International Journal of Healthcare and Medical Sciences
ISSN(e): 2414-2999, ISSN(p): 2415-5233
Vol. 8, Issue. 4, pp: 49-56, 2022
URL: https://arpgweb.com/journal/journal/13
DOI: https://doi.org/10.32861/ijhms.84.49.56

Academic Research Publishing Group

Original Research Open Access

Effects of Marijuana on Weight Changes, Physical Observation and Histology on the Kidney of Wistar Rats

Okobi O. E.
Family Medicine, Lakeside Medical Center, Belle Glade, USA

Iyevhobu K. O. (Corresponding Author)
Lassa Fever Unit, Irrua Specialist Teaching Hospital (ISTH) Irrua, Edo State, Nigeria, Ekpoma, NGA
Email: kennylamai@yahoo.com

Nwanguma A. N.
Medicine and Surgery, Nnamdi Azikiwe University College of Health Sciences, Nnewi, NGA, Public Health, Washington University in St. Louis, St. Louis, USA

Akinsola Z.
Internal Medicine/ Family Medicine, Windsor University School of Medicine, Toronto, CAN

Onyechi N. P.
Internal Medicine, University Hospitals Cleveland Medical Center, Cleveland, USA

Ajibowo A. O.
Internal Medicine, Lugansk Medical University, lugansk, UKR

Odedina J.
Pathology and Laboratory Medicine, University of Jos, Calgary, NGA

Article History
Received: 8 September, 2022
Revised: 7 November, 2022
Accepted: 8 December, 2022
Published: 12 December, 2022

Abstract

Background and Objective: The rate of use of cannabis is rapidly increasing in society. Some of its usefulness has been documented, while its effect on weight changes is still understudied. This study was carried out to evaluate the weight changes, physical changes, and histological effects of cannabis on the kidney of Wistar rats. Materials and Methods: They were divided into five groups, A, B, C, D, and E comprising five rats each. The study involved 25 growing Wistar rats with weight ranges between 250-275g and 50 growing Wistar rats for LD50, with weight ranges between 250-275g. Groups A served as the control and received only growers' mash and distilled water, while groups B, C, D, and E received 65mg/kg body weight, 130mg/kg body weight, 162mg/kg body weight, and 194mg/kg body weight of cannabis respectively. Animal weights were measured before and after the administration of *cannabis*. The control rats and test animals were sacrificed after 28 days, and the kidney was harvested for histological processing. The results obtained were compared with the control using the statistical package for social science (SPSS) version 20 with a level of significance ($P<0.05$). Results: The results on weight recorded were; group B (310.0±14.14), groups C (273.0±2.32), D (295.0±7.07), and E (277.0±3.53) recorded significant ($P<0.05$) lower weight when compared with the control group The control kidney tissue section, however, presented normal cytoarchitectural features of the kidney while the test groups showed histological alterations which were dosage induce. Photomicrograph of group B kidney tissue section shows kidney cytoarchitecture with vacuolations and mild glomerular distortion, photomicrograph of group C kidney tissue section shows kidney cytoarchitecture with vacuolations, photomicrograph of group D kidney tissue section shows shrinking glomeruli which were adjacent to a normal glomerulus and photomicrograph of group E kidney tissue section shows glomerulus surrounded by cellular infiltrates. Conclusion: The study's findings revealed that cannabis has a deleterious effect on the cytoarchitecture of the kidney in a dose-dependent way. Also, more studies are needed to identify the time in relation to dosage consumption effect, knowing which could be harmful to living and the LD_{50} that would produce 100% mortality.

Keywords: Rats; Wistar; Kidney; Histology; Weight; Marijuana; Cannabi.

1. Introduction

Cannabis is a dioecious (having male and female flowers in separate plants), green, leafy plant with characteristic opposite, usually seven-fingered, lance-shaped leaves; on dry, sandy, slightly alkaline soil, it can grow to more than seven meters in height. Glandular hairs develop on the female flower, which secretes a resin. Female plants are more important than male plants for commercial purposes: their fibers are thicker, they form nutritious seeds, and they contain the psychoactive principle tetra hydro cannabinol (THC) [1].

Over the years, Cannabis has remained the most widely used illicit drug worldwide due to its affordability and availability [2]. Besides, cannabis is a major controversial drug as there are numerous conflicting and controversial reports concerning its psychological and physiological effects [3]. Many reports have linked cannabis smoking to the

development of psychosis [4]. Certain studies have also suggested that cannabis smoking is only a form of self-medication in people with psychotic symptoms rather than a causative factor in the development of psychosis [5].

The kidney is a bean-shaped structure having a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ and the renal veins leave. The renal capsule, a thick fibrous structure that encloses the kidney, is surrounded by perinephric fat, renal fascia, and perinephric fat. The peritoneum forms the front boundary of these tissues, and the transversalis fascia forms the posterior border. The kidney is roughly 6 cm wide, 4 cm thick, and 11 to 14 cm long [6, 7].

Furthermore, the kidney is an organ with several functions and essential roles in most animals, including vertebrates and some invertebrates. It is an essential part of the urinary system and serves homeostatic functions, such as regulating electrolytes and maintaining acid-base balance and water balance (via maintaining salt). They serve as the body's natural filter of blood by removing metabolic by-products and wastes (Urea and ammonium) and is responsible for the re-absorption of water, glucose, and amino acid as well as the production of a hormone (calcitriol, erythropoietin) and some enzymes (rennin) [8].

Cannabis could be described as a dry, shredded mixture of the flowers, stems, leaves, and seeds of the cannabis plant. The plant has three species: Cannabis sativa, Cannabis indica, and Cannabis ruderalis. Cannabis sativa leaf is typically green but brown if dried. The primary psychoactive component of cannabis is delta-9-tetrahydrocannabinol (Δ9-THC) [9]. There seems to be an upsurge in reports in recent years that support cannabis psychosis. Further, a review of the evidence surrounding the acute impact on memory concluded that cannabinoids impair all aspects of short-term memory, especially short-term episodic and working memory [10]. Marijuana, like every other substance filtered by the kidney, there is a need to evaluate its effect of this plant on the kidney. Also, most herbal concoctions are not officially regulated like conventional drugs, and reports have shown that their use and abuse are highly prevalent [11].

Numerous studies have demonstrated that cannabis smoking has major physical impacts on the body in addition to its psychological effects [12, 13]. Heart rate has been shown to rise by 20–50% while using cannabis. This most immediate reaction happens shortly after using cannabis. Inexperienced cannabis users frequently experience orthostatic hypotension, or 'light-headedness,' which is commonly the first sign of intoxication, when they suddenly shift their position from laying down to standing up [14]. Other physical effects of cannabis include lowering of body temperature, dry mouth, decreased intraocular pressure, and muscle relaxation [15]. Reddening of the eyes due to conjunctiva blood vessel congestion is another impact of cannabis. The immune system has also been demonstrated to be impacted by cannabinoids [16]. Cannabis enhances appetite, according to clinical research and survey data [17]. It has been demonstrated that the primary component of cannabis, 9-tetrahydrocannabinol (THC), alters both the action and release of insulin [18]. This may help to explain the use of cannabis for diabetes self-medication. Additionally, cannabis has reportedly been used medicinally to treat depression [19]. Leprosy, leucoderma, scabies, smallpox, allergies, burns, cuts, and wounds, as well as sexually transmitted infections and inflammatory conditions have all been treated with cannabis [20]. In Asian traditional medicine, various cannabis preparations have been used to treat a wide range of illnesses, including inflammation, nausea, headaches, hematochezia, diarrhea, and alopecia [21, 22]. These preparations have also shown to have potent anti-inflammatory, analgesic, antipyretic, and antidiarrhea properties [23].

There is a lack of information on potential health effects in developing nations, which may be used to inform health analyses of national development programs. A pre-clinical experimental look at the chronic health effects of cannabis can be provided in the laboratory using experimental animal models in which well-controlled doses are administered over a period of time. Therefore this research is focused on the impact of cannabis on the histology of the kidney in albino rats. The aim of this study was to evaluate the histological effect of marijuana on the Kidney of Wistar rats. This study is limited to the evaluation of the histological effect of Marijuana on the kidney of Wistar rats.

2. Materials and Methods

2.1. Study Duration

The preliminary studies, animal acclimatization, ingredients procurement (Marijuana preparation and production), actual animal experiment, and evaluation of results lasted for a period of three months (July to September, 2021). However, the administration of Marijuana to the test animals lasted for 28 days.

2.2. Ethical Considerations

Approval was obtained from the Research and Bioethics Committee of Ambrose Alli University, Ekpoma, Nigeria, and the study was carried out in strict accordance with the guidelines for the care and use of animals for research.

2.3. Procurement of Plant Material and Authentication

Permission was sought from the National Drug Law Enforcement Agency (NDLEA) at their branch office at Irrua, Edo State. The leaves were also obtained from the NDLEA office and were authenticated at the Department of Botany, Ambrose Alli University, Ekpoma.

2.4. Experimental Animals/Housing Condition

Twenty-five (25) Adult Albino Wistar rats of both sexes and of comparable sizes and weights were procured from the animal house and transferred to the experimental site, where they were allowed two (2) weeks of acclimatization. During this period of acclimatization, the rats were fed growers' mash water provided ad libitum.

2.5. Experimental Design/Animal Grouping

The experimental animals were separated into five groups (A - E). Group A had five rats (n = 5), and groups B - E had five rats (n = 5), each using 5 big cages to house them.

Group A served as the control and received only the normal feed (grower's mash) and water with no administration of Marijuana, while Group B, C, D, and E received different doses of Marijuana. For the LD50, fifty (50) Wistar was used and kept in separate big cages.

2.6. Substance Preparation

Dried leaves of marijuana were grounded and weighed in graded doses; each dose was dissolved in 1mL of water and administered to the rats daily; Group B was given 65mg, Group C was given 130mg, Group D was given 162mg, and Group E was given 194mg daily of marijuana for 28 days.

2.7. Substance Administration

Each dose of powdered cannabis Sativa was dissolved in 1mL of water and administered to all the rats with exception of the control. The administration of the marijuana was given orally as follows:

- Group A (Control) received only normal feed (growers' mash) and distilled water daily for 28days.
- Group B received 65mg/kg bw of Marijuana, feed, and distilled water (5mL) daily for 28days.
- Group C received 130mg/kg bw Marijuana, feed, and distilled water (5mL) daily for 28days.
- Group D received 162mg/kg bw Marijuana, feed, and distilled water (5mL) daily for 28days.
- Group E received 194mg/kg bw Marijuana, feed, and distilled water (5mL) daily for 28days

The animals were weighed on the first day of acclimatization and fed with feed and water given ad libitum. They were housed in well-ventilated labeled wooden cages at the site of the experiment. The cages were designed to properly secure the animals, especially wild animals/insects, and were cleaned daily. The rats were weighed before the administration of the marijuana and just before they were sacrificed, and similar weight measurements were done at the end of each week, and the average weight was recorded accordingly.

2.8. Toxicity Studies

The toxicity LD50 was estimated using fifty (50) Wistar rats, and cannabis was administered orally following Lorke's method [24] dose levels used ranged from 200 mg to 700mg, and toxicity signs such as nervousness, excitement, aggressiveness, and dullness were observed, and no mortality was recorded contrary to Lorke's method [24] where 700mg produced 100% mortality.

2.9. Sample Collection and Analysis

Weight was taken before, and after acclimatization, similar weight measurements were done at the end of the treatment periods, and the average weight was recorded accordingly. Furthermore, the kidney of each rat was obtained at the end of each stage under chloroform anesthesia and fixed in 10% formalin for histological processing.

2.10. Processing Schedule

The tissues were processed using an automatic tissue processor according to the processing schedule used in Histopathology of the Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The fixed plastic cassette tissues in 10% formalin were processed by passing them through different grades of alcohol as follows:

Beaker 1 10% formal saline 1 hour 30mins
Beaker 2 70% alcohol 1 hour
Beaker 3 80% alcohol 1 hour
Beaker 4 90% alcohol 1 hour
Beaker 5 95% alcohol 1 hour
Beaker 6 95% alcohol 1 hour 30 minutes
Beaker 7 absolute alcohol I 2 hours
Beaker 8 absolute alcohol II 2 hours
Beaker 9 xylene I 1hour 30minutes
Beaker 10 xylene II 1hour 30minutes
Wax bath I 2 hours
Wax bath II 2 hours.
As in use in (Histopathology Laboratory AAU)

After the last timing, the tissues were removed from their plastic cassettes, placed at the center of the metallic tissue mold, and filled with molten paraffin wax. They were left to solidify, after which they were now placed in the refrigerator at 5^{o}C for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15

minutes), the blocks were then removed from the metallic case using a knife, after which the paraffin wax at the side of the blocks was removed.

The blocks were trimmed and cut serially at 3nm on a rotary microtome. The sections were floated in a water bath at 55^{o}C and picked up by the use of clean frosted end slides. The frosted end slides were placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides, after which the sections were de-waxed, hydrated, air dried and stored in a slide box ready for the staining process.

2.11. Staining Procedure

Sections for general tissue structure were stained by Haematoxylin and Eosin technique following the steps below.

1. The sections were dewaxed in 3 changes of xylene 5 minutes
2. The sections were hydrated through descending grades of alcohol (absolute, 95%, 80%, and 70%).
3. The sections were stained in Harris's hematoxylin 5 minutes
4. The sections were rinsed in running tap water to remove excess stain
5. The sections were differentiated in 1% acid alcohol 3 seconds
6. The sections were blued in running tap water for 10 minutes
7. The sections were counterstained with 1% eosin 1 minute
8. Sections were finally rinsed in water and dehydrated in
9. ascending grades of alcohol (70%, 80, 95%, and absolute)
10. The sections were cleared in xylene, air-dried, and mounted with dibuthylphthalate propylene xylene (DPX).

The slides were examined under a light microscope, and a photomicrograph was taken.

2.12. Data Analysis

The mean weight ± SD of the test animals and control before and after the administration of marijuana were calculated using SPSS (version 21).

3. Results

3.1. Results of Histological Observations

Sections of tissue were analyzed for histological changes in the kidney, and the results were shown in Figs. 1,2,3,4,5; The photomicrograph of the control kidney tissue section with normal kidney cytoarchitecture were sown in Fig. 1, distinct renal tubules, and glomerulus. The photomicrograph of group B kidney tissue section with kidney cytoarchitecture with vacuolations and mild glomerular distortion were shown in Fig. 2. The photomicrograph of group C kidney tissue section with kidney cytoarchitecture and vacuolations as shown in Fig. 3. The photomicrograph of group D kidney tissue section with shrinking glomeruli adjacent to a normal glomerulus as shown in Fig. 4. The photomicrograph of group E kidney tissue section with glomerulus surrounded by cellular infiltrates as shown in Fig. 5.

Figure-1. Photomicrograph of control kidney tissue section (H&E x400) showing normal kidney cytoarchitecture with distinct renal tubules (white arrow) and glomerulus (black arrow)

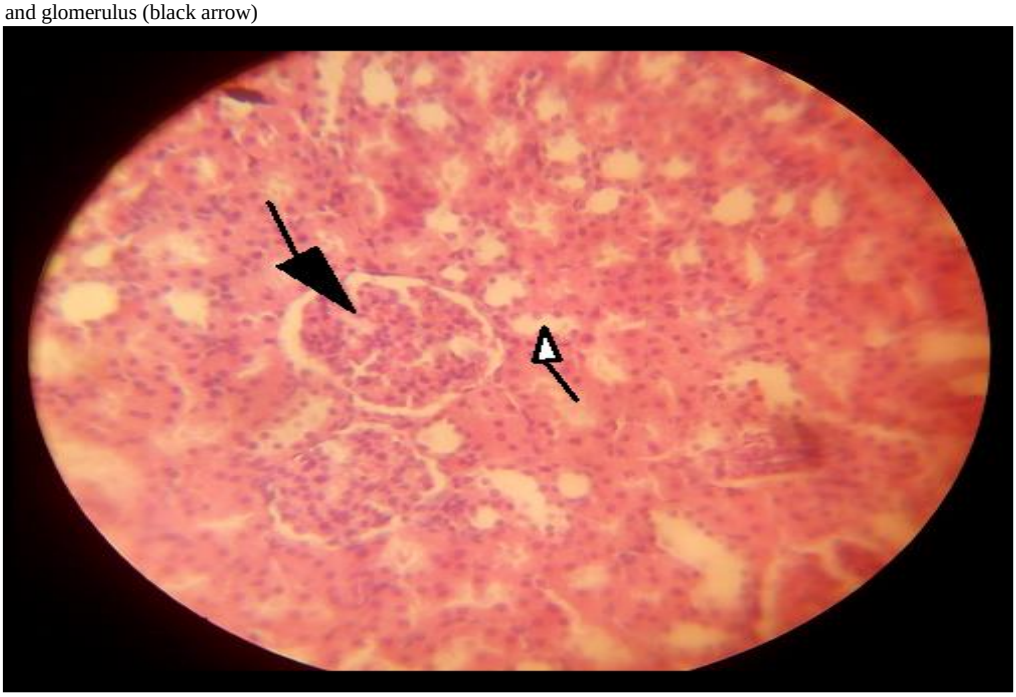


Figure-2. Photomicrograph of group B kidney tissue section (H&E x400) showing kidney cytoarchitecture with vacuolations (white arrow) and mild glomerular distortion (black arrow)

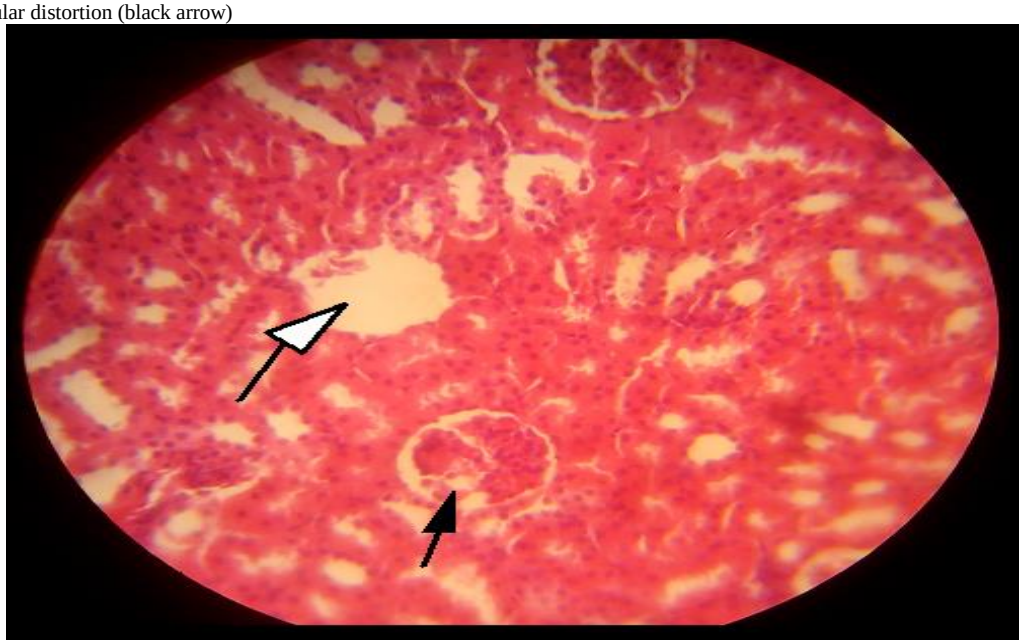


Figure-3. Photomicrograph of group C kidney tissue section (H&E x400) showing kidney cytoarchitecture with vacuolations (black arrow)

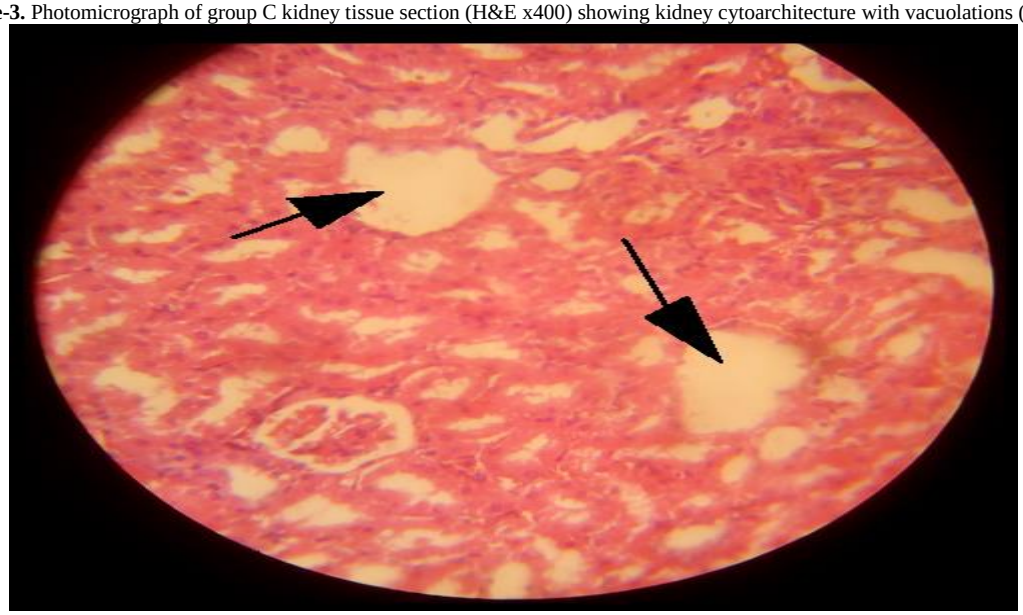


Figure-4. Photomicrograph of group D kidney tissue section (H&E x400) showing shrinking glomeruli (white arrows) which were adjacent to a normal glomerulus (black arrow)

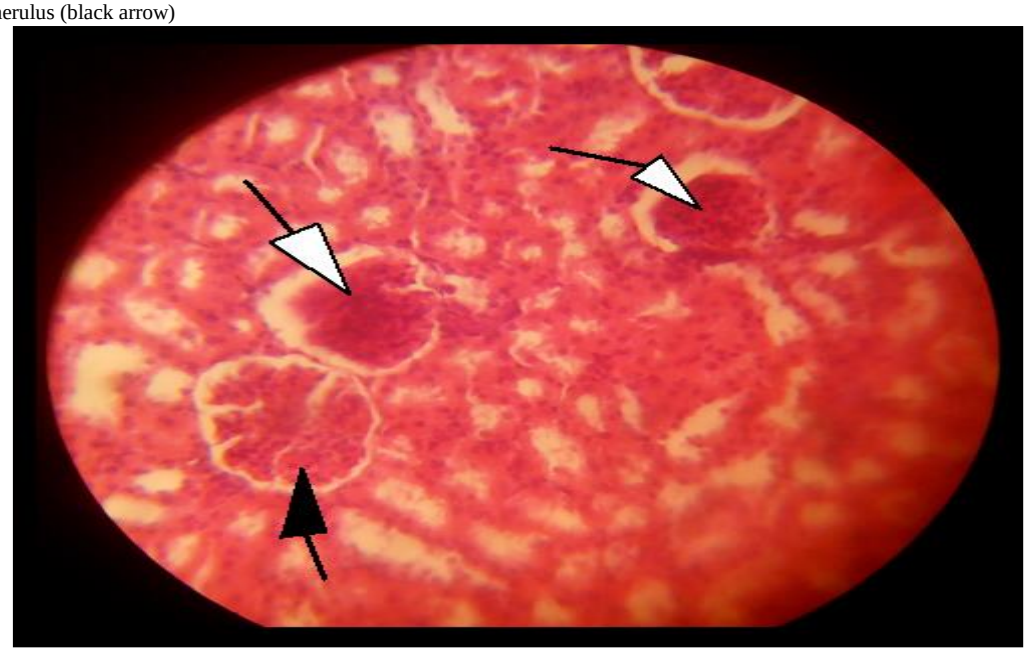
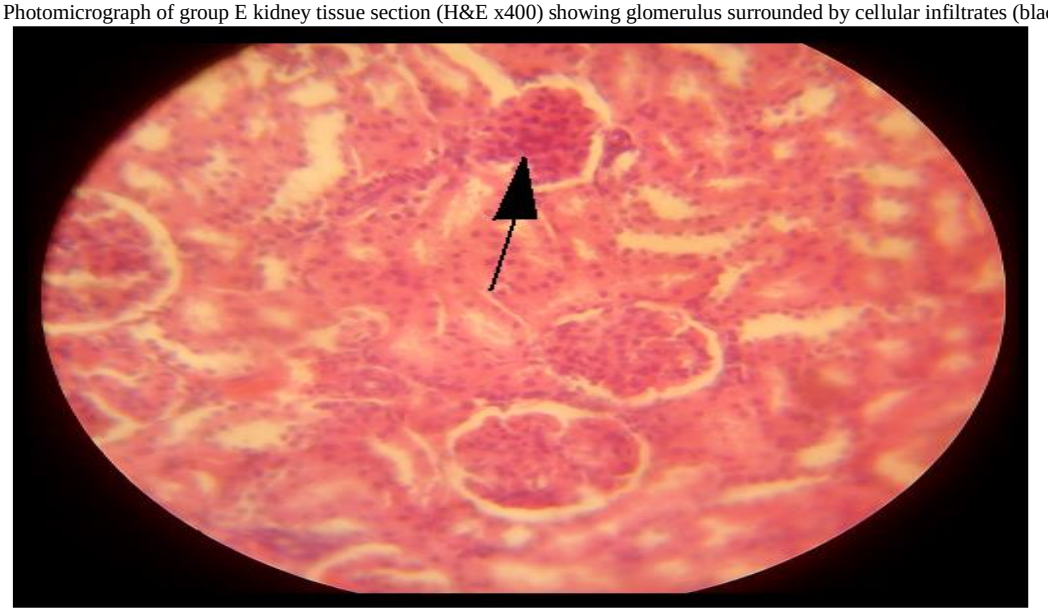


Figure-5. Photomicrograph of group E kidney tissue section (H&E x400) showing glomerulus surrounded by cellular infiltrates (black arrow)



These micrographic histological changes in the kidney were further described in the signing Table 1

Table-1. Histolcal Observations of the Effect of Marijuana on the Kidney of Wistar Rats

Histological effect	GROUP A CONTROL					GROUP B (65mg/mL)					GROUP C (130 mg/mL)					GROUP D (162 mg/mL)		GROUP E (194 mg/mL)							
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Normal histology	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vacuolations	-	-	-	-	-	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glomerular distortion	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	-
Shrinking glomeruli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++	-	-	-	-	-
Cellular infiltrates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++

KEY: - = Negative; ++ = Mild; +++ = Moderate; ++++ = Severe

3.2. Results on Weight

The body weight changes of rats fed with cannabis at various intervals as shown in Table 2. The results showed an increase in the control's weight during the study period. However, the weight of the rats fed with marijuana also increased in all the groups. The mean and standard deviation values of the body weight of rats in all groups before the period of marijuana administration are significant.

Table-2. Body weight changes of rats fed with graded dose of marijuana at various intervals

Weight (g)	A Control (n=5)	B (65mg/mL) (n=5)	C (130mg/mL) (n=5)	D (162mg/mL) (n=5)	E (194mg/mL) (n=5)	F value	P value
WBMA	252.5±3.53	277.5±3.53	252.5±3.53	272.5±3.53	215.5±2.12	29.30	0.001 (S)
WAMA	327.5 ± 3.53	310.0±14.14	273.0±2.32	295.0±7.07	277.0±3.53	18.18	0.004 (S)

KEY: P-value (P<0.05): significant; WBMA: weight before marijuana administration; WAMA: weight after marijuana administration
Values are mean ± standard deviation; n: Number of sample; s: significant; n/s: not significant

3.3. Results of Behavioural Observation

Increased appetite was observed in groups (C - D), and the control group presented normal appetite; there were changes in the color of urine, with groups D and E passing out yellowish-green urine coloration and no changes in the control and group (B and C). Group (B - E) was inactive, with the control group being active. Control groups B and C passed out frequently formed stool, while groups D and E passed out and formed stool infrequently, as illustrated in Table 3.

Table-3. Behavioural Observations during the Study

Observations	Control	GROUP B (65mg/mL)	GROUP C (130mg/mL)	Group D (162mg/mL)	Group E (194mg/mL)
Feeding	Normal	Normal	Appetite increased	Appetite increased excessively	Appetite increased excessively
Stool passage	Frequent formed stool	Frequent formed stool	Frequent formed stool	Infrequent formed stool	Infrequent formed stool
Urine passage	Amber colour	Amber colour	Amber colour	Yellowish green	Yellowish green
Activity	Very Active	Active	Inactive	Inactive	Inactive

4. Discussion

The kidney is an organ that plays several functions in most animals. Some of the most renowned functions are its role in the production of urine, its homeostatic functions, regulating electrolytes, and maintaining acid-base balance and water balance. furthermore, it serves as the body's natural filter for blood, removing metabolic by-products and wastes (Urea and ammonium) and then the re-absorption of water, glucose, and amino acid. It is also involved in the production of hormones (calcitriol, erythropoietin) and some enzymes (rennin) [8]. Marijuana, like many other drugs, is filtered through the kidney; thus, its impact on the kidney is critical. Furthermore, considering the widespread use of these herbal plants, the frequency of misuse, and the continual development of data on dangerous levels, particularly with relation to renal injuries. There is a dearth of scientific literature on the effects of cannabis on the histology of the kidneys.

After exposure to consumption of cannabis Sativa, test groups B-E showed various visual histopathological and morphological features relative to the control groups. These morphological changes include vacuolations, some degrees of glomerular shrinking and distortions, and cellular infiltrates. These histological alterations were apparently dosage induced with no observable dose escalation relationship. Different morphological patterns were seen at different doses. At daily doses lower than < 130mg (Group B and C), vacuolations with glomerular distortions were predominant observed on the H&E slides (97% expression, n= 25), and at higher doses (Groups D and E), glomerular shrinkage and fatty infiltrate were seen (86%, n=25). However, these observations may be similar to various other reported literature on the effect of marijuana on the kidney. In their study, Hsu, *et al.* [25], examined the effect of cannabinoid receptors and signaling on renal vascular and glomerular damage and proteinuria in CB1 transgenic mice. As a potential cause of nephropathy in their rats, they observed increased expression of vascular endothelial growth factor (VEGF) and decreased expression of nephrin in the renal glomeruli. The findings were similar to those seen in severe kidney injury characterized by severe glomerulosclerosis, mesangial expansion, and fibrosis. Renal CB1 signaling has been shown in various studies [Janice] to play a role in apoptotic cell death and renal inflammation by triggering pro-inflammatory markers and oxidative stress. Similar observations were also reported by Twadu [26]. In that study, daily oral treatment of Leptadenia Arborea extracts induced alterations in the essential organs and tissues of rats, including dilation of kidney tubules. Adekomi, *et al.* [27] also reported that extract of *D. stramonium* leaf had detrimental and severe effects on the histopathology of the kidneys of male Sprague Dawley rats as compared to control animals [27]. These data suggest that the renal impact of cannabis is more pronounced in rats given large dosages of the extract for prolonged periods of time.

There were detectable body weight changes for the control rats and those fed with cannabis. However, the large F ratio with significant p values showed that the variation among group means is more than we expect to see by chance. Furthermore, the weight of the rats given marijuana rose in all groups, with mean and standard deviation values of the body weight of rats in all groups before and after marijuana administration being significant. Similarly, and surprisingly other studies have also reported weight changes in rats fed with cannabis compared to wild types [Janice] [Barutta].

On the behavioral observation in the study, there was increased appetite among the group (C - D), and the control group presented normal appetite; there were changes in the color of urine, with groups D and E passing out yellowish-green urine coloration and no changes in the control and group (B and C). Group (B - E) was inactive, with the control group being active. Control groups B and C passed out frequently formed stool, while groups D and E passed out and formed stool infrequently.

5. Conclusions

There were histological abnormalities in the test tissue sections evaluated in this work on the histological effects of marijuana on the kidneys of adult Wistar rats, and the alterations were clearly dose-caused. There were also physical changes in the body weight of the test Wistar rats, which increased after cannabis treatment, and these test rats were equally inactive in greater doses throughout the study period after cannabis administration. The study's findings revealed that cannabis has a deleterious effect on the cytoarchitecture of the kidney in a dose-dependent way. Given these findings, indiscriminate usage of the herb should be avoided. More studies should be conducted to identify the length in connection to dosage consumption effect, the fatality rate, and the LD50 relationship.

Ethics Statement and Conflict of Interest Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** College of Medicine Health and Research Ethical Committee Ambrose Alli University, Ekpoma, Edo State, Nigeria Issued protocol number HREC-3214.

Conflicts of Interest

In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work

Contribution of Author:

- O.E. Okobi, and K.O. Iyevhobu - Research idea and design, drafted the work
- K.O. Iyevhobu – Field work (Feeding of rat and organ harvesting)
- A.N. Nwanguma, and Z. Akinsola - Reviewed the write-up
- N.P. Onyechi, and A.O. Ajibowo – Micrograph review
- J. Odedina - Result analysis

References

[1] Adams, I. B., Martin, B. R., and Cannabis, 1996. "Pharmacology and toxicology in animals and humans." *Wiley Online Library*, vol. 91, pp. 1585-1614.

[2] Bauman, A. and Phongsavan, P., 1999. "Epidemiology of substance use in adolescence: Prevalence, trends and policy implications." *Elsevier*, vol. 55, pp. 187-207.

[3] Richardson, T. H., 2010. "Cannabis use and mental health: A review of recent epidemiological research." *Int. J. Pharmacol.*, vol. 6, pp. 796-807.

[4] Hall, W. and Degenhardt, L., 2008. "Cannabis use and the risk of developing psychotic disorder." *World Psychiatry*, vol. 7, pp. 68-71.

[5] Abdelrahman, S. H., Mousa, I. M., and Khojali, S. M. E., 2012. "Trypanocidal effect of cannabis sativa on experimental camel trypansomosis." *Res. J. Med. Plant*, vol. 6, pp. 281-285.

[6] Glodny, B., Unterholzner, V., and Taferner, B., 2009. "Normal kidney size and its influencing factors-a 64-slice MDCT Study of 1.040 asymptomatic patients." *BMC Urology*, vol. 9, p. 19.

[7] Walter, F. B. and Boulpaep, E. L., 2017. *Medical physiology: A cellular and molecular approach*. Elsevier/Saunders. (ed): Elsevier/Saunders., Elsevier/Saunders.

[8] Guyton, A. C. and Hall, J., 2006. *Metabolic functions of the kidney. Text book of medical physiology. (ed): W.B sanders company*. 3rd ed. Philadelphia.

[9] Ashton, C. H., 1999. "Adverse effects of cannabis sativa and cannabinoids." *Br. J. Anaesth*, vol. 83, pp. 637-649.

[10] Ranganathan, M. and D'Souza, D. C., 2006. "The acute effects of cannabinoids on memory in humans: A review." *Psychopharmacology*, vol. 188, pp. 425-444.

[11] Marchesini, G., Bugianesi, E., and Forlani, G., 2003. "Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome." *Hepatology*, vol. 37, pp. 917-923.

[12] Fant, R. V., Heishman, S. J., and Bunker, E. B., 1998. "Acute and residual effects of marijuana in humans." *Pharmacol. Biochem. Behav.*, vol. 60, pp. 777-784.

[13] Hashibe, M., Morgenstern, H., and Cui, Y., 2006. "Marijuana use and the risk of lung and upper aerodigestive tract cancers: Results of a population-based case-control study." *Cancer Epidemiol. Biomarkers Prev.*, vol. 15, pp. 1829-1834.

[14] Jones, R. T., 2002. "Cardiovascular system effects of marijuana." *J. Clin. Pharmacol Review*, vol. 42, pp. 58-63.

[15] Grotenhermen, F., 1744- 1769. "The toxicology of cannabis and cannabis prohibition." *Chem. Biodivers*, vol. 4, pp. 1744-1769.

[16] Friedman, H., Newton, C., and Klein, T. W., 2003. "Microbial infections, immunomodulation, and drugs of abuse." *Clin. Microbiol. Rev.*, vol. 16, pp. 209-219.

[17] Tehranipour, M. and Ebrahimpour, S., 2009. "Evaluating the effect of aquatic extract of cannabis sativa seed on spatial memory consolidation in rats." *Annals of General Psychiatry*, vol. 9, pp. 884-888.

[18] Frisher, M., White, S., and Varbiro, G., 2010. "The role of cannabis and cannabinoids in diabetes." *Br. J. Diabetes Vasc. Dis.*, vol. 10, pp. 267-273.

[19] Henquet, C. and Kuepper, R., 2010. "Does cannabidiol protect against the negative effects of the THC?" *Br. J. Psychiatry*, vol. 197, pp. 259-260.

[20] Dilaraand, B. and Nath, S. C., 2008. "Ethnobotanical review of medicinal plants used for skin diseases and related problems in northeastern India." *Journal of Herbs, Spices and Medicinal Plants*, vol. 7, pp. 55-93.

[21] Dewan, S., Kumar, S., and Kumar, V. l., 2000. "Anti-pyretic effect of latex of caltropisprocera." *Ind. J. Pharmacol.*, vol. 32, p. 522.

[22] McPartland, M. J., 2004. *Random queries concerning the evolution of cannabis and coevolution with the cannabinoid receptor*. London: The Medicinal Use of Cannabis Royal Society of Pharmacists. Guy G (ed). pp. 702-102.

[23] Kumar, V. l. and Basu, N., 1994. "Anti-inflammatory activity of latex of caltropisprocera." *J. Ethnopharmacol*, vol. 44, pp. 123-125.

[24] Lorke, D. A., 1983. "New approach to practical acute toxicity testing." *Arch. toxicol.*, vol. 54, pp. 275-287.

[25] Hsu, Y. C., Lei, C. C., and Shih, Y. H., 2015. "Induction of proteinuria by cannabinoid receptors 1 signaling activation in CB1 transgenic mice." *Am. J. Med. Sci.*, vol. 349, pp. 162-168.

[26] Twadu, A. S., 1998. *: Studies on leptadenia arborea and syzygium aromaticum toxicity to rats*. Sudan: University of Khartoum. University of Khartoum. pp. 34-42.

[27] Adekomi, D. A., Tijani, A. A., and Adeniyi, T. D., 2011. "Exposure to smoke extract of datura stramonium leaf: Some of its effects on the heart, liver, lungs, kidneys and testes of male sprague dawley rats." *Journal of Pharmacognosy and Phytotherapy*, vol. 3, pp. 6-12.