Histological changes in the vital organs of *male* rats following short term exposure to smoke extract of *Cannabis sativa*.

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Original Article

Abstract

Introduction: Beneficial effects of cannabis intake by any route of administration has since ages been trailed with controversial reports of scientific studies. This study was designed to evaluate the effects of short term exposure to smoke of *Cannabis sativa* on the vital organs (heart, lungs, liver, kidney and testes) of *male rats*.

Methods: Ten (10) male Sprague Dawley (SD) rats with average weight of 140 g were randomly divided into two (2) groups (A and B). Animals in group A (experimental group)were exposed to smoke from a completely burnt 0.74g leaves of Cannabis sativa, wrapped in 0.5g of sterilized cotton wool for 5 minutes three times daily (7am, 10am, and 1pm) while animals in group B (control group) were exposed to smoke from completely burnt 0.5g of sterilized cotton wool. All animals vg.had 5 day exposure to smoke of Cannabis and were sacrificed at least three hours after the last smoke exposure by cervical dislocation. In essence, all animals in the control and treatment groups were exposed to normal air in-between treatments. The rationale behind exposing animals in the control group to cotton wool smoke is to show that sterile cotton wool used as the vehicle for cannabis in the treatment group did not have any extra cytological/histological effect. The vital organs were carefully excised, rinsed, blotted dry and were transferred into (bouin's fluid and 10% formol saline) for at least 72 hours before further histological protocol and analysis.

Discussion: different histological changes observed in these vital organs include mild edema and destruction of myocardial fibers, degeneration of the hepatocytes, slugging off of the germ cells, enlargement of the alveoli and distortion of the renal cortex.

Conclusion: We inferred that short term exposure to smoke of *Cannabis sativa* may be associated with damage to some vital organs in SD rats.

Key words: - *Cannabis, vital organs,* histological changes

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Modifications histologiques dans les organes vitaux de rats mâles à court terme l'exposition à la fumée extrait de Cannabis sativa .

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L'article d'origine

Résumé

Introduction: effets bénéfiques de cannabis d'admission par toute voie d'administration a depuis des âges été tracté avec rapports controversés d'études scientifiques. Cette étude visait à évaluer les effets à court terme l'exposition à la fumée de Cannabis sativa sur les organes vitaux (coeur, poumons, foie, reins et les testicules) de rats mâles.

Méthodes: Dix (10) mâle Sprague Dawley (SD) rats avec poids moyen de 140 g ont été aléatoirement divisés en deux (2) groupes (A et B). Les animaux dans le groupe A (groupe expérimental) ont été exposés à la fumée d'un brûlé complètement 0.74g feuilles de cannabis sativa , enveloppé de 0,5 g de stérilisé coton pendant 5 minutes, trois fois par jour (7h, 10h et 1h) tandis que les autres animaux du groupe B (groupe contrôle) ont été exposés à la fumée de complètement brûlé 0,5 g de stérilisés de coton. Tous les animaux vg.eu 5 jour l'exposition à la fumée de cannabis et ont été sacrifiés au moins trois heures après la dernière exposition à la fumée par dislocation cervicale. En substance, tous les animaux dans les groupes de contrôle et de traitement ont été exposés à l'air normal en entre les traitements. Le raisonnement derrière exposer les animaux dans le groupe contrôle à la ouate de fumée est de montrer que coton stérile de laine utilisé comme véhicule pour le cannabis dans le groupe de traitement n'a pas d'extra cytologique/effet histologique. Les organes vitaux ont été soigneusement excisée, rincés, blotted dry et ont été transférées (bouin liquide et 10% de formol saline) pendant au moins 72 heures avant de continuer protocole histologiques et analyse.

Discussion: Les différentes modifications histologiques observés dans ces organes vitaux comprennent un œdème doux et à la destruction des fibres myocardiques, dégénérescence des hépatocytes, les écoulements hors des cellules germinales, l'élargissement des alvéoles et distorsion du cortex rénal.

Conclusion: nous avons déduit que, à court terme l'exposition à la fumée de Cannabis sativa peut être associée à une détérioration de certains organes vitaux sur des rats SD.

Mots clés :- *Le Cannabis, organes vitaux, modifications histologiques*

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INTRODUCTION

Despite the prohibition of its use in many countries of the world, including Nigeria, Cannabis sativa remains the most widely used illicit natural plant all over. Hall *et. al.* in 1998 reported that in many societies throughout the world, *Cannabis* has been used by a substantial minority, and in some a majority, of young adults. A number of debates about the justification to prohibit the use of *Cannabis* as a result of the seriousness of its adverse effects on health have also been reported (1). The series of debates about prohibition of recreational use of *Cannabis* have entangled the possible therapeutic effects of cannabinoids, making the health effects of *Cannabis* use to remain uncertain. There is also a very little scientific investigation and research with disagreements about the interpretation of the limited laboratory evidences (2). Different *Cannabis sativa preparations* have been used by man for over 5000 years (3) and have numerous and complex effects on the body.

Manifestations of the effects of smoked cannabis are seen within seconds and become apparent and full within a few minutes while they typically last for 2-3 hours (4). *Cannabis* is usually smoked in hand-rolled cigarettes called joints, among other names by its users. Other ways of smoking it are by the use of pipes or water pipes called bongs (5). It is also used in brewing tea and sometimes, it is mixed with foods. It contains over 300 compounds with at least 66 of them being cannabinoids (5, 6). Novak *et. al.* in 2001 reported some of the important chemicals found in *Cannabis sativa* plant to include Ä^{9}-tetrahydrocannabinol, á-pinene, myrcene, trans-â-ocimene, á-terpinolene, trans-caryophyllene, á-humulene, and caryophyllene-oxide (7).

Inhalation of *Cannabis sativa* smoke extract makes the body to interact with it as it attempts to get rid of the harmful toxins which may lead to insult on organs of the body. Such insult usually manifests as alterations in the levels of enzymes and other cellular components. The toxicity which could as well result in tissue or organ damage, commonly affect some vital organs including the brain, heart, liver, pancreas, and kidney to mention but few. The aim of this research work was to study the histological changes in the heart, liver, lungs, kidney and testes of rats following their short term exposure to smoke extract of *Cannabis sativa*.

METHODS

Collection of Plant and Preparation of Plant Extracts

The sample of *Cannabis sativa* used was obtained from the Nigeria Drug Law Enforcement Agencies (NDLEA), Ilorin Command, Ilorin, Kwara State, Nigeria, air-dried under standard laboratory conditions and was weighed using electronic weighing balance (Gallenkamp FA2104A, England). A measure of 0.74 g of the dried cannabis was wrapped with 0.5 g of refined and sterilized cotton wool following the protocol of Adekomi *et al* in 2011 and burnt to produce the smoke extract for animal exposure (8).

Animal Care

Ten male Sprague Dawley (SD) rats with average weight of 140 g were procured from the central animal laboratory of the College of Health Sciences of Osun State University, Osogbo. They were housed in standard laboratory cages in the animal holding of the Department of Anatomy of the University, allowed to acclimatize to the standard laboratory conditions of good ventilation and lighting, moderate temperature and adequate humidity for two weeks before the commencement of the experiment, while they also have free access to standard rat chow and water *ad libitum*. They were adequately cared for before and during the experiment following the standard humane animal care of the Institute of Laboratory Animal Resource, National Research Council, DHHS, Pub. No NIH 86 – 23 (9).

Experimental Design

These animals were randomly and equally divided into experimental and control groups with designations A and B

respectively. They were placed in closed glass chambers of approximately 0.1 m^3 volume (38cm X 88cm X 30cm) with an opening of 1cm in its upper surface, according to the protocol of Onarlioglu *et. al* in 1999 (10). The animals in group A were exposed to smoke of burnt 0.74g leaf of Cannabis sativa wrapped with 0.5g of refined and sterilized cotton wool for 5 minutes while animals in group B were exposed to smoke of burnt 0.5g of refined and sterilized cotton wool for 5minutes. All animals had three exposures per day during the hours of 07.00, 10.00, and 13.00 for 5 days.

Animal Sacrifice

All animals were sacrificed by cervical dislocation at least three hours after the last exposure to smoke extracts of cannabis and sterile cotton wool. They were laid down on the dissection board in a supine position and their anterior thoraco-abdominal and pelvic wall with peritoneum was carefully dissected in the midline to expose the organs of interest. The heart, liver, lungs, kidney and testes were carefully excised from the animals, rinsed in cold sucrose solution and blotted dry with filter paper. Liver, lungs, kidney, testes and heart were transferred into 10% formol saline for liver lungs, kidney and heart and bouin's fluid for testes and were allowed to fix for at least 72 hours before further histological protocol and analysis.

Histological Protocol

Fixed tissues cut to 4-6 mm were processed for Haematoxylin and Eosin staining. They were thoroughly rinsed in tap water, dehydrated and cleared of the dehydrating agent. They were infiltrated with wax and were made in blocks of wax for trimming and sectioning. Thin sections of 7 microns were stained with Haematoxylin and Eosin (H&E) and were studied histologically using Olympus binocular light research microscope (XSZ-107BN, No. 071771). Micrographs of the sections were taken with a Kodak Digital Camera (Kodak Easyshare C183) for subsequent histological analysis.

RESULTS

Histological Observations

Heart: Histological changes seen in the sections of the heart of group A animals include mild edema and myocardial destruction as indicated by arrow in Fig 1. This is as opposed to the sections of the heart of group B animals with normal histological profile (Fig 2).

Kidney: The histological profile of the kidney sections (Fig 3) of the animals in group A exposed to the smoke extract of *Cannabis sativa* showed significant degenerative changes. In the renal cortex, the glomerulus was observed to consist of interstitial and diffused glomerular hemorrhage (distorted renal cortex arrowed). The tubular and collecting system of the kidney were gradually becoming vacuolated (degeneration) Sections of the kidney of group B animals also had normal histological profile (Fig 4).

Lungs: The histological outline of the lungs of the animals in group A revealed significant cytoarchitectural disruptions (Fig 5). Bronchial occlusions (arrowed) with enlargement of the alveoli and the alveolar sacs could be depicted. Fig 6 is the micrograph of the lungs of animals in group B with normal lung cytoarchtecture.

Liver: Liver sections of animals in group A showed degeneration and vacuolations in the hepatocytes (arrowed) with scanty sinusoidal lining cells (Fig 7). Micrograph of the liver sections of animals in group B showed normal histological profile (Fig 8).

Testes: Thin **s**ections of the testes of the animals in group A **(**Fig 9**)** showed slugging off of the germ cells, degeneration of Leydig cells and germ cells in the seminiferous tubules, with evidenced cytoarchitectural distortions and vacuolations. Sections of the testes of the animals in group B have preserved histological profile (Fig 10).

DISCUSSION

There are several scientific debates on how *Cannabis* affects the various organs

of the body. Several scientific literatures have discussed how *C. sativa* affects the brain of laboratory animals (11, 12, 13, 14, 15). However, there are few scientific literatures on the histological effects of *C. sativa* on the vital thoraco-abdominal and pelvic viscera organs of Sprague Dawley rats.

This study, in which some of the effects of the smoke extract of *Cannabis sativa* on the heart, liver, lungs, kidney and testes of male Sprague Dawley rats were examined using histological techniques and light microscopy**,** showed that *Cannabis sativa* smoke extract has severe adverse histological effects on the visceral organs examined.

Cannabis sativa did not show any specific pattern of toxicity on the lungs. Massive alveolar enlargement and bronchiolar occlusion could have resulted from direct cannabis toxicity.

All sections of the liver, kidney and testes obtained from animals in group A have easily recognizable altered histological profiles when compared with the sections from animals in group B. Degeneration and disruption of the hepatocytes and cells lining the bile ducts with central portal vein occlusions are few of the histological derangements seen in the liver sections of cannabis treated animals. These histological abnormalities will most likely be accompanied with compromise in the physiological and biochemical activities of the liver. Hepatocytes are known to play very important roles in liver functioning. They frequently contain glycogen and maintain a steady level of blood glucose by the processes of glycolysis, glycogenesis and gluconeogenesis as one of the main sources of energy for use by the body (16, 17)**.** Such micro anatomical compromise in the integrity of the hepatocytes, seen in this research work could lead to improper functioning of the liver.

Renal cortices of *Cannabis* exposed animals were characterized with histological alterations. The varying degrees of vacuolations seen in the proximal convoluted tubules may compromise the functional and structural integrity of the brush border. This may lead to the retention of waste products of metabolism and if it persists, may result in the loss of the sensitive homeostatic mechanisms of the kidney (16).

Testes of the *Cannabis* exposed animals were also characterized by varying degree of changes in the histological profile of the tissues. Degeneration and disruption of the germ cells in the seminiferous tubules, with the degeneration of the Leydig cells obtained in this group of animals could imply reduction of sperm cells which could lead to infertility.

According to Vaux *et al* in 1994 (18), degenerative changes which have been proved scientifically to result in cell death, cause two types of cell death-apoptosis and necrosis. Bose and Sinha (1994) (19) reported that these two types of cell death differ structurally and biochemically. Many have described apoptosis as a non-inflammatory response to tissue damage which is characterized by a series of structural and biochemical changes (20, 21). Schulte-Hermann et al in 1999 (22) reported that these changes in apoptosis can be triggered by either toxic chemicals or injury that can lead to damage of important cells, tissues and organs. Apoptosis is initiated by insults from multiple stimuli such as heat, toxins and chemical (drugs), reactive oxygen species (ROS), growth factor withdrawal, cytokines such as transforming growth factor- beta, loss of matrix attachment, glucocorticoid, nitric oxide, and exposure to huge amount of radiation (23). McConkey and Orrenius in 1991 (24) argued that these insults function in conjunction with other intrinsic factors that determine the cells' potential for apoptosis.

In male reproduction, apoptosis in the testes helps in elimination of abnormal spermatozoa to maintain the nursing capacity of the sertoli cells. Reactive Oxygen Species (ROS) disrupt the inner and outer mitochondrial membranes to induce the release of the cytochrome-C protein and activate the caspase cascade with ultimate results in the fragmentation of a cells' DNA (25).

Cellular necrosis is induced by a

sudden environmental perturbation and departure from the normal physiological conditions. This is as against the induction of apoptosis by stimuli intrinsic to the cells as in programmed cell death (PCD**)**. The rate of progression of cell death in cellular necrosis depends on the severity of the insults and the more severe the insults, the more rapid the progression of the injury (26).

CONCLUSION

Our study suggests a strong causal relationship of the toxic and destructive interference of smoke extract of *Cannabis sativa* on cellular integrity of the heart, liver, lungs, kidney and testes of male Sprague Dawley rats. Considering these effects on the histological integrity of the organs studied in these rats every one especially our youths, who have become addicts of cannabis need to be made aware and educated on the danger attached to the use of the plant. Further investigations are required to determine the mechanism of histological changes caused by cannabis on these vital visceral organs.

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HISTOLOGICAL ILLUSTRATIONS

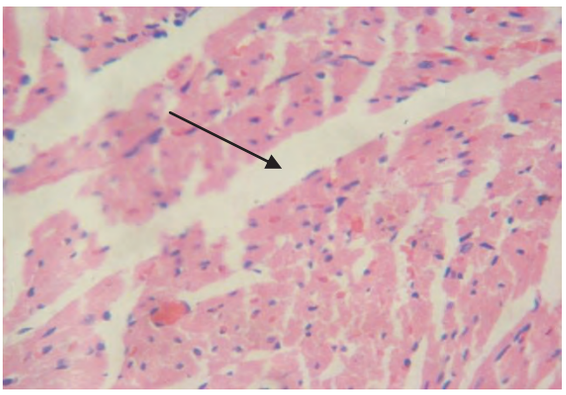

Fig 1. Section of the heart of the animals in the treatment group showing destruction of mild edema and myocardial fibers (H&E) x 480

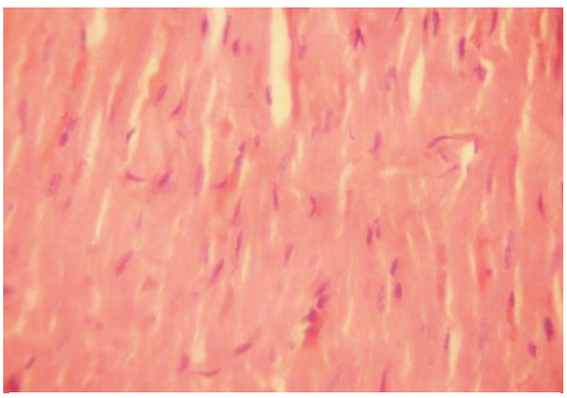

Fig 2. Section of the heart of the animals in the control group with well preserved outline of the heart (H&E x 480)

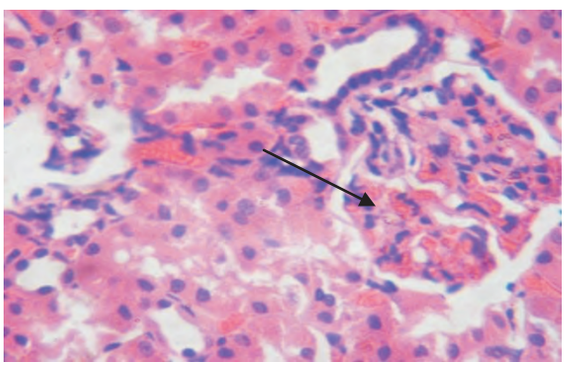

Fig 3. Section of the kidney of the animals in the treatment with distortion of the renal cortex (H&E x 480)

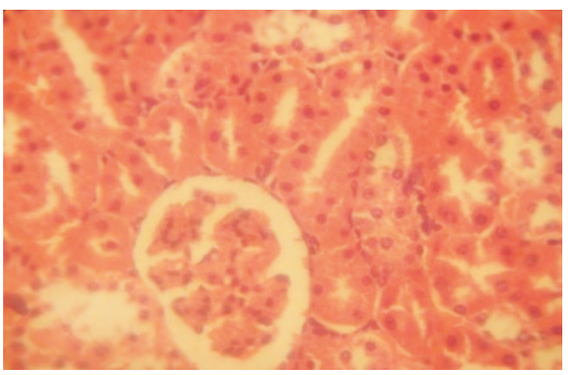

Fig 4. Section of the kidney of the animals in the control group with preserved outline of the kidney (H&E x 480)

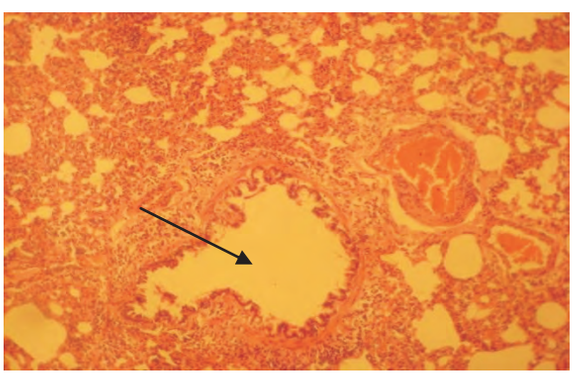

Fig 5. Section of the lungs of the animals in the treatment group showing enlargement of the alveoli (H&E x 480)

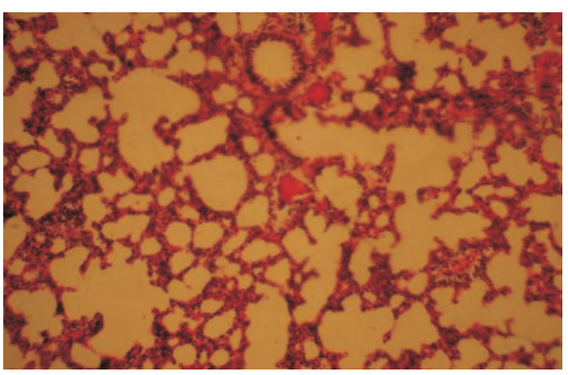

Fig 6. Section of the lungs of the animals in the control group with preserved outline of the lungs (H&E x 480)

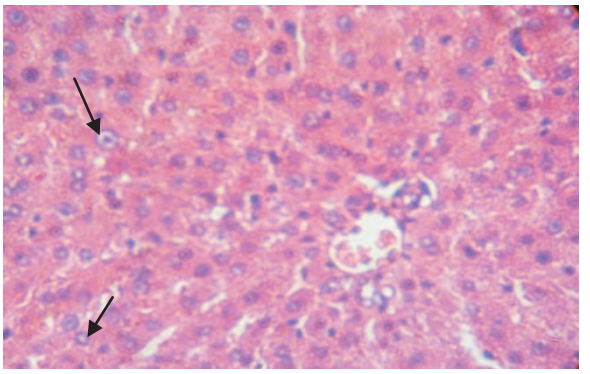

Fig 7. Section of the liver of the animals in the treatment group showing degeneration of the hepatocytes (H&E x 480)

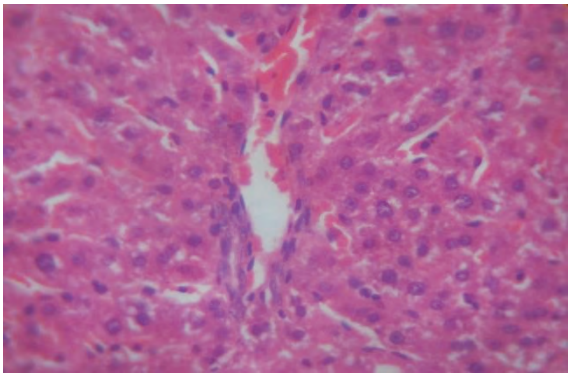

Fig 8. Section of the liver of the animals in the control group with well preserved outline of the liver in the control rats (H&E x 480)

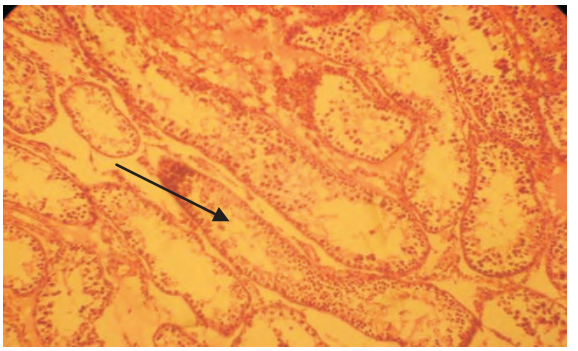

Fig 9. Section of the testes of the animals in the treatment group showing slugging off of the germ cells (H&E x 480)

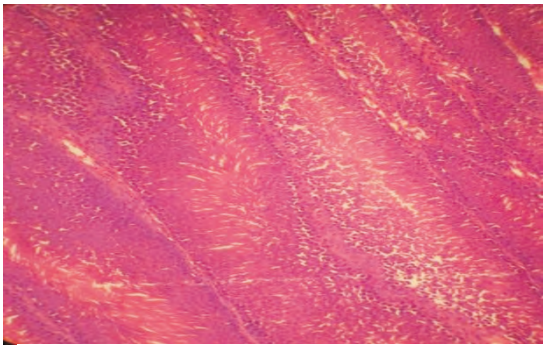

Fig 10. Section of the testes of the animals in the control group showing well preserved outline of the testes in the control rats (H&E x 480)

LEGEND

Fig 1 showing destruction of mild edema and myocardial fibers (black arrow) compare the well preserved outline of the heart in the control rats (Fig 2)

Fig 3 showing distortion of the renal cortex (black arrow) compared with the well pres outline of the kidney in the control rats (Fig 4)

Fig 5 showing enlargement of the alveoli (black arrow) compared with the well pres outline of the lungs in the control rats (Fig 6)

Fig 7 showing degeneration of the hepatocytes (black arrow) compared with the well pre outline of the liver in the control rats (Fig 8)

Fig 9 showing slugging off of the germ cells(black arrow) compared with the well preserv outline of the testes in the control rats (Fig 10)