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NEUROLOGICAL BEHAVIOUR OF ALBINO RATS TREATED SEPARATELY AND IN COMBINATION WITH *CANNABIS SATIVA* L. AND *CANNABIS INDICA L.*

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ABSTRACT

There has been an increasing rate of cannabis consumption globally, especially among the youths. This study therefore evaluated the neurological behaviours and some brain marker hormones and enzymes of cannabis administered rats. Twenty six albino rats were divided into four groups based on oral cannabis administration (control, Cannabis sativa, Cannabis indica and the combination of the two). At the end of seven days, open field test was conducted on the rats. Also, brain neuro-chemicals, activities of antioxidant enzymes and lipid peroxidation were evaluated using spectrophotometry. The results of the Open-Field Test showed an appreciable increase in the level of ambulation (line crossing), grooming, urination and stretched attend posture in the rats administered with Cannabis indica, Cannabis sativa and the combination when compared with the control. Norepinephrine was significantly lower ($p < 0.05$) in the rat groups administered with the combination of Cannabis indica and Cannabis sativa. The control group however had the lowest dopamine level. Superoxide dismutase (SOD) was significantly lower ($p < 0.05$) in the rats administered the combination of both Cannabis indica and Cannabis sativa. The brain level of reduced glutathione (GSH) was significantly higher in the rats administered with Cannabis indica. Malondialdehyde (MDA) was significantly higher in the rats administered with Cannabis sativa than the other rat groups. Histopathological evaluation of the brain also revealed various damages in the brain cells of rats administered with cannabis compared to the normal brain structure of the control rats. It is thus said that consumption of C. sativa or C. indica alone produced mild effect on the brain cells and physiology in rats. However, combination of C. sativa and C. indica produced a severe synergistic effect on the neurological function of the exposed rat.

Keywords: neurotransmitters, neurological behaviours, brain histopathology, *Cannabis sativa*, *Cannabis indica*

INTRODUCTION

The genera Cannabis belongs to the family (Cannabaceae, sometimes known as Cannabinaceae) (Srivastava and Yadav, 2013). Gigliano (2001) described cannabis as a tall, erect, annual herb, which grows well in an open sunny environment with light well drained composted soil and ample irrigation. It is a coarse rangy, annual plant that grows 1.8-3.7 m in height with palmate leaves divided into 3-7 harrows and about 5.2-7.6 cm long. Its stems are rough with fibrous inner bark (Tijani *et al.*, 2014). It is normally a dioecious species, with male and female

flowers on separate plants, but sometimes bisexual plants occur (Moliterni *et al.*, 2004). According to Reichard (2014), there are two main varieties or classifications of cannabis that make up the majority of all marijuana strains appearing on dispensary, delivery service, and collective menus. The two main strains are popularly known as *Cannabis sativa* and *Cannabis indica*.

Marijuana has long been used as medicine and spiritual tool by humans (Brady *et al.*, 2009). Such include the preparation of flowers and leaves of marijuana and are consumed by smoking, vaporizing and oral ingestion (West, 2007). Foster and James (1990) identified some of the medicinal use of this plant to include the treatment of glaucoma, depression and to relieve the nausea associated with the cancer treatment. Other ailments which have been treated with marijuana include menstrual fatigue, gout, rheumatism, malaria, beriberi, constipation, pain, and absentmindedness (Abel, 1980). Similarly, Van Roekel (1994) reported that hemp foods show no known allergies. However, cannabis has been reported as the most widely used illicit drug in the world (Bruijnzeel *et al.*, 2016; Degenhardt *et al.*, 2008) which its use often starts during teenage years (Terry-McElrath *et al.*, 2005). The report of the United Nations Office on Drugs and Crime (UNODC, 2014) showed that about 2.7 – 4.9% of adults worldwide use cannabis. Similarly, the prevalence of cannabis use has been on the increase in several countries including Ghana, Zambia, Canada, United States of America, New Zealand and Nigeria (UNODC, 2014; Adamson *et al.*, 2015).

As reported by Elsohly and Slade (2005), the cannabis plant consists contains more than one hundred C21 terpenophenolic compounds, known collectively as phytocannabinoids. Similarly, Ashton (2001) submitted that the primary psychoactive constituent of cannabis is Δ9-tetrahydrocannabinol (THC). The subjective effects THC in cannabis have been shown to include relaxation, mild euphoria, perceptual changes, intense laughter, and talkativeness (Green *et al.*, 2003; Hall and Degenhardt, 2009), impaired memory function and paranoia (Crean *et al.*, 2011; Freeman *et al.*, 2015). Similarly, chronic cannabis use may lead to dependence (Grant and Pickering, 1998) while cessation of chronic use can lead to affective withdrawal symptoms such as increased anxiety, irritability, aggression, intense craving for cannabis, difficulty in sleeping, and somatic complaints (Badney *et al.*, 2001; Haney *et al.*, 1999).

Although, the use of psychoactive drugs has long been of interest to researchers especially in Nigerian, limited work has been conducted on the use of tobacco and cannabis (Ibeh and Ele, 2003). Also, most of the adverse effects of cannabis have been identified on various cognitive and nervous functions. However, there is still the need to monitor the behavioural, antioxidant enzymes and hormonal response of the brain to the oral consumption of Cannabis. This present study therefore evaluated the behavioural modification, antioxidant enzymes and hormonal response of the brain to the oral consumption of the two main strains of Cannabis, *Cannabis sativa* and *Cannabis indica* in Albino rats.

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MATERIALS AND METHODS

Study site

This study was carried out at the animal house of the Department of Zoology and Environmental biology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Plant Collection

The plants were purchased in Ago-Iwoye and authenticated in the EliKaf Herbarium of the Department of Plant Science, the Voucher Specimen were deposited in same Herbarium as EH/2019/19001 and EH/2019/19002 for *Cannabis sativa* and *Cannabis indica* respectively.

Experimental set-up

A total of twenty-six (26) albino rats were used for this research. These were divided into four experimental groups as described below:

Group 1 (Control)	Administered with 180 g of feed with water
Group 2	60 g of dried grinded *Cannabis sativa* + 120 g of feed + water
Group 3	60 g of dried grinded *Cannabis indica* + 120 g of feed + water
Group 4	30 g of dried *Cannabis sativa* + 30 g of *Cannabis indica* + 120 g of feed + water

The rats were initially acclimatized under the laboratory conditions of 25±5°C and 65±5% Relative Humidity in a well-ventilated experimental animal house for one week before the commencement of the study. The diets used for this study were formulated with the following compositions: fishmeal (29.78%), GNC (17.98%), wheat offal (19.61%), bone meal (0.50%), soybeans meal (2.00%), dicalsium phosphate (0.10%), corn meal (29.78%) and mineral premix (0.25%). Appropriate quantity of *C. sativa* and *C. indica* was added to the feed either alone or in combination and fed the experimental rats for a period of 7 days.

Sample collection

At the end of the seven days experimental period, four rats were randomly selected and sacrificed from each of the rat groups and dissected following internationally accepted principles for laboratory use and care of European Community (EEC-directive of 1986: 86/609/EEC) and the regulations of the local ethics committee in animal care Unit of Olabisi Onabanjo University, Ago-Iwoye. Brain samples were carefully collected from the sacrificed rats and taken to the laboratory for other laboratory analyses.

Assessment of Behavioural Response in Open-Field Apparatus

The effects of the plants on locomotion, exploratory activity and anxiety behaviour of each rat were studied using open-field test as described by Zhu *et al.* (2001) with little modification, which is a central square in the middle of the open-field apparatus as described by Brown *et al.* (2004). Briefly, the open-field apparatus was constructed using a plastic box (50 cm × 50 cm × 46 cm high) with clear Plexiglas on the inner surface. The floor was divided into 24 equal squares with a central square (18 cm × 18 cm), drawn in the middle of the open-field. Each rat was placed in the central square and observed for 5 minutes

and the following behaviours were recorded during the next 3 minutes after initial 2 minutes of habituation:

1. Ambulation: the number of grid lines it crossed with all the four paws
2. Central square duration: time spent in the central square.
3. Grooming: number of times the animal made the following responses: grooming of the face, licking/cleaning and scratching the various parts of the body
4. Stretch-attend postures: the number of times there is forward elongation of the head and shoulders (head is usually low to the platform) followed by retraction to the original position
5. Defecation and urination: the number of faecal boli excreted and urine streak produced respectively, and
6. Immobilisation (freezing): It was recorded to have occurred, when a rat had its eyes open, holding its head against the gravity, but without any head, body or limb movement.

Assessment of Excitability Score

The excitability score of each rat was assessed using excitability score test as described by Maria *et al.* (2002) with little modification by Ayo *et al.* (2006) and Adeiza and Minka (2010). Briefly, each rat was held by the tail upside down and kept in such position for 30 seconds. The level of reaction of the animal was graded and scored as follows:

1. Score 1 (calm): Rat did not show any sign of wriggling and paw movement.
2. Score 2 (occasional shakes): Rat responded through gentle wriggling and movement of forepaw.
3. Score 3 (repeated shakes): Rat responded through stronger wriggling and movement of forepaw.
4. Score 4 (violent shakes): Rat responded through vigorous wriggling, strong movement of fore- and hind limb as well as successfully climbed the tip of its tail.

Assessment of brain antioxidant system

Lipid peroxidation assay

The levels of lipid peroxidation in the brain tissues were measured as malondialdehyde (MDA) according to the method of Ohkawa *et al.* (1979). To 0.1 ml brain homogenate, 0.2 ml of 8.1% (w/v) sodium dodecylsulphate (SDS), 1.5 ml of 20% (v/v) glacial acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid (TBA) were added. The mixture was made up to 4 ml with the addition of 0.7 ml of distilled water. The test tubes were heated at 95°C for an hour with a marble on top of each test tube. After incubation, the test tubes were cooled and then centrifuged at 3000 × g for 10 minutes. After centrifugation, the concentrations of MDA were measured spectrophotometrically at 532 nm. 1,1,3,3-tetraethoxypropane (TEP) was used as standard prepared in concentration range of 0.1 to 1 mM.

Superoxide dismutase assay

Assay of superoxide dismutase (SOD) was performed using Cayman assay kit according to manufacturer's instructions. SOD activity was assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine in a convenient 96 well format. A standard curve was generated using a quality-controlled SOD standard. The activity of the SOD was accurately

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quantified from the standard curve and was expressed in Unit/ml. One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Glutathione peroxidase (GPx) assay

GPx activity was assayed using Cayman assay kit according to manufacturer's instructions. This kit measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of organic hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in absorbance is directly proportional to the GPx activity in the sample.

Catalase (CAT) assay

The activity of CAT was measured spectrophotometrically as described by Goth (1991). Briefly, the procedure entailed incubating 100μl of brain homogenate with 500μl of hydrogen peroxide at 37°C for a minute. The addition of 500μl of ammonium molybdate solution stopped the reaction with a formation of a yellow complex. The absorbance was then measured at a wavelength of 405 nm using a spectrophotometer.

Reduced glutathione (GSH) assay

GSH was estimated using assay kit (Calbiochem, USA) according to the manufacturer's instructions. Briefly, the brain homogenates were deproteinized in 5% metaphosphoric acid, centrifuged and the glutathione contents of the supernatants were measured by the rate of colorimetric change of 5, 5'-dithiobis(nitrobenzoic acid) at 412 nm in the presence of glutathione reductase and NADPH.

Estimation of dopamine, serotonin and noradrenaline content in the brain

Immediately after the behavioural task, the rats were killed by cervical dislocation, and the brain transferred and was homogenized within 1 min to ice-cold isolation buffer (0.23 M mannitol, 0.07 M sucrose, 10 Mm Tris-HCl, and 1 mM EDTA, pH 7.4) at a concentration of 15% (w/v). This would release soluble protein, leaving only membrane and nonvascular matter in a sentimental form. It was then centrifuged in cooling centrifuge at 620 g for 5 min. The supernatant was separated and further centrifuged at 5000 g. The noradrenaline, dopamine and serotonin activity was assayed according to the method of (Schlumpf *et al.*, 1974).

Nor-adrenaline and dopamine assay

The assay represents a miniaturization of the trihydroxyindole method. To 0.02 ml of HCl phase, 0.005 ml 0.4 M HCl and 0.01 ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution for oxidation. The reaction was stored after two minutes after adding 0.01 ml Na_2SO_3 in 5 M NaOH. 0.01 ml of acetic acid was added 15 min later, the solution was then boiled to 100 °C for 6 min. When the sample again reached room temperature, emission spectra were read in the microcuvette at 485 nm for Nor-adrenaline and 375 nm for dopamine in a spectrofluorimeter.

Serotonin (5-HT) assay

Some modification in reagent concentration became necessary together with changes in the proportions of the solvent, in order to obtain in a well fluorescence yield with

reduced volumes. For 5-HT determination, the O-phthaldialdehyde (OPT) method was employed. From the OPT reagent 0.025 ml was added to 0.02 ml of the HCl extract. The fluorophore was developed by boiling at 100 °C for 10 min. After the sample reached equilibrium with the ambient temperature, emission spectra or intensity reading at 470 nm were taken in the microcuvette.

Aetylcholinestrase activity estimation

Cholinesterase activity was also determined in the supernatant obtained from the brain samples as described by Ellman *et al.* (1961) using acetylthiocholine iodide (30 µl final concentration = 0.5 mmol) as substrate and 5,5-dithiobis-2-nitrobenzoeic acid (DTNB; 200 µl; final concentration = 0.33 mmol). Assay tubes were completed to 1 ml with sodium phosphate buffer (pH 8) as described by Canadas *et al.* (2005). The enzyme activity was calculated relative to protein concentrations.

Statistical analyses

Data collected was analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011). Analysis of Variance (ANOVA) was used to test for significant in the levels of brain neurochemicals, antioxidant enzymes and lipid peroxidation parameters between the four treatment groups. Post Hoc test was conducted utilising the Student Newman Keuls (SNK) test. *p* value was set at 0.05.

RESULTS

Behavioural response of the rats

Open-Field Test: The behavioural response of the rats administered with *Cannabis indica*, *Cannabis sativa* and the combination in Open-Field Test is represented in Table 1. When compared with the control, there was an appreciable increase in the level of ambulation (line crossing), grooming, urination and stretched attend posture in the rats administered with *Cannabis indica*, *Cannabis sativa* and the combination of *Cannabis indica* and *Cannabis sativa* in the Open-Field Test. These were however highest in the rats administered with only *Cannabis sativa*. *Cannabis indica* and *Cannabis sativa* were observed to show reduced defecation when compared with the control. On the other hand, rats administered with the combinations of *Cannabis indica* and *Cannabis sativa* showed a higher level of defecation than the other rat groups.

Table 1: Behavioural Response of the rats administered with Cannabis indica, *Cannabis sativa* and the combination in Open-Field Test in 5 minutes

	Control	*CS*	*CI*	*CS + CI*
Ambulation (line crossing)	12.52	29.81	22.10	25.30
Centre square duration	0.00	0.00	1.20	2.72
Grooming	0.87	3.01	2.71	2.96
Urination	0.34	0.85	0.82	0.65
Defeacation	2.65	1.82	2.11	3.62
Immobilization (Freezing)	9.35	6.21	10.21	12.32
Stretched attend posture	18.20	23.61	22.01	19.29

CS= *C. sativa*, SI = *C. indica*, CS + CI = *C. sativa* + *C. indica*

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Percentage excitability scores: Rats administered with *Cannabis indica* had the highest percentage calmness (Figure 1). This was followed by those administered with the combination of *C. sativa* and *C. indica*. However, none of the animals administered with *C. sativa* and those of the control showed any degree of calmness. On the other hand, occasional shakes were only recorded in the control rats and those administered with *C. sativa* (Figure 1). Percentage occasional shakes were however higher in the rats administered with *C. sativa* than those of the control.

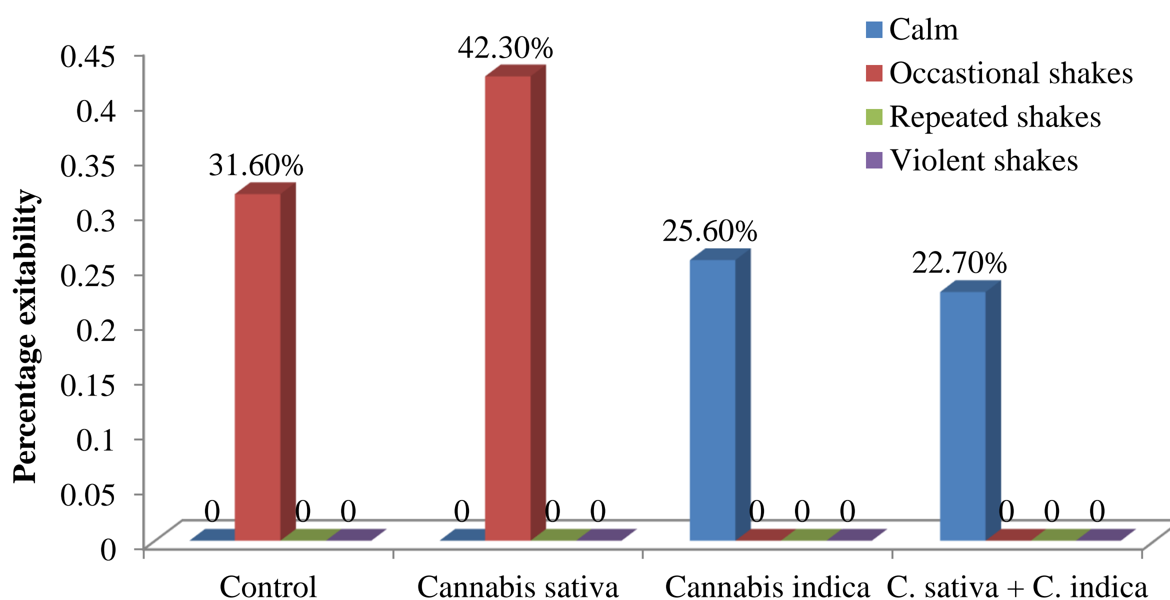

Figure 1: Percentage excitability scores of the rats administered with *Cannabis indica*, *Cannabis sativa* and the combination

Levels of brain neurochemicals

Table 2 show the levels of norepinephrine, dopamine, serotonin and acetylcholinesterase in the brain of the experimental rats. The level of norepinephrine was significantly lower ($p < 0.05$) in the rat groups administered with the combination of *Cannabis indica* and *Cannabis sativa*. However, there was no significant difference ($p > 0.05$) recorded in the levels of norepinephrine recorded in the control rats and those administered with *Cannabis indica* and *Cannabis sativa* respectively.

Levels of dopamine recorded in the rats administered separately with *Cannabis indica* and *Cannabis sativa* were significantly higher than those administered with a combination of *Cannabis indica* and *Cannabis sativa* (Table 2). The control group however had the lowest dopamine level. On the other hand, the level of serotonin was significantly higher ($p < 0.05$) in the rats administered with *Cannabis sativa*. However, there was no significant difference ($p > 0.05$) recorded in the serotonin levels between the other experimental groups. Similarly, the level of acetylcholinesterase was significantly higher ($p < 0.05$) in the rats administered

with *Cannabis sativa* and lowest in those administered with a combination of *Cannabis indica* and *Cannabis sativa*.

Table 2: Levels of some brain neurochemicals in the rats administered with Cannabis indica, *Cannabis sativa* and the combination

	Norepinephrine (ng/mg tissue)	Dopamin (ng/mg tissue)	Serotonin (ng/mg tissue)	Acetylcholinesterase (IU/mg protein)
Control	0.55±0.04[a]	1.39±0.02[b]	0.57±0.01[b]	20.05±0.18[b]
Cannabis sativa	0.60±0.02[a]	1.44±0.01[a]	0.76±0.06[a]	21.99±0.74[a]
Cannabis indica	0.58±0.01[a]	1.43±0.01[a]	0.60±0.01[b]	20.56±0.35[b]
Sativa + indica	0.50±0.02[b]	1.40±0.01[b]	0.55±0.03[b]	18.20±0.55[c]

[abc]Mean (±Standard deviation) in the same column having similar superscripts are not significantly different at $p < 0.05$

Activity levels of antioxidant enzymes in the brain

The levels of superoxide dismutase (SOD), reduced glutathione (GSH), catalase and glutathione peroxidise (GPx) in the brain of the experimental rats were presented in Table 3. There was no significant difference ($p > 0.05$) in the activity of SOD recorded in the control rats and those administered with *Cannabis indica* and *Cannabis sativa*. However, SOD was significantly lower ($p < 0.05$) in the rats administered the combination of both *Cannabis indica* and *Cannabis sativa*.

The brain level of GSH in the experimental rats was significantly higher ($p < 0.05$) in the rats administered with *Cannabis indica* (Table 3). On the other hand, the levels of catalase and GPx in the brain of the experimental rats followed the same trend. These were significantly higher ($p < 0.05$) in the rats administered with *Cannabis sativa* and lower in those administered with the combination of *Cannabis indica* and *Cannabis sativa*.

Table 3: Activity levels of some antioxidant enzymes in the brain of rats administered with Cannabis indica and*Cannabis sativa* alone and in combination

	SOD (U/ml)	GSH (U/g tissue)	CAT (nmol/min/ml)	GPx (nmol/min/ml)
Control	47.44±0.59[a]	9.71±0.47[ab]	45.70±0.45[b]	57.54±0.56[b]
Cannabis sativa	51.82±1.48[a]	10.08±0.29[ab]	50.62±0.73[a]	59.99±0.69[a]
Cannabis indica	49.64±1.80[a]	11.16±0.22[a]	43.79±0.69[b]	57.66±0.50[b]
C. sativa + indica	41.77±3.52[b]	8.78±1.50[b]	38.42±3.14[c]	56.14±0.70[c]

[abc]Mean (±Standard deviation) in the same column having similar superscripts are not significantly different at $p < 0.05$

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Levels of lipid peroxidation

The level of lipid peroxidation as measured using malondialdehyde (MDA) in mM is represented in Figure 2. Mean level of MDA was significantly higher ($p<0.05$) in the rats administered with *Cannabis sativa* and those administered with a combination of *Cannabis indica* and *Cannabis sativa*. However, there was no significant difference ($p > 0.05$) in MDA levels recorded in the control rats and those administered with *Cannabis indica*.

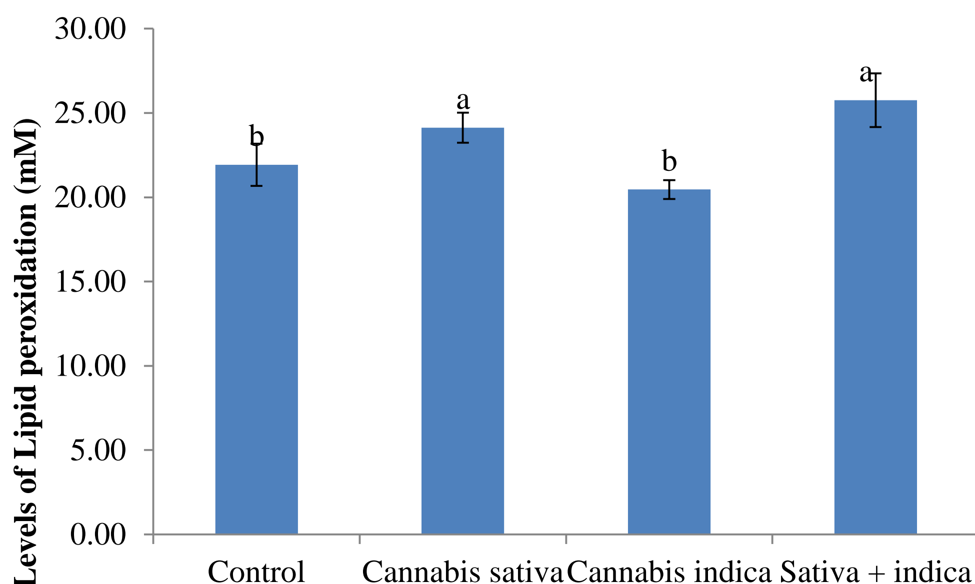

Figure 2: Levels of lipid peroxidation in the rats administered with *Cannabis indica* and *Cannabis sativa* alone and in combination

Histopathological evaluation of the brain

The result of the histopathological evaluation of the brain of the experimental rats is represented in Figure 3. Normal layering, cerebral cortex, pyramidal neurons and blood capillaries were observed in the control rats. However, the brain of *C. sativa* administered rats showed distorted layers and increased eosinophilia, distorted neuropile and vacuolated neurons. The brain of *C. indica* administered rats showed distorted layers and apparent reduction in neuronal density, dilated capillaries, chromatolytic neurons and degenerated neurons. Similarly, the brain of rats exposed to combined consumption of *C. sativa* and *C. indica* showed distorted layers, apparent hypertrophied neurons and micro-vacuolations.

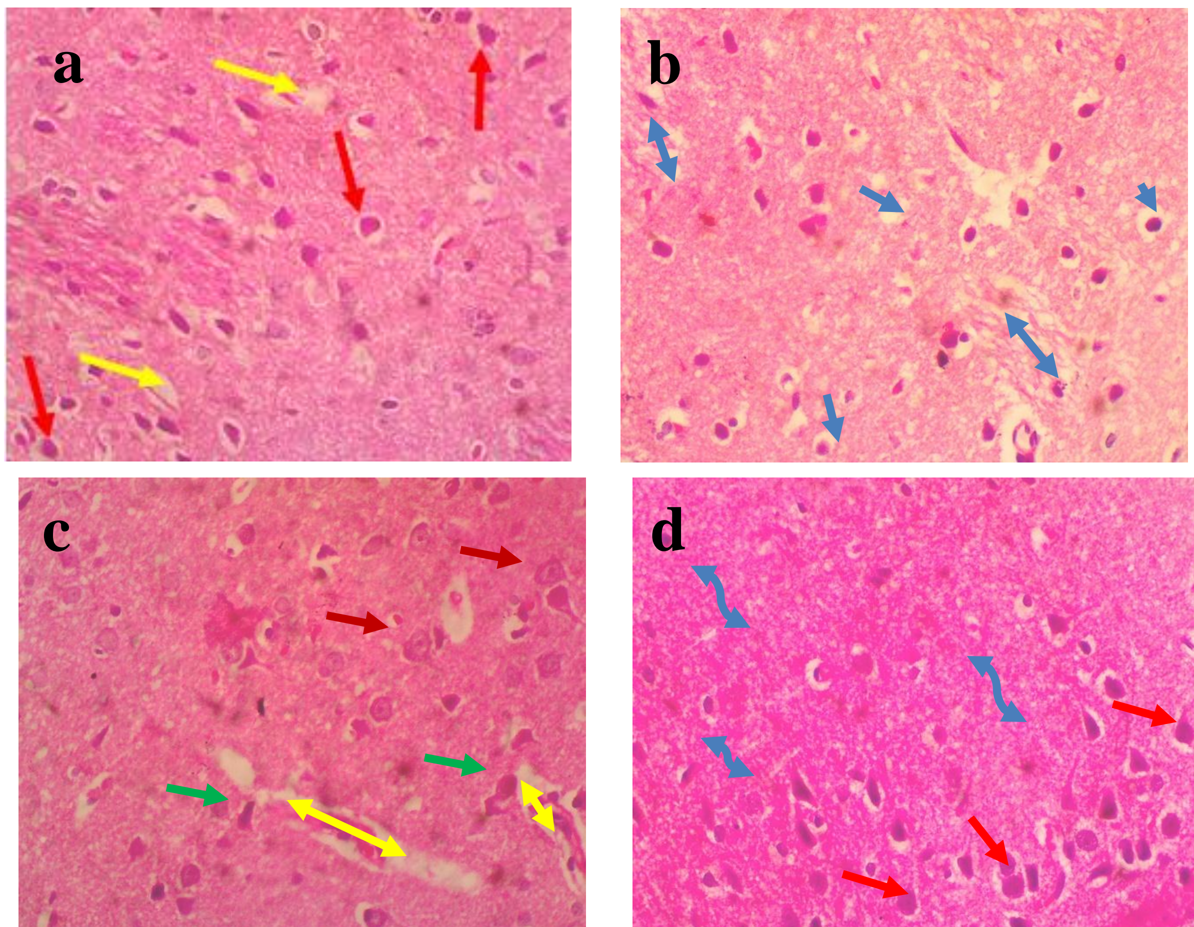

Figure 3: The photomicrographs of the experimental rats

(a) Control rat cerebral cortex showing normal pyramidal neurons (red arrows) and blood capillaries (yellow arrows), stained with H&E X400;

(b) *C. sativa* treated rats showing distorted neuropile (double blue arrow) and vacuolated neurons (blue arrows), stained with H&E X400.

(c) *C. indica* treated rats showing dilated capillaries (double yellow arrows), chromatolytic neurons (wine arrows) and degenerated neurons (green arrows),

(d) *C. indica* and *C. sativa* treated rats showing apparent hypertrophied neurons (red arrows) and micro-vacuolations (sigmoid arrows), stained with H&E X400.

DISCUSSION

This study has shown that the consumption of *Cannabis sativa*, *Cannabis indica* or the combination of the two has the potential of facilitating exhibited behaviours in albino rats. The levels of ambulation (line crossing), grooming, urination and stretched attend posture as shown by the open field test were found to be higher in the rat groups administered with *Cannabis sativa*, *Cannabis indica* and the combination (*C. sativa* + *C. indica*) than in the normal control rats. According to Bruijnzeel *et al.* (2016), the small open field has been widely used to assess the stimulant-like effects of drugs. Similarly, it has been recorded that the cannabis rats displayed an increase in locomotor activity during the first minutes of a small open field test (Bruijnzeel *et al.*, 2016). Therefore, the exhibited behaviours in the cannabis administered rats of this study

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could therefore be linked to the drug effects of cannabis on the brain.

The results obtained for the open field test could have resulted from fluctuations of the brain neurotransmitters. Although, no significant difference was observed in the levels of brain norepinephrine between the control rats and those administered with *C. sativa* and *C. indica*, rat groups administered with the combination of *C. sativa* and *C. indica* showed a significant reduction in brain norepinephrine level. Also, the rat groups administered with the cannabis plants and the combination showed significantly higher levels of dopamine than the control. The role of dopamine systems in motivated behaviour is of particular importance. It is proposed to mediate a performance activating effect of motivated behaviour, as well as conveying internal reward signals. In the central nervous system, high concentrations of dopamine are linked to love alongside attention, motivation and goal-directed behaviour (Fisher, 2000). In addition, the ability to focus, remember, cherish of a beloved indicates that dopamine is involved in this phenomenon (Fisher, 2000). Similarly, high concentrations of dopamine in the brain was associated with euphoria, loss of appetite, hyperactivity, increased mental activity, less likely to feel fatigue, the lack of need to sleep, hyperactive fear-like state, anxiety and panic (Wikiversity, 2017). As reported in Uppala *et al.* (2015), dopamine is a neurotransmitter involved in decision-making. However, low levels of dopamine or impaired dopamine function has been associated with depression in individuals (William and Michael, 2004). It is therefore possible that the low dopamine level recorded in this study for rats administered with the different species of cannabis poses significant threat on the secretion of dopamine. Hence, consumption of *Cannabis sativa* and *Cannabis indica* or the combination of both has the potential to induce depression in the individuals.

The results of this study also showed that combined consumption of *C. sativa* and *C. indica* could significantly reduce the levels of serotonin and acetylcholinesterase. The roles of serotonin and acetylcholinesterase (AChE) in the body are very vital. According to Uppala *et al.* (2015), adequate amounts of serotonin are necessary for a stable mood and to balance any excessive excitatory neurotransmitter firing in the brain. In addition to mood control, serotonin was linked with a wide variety of functions, including the regulation of sleep, pain perception, body temperature, blood pressure and hormonal activity. Similarly, acetylcholinesterase is a type B carboxylesterase that rapidly hydrolyzes the neurotransmitter acetylcholine (ACh) at brain cholinergic synapses as well as at neuromuscular junctions (Taylor and Radiac, 1994). It was also reported as a specialized enzyme whose main physiological function is hydrolysis of acetylcholine (ACh), a mediator of neuro-transduction in cholinergic synapses (Jebali *et al.*, 2013). It is transmitted within cholinergic pathways that are concentrated mainly in specific regions of the brainstem and are thought to be involved in cognitive functions, especially memory (Uppala *et al.*, 2015). The reduction in the levels of these two essential neurotransmitters in the rats subjected to combined consumption of *C. sativa* and *C. indica* is therefore an indication that combined consumption of

C. sativa and *C. indica* threatens the normal physiological functions of the neurotransmissions as well as affect the mood of individuals.

Similarly, activities of antioxidant enzymes (SOD, catalase and GPx) and concentration of non antioxidant enzyme (GSH) were significantly lower in the rat exposed to the combined consumption of *C. sativa* and *C. indica*. Antioxidant enzymes have been reported to play major primary antioxidant defence roles in catalyzing the dismutation of superoxide radical (O^{2-}) to H_2O_2 and decomposition of H_2O_2 to H_2O, respectively (Chelikani *et al.*, 2004; Goodsell, 2010). In most cases, the abnormal generation of reactive oxygen species (ROS), which results in significant damage to cell structure, is considered an important signal of oxidative damage (Barzilai and Yamamoto, 2004). Reactive oxygen species (ROS) are generated from molecular oxygen/nitrogen through the Electron Transport Chain (ETC), cytochrome P450, and other cellular and sub-cellular functions (Noori, 2012). According to Damien *et al.* (2004), reactive oxygen species (ROS) which includes the superoxide radical ($^{\bullet}O^{2}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) affect mainly lipids, proteins, carbohydrates and nucleic acid. Owagboriaye *et al.* (2016) therefore concluded that the depletion of antioxidant enzymes impairs the cell against the toxic actions of xenobiotics which could lead to cell injury or death. Hence, the lower level of these antioxidant enzymes observed in the brain of the rats administered with the combination of *C. sativa* and *C. indica* as reported in this study could predispose the brain cells to injury or death. This is also evident in the various distortions observed in the histopathological structures of the brain.

The results of this study have shown that the consumption of *C. sativa* or *C. indica* alone could induce a mild alteration to the behaviour of individuals within one week of consumption. However, consumption of *C. sativa* and *C. indica* in combination could pose a deleterious threat to the brain cells, brain neurotransmitters as well as brain antioxidant systems.

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