. 13(3), August 2024: 90-94
7. Oluwasola A, Ayoola OE, Garba S, Adepoju MA, Biliaminu SA, Olayaki LA. Melatonin Mitigates Hormonal Toxicity in Cannabis-Treated Female Wistar Rats. The Tropical Journal of Health Sciences. Vol. 30(2), July 2023a: 14-19.
8. Oluwasola A, Ayoola OE, Garba Saa'du, Adepoju MA, Balogun ME, Biliaminu SA, Olayaki LA.The Ameliorative effects of Melatonin on Cannabis-induced Liver toxicity in male and female Wistar rats. *The Tropical Journal of HealthSciences*.Vol.30(3), July 2023b:20-26.
9. Oluwasola A, Ayoola OE, Odetayo AF, Garba S, Olayaki LA. Ameliorative Effect of Melatonin on Reproductive Hormones in Ethanol Extracts of Cannabis sativa-Treated Female Wistar Rats. NISEBjournal. Vol. 22(2), September/December 2022a: 27-32.10.
10. Oluwasola A, Balogun ME, Muzzammill SS, Zainab SO, Adekunle OJ, Mariam EB, Nkemdili VJ, Aishat NH, Comfort AM. Effect of Acute Administration of Ethanol Extract of *Cannabis sativa* Leaf on Liver Function Biomarkers in Male Wistar Rats'. *NISEB journal*. Vol. 22(1), September/December 2022b: 20-26
11. Abdulrahim HA, Alagbonsi IA, Oluwasola A, Omeiza NA, Feyitimi AA, Olayaki LA.*Cannabis sativa* and/or melatonin do not alter brain lipid but alter oxidative mechanismsin female rats. Journal of Cannabis Research, 2021, Vol. 3(38): 1-10

12. Oluwasola A, Olayaki LA, Ayinde TO. Effects of Melatonin and Ethanolic Extract of Cannabis sativa Leaves on Sexual Behaviour Parameters of Female Wistar Rats. Bioscience Research Journal. Vol. 32(1), Feb 2020a: 29-36.
13. Olayaki LA. Oluwasola A. Melatonin Mitigates Ovarian Toxicityin Cannabis-Treated Female Witar Rats. Federation of American Societies for Experimental Biology Journal. Vol. 34(S1), March 2020b: 1-1.
14. Oluwasola A, Olayaki LA, Ayinde TO. Effects of Melatonin on Estrous Cycle Changes Induced by Etanolic Extract of Cannabis sativa in Female Wistar Rats. Nigeria Society of Experimental Biology Journal. Vol. 19(12), June 2019a: 55-60
15. Oluwasola A, Ayinde TO. Comparative Study on the Antioxidant Potentials of Honey and Some Selected Beverages in Male Wistar Rats. *Al-Hikmah Journal of Education.*Vol.6(2), December 2019:1-7
16. Madhavan UN, Zarina A, Beena V. Fluid and electrolyte balance. In Textbook of Veterinary. 2023, Physiology (pp. 193-211). Singapore: Springer Nature Singapore.
17. Rein JL, Zeng H. A retrospective cohort study that examined the impact of cannabis consumption on long-term kidney outcomes. Cannabis and Cannabinoid Research. Vol. 9(2), May 2024: 635-45.
18. Collister D, Pyne L, Bhasin AA. Sex and gender in randomized controlled trials of adults receiving maintenance dialysis: A meta-epidemiologic study. American Journal of Kidney Diseases. Vol. 81(5), July 2023: 575-82.
19. Gupta A, Madhavan MV, Sehgal K. Extrapulmonary manifestations of COVID-19. Nature medicine. Vol. 26(7), March 2020: 1017-32.
20. Ali SM, Ibupoto ZH, Salman S, Nur O, Willander M, Danielsson B. Selective determination of urea using urease immobilized on ZnO nanowires. Sensors and Actuators B: Chemical. Vol. 160(1), December2011: 637-43.
21. Miller Z, Miller BF. Specific *determination of serum creatinine.* Proc Soc Exp Biol Med.Vol. 78(2), November 1951: 471–473*2*
22. Fokam Tagne MA, Akaou H, Noubissi PA, Foyet Fondjo A, Rékabi Y, Wambe H, Kamgang R, Essame Oyono JL. Effect of the Hydroethanolic Extract of Bixa orellana Linn (Bixaceae) Leaves on Castor Oil-Induced Diarrhea in Swiss Albino Mice. Gastroenterology Research and Practice. Vol. 1, 2019:6963548.https://pmc.ncbi.nlm.nih.gov/articles/PMC6914941/
23. Prakash J, Patrikar RM, Palekar S, Kalambe J. Potassium Detection in Blood Serum By Image Processing. In2024 IEEE International Conference on Electronics, Computing and Communication Technologies (CONECCT). July 2024: 1-6.https://ui.adsabs.harvard.edu/abs/2024cone.conf..244P/abstract
24. Potukuchi PK, Moradi H, Park F. Cannabis use and risk of acute kidney injury in patients with advanced chronic kidney disease transitioning to dialysis. Cannabis and cannabinoid research. Vol. 8(1), August 2023: 138-47.
25. Tonial N. Non-Renal Alterations of Drug Disposition in Chronic Kidney Disease (Doctoral dissertation, The University of Western Ontario (Canada)). 2023.
26. Gitau K, Howe HS. Therapeutic cannabis use in kidney disease: A survey of Canadian nephrologists. Kidney Medicine. Vol. 4(5), July 2022: 100453.
27. Golosova D, Levchenko V. Acute and long-term effects of cannabinoids on hypertension and kidney injury. Scientific Reports. Vol. 12(1), May 2022: 6080.

28. Pressly JD, Soni H. Activation of the cannabinoid receptor 2 increases renal perfusion. Physiological genomics. Vol. 51(3), August 2019: 90-96
29. Rein JL. The nephrologist's guide to cannabis and cannabinoids. Current Opinion in Nephrology and Hypertension. Vol. 29(2), June 2020: 248-57.
30. Nedumaran B, Rudra P, Gaydos J. Impact of regular cannabis use on biomarkers of lower urinary tract function. Urology. Vol. 109, January 2017223-e9.
31. Onyema JO, Onyieke EN, Amadi BA. Mitigating Marijuana-Induced Nephrotoxicity: The Role of Selected Local Spice Diets in Wistar Rats. International Journal of Innovative Biochemistry & Microbiology Research. Vol. 12(4), June 2024: 1-15
32. Mobisson SK, Onyebuagu PC, Wopara I. Cannabidiol Oil and Prednisolone Treatment Altered Hematologic Indices, Serum Urea, Creatinine and Cellular Architecture of Kidney on Cadmium Induced Toxicity in Male Wistar Rats. Journal of Pharmaceutical Research International. Vol. 35(21), December 2023: 14-27.
33. Okon VE, Obembe AO, Osim EE. ecreased biliary secretion, biliary composition and serum electrolytes with chronic administration of Cannabis sativa in albino wistar rats. National Journal of Multidisciplinary Research and Developmen. Vol. 8(2), February 2023: 21-25
34. Stolić R, Milić M, Mitrović V. The Influence of Psychoactive Substances on Nephrotoxicity of the Kidneys. Acta facultatis medicae Naissensis. Vol. 41(2), July 2024: 294-309.