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BIOCHEMICAL AND HISTOLOGICAL CHANGES IN KIDNEY AND HEART OF MALE RATS FOLLOWING ORAL ADMINISTRATION OF AQUEOUS AND METHANOLIC EXTRACT OF *CANNABIS SATIVA* LEAVES

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ABSTRACT
The biochemical and histological changes in kidney and heart of male rats was evaluated following oral administration of aqueous and methanolic extract of *Cannabis sativa* (Marijuana) leaves. Twenty seven (27) male albino rats were divided into three groups (A, B and C) comprising nine rats each. Animals in group A (115.43±2.45g), B (135.56±3.27g) and C (145.92±2.13g) were administered 0.5 ml of distilled water, 700 mg/kg body weight aqueous and methanolic extract of *C. sativa* leaves respectively. Treatment was done once daily until the 21st day. Secondary metabolites present in the methanolic extract are alkaloids, steroids, phenolics, terpenoids, flavonoids, tannins, cardiac glycosides, starch and reducing sugars while the aqueous extract contains all except phenolics, tannins and starch. Although treatment with the aqueous and methanolic extract significantly ($p<0.05$) decreased WBC, MCV, monocyte and lymphocytes in the aqueous and methanol group when compared with the control group, it increased the levels of platelet and Na^+. However, treatment with both extract did not significantly ($p>0.05$) affect the levels of PCV, haemoglobin, uric acid, creatinine, K^+, Cl^-, HCO_3^-, PO_4^{3-} when compared with the control group. Histological section of the kidney and heart were normal for the control group. However, it showed damaged pyknotic cells, degenerated distal and proximal convoluted tubule as well as disarranged glomerulus and narrowed Bowman's capsule in both aqueous and methanol treated groups respectively. In the heart, it showed visible tissue inflammation, necrosis of heart muscle fibre and hypoplesia respectively in both groups throughout the exposure period. The effect of methanolic extract of *C. sativa* leaves was greater than that of the aqueous extract both in kidney and heart, suggesting that methanol is a better solvent for extraction of marijuana leaves than water. The alterations in biochemical indices and histology indicate that marijuana leaves can do more harm than good. Caution should therefore be exercised during consumption especially at the dose investigated in this study.

KEYWORDS: *Cannabis sativa*, cannabaceae, marijuana, histology, pyknotic cells, hypoplesia.

INTRODUCTION
Medicinal plant is an important element of indigenous medical systems all over the world (Farnsworth, 1990). They are resources of traditional medicines and many of the modern medicines are produced directly or indirectly from plants. The goals of using medicinal plants as sources of therapeutic agents are a) to isolate bioactive compounds for direct use as drugs, e.g., digitoxin, morphine, reserpine, taxol, vincristine; b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, e.g., metformin, nabilone, oxycodon (and other narcotic analgesics), teniposide, verapamil, and amiodarone, which are based, respectively, on galegine, Δ^9-tetrahydrocannabinol, morphine, and khellin; c) to use agents as pharmacologic tools, e.g., lysergic acid diethylamide, mescaline, yohimbine; and d) to use the whole plant or part of it as a herbal remedy, e.g., cranberry, echinacea, feverfew, garlic, ginkgo biloba, saw palmetto (Farnsworth *et al*., 1985; Fabricant and Farnsworth, 2001). The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance (Hosseinzadeh *et al*., 2015).

Medicinal plants give rise to natural products, which have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Newman *et al*., 2000) and its importance

in modern medicine has been discussed in different reviews and reports (Jones *et al*., 2006). The value of natural products in this regard can be accessed from: 1) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification, 2) the number of diseases treated or prevented by these substances, and 3) their frequency of use in the treatment of disease. The World Health Organization (WHO, 2004) has therefore recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines (Hosseinzadeh *et al*., 2015). Despite the enormous importance and use of herbal concoction from medicinal plants, there is little evidence to scientifically verify the shortcomings of their usage or knowing the possible adverse effect they pose. One of such medicinal plants is *Cannabis sativa*.

Cannabis sativa, an annual herbaceous plant, an angiosperm belonging to the *Cannabaceae* family (Burkill, 1985; John, 2010), is called Indian hemp or Ganja in English. Its preparation is known by various names worldwide. It is called Marijuana in America; Bhang, Ganja and Charas in India; Kif in North Africa; Dogga in South Africa; Krori in Tunisia, Habak in Turkey; Hashish in Middle East; Djomba or Liamba in Central Africa and Brazil; Sodom, Tampl, Gum, Gauge and stuff in Kinshasa; Swala and Whiskt in Ghana; Grifa in Mexico and Ma-cohna in some parts of South America (Sachindra and Pradhan, 1977). In the native Nigerian languages, it is called igbò in Yoruba; Nwonkaka in Igbo and Ikya in Tiv (Saalu, 2016). It is an annual, greenish or brownish herb attaining as much as 5 m tall (Ontario Hemp Alliance, 2010). Its leaves are bitter, astringent, tonic, aphrodisiac, alterative, intoxicating, stomachic, analgesic and abortifacient. It is grown and processed for many uses; they are used in abdominal disorders, malarial fever, dysentery, diarrhoea, skin diseases, hysteria, insomnia, gonorrhoea, colic, tetanus and hydrophobia (Brady *et al*., 2009).

Previous studies by Adams *et al* (2017) have reported the toxic effect of *C. sativa* leaves in liver and brain in male rats. Mukhtar and Elbagir (2011) have also evaluated the effect of *C. sativa* on hematological indices in rats. Obembe *et al* (2013) and Obembe *et al* (2015) worked on the effect of chronic consumption of *C. sativa* on bleeding time, prothrombin time and platelet count and haematological and immunological effect of *C. sativa* in rats respectively. Aizpurua-Ppolaizola (2014) worked on identification and quantification of cannabinoids in *Cannabis sativa* by HPLC-MS while Odokuma and Ogbor-Omorie (2015) saw the histomorphologic effects of *C. sativa* on the brains of adult Wistar rats. Furthermore, Al-Alem (2003) saw the nephrotoxic effect of *Cannabis sativa* plant in male rats. Despite the studies carried out on *C. sativa* leaves, there is dearth of information in the open scientific literature on the toxicity of the aqueous and methanolic extract of *C. sativa* leaves in the kidney and heart of rats at the dose of 700 mg/kg body weight. Therefore, this study was carried out to evaluate the toxicological effect of the aqueous and methanolic extract of *Cannabis sativa* leaves at the dose of 700 mg/kg body weight on some biochemical parameters and histology of the kidney and heart of male rats.

MATERIALS AND METHODS

Materials

Plant Materials and Authentication

Cannabis sativa leaves which were purchased from Pyata village, Bosso Local Government Area, Niger State, Nigeria were authenticated at National Tropical Botanical Garden, French Polynesia, France with Voucher Specimen Number (PTBG0000040397).

Experimental Animals

Male Wistar rats (*Rattus norvegicus*) weighing 115.43±2.45g, 135.56±3.27g, 145.92±2.13g were obtained from the Animal House of Bingham University, Karu, Nasarawa State, Nigeria.

Assay Kits and Chemicals

The assay kits for the determination of creatinine, uric acid, Na^+, K^+, Cl^-, PO_4^{3-}, and HCO_3^- were products of Randox Laboratory Ltd, Co-Atrium, Uk.

Other Reagents

All other chemicals and reagents used which were of analytical grade were products of Sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water.

Methods

Preparation of Aqueous Extract of *Cannabis sativa* Leaves

Dried leaves of *Cannabis sativa were* pulverized in a blender (Philips, Model HR-1724, Brazil) to obtain smooth powder. A known weight (100 g) of the powdered sample was extracted in 500 ml of distilled water for 72 hours at room temperature. The mixture was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a water bath (Model: NL-420S, NEWLIFE® Medical Instrument, England) to give a brownish-black residue (extract). The extract was then reconstituted in distilled water to give the required dose of 700 mg/kg body weight used throughout the experimental period.

Preparation of Methanolic Extract of *Cannabis sativa* Leaves

Dried leaves of *Cannabis sativa* were pulverized in a blender (Philips, Model HR-1724, Brazil) to obtain smooth powder. A known weight (100 g) of the powdered sample was extracted in 1000 ml of methanol in a Soxhlet extractor. The resulting mixture was concentrated in a Rotary Evaporator (Model: RE-52A, Shanghai Ya Rong Biochemistry Instrument Factory, China) to obtain an extract. The extract was transferred into water bath (Model: NL-420S, NEWLIFE® Medical

Instrument, England) where it was further evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required dose of 700 mg/kg body weight used throughout the experimental period.

Screening of Secondary Metabolites

Screening of secondary metabolites to detect the presence of alkaloids, tannins, flavonoids, cardiac glycosides, reducing sugars, starch, steroids, terpenoids and phenolics were carried out by adopting the procedures described by (Harborne, 1973; Odebiyi and Sofowora, 1978; Trease and Evans, 1989; Sofowora, 1993; El-Olemy *et al*., 1994; Walls *et al*., 1996; Awe and Sodipo, 2001; Edeoga *et al*., 2005; Ganesan and Bhatt, 2008).

Animal Grouping and Extract Administration

The animals which were housed in aluminium cages placed in well ventilated standard housing conditions (temperature: 28-31 ^{0}C; photoperiod: 12 hours; humidity: 50-55%) were allowed free access to rat pellets (Vital Feed®, Grand Cereals Ltd, Jos, Plateau State, Nigeria) and tap water. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. Twenty seven (27) male albino rats were divided into three groups (A, B and C) comprising nine rats each. Animals in group A (115.43±2.45g), B (135.56±3.27g) and C (145.92±2.13g) were administered 0.5 ml of distilled water, 700 mg/kg body weight aqueous and methanolic extracts respectively. Treatment was done once daily Treatment was done once daily until the 21st day while the animals were sacrificed on the 22nd day. This experimental investigation was carried out in accordance with the standard humane animal care as outlined in the "Guide for the Care and Use of Animals in Research and Teaching", as approved by the Institute of Laboratory Animal Resource, National Research Council, DHHS, Pub. No NIH 86-23 (National Institute of Health, 1985).

Preparation of Plasma, Serum and Tissue Supernatant

The rats were anaesthesized in a glass jar containing cotton wool soaked in diethyl ether. Thereafter, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scapel blade and an aliquot (2 ml) of the blood was collected into EDTA sample bottles to give plasma for the haematological analysis. Blood (5 ml) collected in plain bottles was centrifuged (using High Speed Centrifugal Machine, Model: YXJ-2, Essex, England) at 2000 g for 10 minutes and the serum used for the biochemical analyses. Animals were dissected during which the kidney and heart were removed, weighed, homogenized, centrifuged (3000 g at 15 minutes) and the resulting supernatant kept frozen for 24 hours before being used for the determination of selected biochemical parameters.

Determination of Haematological Parameters

Haematological Autoanalyzer (Beckman Coulter, Inc. Fullerton, CA, USA) was used for the determination of haematological parameters: Packed cell volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count, mean corpuscular volume (MCV), monocyte and lymphocyte by adopting the procedures described by Dacie and Lewis (1995) and Lewis *et al* (1995).

Histological Examination

The tissues of animals (kidney and heart) were fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56 ^{0}C) (Krause, 2001; Avwioro, 2010). Tissue sections were then prepared according to the procedures described by Disbrey and Rack (1970) and Drury and Wallington (1973) and stained with haematoxylin/eosin (H&E). The histology slides was read with a binocular light research microscope (Olympus, Model: XSZ-209BN, New York Microscope Company Inc., New York). Cross section of the kidney was captured at x400 while that of the heart was captured at x100 with Kodak Digital Camera package (Model: Powershot M4505, Germany).

Data Analysis

Data were expressed as the mean ± SD of 9 determinations. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Data were considered statistically significant at $p<0.05$. All these analyses were done using SPSS 20.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

RESULTS

Phytoconstituents present in the methanolic extract of *Cannabis sativa* leaves are alkaloids, steroids, phenolics, terpenoids, flavonoids, tannins, cardiac glycosides, starch and reducing sugars while the aqueous extract contains all except phenolics, tannins and starch (Table 1).

Table 1: Secondary metabolite content of aqueous and methanolic extract of *Cannabis sativa* leaves.

Class of compounds	Methanolic extract	Aqueous extract
Alkaloids	+++	+
Steroids	++	+
Phenolics	++	_
Terpenoids	+	+
Flavonoids	++	+
Tannins	+	-
Starch	+	-
Cardiac glycosides	++	+
Reducing sugars	++	+

(+) = Present in minute amount, **(++)** = Present in moderate amount, **(+++)** = Present in appreciable amount, **(-)** Not present.

Treatment of animals with both extract of *C. sativa* leaves at 700 mg/kg body weight which significantly ($p<0.05$) decreased the white blood cell (WBC) count, mean corpuscular volume (MCV), monocyte, lymphocyte, significantly ($p<0.05$) increased the platelet count in the aqueous and methanol group when compared with the control group (Table 2). Throughout the experimental period, treatment of animals with both aqueous and methanolic extract at 700 mg/kg body weight did not significantly ($p>0.05$) affect the levels of packed cell volume (PCV) and haemoglobin (Hb) when compared with the control group (Table 2).

Table 2: Haematological parameters of male rats following oral administration of aqueous and methanolic extract of *Cannabis sativa* leaves.

Parameters	Control Group (MEAN±SD)	Aqueous Group (MEAN±SD)	Methanol Group (MEAN±SD)
Platelets (10^3/mL)	281.01 ± 1.95^a	488.02 ± 15.19^b	528.24 ± 11.93^c
Monocytes (%)	4.17 ± 0.39^a	1.63 ± 0.63^a	1.14 ± 0.53^a
Haemoglobin (g/dl)	14.43 ± 0.17^a	14.49 ± 0.06^a	14.50 ± 0.06^a
PCV (%)	43.43 ± 0.18^a	43.50 ± 0.20^a	43.50 ± 0.20^a
WBC (10^6/µL)	7.20 ± 0.37^a	2.77 ± 1.10^b	1.94 ± 1.05^c
Lymphocytes (%)	93.31 ± 1.06^a	75.66 ± 6.21^b	70.62 ± 6.11^c
MCV (flµm^3)	56.58 ± 2.48^a	34.72 ± 9.16^b	30.61 ± 8.74^c

Data are mean ± SD of nine determinations. Values with superscripts **b** and **c** different from their respective control group **a**, across the row are significantly different ($p<0.05$).
PCV= Packed Cell Volume; **WBC=** White Blood Cell; **MCV=** Mean Corpuscular Volume.

Treatment of animals with the aqueous and methanolic extract at 700 mg/kg body weight significantly ($p>0.05$) increased the concentration of Na^+ when compared with the control group (Table 3). However, treatment of animals with both extract at 700 mg/kg body weight did not significantly ($p>0.05$) alter the levels of packed cell volume (PCV), haemoglobin (Hb), uric acid, creatinine, K^+, Cl^-, PO_4^{3-} and HCO_3^- when compared with the control group (Table 3).

Table 3: Effect of aqueous and methanolic extract of *Cannabis sativa* leaves on selected kidney biomarkers of male rats.

Parameters	Control Group (MEAN±SD)	Aqueous Group (MEAN±SD)	Methanol Group (MEAN±SD)
Cl^- (mmol/L)	100.45 ± 0.92^a	100.70 ± 0.38^a	100.70 ± 0.44^a
HCO_3^- (mmol/L)	26.44 ± 5.10^a	27.33 ± 4.35^a	25.33 ± 4.82^a
K^+ (mmol/L)	6.23 ± 0.76^a	5.78 ± 0.34^a	6.15 ± 0.62^a
Uric acid (mg/dl)	0.34 ± 0.22^a	0.48 ± 0.29^a	0.48 ± 0.22^a
PO_4^{3-} (mmol/L)	10.12 ± 0.33^a	9.75 ± 0.51^a	9.87 ± 0.42^a
Creatinine (µmol/L)	0.38 ± 0.09^a	0.34 ± 0.05^a	0.25 ± 0.12^a
Na^+ (mmol/L)	135.3 ± 44.8^a	150.4 ± 0.28^b	135.3 ± 45.02^c

Data are mean ± SD of nine determinations. Values with superscripts **b** and **c** different from their respective control group **a**, across the row are significantly different ($p<0.05$).
Na^+ = Sodium ion; **K^+** = Potassium ion; **Cl^-** = Chloride ion; **PO_4^{3-}** = Phosphate ion; **HCO_3^-** = Bicarbonate ion.

Histological section of the kidney showed normal glomerular tuft (G), proximal convoluted tubule (PCT) (Plate 1a). Histology of the heart revealed normal sinusoids (S) and Kuppfer cells (KC) for the distilled water treated control group (animals) (Plate 1b).

The cross section of kidney of animals treated with 700 mg/kg body weight of the aqueous extract of *C. sativa* leaves revealed disarranged glomerulus (G), pyknotic cell (PK), as well as damaged distal and proximal convoluted tubule (DCT)/(PCT) (Plate 2a) while the kidney of animals treated with 700 mg/kg body weight of the methanolic extract showed severe coagulative necrosis in the epithelium lining the proximal convoluted tubules as well as narrowed Bowman's capsule and deranged glomerulus (Plate 2b). The cross section of heart of animals treated with 700 mg/kg body weight of the aqueous extract of *C. sativa* leaves revealed severe tissue fragmentation (TF)/inflammation as well as necrosis of the muscle fibre (NMF) (Plate 3a) while the heart of animals treated with 700 mg/kg body weight of the methanolic extract showed severe tissue fragmentation (TF), severe necrotic changes (SNC) and hypoplesia (HPP) (Plate 3b).

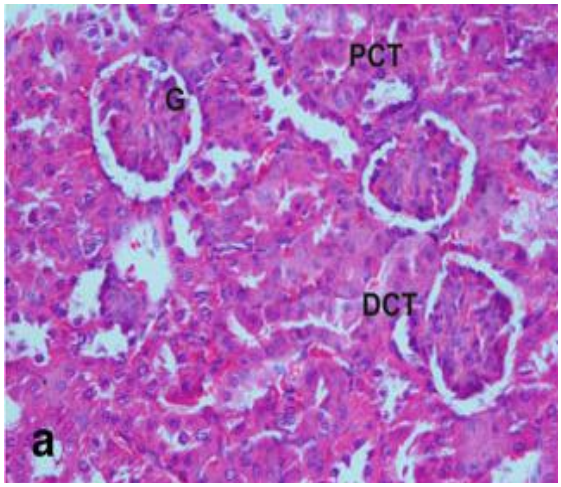

Plate 1a: Cross section of kidney of rat (control group) administered distilled water indicating: normal glomerular tuft (G), proximal convoluted tubule (PCT) (x400; H&E).

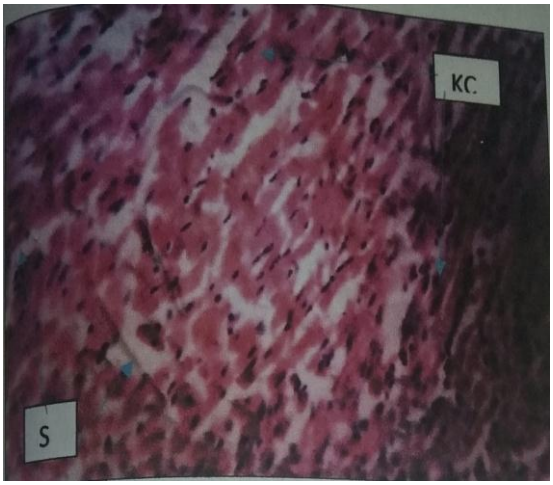

Plate 1b: Cross section of heart of rat (control group) administered distilled water showing normal Kuppfer cells (KC) and sinusoids (S) (x100; H&E).

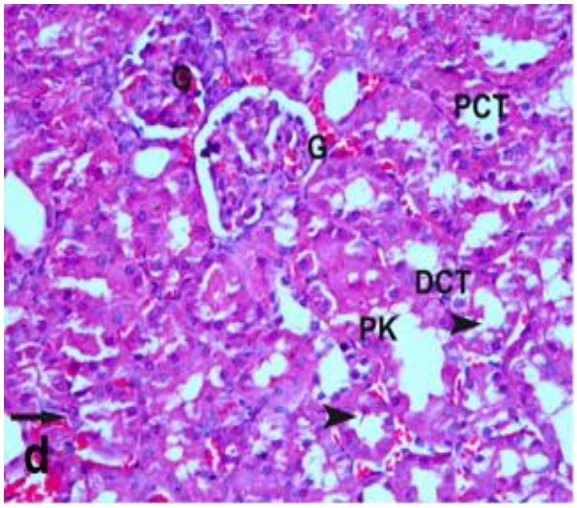

Plate 2a: Cross section of kidney of rat (aqueous group) administered 700mg/kg body weight of aqueous extract of *C. sativa* leaves showing: glomerulus (G)/disarranged, proximal convoluted tubule (PCT), pyknotic (PK) cell, distal convoluted tubule (DCT) (x400; H&E).

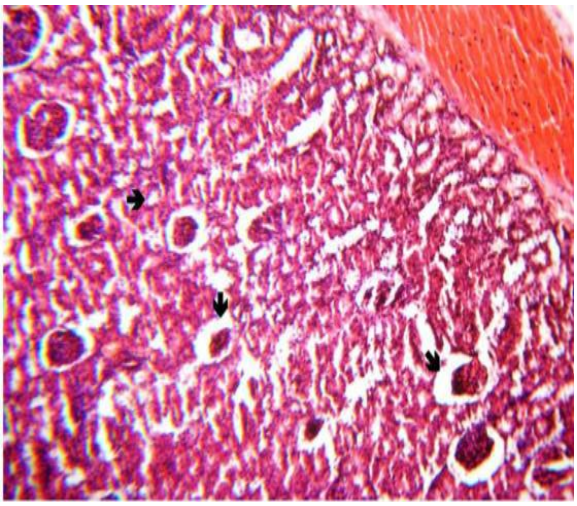

Plate 2b: Cross section of kidney of rat (methanol group) administered 700mg/kg body weight of methanolic extract of *C. sativa* leaves indicating: many lesions characterized by severe coagulative necrosis in the epithelium lining the proximal convoluted tubules as well as narrowed Bowman's capsule and deranged glomerulus (x400; H&E).

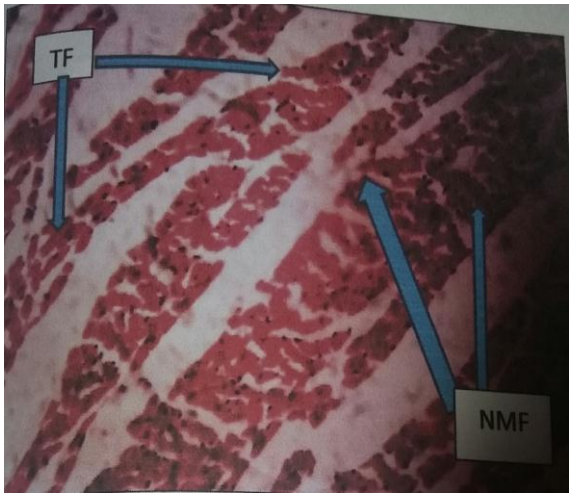

Plate 3a: Cross section of heart of rat (aqueous group) administered 700 mg/kg body weight of aqueous extract of *C. sativa* leaves indicating: severe tissue fragmentation (TF)/inflammation, and necrosis of the muscle fibre (NMF) (x100; H&E).

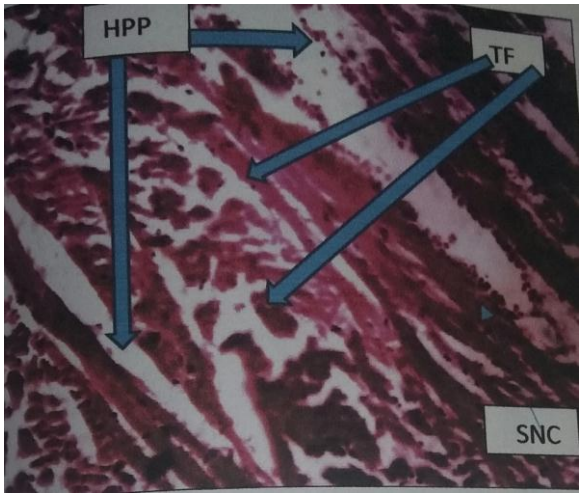

Plate 3b: Cross section of heart of rat (methanol group) administered 700 mg/kg body weight of methanolic extract of *C. sativa* leaves indicating: severe tissue fragmentation (TF), severe necrotic changes (SNC) and hypoplesia (HPP) (x100; H&E).

DISCUSSION

Treatment of experimental animal with plant extracts without any standard dosage coupled with non-availability of adequate scientific validation on their safety has raised issues on their toxicity (Saad *et al*., 2006).

Screening of secondary metabolite of aqueous and methanolic extract of *C. sativa* leaves which revealed the presence of significant amount of secondary metabolite contents might be responsible for some of the various biochemical and histological effects of the plant. The number of secondary metabolite obtained from methanolic extract of *C. sativa* leaves was greater than the aqueous extract suggesting that methanol is a better solvent for extraction of *C. sativa* leaves than water.

The analysis of blood indices has proven to be a valuable approach for analyzing the health status of animal models as these indices provide reliable information on metabolic disorders, deficiencies, chronic stress status and blood relating functions before they are present in a clinical setting (Bahmani *et al*., 2001). Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals (Hoffbrand, 1997; Edet *et al*., 2013). The non-significant effect in packed cell volume (PCV) by both extracts at 700 mg/kg body weight suggests that the extracts may not affect the rate of production of RBCs (erythropoiesis). The non-significant effect in haemoglobin (Hb) by both extracts at 700 mg/kg body weight suggests that the extracts may not affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues (Seigler, 2003). The non-significant effect in haemoglobin (Hb) and packed cell volume (PCV) by both extracts at 700 mg/kg body weight in the rats suggest that the extract could possess erythropoetic activity which will enhance the PCV and Hb level and thereby correct anaemia. The significantly high level of platelet count in the aqueous and methanol groups following administration of 700 mg/kg body weight when compared with control group may imply better blood clotting ability. It may also be due to stimulatory effect on thrombopoietin (Kaushansky, 1995; Li *et al*., 1999).

The decrease in white blood cell (WBC) count in the aqueous and methanol group at 700 mg/kg body weight may suggest poor defensive mechanisms against infection resulting to decrease in ability of the body to respond to infection. The decrease in WBC count by both extracts at 700 mg/kg body weight may also indicate a weak immune system. The decrease in level of MCV following administration of both extracts at 700 mg/kg body weight suggests that the animals may be predisposed to anaemia (Coles, 1986). The reduction in monocyte by both extracts at 700 mg/kg body weight could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi *et al*., 2010). Lymphocytes are the main effectors cells of the immune system (McKnight *et al*., 1999). The decrease in lymphocytes by both extracts at 700 mg/kg body weight suggests inhibitory effect on the effectors cells of the immune system.

Renal function is an indication of the state of the kidney and its role in removing wastes like creatinine and urea, controlling the body's fluid balance, and regulating the balance of electrolytes. Renal function indices are

usually required to determine the normal glomerular and tubular functioning of the nephrons (Panda, 1989; Abolaji *et al*., 2007). Creatinine is the major catabolic product of the muscle (Samra and Abcar, 2012). Therefore, the non-significant effect in serum creatinine by the aqueous and methanolic extract at 700 mg/kg body weight suggests that it may not affect muscle catabolism. Creatinine clearance measures how much creatinine is cleared out of the body, or how well kidneys filter creatinine. Therefore, the non effect of serum creatinine by both extract may also imply normal clearance of creatinine by the kidney. Uric acid is the metabolic product of purine catabolism (Cirillo *et al*., 2006). The non-effect in serum uric acid concentration by the aqueous and methanolic extract at 700 mg/kg body weight implies that the extracts may not affect purine catabolism.

The serum concentrations of electrolytes (Na^+, K^+, Cl^-, HCO_3^- and PO_4^{3-}) could give an insight into the effect of a compound/plant extract/fraction on the tubular and or glomerular part of the kidney. Therefore, the non effect of the aqueous and methanolic extract of *C. sativa* leaves at 700 mg/kg body weight investigated on the renal function indices may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected. This is because these parameters of organ function, if altered, would impair the normal functioning of the organs. Sodium ion (Na^+) is the major cation of extracellular fluids (Naik, 2007; Smyth *et al*., 2014). The rate of Na^+ excretion by the kidney is related to the glomerular filtration rate (GFR). When the GFR falls, less Na^+ is excreted and vice versa (Horton *et al*., 1993; Naik, 2007). Therefore, the non-significant effect in serum Na^+ concentration by both extract at 700 mg/kg body weight may imply normal glomerular filtration rate (GFR). The kidney plays important role in reabsorption of filtered potassium at the proximal convoluted tubule. Nearly all the potassium filtered at the glomerulus is reabsorbed in the proximal tubule. Less than 10% reaches the distal tubule, where the main regulation of potassium excretion occurs. Thus, during potassium reabsorption, there is an obligatory loss of potassium (Naik, 2007). Therefore, the non-significant effect in serum K^+ concentration by both extract at 700 mg/kg body weight may imply that the filtrative, reabsorptive and excretory capacity of the kidney was not compromised (Pohl *et al*., 2013).

Chloride is essential for water balance, regulation of osmotic pressure and acid-base balance. Therefore, the non-significant effect in serum Cl^- concentration by both extract at 700 mg/kg body weight may imply normal functioning capacity of the kidney. Bicarbonate ion (HCO_3^-) is alkaline, and a vital component of the pH buffering system of the human body (Bray, 1999; Vasudevan *et al*., 2011). It is a measure of the base that remains after all acids, stronger than carbonic acid, have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids. The rise in blood HCO_3^- is compensated by increased renal excretion of HCO_3^-. Therefore, the non-significant effect in serum HCO_3^- concentration by both extract at 700 mg/kg body weight may imply normalcy in renal excretion of HCO_3^- (Widmaier *et al*., 2014). Phosphate ion (PO_4^{3-}) is useful in animal cells as buffering agents as well as the normal function of the nephron. The non-significant effect in serum PO_4^{3-} concentration by both extract at 700 mg/kg body weight implies that it may not adversely affect the tubular and glomerular function of the nephron (Kulaev *et al*., 2004).

The absence of significant histoarchitectural changes in the kidney and heart of the distilled water treated control animals as revealed by histological examination in the present study indicate absence of structural toxicity. Administration of 700 mg/kg body weight of both extract which caused disarrayed glomerulus, narrowed Bowman's capsule as well as damaged distal and proximal convoluted tubules in the kidney of the aqueous and methanol group following treatment with 700 mg/kg body weight of *C. sativa* leaves is an indication of compromise in kidney's histoarchitectural integrity (Hook and Goldstein, 1993; Schnachenberg, 2002; Ashraf *et al*., 2004). The severe tissue fragmentation, hypoplesia and severe necrosis of the muscle fibre in the aqueous and methanolic groups following administration of 700 mg/kg body weight of both extract is an indication of histoarchitectural damage on the heart which would have adverse effect on the structural architecture of the tissues (Davis and Tikunova, 2008; Senyo *et al*., 2013).

CONCLUSION

Alterations in the biochemical indices as well as histology of the kidney and heart by the extract may impair the normal functioning of the organs. The extract caused functional and structural toxicity, hence should be consumed with caution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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