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Research Article

Route of exposure influences the cardiovascular effects of *Cannabis sativa* in salt-induced hypertensive male Wistar rats

***A.O. Ige, M.O. Olaoye, D.T. Oluwole, G.T. Ayeni, E.O. Adewoye**

Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan

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Hypertension, Cannabis sativa, blood pressure, aorta

ABSTRACT

Background: Exposre to *Cannabis sativa* (CS) has been suggested to exert ameliorative effects in hypertensive conditions. Using various exposure routes, this study investigated the likely cardiovascular protective effect of CS in high salt diet (HSD) induced hypertensive male Wistar rats. **Methods**: Exposure routes investigated include dietary incorporation (10%CS+90%HSD), ethanol extract of C. *sativa* intake (ECS, 3mg/kg p.o.), and inhalation of *C. sativa* fumes (1g/day/animal). GC-MS analysis of CS was evaluated, and forty animals were equally divided into 5 groups as follows; Group I (control) received normal diet, Groups II-V received HSD alone, CS+HSD, ECS+HSD, and CS fumes+HSD for 28days, respectively. Thereafter, systolic, diastolic, mean arterial blood pressure, and electrocardiographic readings were assessed. Haematological analysis of retro-orbital sinus blood samples after light anaesthesia was also evaluated for full blood cell counts, erythrocyte sedimentation rate, fibrinogen concentration, and blood viscosity. Aortic samples were harvested for histology. **Resulte**: The GC-MS showed Δ9-Tetrahydrocannabinol, Δ9-Tetrahydrocannabivarin, Cannabidiol and Cannabinol, as prevalent in CS. The HSD only exhibited elevated ($P<0.05$) RBC, PCV, haemoglobin, MCV, platelets, WBC, neutrophil, blood viscosity, systolic, diastolic and mean arterial blood pressure compared to control. CS exposure groups (III-V) exhibited reduced ($P<0.05$) RBC, PCV, haemoglobin, WBC, blood viscosity, systolic, diastolic and mean arterial blood pressure compared to HSD only. However these values were elevated compared to control. ECG tracings seen in group II suggests myocardial electrical signal dysfunction while tracings in the CS exposure groups suggest partial amelioration of myocardial signalling pathways. Histology showed hypertension-induced aortic structural alterations that were not ameliorated by exposure to *CS.* **Conclusion**: Data obtained suggest that controlled exposure to *Cannabis sativa* either in diet, as ethanol extract or inhalation may mediate elevated blood pressure and impaired cardio-electrical signalling in salt (NaCl)-induced hypertension. However, hypertension-induced cardiac structural and vascular impairments are not ameliorated by exposure to *Cannabis sativa*.

INTRODUCTION

Globally, United Nations Office on Drugs and Crime (UNODC) estimates that *Cannabis sativa* users comprise the largest number of illicit drug users accounting for about 129-190 million people worldwide (UNODC, 2010). The popularity and use of *Cannabis sativa* has grown over the years more so there is increased awareness of its ameliorative effects in the management of some disease conditions (Russo *et al.*, 2007, Kogan *et al.*, 2007). *Cannabis sativa* can be consumed via various routes, which include smoking, eating (Schauer *et al.*, 2016), vaporization (Abrahms *et al.*, 2007), and following solvent extraction, drank as a decoction (Ige *et al.*, 2020). These various routes of *cannabis* intake have been reported to affect plasma levels of cannabinoids and affect the onset, degree and duration of its clinical effects (Russell *et al.*, 2011).

The cannabis plant is said to contain about 60 different cannabinoids with the major ones being tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV) and cannabichromene (CBC) (Andre *et al.*, 2016). Cannabinoids (endogenous cannabinoids) are also, naturally produced by the body

*Address for correspondence:
Email: aby_ige@yahoo.com; ao.ige@mail1.ui.edu.ng

where they exert their actions through the endocannabinoid system (ECS) whose main function is to maintain bodily homeostasis i.e. biological harmony in response to changes in the environment (de Laurentiis *et al.*, 2014). This system is said to be involved in a wide variety of processes including pain, memory, mood, appetite, stress, sleep, metabolism, immune function, and reproductive function (Maccarrone *et al.*, 2015). The effects of cannabinoids on the ECS are mainly mediated through two cannabinoid receptors often referred to as CB1 and CB2 receptors respectively (Montecucco and Di Marzo, 2012).

Cannabinoids have been reported to exert pleiotropic effects on the cardiovascular system (Montecucco and Di Marzo, 2012). *et al* Hence the exact effects of cannabis on the cardiovascular system is yet to be fully comprehended. Studies have demonstrated that *cannabis* consumption may be associated with the development of arrhythmias including ventricular tachycardia, and increased risk of myocardial infarction (Goyal *et al.*, 2019). Conversely, due to increased expression of the ECS in hypertension (Hiley, 2009) and the observed hypotensive effect of *C. sativa* (Booz, 2011; Stanley *et al.*, 2013), a likely therapeutic role has been suggested for C. sativa in hypertensive conditions (Bátkai *et al.*, 2004; Malinowska *et al.*, 2019). Cannabinoids, acting through the ECS, are also known for their vasodilatory effects (Stanley and O'Sullivan, 2014) and hence it has been suggested that they might play a protective and therapeutic role in hypertension.

In this study, it is postulated that the various routes of *Cannabis* intake may affect its clinical and therapeutic use. Hence, this study was therefore designed to investigate the cardiovascular function in salt-induced (8% NaCl-diet) hypertensive male Wistar rats exposed to *Cannabis sativa* through various routes (dietary incorporation, orally following solvent (ethanol) extraction, and inhalation).

MATERIALS AND METHODS

Preparation of Plant material

Cannabis sativa plant was obtained and identified (UIH-22809) as previously described (Ige *et al.*, 2020). The plant material was air-dried, pulverized and divided into 3 portions based on the *C. sativa* exposure routes being studied. For dietary exposure, the pulverized plant material was mixed with hypertensive diet in the ratio 10:90 and pelletized (Ebuehi *et al.*, 2016). For oral extract exposure, Soxlet ethanol (95%) extraction of the pulverized C. sativa was carried out as described by Alagbonsi and Olayaki (2016). The ethanol extract (ECS) obtained was stored at 4^0C until use. For inhalational exposure, dried pulverized plant material was placed on a rolling paper (1gm/paper), rolled into cigarettes. Animals were exposed to fumes from this cigarette (Hunault *et al.*, 2013) in an exposure chamber.

Formation of High Salt Diet (HSD)

The high salt (8% NaCl) diet (HSD) model was used to induce hypertension (Gu *et al.*, 2008) as follows: Pelletized standard rat chow obtained from Ladokun Feeds Nigeria Limited (composition: carbohydrates 67%, proteins 21%, fats 3.5%, fibre 6%, calcium 0.8%, NaCl 0.25% and phosphorus 0.8%) was milled into powdery form. The milled rat chow was mixed with sodium chloride (NaCl) salt in the ratio 8.25:91.75 to make a final HSD diet consisting of 8% NaCl. This combination was then properly mixed and re-pelletized.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of the ethanol extract of *C. sativa* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl /95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μL was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of phytocomponents

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known

components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Experimental animals

Forty -(40)- male Wistar rats weighing between 180-200g housed in well-aerated plastic cages at room temperature with alternating day and night natural cycles. They were allowed free access to food (standard rat chow) and water. The rats were handled according to the ethics of animal handling. Animals were given humane care and treatment and all experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). The animals were randomly grouped into 5 equal groups as follows. Group I was control and was exposed to only standard rat chow and water *ad libitum*. Animals in group II were exposed to HSD (hypertensive control) only while group III was exposed to 10% *C. sativa* incorporated HSD. Group IV animals where treated daily with ethanol extract of *C. sativa* (ECS; 3mg/kg p.o. Okwari *et al.*, 2014) and exposed to HSD. Animals in group V were each exposed to the fumes of 1gm of *C. sativa* in an exposure chamber (42x45x42cm) and HSD daily. All treatments and exposures were for 28 days respectively.

Blood Pressure analysis

Five animals from each treatment group were randomly selected for blood pressure analysis. The systolic, diastolic and mean arterial blood pressures were measured simultaneously non-invasively by tail-cuff method using a CODA blood pressure machine (Kent Scientific Co., USA). The CODA tail-cuff system used volume pressure recording (VPR) to evaluate tail blood volume and hence blood pressure as its method of analysis. The animals were allowed to enter the holders on their own freewill and were maintained therein for at least 10-15 minutes for acclimatization test runs, prior to actual recording of blood pressures.

Electrocardiography

The remaining 3 animals in each group were evaluated for electrocardiography non-invasively as follows: Subcutaneously, ketamine (75mg/kg) and xylazine (0.75mg/kg) were used as anaesthesia and thereafter, electrocardiograph (ECG) leads were arranged on the body of the animal as described by Normann *et al.*, (1961). Briefly, the electrodes are placed under the skin of left and right arms and the tail. An additional electrode was placed on the left leg while a grounding electrode was placed on the right leg of the rat. The 4 Channel Small Animal ECG/EMG Recorder with LabScribe ECG analysis Software The IX-BIO4-SA system was used to capture and analyse ECG tracings obtained.

Evaluation of haematological indices

Following blood pressure evaluation, animals were mildly anaesthetized using diethyl ether and blood samples were obtained from the retro orbital sinus using a capillary tube into plain and ethylene diamine tetracetic acid- (EDTA)-lined sample bottles. Samples collected into plain bottles were analysed for whole blood viscosity as described by Elblbesy (2014). Blood samples collected into EDTA lined bottles were analysed for haematology using standard laboratory techniques as follows: Packed cell volume (PCV) was determined by allowing whole blood to enter heparinised microhaematocrit capillary tubes by capillary action, sealed at one end with plasticin and centrifuged for 3 minutes using a microhaematocrit centrifuge at 10000rpm. The separated cells were read off a haematocrit reader. Haemoglobin (Hb) concentration was assessed using Oxyhaemoglobin method. Red blood cell (RBC) and total white blood cell (WBC) counts were determined using the haemocytometer; the Wright's stain (Conn,1969) was used for differential WBC (lymphocytes, neutrophil, monocytes, eosinophil) count and the Ress-Ecker method for platelet count. The Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were thereafter calculated. The electrolyte sedimentation rate (ESR) was measured using the Westergren method (Westergren, 1957) while fibrinogen was assayed using the heat precipitation method (Millar *et al.*, 1971).

Histological evaluation

The descending aorta (to the iliac bifurcation) was harvested from 5 animals in each group after blood collection and analysed for structural changes using Haematoxylin and Eosin (H and E) stains as described by Thent, et al. (2012).

Statistical analysis

Data were expressed as mean ± S.E.M. One-way Analysis of variance (ANOVA) and Tukey Post Hoc test were used to establish statistical significance at $P<0.05$.

RESULTS

GCMS analysis and Identification of compounds in ethanol extracts of Cannabis sativa

GC-MS chromatogram analysis of the ethanolic extract of *C. sativa* (Figure 1) showed eighteen (18) peaks,

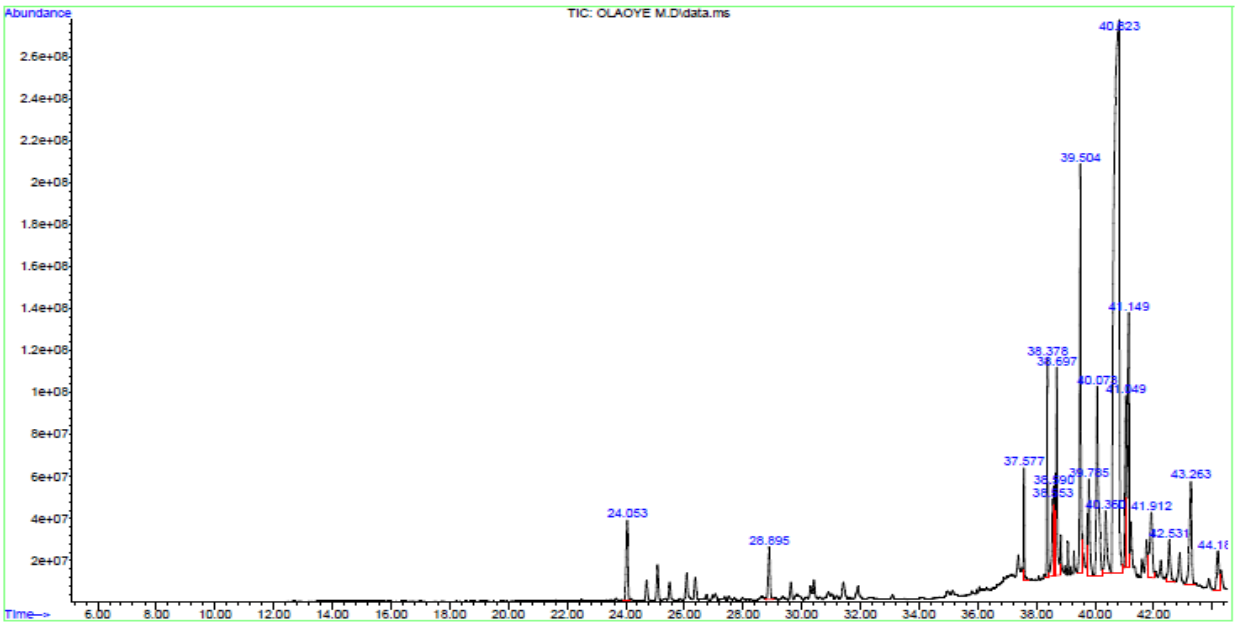
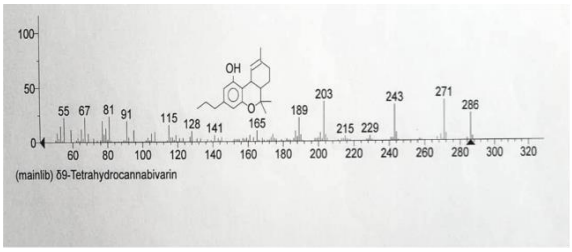
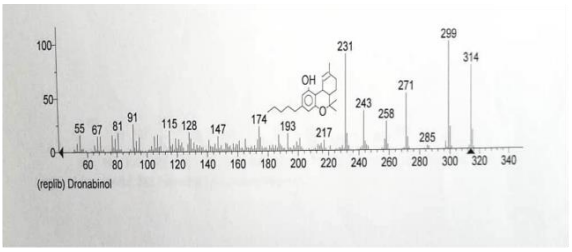


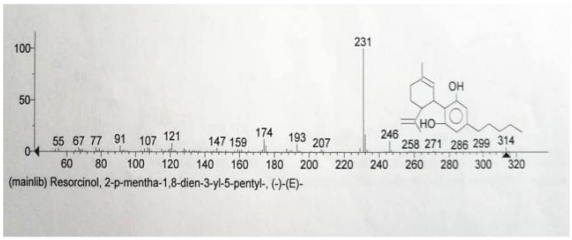
Fig. 1. Gas Chromatography Mass Spectrometry (GC-MS) Chromatogram of Cannabis sativa



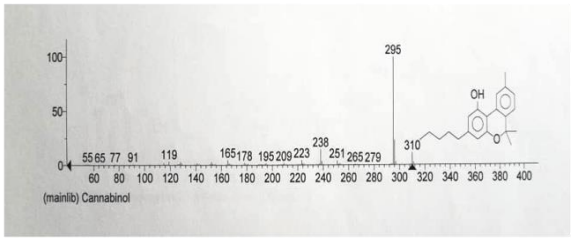
δ9-Tetrahydrocannabivarin



Dronabinol



Resorcinol



Cannabinol

Fig. 2. Mass spectrum and structure of phytocomponents identified by GC-MS in the ethanol extract of *Cannabis sativa*

which indicated the presence of a large number of phytochemicals. On comparison of the mass spectra of the constituents with the NIST library, the 18 phytocompounds were characterized and identified (Table 1). The most prevailing compounds indentified in the ethanol extract of *C. sativa* were Dronabinol (Δ9-Tetrahydrocannabinol) (36.7%), δ9-Tetrahydrocannabivarin (Δ9-Tetrahydrocannabivarin) (9.2%), Resorcinol (Cannabidiol) (7.9%), and Cannabinol (7.8%). These compounds have been reported to contribute to the biological activities of *C. sativa* and their mass spectra are presented in (Figure 2).

Body weight changes in control and experimental groups

Body weight (gm.) on day 28 (212.8 ± 3.5) in the group I (control) was significantly ($P<0.05$) higher(11.5%) compared to day 1 (190.8 ± 3.4) within the same group. On day 28, animals in groups II, III, IV and V exhibited 5.1%, 16.6%, 16.0%, and 26.0% reductions ($P<0.05$) in body weight when compared day 1 values in their

Table 1. Phytocomponents identified in the ethanol extract of *Cannabis sativa* by Gas Chromatography Mass Spectrometry (GC-MS) analysis

S/N	Compound	Quantity (%)	Retention Time (Min)
1	Caryophyllene	2.731	24.053
2	Caryophyllene oxide	1.892	28.895
3	Hexadecanoic acid	1.733	37.577
4	Phytol	3.393	38.378
5	1-Heptatriacotanol	1.817	38.553
6	9,12-Octadecadienoic acid	2.047	38.590
7	9,12,15- Octadecadienoic acid	3.499	38.697
8	δ9-Tetrahydrocannabivarin	9.153	39.504
9	2-tert-Butyl-4-methyl-6-(1methyl-1-phenylethyl)phenol	2.684	39.785
10	Resorcinol (Cannabidiol)	7.927	40.073
11	Benzeazulene-3,8-dione	2.625	40.360
12	Dronabinol (Δ9-Tetrahydrocannabinol)	36.645	40.823
13	1,3-Benzenediol	4.441	41.049
14	Cannabinol	7.773	41.149
15	(5β)3-Acetooxypregnan-20-ol-6one-18-oic acid lactone	3.523	41.912
16	6H-Dibenzo[b,d]pyran-1,8-diol	1.858	42.531
17	Obacunone	4.378	43.263
18	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.882	44.189

respective groups. Body weight values obtained on day 28 in all experimental groups were significantly reduced (P<0.05) compared to control. Values obtained in groups III, IV and V were also significantly reduced (P<0.05) compared to group II (Hypertensive control) (Table 2).

Changes in red cell indices of control and experimental animals

Packed cell volume (%) was significantly increased (P<0.05) in all experimental groups compared to group I, control group. However values obtained in groups III (54.6 ± 1.57), IV (53.6 ± 0.93) and V (53.0 ± 1.90) were reduced significantly (P<0.05) compared to group II, hypertensive control (60.6 ± 1.89). Red blood cell count (RBC) ($x10^6/mm^3$) was significantly increased (P<0.05) in groups II and III compared to control. Values in group III-V where however reduced (P<0.05) compared to group II. Haemoglobin (g/dL) and Mean corpuscular volume (MCV) (fL) were also increased significantly (P<0.05) in all experimental groups compared to group I, control. However, Haemoglobin levels in groups III-V were reduced (P<0.05) compared to group II, hypertensive controls. No significant difference was observed in the Erythrocyte sedimentation rate (ESR) (mm/hr.) between control and all other experimental groups (Table 3).

Blood viscosity and Fibrinogen levels in control and experimental groups

Blood viscosity increased significantly (P<0.05) in groups II (16.28 ± 0.45), III (13.06 ± 0.34), IV (10.92 ± 0.30) and V (8.29 ± 0.20) compared to group I (6.94 ± 0.23). However, values obtained in groups III, IV and V were 19.8%, 32.9% and 49.1% reduced (P<0.05) respectively, compared to group II (Hypertensive control) (Figure 3). Fibrinogen (mg/dL) in groups II, III, IV and V was 50%, 33.3%, 25%, and 16.7% increased significantly compared to control (Figure 4).

Changes in white cell indices of control and experimental animals

Total white blood cell count (10^3 cells/mm^3) was increased (P<0.05) in all experimental groups compared to group I, control. Values obtained in groups III (3690 ± 136.4), IV (3330 ± 196.6) and V (3200 ± 70.7) were significantly reduced (P<0.05) compared to group II (4060±96.7), hypertensive controls. Neutrophils increased signifcantly (P<0.05) only in group II compared to control, while no differences across the groups were observed for lymphocytes, monocytes and eosinophil respectively (Table 4).

Blood pressure changes in control and experimental groups

Systolic blood pressure (mmHg) was significantly increased (P<0.05) in all experimental groups compared to group I control, while values observed in groups III (151.0 ± 2.92), IV (144.6 ± 2.29) and V (130.2 ± 2.44) where significantly reduced (P<0.05) compared to group II (185.4 ± 2.64), Hypertensive control (Figure 5). Diastolic and mean arterial blood pressures (mmHg) were increased (P<0.05) only in group II compared to control. Values for diastolic and mean arterial blood pressures in groups III, IV, and V were however reduced (P<0.05) compared to group II (Figure 6-7).

Electrocardiograph (ECG) reading in control and experimental groups

The heart rate (beats/min) and P-wave duration (ms) in all experimental groups was reduced compared to

Table 2. Body weight changes in control and experimental groups

GROUPS	Body weight (g)					% Change in body weight
	Day 1	Day 7	Day 14	Day 21	Day 28	
I	190.8 ± 3.4	194.4 ± 3.0	198.6 ± 3.8	205.2 ± 3.9	212.8 ± 3.5[a]	11.5%
II	195.6 ± 2.5	194.4 ± 2.1	189.4 ± 2.3	188.4 ± 1.8	185.6 ± 1.9*[a]	-5.1%*
III	191.4 ± 3.4	188.8 ± 2.8	177.8 ± 3.5	169.6 ± 4.1	159.6 ± 4.6*[#a]	-16.6%*[#]
IV	188.6 ± 3.4	187.4 ± 3.1	180.0 ± 2.4	170.8 ± 3.1	158.4 ± 2.1*[#a]	-16.0%*[#]
V	192.2 ± 3.9	182.8 ± 2.9	167.6 ± 2.4	156.6± 2.6	142.2 ± 2.1*[#a]	-26.0%*[#]

Values are mean ± SEM and *indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control). [a] indicates values that are significantly different on day 28 compared to initial body weights within the same group.

Table 3. Red blood cell indices in control and experimental groups

Groups	Packed cell Volume (%)	Red blood cell count ($x10^6/mm^3$)	Haemoglobin (g/dL)	Erythrocyte sedimentation rate (mm/hr.)	Mean corpuscular volume (fL)
I	43.0 ±0.71	8.31 ± 0.21	14.54 ± 0.12	1.06 ± 0.11	51.9 ± 1.66
II	60.6 ± 1.89*	9.91 ± 0.15*	18.76 ± 0.27*	1.38 ± 0.11	63.7 ± 2.05*
III	54.6 ± 1.57*[#]	9.21± 0.19*[#]	17.20 ± 0.20*[#]	1.30 ± 0.16	59.4 ± 2.29*
IV	53.6 ± 0.93*[#]	8.73 ± 0.13[#]	16.7 ± 0.45*[#]	1.22 ± 0.11	61.4 ± 0.92*
V	53.0 ± 1.90*[#]	8.52 ± 0.06[#]	16.46 ± 0.35*[#]	1.18 ± 0.07	62.3 ± 2.54*

Values are mean±SEM and *indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

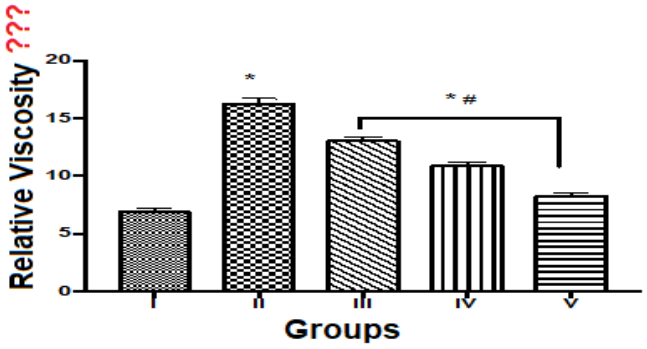


Fig. 3. Blood viscosity in control and experimental groups. Values are mean±SEM and * indicates values that are different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

control. However, groups III, IV and V showed increased heart rates and P-wave duration compared to group II, hypertensive control. Compared to control (group I), P-R interval (ms) reduced significantly only in groups II and V. However values obtained in groups III-V were increased compared to group II. No significant difference was observed for the duration of the QRS complex across all groups while the QT segment was decreased in groups II, IV and V compared to control. QT segment values for groups III-V were however increased compared group II,

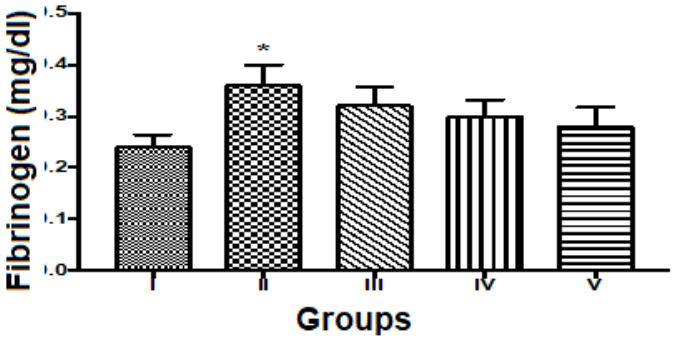


Fig. 4. Fibrinogen (mg/dL) in control and experimental groups. Values are mean±SEM. * indicates values that are significantly different from group I (control).

hypertensive control. The QTc also reduced in groups II, IV and V compared to control. However, compared to group II, hypertensive control, groups III-V were increased. The R amp was increased in groups III –V compared to both control and group II, hypertensive control (Table 5). Representative ECG tracing from each group is shown in Figure 8.

Histology of the aorta in control and experimental groups

Histology of the aortic samples in the control and experimental groups are shown in figure 9(I-V).

Table 4. White blood cell indices in control and experimental groups

	Groups				
	I	**II**	**III**	**IV**	**V**
Total WBC count (cells/mm^3)	2188 ± 29.9	4060 ± 96.7*	3690 ± 136.4*#	3330 ± 196.6*#	3200 ± 70.7*#
Neutrophils (%)	16.4 ± 0.51	19.6 ± 0.81*	18.6 ± 0.51	18.2 ± 0.37	17.8 ± 0.37
Lymphocyte (%)	78.8 ± 0.86	79.6 ± 1.86	76.6 ± 0.93	77.0 ± 0.63	75 ± 0.84
Monocyte (%)	2.6 ± 0.25	2.8 ± 0.37	2.8 ± 0.20	2.4 ± 0.25	2.4 ± 0.25
Eosinophil (%)	2.2 ± 0.20	3.0 ± 0.32	2.4 ± 0.25	2.2 ± 0.20	2.2 ± 0.37

Values are mean±SEM and * indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

Table 5. Electrocardiogram recordings in control and experimental groups

	Groups				
	I	**II**	**III**	**IV**	**V**
Heart Rate (beats/min)	258.8 ± 5.97	177.3 ± 7.93*	228.8 ± 6.50*#	233.25 ± 2.78*#	209.75 ± 6.54*#
P-wave (ms)	26.0 ± 1.08	11.8 ± 2.36*	18.5 ± 0.50*#	20.0 ± 0.71*#	20.0 ± 2.68*#
P-R Interval (ms)	57.5 ± 2.40	23.25 ± 2.36*	49.4 ± 3.30#	54.4 ± 0.99#	41.8 ± 4.09*#
QRS Complex (ms)	17.0 ± 0.71	15.7 ± 0.85	15.5 ±0.29	15.3 ± 1.12	14.5 ± 0.29
QT segment (ms)	80.0 ± 0.82	59.3 ± 6.12*	83.3 ± 4.75#	71.4 ± 1.72*#	63.8 ± 3.84*
QTc	156.3 ± 0.85	102.3 ± 13.47*	163.5 ± 13.88#	141.4 ± 5.96*#	116.2 ± 8.86*
R amp	0.35 ± 0.02	0.36 ± 0.03	0.72 ± 0.07*#	0.53 ± 0.05*#	0.56 ± 0.08*#

Values are mean±SEM and *indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

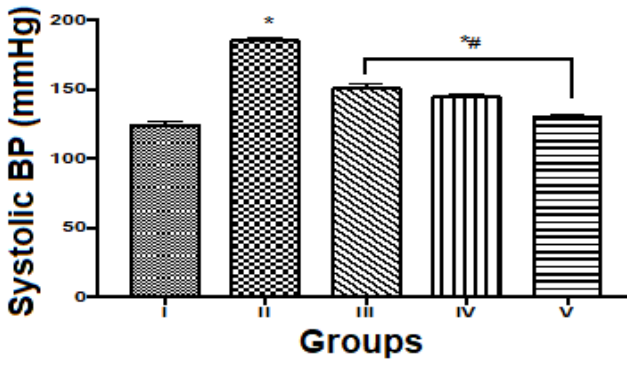


Fig. 5. Systolic blood pressure in control and experimental groups. Values are mean±SEM and * indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

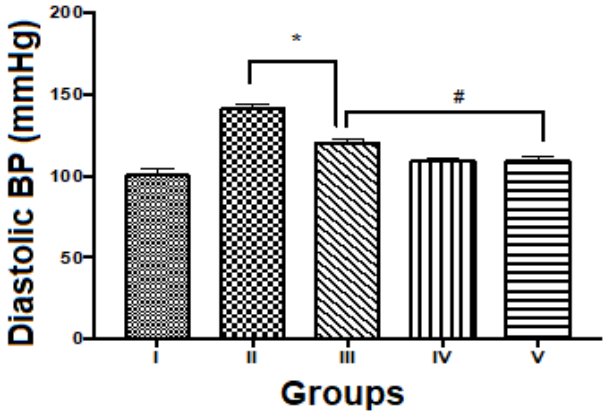


Fig. 6. Diastolic blood pressure in control and experimental groups. Values are mean±SEM and * indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

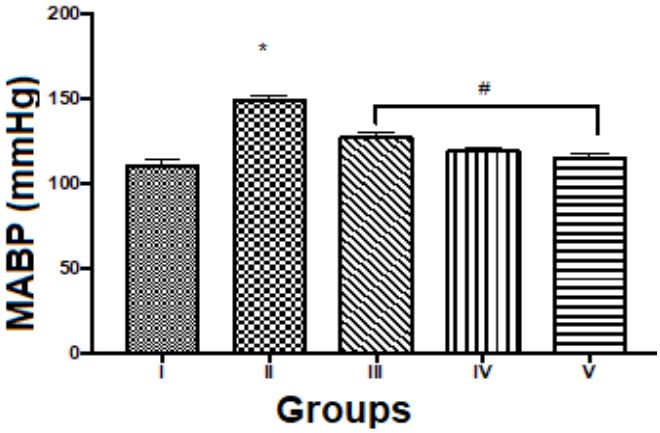


Fig. 7. Mean arterial blood pressure (MABP) in control and experimental groups. Values are mean±SEM and *indicates values that are significantly different from group I (control) while # indicates values that are significantly different from group II (hypertensive control).

Normal control group showed normal tunica intima, media and adventitia with no lesions observed. Group II, the hypertensive control group showed marked thickening of the tunica media due to hyperplasia of the myofibres (a) and fat deposits in the adventitia (b). Aorta in group III exhibited tunica intima with haemosiderin laden macrophages (c), the tunica media in this group is moderately thickened (d) due to hypertrophy and there was congestion of the adventitia capillaries (e). Group IV exhibited aorta with myofibre degeneration (f) and slight atrophy of the tunica media (g) while in group V, aorta samples showed abundant fat deposits on the aortic wall (h).

DISCUSSION AND CONCLUSION

Worldwide *Cannabis* use, either for recreational or medicinal purposes, is on the increase (Bridgeman and Abazia, 2017).

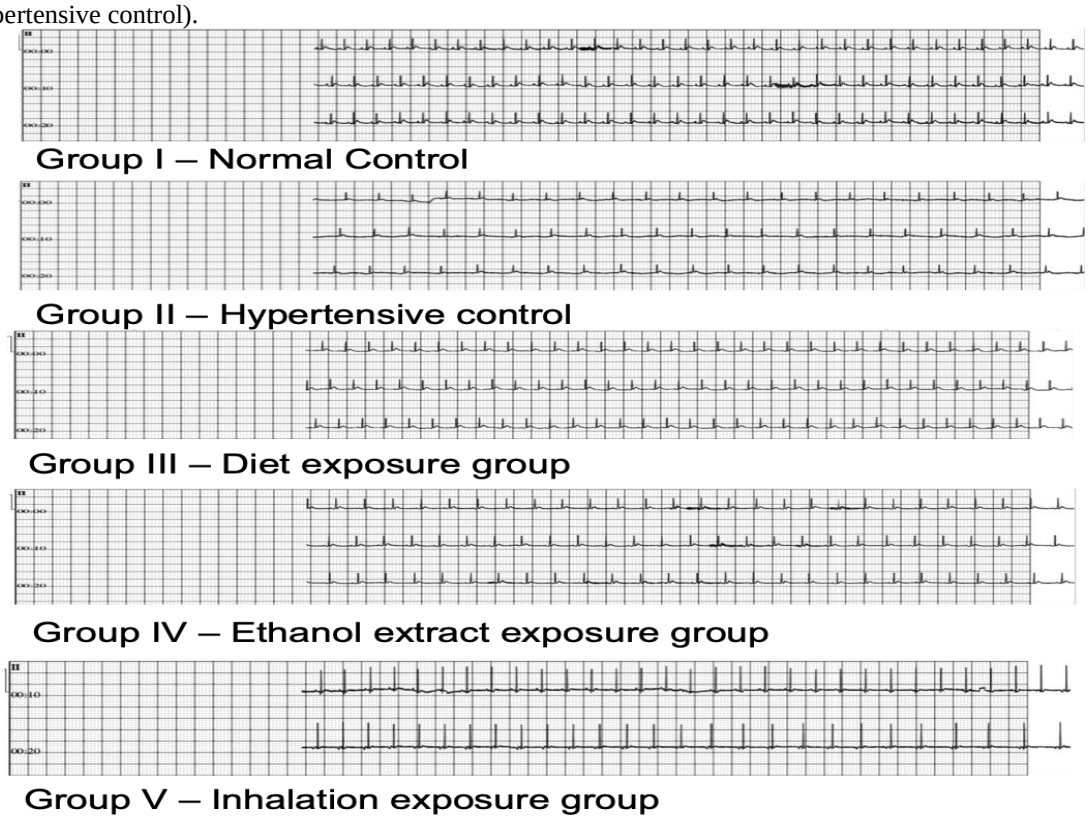


Fig. 8. Electrocardiograph tracings in control and experimental groups. Each tracing is a representative for 3 readings in each group.

Hence there is a need for evaluation of the risks associated with *Cannabis* intake beyond its additive properties. Investigations into the effects of *C. sativa* on cardiovascular function have shown conflicting results with impairment reported in normotensive conditions (Pacher *et al.*, 2005) and amelioration observed under hypertensive conditions (Kicman and Toczek, 2020). These ameliorative effects in hypertension is said to depend on the model of hypertension studied, *C. sativa* prior intake or otherwise, and duration of exposure to *C. sativa (*Sultan *et al.*, 2018*)*. Cardiovascular ameliorative effects of *C. sativa* have been demonstrated in deoxycorticosterone acetate (DOCA)-salt induced hypertension model

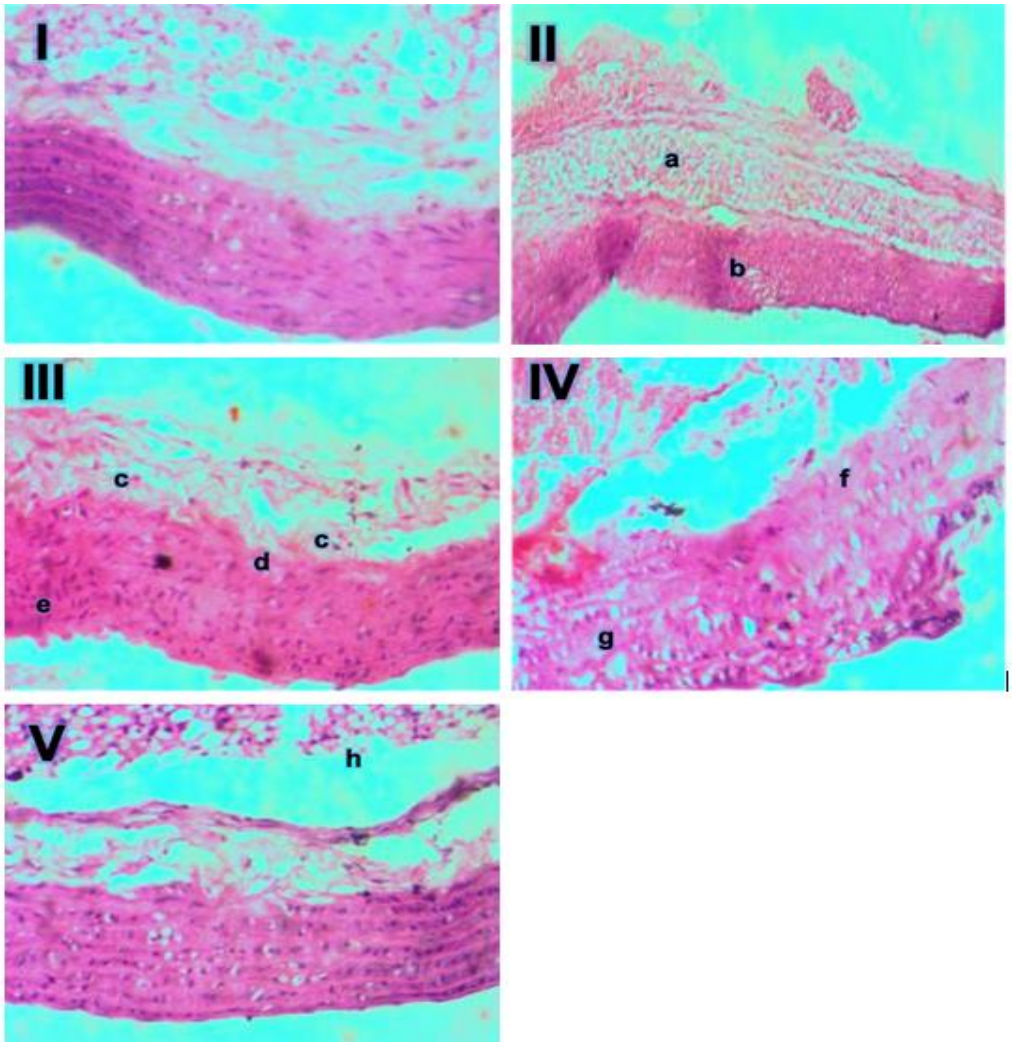


Fig. 9 (I-V) Histology of the Aorta in control and experimental groups. I - Normal Control group; II - Hypertensive control group; III – Diet exposure group; IV – Ethanol exposure group; V – Inhalation exposure group. Normal control group showed normal tunica intima, media and adventitia with no lesions observed. Group II, the hypertensive control group showed marked thickening of the tunica media due to hyperplasia of the myofibres (a) and fat deposits in the adventitia (b). Aorta in group III exhibited tunica intima with haemosiderin laden macrophages (c), the tunica media in this group is moderately thickened (d) due to hypertrophy and there is congestion of the adventitia capillaries (e). Group IV exhibited aorta with myofibre degeneration (f) and slight atrophy of the tunica media (g) while in group V, aorta samples showed abundant fat deposits on the aortic wall (h).

(Malinowska *et al.*, 2019), while studies using high salt (8% NaCl) diet induced-hypertension model in normal rats are sparse.

This study shows reductions in body weight, increased systolic, diastolic and mean arterial pressures in the group exposed to only high salt (8% NaCl) diet for 28 days thus confirming the presence of hypertensive conditions in this group. High salt induced hypertension, has been observed to result from increase arterial constriction and perivascular resistance (Blaustein *et al.*, 2012) as well as uniform expansion of extracellular fluid volume and increased cardiac output (Feng *et al.*, 2017),

This study shows a reduction in systolic, diastolic and mean arterial blood pressure in the *Cannabis sativa* exposure groups compared to the hypertensive control (HSD) group, which suggests an amelioration of hypertensive conditions by *Cannabis sativa* exposure either with diet, as an ethanol extract decoction, or when smoked. This observation is in accordance with other reports that suggest a hypotensive effect of Cannabis sativa treatment under hypertensive conditions (Kicman and Toczek, 2020). The hypotensive effect of *C. sativa* treatment has been attributed to stimulation of the endocannabinoid system (ECS), specifically decreasing the expression of CB1

receptors and stimulating CB2 receptors, resulting in hypotension that is mediated through bradycardia and arterial vasorelaxation (Cunha *et al.*, 2011). The hypotensive effect of *C. sativa* observed in this study did not however restore normotensive conditions as values for systolic blood pressure were still elevated in the *C. sativa* exposure groups compared to control. They were however within the permissible limit for blood pressure elevation and suggests the likelihood that chronic use of cannabis may elicit prolonged reductions in blood pressure as already suggested by Malinowska *et al.*, (2012). However, except for the diet exposure group (group III), diastolic blood pressure was restored to normotensive conditions in the *C. sativa* extract and inhalation groups. This suggests the likely presence and persistence of elevated stroke volume and arterial stiffness in the diet exposure group and an amelioration of same indices in the other exposure groups. It is likely that this difference in effect may be due to the longer duration for the release of phyto-active compounds as a result of the digestive process in the case of the diet group as against the other treatments groups that may have had active compounds absorbed immediately on exposure through either the stomach (extracted treated group) or nasal cavity (fumes exposed group). This observation again re-emphasises that the route of exposure to the plant may influence the pharmacological and clinical effects of cannabis. Furthermore, the mean arterial blood pressure in all *C. sativa* exposure groups was restored to normotensive conditions compared to control suggesting an amendment of blood pressure perturbations in the arterial circulation in these groups compared to hypertensive controls.

Using the GC-MS in this study, the major phytochemical constituents identified in *Cannabis sativa* were Dronabinol (Δ9-Tetrahydrocannabinol), δ9-Tetrahydrocannabivarin (Δ9-Tetrahydrocannabivarin), Resorcinol (Cannabidiol), and Cannabinol. These phytochemicals are referred to as cannabinoids (Gonçalves *et al.*, 2019) and act via a variety of receptors, including CB1, CB2 and transient receptor potential vanilloid type- 1 (TRPV1), in exerting their effects on the cardiovascular system (Alfulaij *et al.*, 2018). The most abundant cannabinoid in *C. sativa* is Δ9-Tetrahydrocannabinol (Δ9-THC) and it is the principal psychoactive constituent of *C. sativa*. It targets primarily CB1 receptors in the central, peripheral and autonomic nervous system (Singh *et al.*, 2017). Neglecting the psychotropic effects, the collective effect of THC on the autonomic nervous stimulation has been reported to be an increase in cardiac workload and myocardial oxygen demand. It is also believed to cause vasodilatory responses possibly through activation of transient receptor potential ankyrin type-1 (TRPA1) ion channels on perivascular sensory neurons (Singh *et al.*, 2017). Tetrahydrocannabivarin (THCV), another active agent identified using GC-MS in this study, is a propyl analogue of Δ9-THC that has shown potential therapeutic effects demonstrating anticonvulsant, antipsychotic and antiepileptiform activities (National Center for Biotechnology Information, 2020). Its effects are contrary to that of Δ9-THC as it acts as a CB1 antagonist and a partial agonist of CB2. Furthermore, it has been reported to interact with different transient receptor potential (TRP) channels including TRPV2, which may contribute to the reported analgesic, anti-inflammatory and anti-cancer effects of cannabinoids and *Cannabis* extracts (De petrocellis *et al.*, 2010). Cannabidiol a phytocannabinoid from *Cannabis* species has been observed to be devoid of psychoactive activity and shows anti-inflammatory, analgesic, and chemo-preventive effects (National Center for Biotechnology Information, 2020). Its administration in experimental studies have showed that it may exert anti-proliferative, anti-angiogenic and pro-apoptotic activity through a variety of pathways that do not involve CB1, CB2 and TRPV receptors (Massi *et al.*, 2013). Cannabinol, another *Cannabis* compound observed in this study, has been associated with potential immunosuppressive and anti-inflammatory activities (National Academies of Sciences, Engineering, and Medicine 2017). It has been reported to bind to the CB2 receptors, which are mainly expressed on a variety of immune cells, such as T-cells, B-cells, macrophages and dendritic cells. Taken together, it is likely that the hypotensive effects of *Cannabis sativa* seen in this study could be a synergistic effect of CB1 receptor stimulation by Δ9-THC resulting in initial increase in cardiac activity and CB1 receptor antagonism accompanied by CB2 receptor stimulation by Δ9-Tetrahydrocannabivarin, Cannabidiol and Cannabinol which were all present in the *Cannabis* plant used.

Studies have shown that rodents, particularly rats and mice, represent the most used species for experimental cardiac electrophysiology studies (Milani-Nejad and Janssen, 2013). These electrocardiogram (ECG) tracings are used in determining and monitoring arrhythmias and cardiac function in several disease models (Becker, 2006). However, there appears to be a deficit of standardized reference values for ECG tracings for different species (Milani-Nejad and Janssen, 2013). ECG tracings obtained in experimental studies have however been shown to provide adequate

information to reveal the effects of drugs and treatments on electrical activity in the heart. An investigation of ECG tracings in this study shows manifestations in the hypertensive group that are consistent with the condition. These include increased activation and stimulation of sinoatrial node (decreased P wave duration), increased impulse conduction throughout atrial muscles and to the atrioventricular node (shortened P-R interval), likely predisposition to myocardiac infarction (shortened QT segment and QTc). Surprisingly, R wave amplitude seen in the hypertensive group was similar to that in control suggesting no difference in ventricular function between these groups. The heart rate values observed in the hypertensive group were also reduced compared to control, which seemed to contradict tachycardia that is often noted in hypertensive conditions. It is however likely that the reduced heart rate in the hypertensive groups may be a manifestation of either impairment in electrical signalling following myocardial hypertrophy in hypertension (Bornstein *et al.*, 2020) or an increase in central aortic pressure which can also accompany hypertensive conditions (Williams *et al.*, 2006).

Exposure to *Cannabis sativa* either as part of diet, orally taken solvent extract or when inhaled through smoking in this study appeared to ameliorate increased stimulation of the SA node though values obtained were still significantly different from control. This was also reflected in the heart rate values seen in the treatment groups which though increased compared to hypertensive group were reduced compared to control. Furthermore, PR intervals in the *Cannabis sativa* exposure groups exhibited values comparable to control and different from the hypertensive group suggesting a correction of impulse transmission between the atria and ventricular muscles. Though QRS complex was not affected in this study, the possibility of myocardial infarction seem reduced in the *C. sativa diet* and ethanol extract groups (comparable QT segment and QTc with control) but not in the *C. sativa* fumes exposure group (reduced QT segment and QTc compared to control) which suggests a route dependent effect of *C. sativa* on electrical signalling in hypertensive conditions. The amplitude of the R wave, a measure of ventricular function, was however increased in the *C. sativa* exposure groups, especially the diet exposure group, suggesting the likely presence of ventricular dysfunction and coronary narrowing.

Evaluation of the aorta in the untreated hypertensive groups suggests the presence of aortic remodelling with loss of elasticity, a thickening of the myofibres and presence of fat deposits suggesting a likely predisposition to atherosclerosis (Rafieian-Kopaei *et al.*, 2014). Treatment with *C. sativa* did not appear to ameliorate the aortic wall remodelling caused by the hypertensive condition as additional pathologies seen included macrophage invasion and congested adventitia capillaries (diet exposure group), myofibre degeneration (extract exposure group) and increased fat deposition (inhalation exposure group). Taken together this implies that exposure to *Cannabis sativa* exacerbated the aortic structural pathologies that usually accompanies hypertension which include loss of elasticity (Cernes *et al.*, 2008), thickening of tunica media (Martinez-Quinones *et al.*, 2018) and atherosclerosis (Zaheer *et al.*, 2016).

Several studies have evaluated the relationship between biochemical risk factors and hypertension with most studies focussing on the lipid profile and its predisposition to cardiovascular diseases, especially hypertension (Kotis, 2007). However, studies on haematological variables in hypertension are few (Emamian *et al.*, 2017) with reports available showing elevated white blood cell (WBC) count, especially neutrophils (Shankur, et al 2004). Furthermore, increased erythrocyte distribution, circulating size and volume (Liu *et al.*, 2015), increased haemoglobin levels have also been implicated in the pathogenesis of hypertension (Atsma *et al.*, 2012; Lee *et al.*, 2015). Observations in this study from the hypertensive group show elevated blood indices (packed cell volume (PCV), red and white blood cell counts, haemoglobin, mean corpuscular volume), increased blood viscosity, which are consistent with previous findings in hypertension. Exposure to *C. sativa* in this study appeared to, in-part, ameliorate haematological perturbations observed in this study, as PCV, RBC, haemoglobin, mean corpsular volumes and blood viscosity, though elevated compared to control, were reduced compared to animals in the hypertensive untreated group. Cannabinoids have been reported to act through CB2 receptor stimulation of colony-stimulating factor (CSF), resulting in the stimulation of hematopoiesis and therefore increased red cell counts, PCV (Mukhtar and Elbagir, 2011) and haemoglobin (Karimi *et al.*, 2007). The elevations of PCV and haemoglobin would also lead to increased blood viscosity (Martini, 2005). This was observed in the experimental groups compared to control and may be ascribed to the cannabinoids present in *Cannabis sativa* regardless of the route of exposure as all animals exhibited similar patterns of haematological perturbations. It would be expected that the greater hematocrit concentrations would significantly thicken the blood, slow its flow rate throughout the body, raise the peripheral resistance, reducing blood flow and

perfusion to various tissues including the brain (Stack and Berger, 2009) and thus further worsen cardiovascular function. However lower systolic, diastolic, and mean arterial pressures were noted in the experimental groups, which may be ascribed to the reported vasorelaxant activity of *Cannabis sativa* exposure acting through its cannabinoid content stimulation of CB1 receptors (Stanley *et al.*, 2015). The influence of central nervous stimulation by *C. sativa* cannot also be overlooked in this study as stimulation of the rostral ventrolateral medulla (RVLM) by cannabinoids have been reported to result in reduced blood pressure and increased heart rate (Penumarti and Abdel-Rahman, 2014) which was similarly observed in the present study.

Findings from this study also showed increased risk of cardiovascular disease (elevated fibrinogen) and infection (elevated total WBC and neutrophil) in the salt-induced hypertensive group. Exposure to *C. sativa* in the experimental groups did not improve the risk of cardiovascular as fibrinogen values obtained were still increased compared to controls. Furthermore, there was a persistence of inflammation (elevated WBC) in the *C. sativa* exposure groups however the neutrophil count (marker of infection susceptibility) was reduced in the experimental groups compared to hypertensive untreated and comparable to controls suggesting a reduction in the risk of infection development in these groups.

Substantial reports have demonstrated that increases or decreases in body weight are major determinants in the modulation of blood pressure (DeMarco *et al.*, 2014). Weight gain and obesity have been positively correlated with an increase in blood pressure. While control animals showed an increase in weight gain, hypertensive untreated group exhibited marginal reductions in body weight which may be ascribed to initial reduction in feed acceptance as observed by Awobajo *et al.* (2018), in salt-induced hypertensive rats. *Cannabis sativa* has been reported to stimulate appetite via the activation of cannabinoid receptor type 1 (CB1) leading to an increase in ghrelin (Riggs *et al.*, 2011). However, studies have also shown that the Cannabis-induced appetite stimulation is not accompanied with weight gain (Le Foll *et al.*, 2013) except in the presence of pathological conditions (Ben Amar, 2006). Furthermore, both tetrahyrocannabinol and cannabidiol have been reported to stimulate weight loss via CB1 and CB2 receptor stimulation respectively (Le Foll *et al.*, 2013) resulting in reduced energy storage and increased metabolic rates (Clark, 2018). This may account for the reductions in body weight observed in the *Cannabis sativa* exposure group and constitute a likely cardio-protective mechanism in these exposure groups as reductions in body weight have been correlated with cardio-protection against the development of hypertension.

In conclusion, this study has demonstrated that in the salt (NaCl) induced hypertensive rat model, exposure to *Cannabis sativa*, whether when incorporated in diet, taken as an ethanol extract decoction or smoked, at moderate and low doses may alleviate elevations in blood pressures seen in the hypertensive state possibly by facilitating vascular vaso-relaxation. This effect is likely to be modulated by a synergy of CB1 (Δ9-Tetrahydrocannabinol) and CB2 (Δ9-Tetrahydrocannabivarin, Cannabidiol, Cannabinol) receptor stimulation by the major phytochemical constituents observed in this study. Myocardial electrical signaling also appear to be improved following exposure to Cannabis sativa however the presence of hypertension-induced structural aberrations in the ventricles and aorta where not improved. Hypertension-induced inflammatory processes were also not ameliorated by exposure to *Cannabis sativa*, however susceptibility to infection was reduced.

It is suggested that further investigations should focus on separation of the major constituents of *Cannabis sativa*, and evaluation of their specific and individual effects on cardiovascular function and electrical activity. Due to the multifaceted acting points of cannabinoids in *Cannabis sativa*, care should be taken while advocating for its likely use in hypertensive conditions.

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