World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

HISTOPATHOLOGICAL EFFECTS OF CANNABIS ON THE LIVER AND KIDNEYS OF WINSTER RATS

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Article Received on 01/12/2022 **Article Revised on 22/12/2022** **Article Accepted on 11/01/2023**

ABSTRACT

Background: Some zenobiotics can cause direct structural affectation and/or minor or major histological changes to tissues and organs of the body. The central nervous system (CNS) physiological effects of cannabis have been extensively studied. However, very little is known about the histopathological changes in the brain both in short time and chronic cannabis users. In this study, toxic effects of cannabis on the kidneys of rat were investigated. **Aim.** The study aimed to investigate any dose dependent toxic effects of cannabis on the liver and kidneys of rats. **Methodology**: Thirty (30) rats with average weights 150-200g were used for the study. The animals were divided into groups A, B, C and D. They were all acclimatized for 5 days and fed on the same feed. Animals in groups B to D were administered with different doses of cannabis—with Group D— the highest dose—4Omg/kg/day, Group C—20mg/kg/day and Group B—l0mg/kg/day. Group A was used as the control. The cannabis substance was administered to the rats daily for 28 days in the laboratory in addition to their usual daily feeds and water. The animals each from groups A, B, C and D were sacrificed on days 7, 14, 21, and 28. The liver and kidneys were excised after dissecting the rats and tissues were collected for histology. **Results:** The test group rats had increased appetite with subsequent weight gain. Group D from 170 ± 10g in the first week to 200 ± 20g in the fourth week, Group C from 170± l0g to 200± l0g, Group B from 160± 5g to 180± 5g while the Control Group 150 ± 5g to 220 ± 20g. Various dose dependent histological changes were noted in the organs studied. In the liver changes range from diffuse inflammation, portal triaditis to various degrees of fatty changes which became marked with prolonged administration. In the kidney, there was marked inflammation with collapsed glomeruli, widening of Bowman spaces and edematous tissues. **Conclusion**: This study has revealed that, in addition to issues of intoxication, cannabis can also produce histopathological changes in the liver and kidneys. It is therefore recommended that in addition to advice based on intoxication as reasons for abstinences, p0ssible liver and kidney damages- hepathotoxicity, nephrotoxicity, and nephropathies, should also be given as reasons for abstinence and furthermore treatment of cannabis abuse should also involve possible investigations and treatment of liver and kidney damages.

KEY WORDS: Histopathological, cannabis, liver, kidneys, rats.

INTRODUCTION

Different plant substances produce specific pharmacological effects on humans (Ashton, 2001, Berham, 2001). Such effects may alter the normal physiological functions of the body, and even greater alteration at toxic levels (Rajput 2018; Akinola et al, 2019). Such alterations may include respiratory difficulty, changes in cardiac function, impaired renal functions and an altered perception. Cannabis comsuption is also associated with a number of psychological effects in humans (Thornicroft, 1990; Louise et al, 2002; Patton et al, 2002; Odejide, 2003; Stanley and Eneh, 2004; Zou and Kumar, 2018).

In addition to the physiological alteration, at normal or otherwise toxic levels, some zenobiotics can cause direct structural affectation and/or minor or major histological changes to tissue and organs of the body (Awolabi, 2017). Control/regulations for cannabis use is still relatively in effective in many countries (Obot, 1993). In spite of all these, a number of studies have laid claims to the medical usefulness of cannabis (Blandchard, 1992; Hollister, 1998; Hubbard et al, 1999).

The central nervous system (CNS) physiological effects of cannabis have been extensively studied (Higuera-Matas et al, 2009; Ujah, 2014; Broyd et al, 2016; Cohen et al, 2017; Cohen and Weinstein, 2018; Roddriques et

al, 2019). However, very little is known about the histopathological changes in the brain and other important organs of both short time and chronic cannabis users (Awolabi, 2017).

Cannabis is a genus of flowering plant that belongs to the kmgdom-plantae, division magnolioghyta, class magnohopsidea, order Rosales, family-cannabareae (Hangman et al, 2006). Cannabis has three putative species, *cannabis sativa linnaeus subsp.* Indica L., *Cannabis sativa subsp. Sativa, L.* (cannabis sativa information, 2008), and *Cannabis ruderalis* Janiseh (Berham, 2001; Booth, 2005; Audu et al, 2014). These three texas are indigenous to central Asia and surrounding regions. *C. Rucleralis* is commonly described as 'auto-flowering and may be day-neutral. All known strains of cannabis are wind-pollinated (Clark et al, 1991; Ainsworth, 2000) arid produce 'feed' that are technically called achenes. Most strains of cannabis are short day plant (Clark *et al.,* 1991) with the possible exception of Cannabis *sativa subsp. Saliva* var. *Spontanea (=C. ruderalis).* Cannabis is an annual dioecious, flowering herb. The leaves are palmately comp and, with serate leaflets.

There are 3 consumable forms of cannabis: Hashish or Charas (Kief and Hash oil), Bhang and Marijuana or ganja (Faeti et al, 1996)

Hashish or Charas is The herbal form of the drug consists of dried mature flowers and subtending leaves of pistillate female plants. Hashish is the most potent form of cannabis (Lebel hardenack, S. and S. R. Grant, 1997). The active constituents are various isomers of tetrahydrocannabinoid (THC). It contains over eighty THC. The resinous form known as hashish, consists primarily of glandular trichomes collected from the same plant material hashish (pressed kiel) or charas is a concentrated resin that have been physically extracted (). (Hashish def. From dictionary. Com), usually by rubbing, shifting or with rice.

Bhang is the cheapest and it is obtained from cuts of uncultivated plant, has low resin content. It is found commonly in the U.S.A.

Marijuana or ganja is derived from flowering tops and leaves of cultivated female plants. It has high resin content. (Marijuana Def. From dictionary Corn 2008) It forms less than 1% THC to 22°/a THC, the wide range is probably one of the reasons for the conflicting results from different studies. It is available in Nigeria.

The quality and potency of cannabis depend on a number of factors; the part of the plant, the planet, the soil Cultivation and method of preparation (Pertwee et al, 2010; Ujah, 2014; Oladimeji and Valan, 2020). The psychoactive potency of cannabis plant is approximately as follows (descending order). (Marijuana potency http:64233. 167.104/search?), Trichomes, Female flowering buds, Male flowering buds, New shoots, Leaves from flower buds, Leaves in ascending order of size, Stems of leave (petioles) in ascending order of size, Stem in ascending order of size, Roots and seeds. The potency of herbal cannabis decreases over time in storage and is affected by what parts of the plant have been included in the product (Chen et al, 2017; Zou and Kumar, et al, 2018). Hence, a user has little guarantee about the intensity of the high (Anthony and Heizer, 1991; Beautrais et al, 1999; Higuera-Matas et al, 2009; Ujah, 2014; Broyd et al, 2016; Cohen et al, 2017; Cohen and Weinstein, 2018).

Also, it has been found that the intensity of the smell of skunk appears to be no guide to the actual strength either. Various forms of herbal cannabis e.g 'Sinsemilla' (a bud grown in the absence of male plant which has no seed), 'homegrown', 'skunk' (which has a particular strong smell) and 'netherweeds' are often grown from selected seeds by intensive indoor methods (e.g using hydroponic methods, artificial lightening etc) to optimize their potency (Choudhary et al, 2013).

Some of the strains' names such as chocolate Thai, (Hirsch *et at.,* 1997), popular in the early 1990s due to its supposed high potency (Short D.J. 1990), entered the mass culture. The liver may be regarded as a modified exocrine gland that also has other functions. It is made up, predominantly, of liver cells or hepatocytes. Each hepatocytes is a large cells with a round open-faced nucleus with prominent nucleoli (Singh, 2001).

The liver, substance is divisible into a large number of large lobes, each of which consists of numerous lobules Bile is poured out from liver cells into very delicate bile canahculi that are present in intimate relationship to the cells From the canaliculi bile drains into progressively larger ducts which end in the **bile duct**(Singh, 2001)**..** This duct conveys bile into the duodenum where bile plays a role in digestion of fat.

All blood draining from the stomach and intestine (and containing absorbed food materials) reached the liver through the portal vein and its branches(Singh, 2001).. Within the liver this blood passes through sinusoids and comes into very intimate relationship with liver cells. The liver is thus able to 'screen' all substances entering the body through the gut. Some of them (e.g amino acids) are used for synthesis of new proteins needed by the body. Others (e.g, glucose, lipids) are stored in liver cells for subsequent use; while harmful substances (e.g drugs, alcohol) are detoxified.

Each kidney has a characteristic bean-like shape. Kidney tissue consists of an outer part called the cortex, and an inner part called the medulla(Singh, 2001).

The medulla is made up of triangular areas of renal tissue that are called the renal pyramids. Each pyramid has a base directed towards the cortex; and an apex (or papilla)

which is directed towards the renal pelvis, and fits into a minor calyx(Singh, 2001).. Pyramids show striations that pass radially towards the apex.
The renal cortex consists of the following: medullary rays and renal **columns.**

Kidney tissue is intimately covered by a thin layer of fibrous tissue which is called the capsule. The capsule of a healthy kidney can be easily stripped off, but is becomes adherent in some diseases. From a functional point of view the kidney may be regarded as a collection of numerous uriniferous tubules that are specialized for the excretion of urine.

OBJECTIVES

i. To examine the possible histopathologrcal effect of cannabis on the liver and kidney of rats.
ii. To determine any dose dependent effects of cannabies on these tissues.
iii. To find out any alteration in the histological architecture of the liver and kidney of rat using histopathological examination.

METHODOLOGY

Thirty (30) rats with average weights 150-200g were used for the study. The animals were divided into groups A, B, C and D. They were all acclimatized for 5 days and fed on the same feed. Animals in groups B to D were administered with different doses of cannabis—with Group D— the highest dose—4Omg/kg/day, Group C—20mg/kg/day and Group B—l0mg/kg/day. These doses were chosen mainly from extensive literature review. Group A was used as the control. The cannabis substance was administered to the rats daily for 28 days in the laboratory in addition to their usual daily feeds and water. The animals from the different groups were sacrificed on days 7,14,21, and 28. The liver and kidney were excised after dissecting the rats and the tissues were collected for histology.

RESULTS

i. Effect of Cannabis on Weight

Table 1: Effect of Cannabis on Weight.

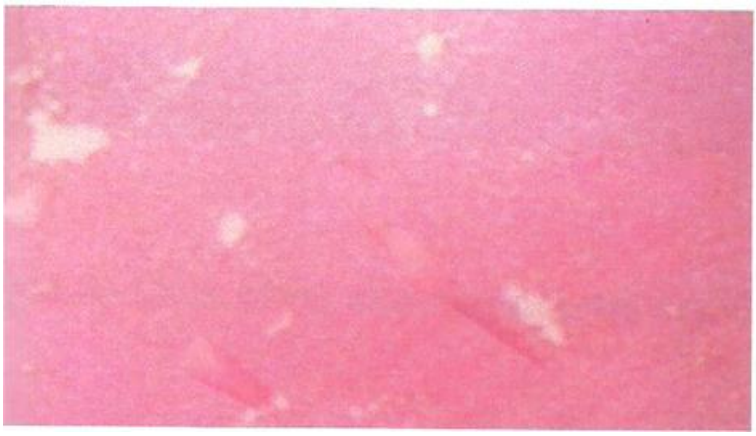
Group	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21(g)	Day 28 (g)
Control (A)	*155±5*	180±20	200±20	125±5	135±5
10mg/kg/day	160±5	200±5	190±5	195±5	*195±5*
20mg/kg/day	*170±5*	210±5	210±10	195±10	180±5
40mg/kg/day	170±5	210±5	220±10	185±10	175±5

From the study, it was observed that the rats gained weight in the first and second weeks, but most of the rats, lost some weight in the last week of the experiment. The later observation was not noticed in the rats in group C that receive the lowest dose as well as in the control animals.

ii. Effects of cannabis on the histology of the liver

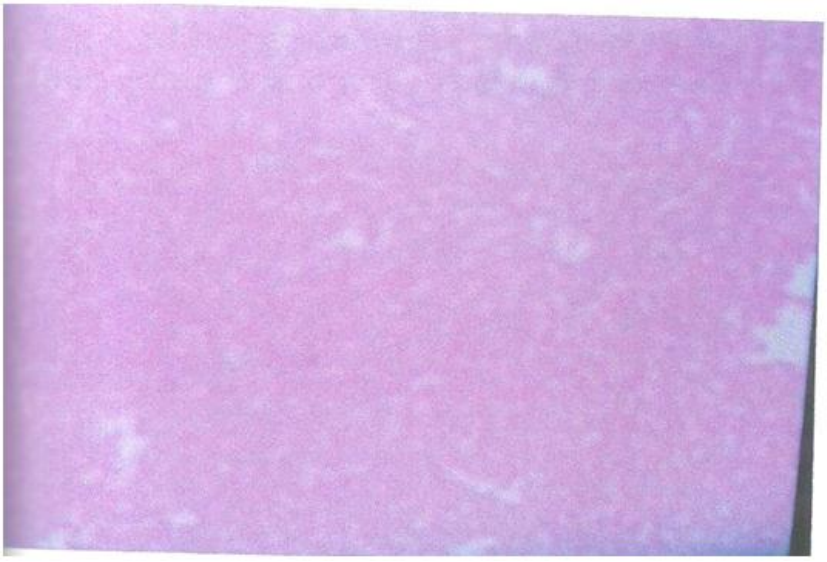
Some interesting histological changes were found in the liver during the experiment.

Figures 1a, 1b and 1c below the photomicrographs of the liver of the control groups on days 7, 14 and 28 showing normal histology



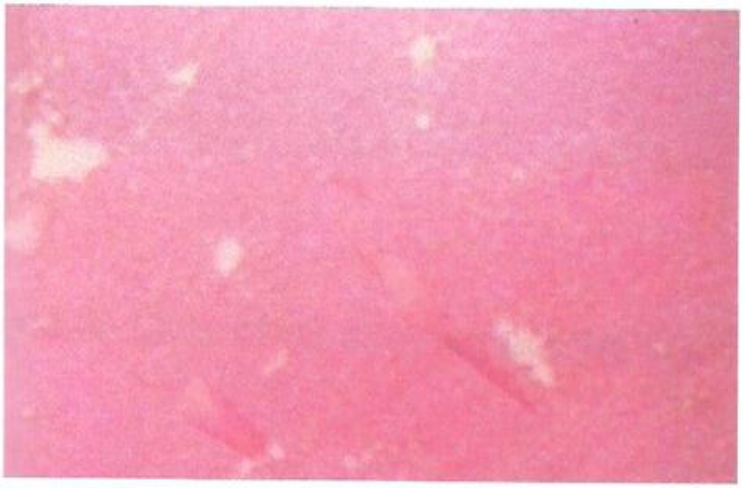
Control group day 7

Fig 1a: liver of rat showing normal cellular architecture.



Control Group A day 14

Fig 1b: Liver of rat showing normal cellular architecture.

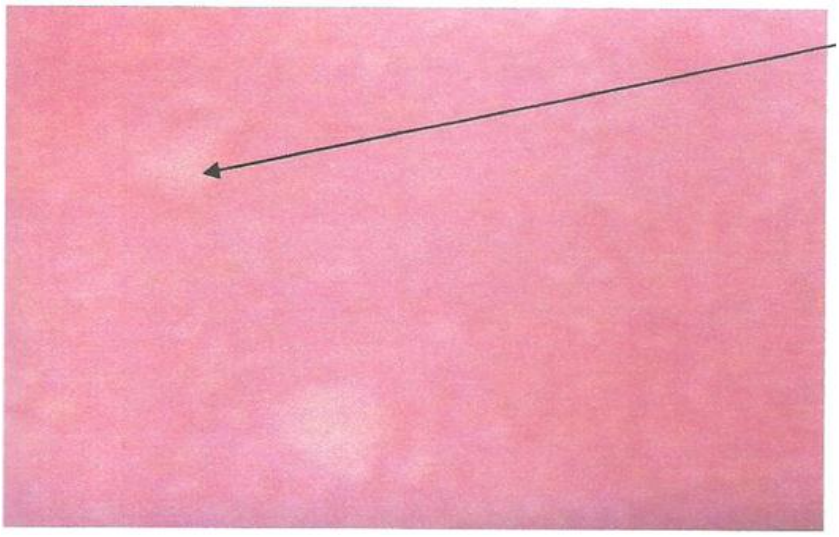


Control Group A day 28

Fig 1c: Liver of rat showing normal cellular architecture.

Fig 2: Liver of rat showing normal cellular architecture

Fig 4.3d below shows the photomicrograph of the liver of the test group B at day 14 showing



Congestion with real blood cells

Fig 3. liver of rat showing congestion of venous sinusis.

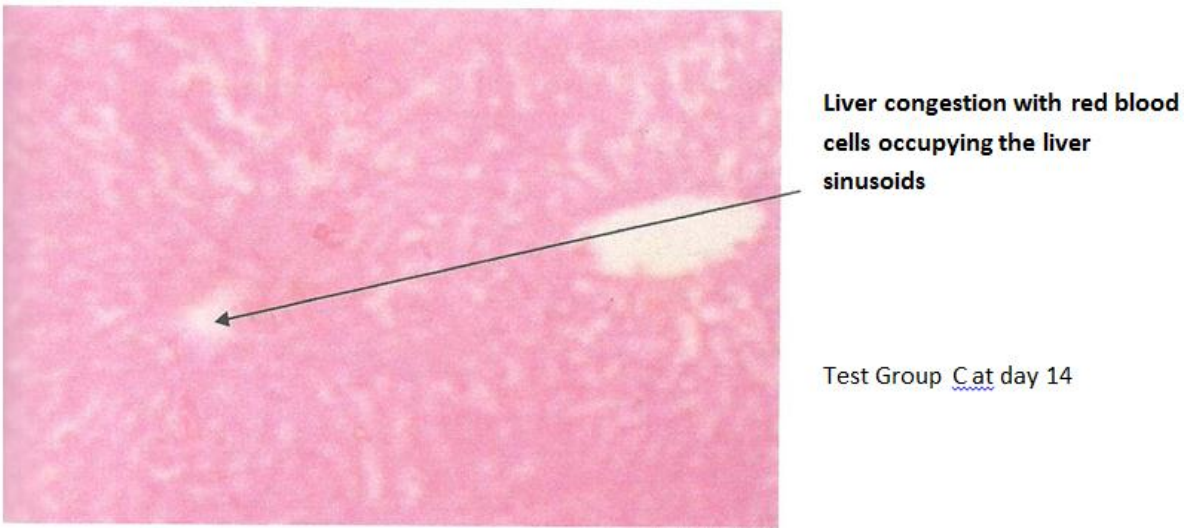


Fig 4: Liver of rat showing congestion with red blood cells occupying the liver sinusoids.

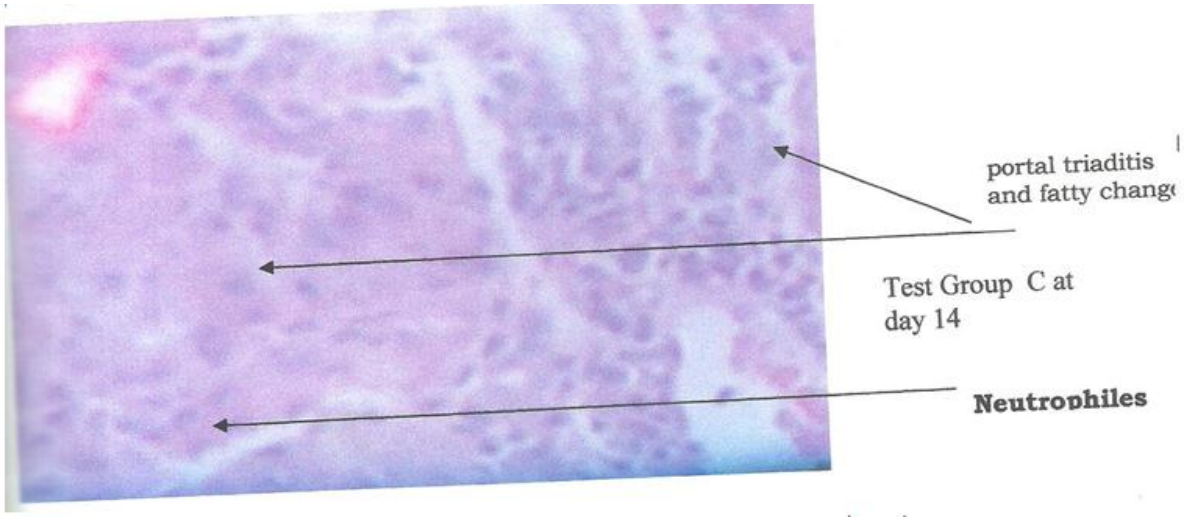


Fig 5: Liver of rat showing portal traditis and fatty change (+++) with numerous inflammatory cells-neutrophiles and lymphotyes.

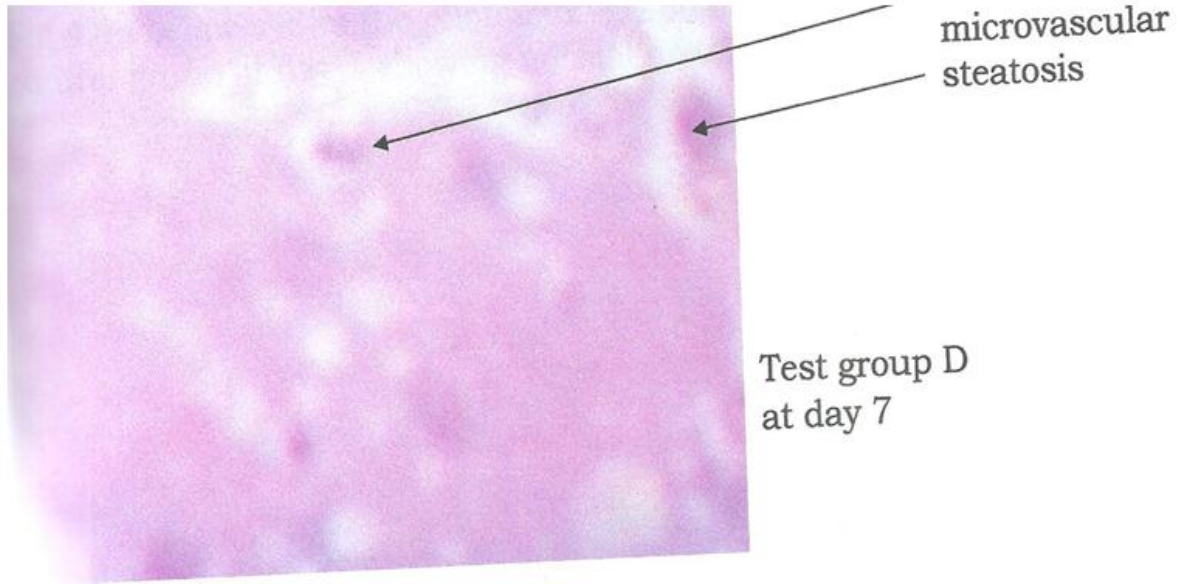


Fig 6: below shows the photomicrograph of the liver of the test group D at day 7 showing microvascular steatosis.

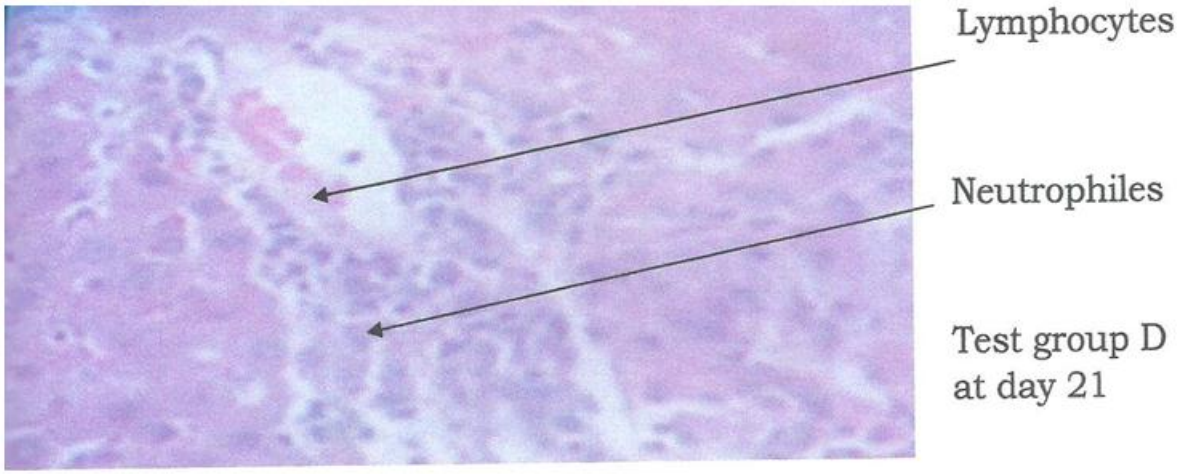


Fig 7: Liver of rat showing marked fatty change with compression of the sinusoidal spaces with bile staining with numerous inflammatory cells –Neutrophiles and Lymphocytes.

iii. Effects of cannabis on the Kidneys (Variable result)
Kidney of rat showing Normal cellular architecture

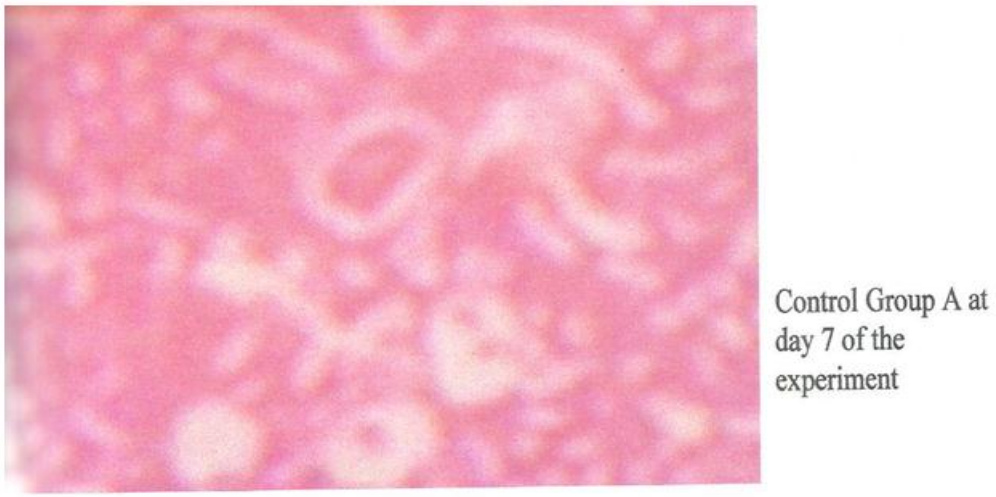


Fig 8: below shows the photomicrograph of the kidney of the control group at day 17 showing normal histology.

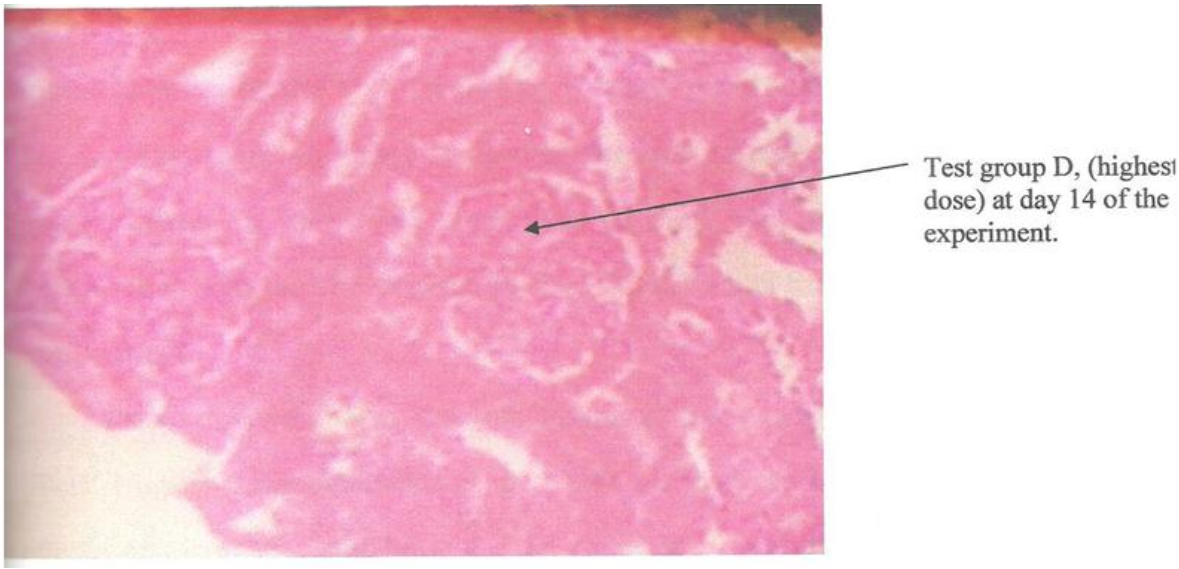
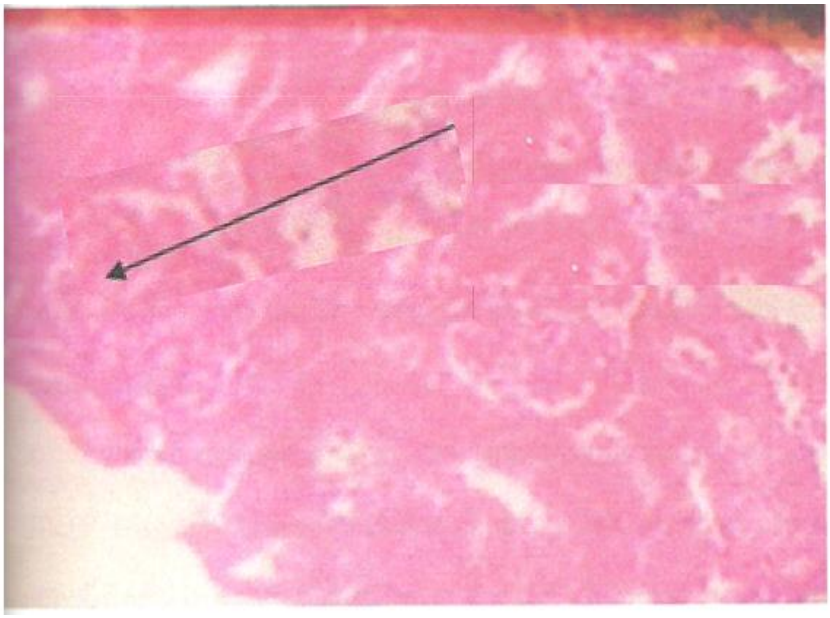


Fig 9: below shows the photomicrograph of the kidney of the test group D at day 14 showing collapsed glomerulli, widening of bowman spaces, edematous tissue.



Test group D, (highest dose) at day 21 of the experiment.

Fig 10: Kidney of rat showing Inflammatory collapsed glomerulli, widening of bowman spaces, edematous tissue.

DISCUSION

The central nervous system (CNS), particularly the limbic system constitutes the center of emotion and appears to be the pharmacodynamic target of cannabis (Gardner et al, 1991; Hillig et al, 2004; Hangmen et al, 2005; Friedman et al, 1995; Musty et al, 1995; Onaivi et al, 1996; Beale et al, 2018). As stated earlier, the principal component of cannabis is A9 THC (Murphy et al, 1994), however, the cannabis plant contains more than 400 chemicals, of which about 60 are chemically related to A9 THC (Ujah, 1014; Rajput and Kumar, 2018; Oladimeji and Valan, 2020). In humans, A-THC, is the metabolite that is active in the central nervous system (CNS). The receptor for the cannabinols has been identified, cloned and characterized (Friedman et al, 1995; Ujah, 1014; Rajput and Kumar, 2018; Oladimeji and Valan, 2020)) The distribution of cannabinoid receptors in rats is similar to that of humans (Munros, 1993). This may be the reason why similar effects are produced in humans with acute or chronic cannabis use (Arnt et al, 1992; Chait et al, 1992; Cantwell et al, 1999; Adamson, 2005;

This study was undertaken to determine, if in the addition to the usual intoxication, euphoria and the feeling of "high" experienced by the users of cannabis, there are other structural changes caused by substance in some organs in the body and also to find any associated dose dependency effects (Curran et al, 2002). The organs studied include the brain, heart, liver and kidneys.

Surveys have shown that marijuana is the most commonly used illicit drug worldwide (Lowinson et al, 1992; McKim, 2002; M,oral et al, 2002; Kessler et aL,1994,). Several studies done on the effects of cannabis used have limited themselves mainly to the central nervous system (CNS) effects of this psychoactive substance. A dose dependent intoxication effect was observed in the experimental animals following each administration of cannabis probably due to the high". This is consistent with many other earlier works that administration or consumption of large doses of cannabis causes acute intoxication ((Curran et al, 2002).

Also, Gbanai et al in 2008 noted that the wide therapeutic index is largely due to the fact that cannabis can bind with mirage of receptors, has prolonged half — life and can also accumulate in the tissue for a long time (Friedman et al, 1995; Onaivi, 1996).

Secondly, direct involvement of the liver and the kidneys in the metabolism and excretion of ingested cannabis respectively could also account for the marked histopathological changes seen in these organs. Other structural changes seen in the liver include marked fatty changes alone or with compression of the sinusoidal spaces and around the centrilobular vein. It was found out that abnormalities were more marked in the group that received the highest dose. Also, the structural changes progressed from mild acute inflammatory changes and portal triaditis in the first and second weeks to more extensive structural change, fatty changes in the last 2 weeks of the study.

An understanding of the pharmacokinetics of cannabis reveals that it is rapidly absorbed following oral administration, metabolized by the liver with marked first pass metabolism and limited system bioavailability after single doses. Both active and inactive metabolites are formed in the liver: the principal active metabolite is 11 —detla-9- tetrahydrocannabinol. Both cannabis and its metabolites are 95% bound to plasma proteins. The drug is excreted primarily via the biliary-fecal route, with

only 10% to 15% excreted in the urine (Hordmen and Human, 2006).

From the forgoing, the liver appears to be the main organ that metabolizes ingested cannabis and its metabolites. This will explain why there could be these marked histological changes in either prolonged use or intake of higher doses.

The kidney excretes about 10% to 15% of the metabolites of cannabis. In the study it was found that the kidneys showed no abnormality on histology upto day 14, but began to show focal areas of inflammation around the collecting ducts with collapse of most of the glomeruli and widening of bowman's space from day 21. This structural changes or abnormality were not seen in the control group. Although this finding did not clearly indicate a dose dependent effect, it clearly pointed to a prolonged used as all the animals, sacrificed at the end of the first week did not show any such histological alteration.

CONCLUSION

From the study, it was observed that cannabis use causes various histological alterations in the liver and kidney of rats.

RECOMMENDATIONS

From the observations and results from this study, it has become appropriate to make the following recommendations:

i. Abusers of cannabis should abstain from the psychoactive substance as it is capable of causing structural damage(s) to such organs like the liver and kidney.

ii. The management of patient with cannabis abuse should necessarily involve appropriate investigations and treatment of these organs studied as may be unique to the patient.

iii. The health sector, government as well as nongovernmental organizations should increase awareness and advocacy campaign about the inherent danger as well as the need to abstain from cannabis consumption

iv. Such campaign should be focused on at risk group like the youth, motor park drivers, secondary as well as university students

v. The already existing legislations by the NDLEA prohibiting the cultivation, handling and supply as well as smoking of cannabis should be well structured.

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