EFFECTS OF WHOLE *CANNABIS SATIVA* INGESTION ON BEHAVIOURAL PATTERNS AND OXIDATIVE STRESS IN MICE BRAIN TISSUES

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Received: *February 22, 2019* ***Revised:*** *March 12, 2019* ***Accepted:*** *April 11, 2019*

ABSTRACT

The unregulated habitual use of whole Cannabis sativa remains a challenge for the potential medical usefulness of the plant. As a psychoactive substance with different physiological properties, the onset and extent of its effects are often a factor of the mode of consumption. This study evaluated the neuro-behavioural effects of daily oral ingestion of C. sativa and its modulatory changes in oxidative stress parameters in mice brain tissues. Twenty-five male Swiss albino mice were separated into 5 groups of 5 animals each. Cannabis-diet were prepared from whole dried cannabis and standard mice feed. Groups I – IV, were fed with 40, 20, 10 and 1 % cannabis-diet ad libitum for 14 days, while group V animals were fed the standard mice diet ad libitum for 14 days and served as control. Neuro-behavioural activities were assessed by observing animals rearing, grooming, ambulation, head dipping and freezing times. The brain oxidative stress parameters were assayed to determine the effect of cannabis oral consumption on activity in mice brain. The animals fed with cannabis-diet displayed significantly reduced anxiety but statistically insignificant locomotory function, exploratory tendencies and neophilia, in a quantity dependent manner relative to the controls. Cannabis demonstrated both antioxidant and oxidative stress tendencies. Ingestion of whole cannabis plants may not adversely influence neuro-behavioural patterns in animals. A trade-off between oxidative stress induction and brain tissue injury repair mechanisms may have been elicited by different constituents of Cannabis. Thus, oral ingestion of cannabis may not readily cause changes in neuro-behavioural patterns.

Keywords: *Cannabis sativa,* Neuro-behaviour, Oral ingestion, Locomotory function, Oxidative stress

INTRODUCTION

Cannabis sativa is one plant of uncommon controversies arising from its medicinal potentials, economic value, socio-cultural uses and political grand standings. *Cannabis*, also known as *gbanaa* or *igbo* in Nigeria is primarily used for its psychoactive effects but also it possesses other activities (Welty *et al.,* 2014; Russo, 2016). Approximately 13.1 million people are *Cannabis* dependent globally (Degenhardt, *et al.,* 2013), and it is the most used illicit substance worldwide (ElSohly and Slade, 2005). The actual prevalence of *cannabis* use by persons remains unstable due to limited and unreliable data from most countries. However, estimated figures show a high rate (>10 % of population) of use in 19 out of 163 countries where data was available (UNODC, 2009).

The psychoactive and many other actions of *C. sativa* are majorly attributed to its constituents known as cannabinoids (Wang *et al.,* 2014). The plant contains at least 750 chemicals, among which are 104 different cannabinoids or phytocannabinoids (Radwan *et al.,* 2015) and 525 natural compounds belonging to several classes of chemicals (Russo and Marcu, 2017). Cannabinoids, including both phytocannabinoids and synthetic cannabinoids, mediate their effect through binding to specific receptors called cannabinoid receptors 1 and 2, members of the G-protein-coupled receptor super-family (Munro *et al.,* 1993). Cannabinoid-1 (CB1) receptors are found throughout the central and peripheral nervous system, their major role is to modulate neurotransmitter release, whereas the cannabinoid-2 (CB2) receptors are found mainly on immune cells and are known to play a role in immune responses and regulation of inflammatory processes (Van Sickle *et al.,* 2005).

Understanding the multiple functions of endocannabinoid signaling in the brain offers insight into the pharmacological effects of *Cannabis* and other exogenous cannabinoids, their therapeutic potential and undesirable adverse effects. The endocannabinoid signaling is crucial for brain development, and guides neural stem cell survival and proliferation, cell fate decisions and the motility and differentiation of ensuing neuronal and glial cells (Földy *et al.,* 2013). Cannabis-altered endocannabinoid signaling may contribute to neuropsychiatric diseases that are of developmental origins and where modifications to signaling have been observed; autism (Földy *et al.,* 2013), schizophrenia (Eggan *et al.,* 2010), bipolar disorder (Minocci *et al.,* 2011) and depression (Steel *et al.,* 2014). The central role of the cannabinoid system in promoting adult neurogenesis in hippocampus and the lateral ventricles provides insight into the processes underlying post-developmental neurogenesis in the mammalian brain. Both THC (Schiavon *et al.,* 2016) and CBD (Prenderville *et al.,* 2015) inhibit neurogenesis in adolescent or adult rodent brain, a process of potential relevance to a wide range of cannabis-induced adverse events (Akirav, 2011).

The endocannabinoid system has mood elevating, anti-depressant and anxiolytic effects. The anxiolytic response to *Cannabis* is biphasic, implying that *Cannabis* dosing is a critical factor in minimizing risk of anxiety, depression and maximizing benefit (Hirvonen *et al.,* 2012). *Cannabis* at high doses increases the risk for depression or anxiety possibly by down-regulating CB1 receptors (Shollenbarger, *et al.,* 2015; Choi *et al.,* 2016). The endocannabinoid system plays a complex role in regulating motor pathways, which conceivably are relevant to symptomatic relief, or to addressing the underlying pathology in a wide range of neurological diseases characterized by motor impairment (Fernández-Ruiz, 2009). CB1 receptors are abundant in brain regions that regulate motor function and coordination, including the basal ganglia and cerebellum. CB1 receptors are down-regulated in several neurological conditions (Youssef and Irving, 2012).

Though, whole *C. sativa* has also been used for the treatment of numerous diseases, its current medicinal usage is highly restricted due to its psychoactive properties (Marchalant *et al.,* 2009). In the last decade, cannabinoids have emerged as putative modulators of the central nervous system (CNS) immune and plastic events as well as behavioural and cognitive functions (Takeda *et al.,* 1998;

Campos *et al.,* 2012; Kaplan, 2013). The unregulated habitual use of whole *C. sativa* remains a challenge for the potential usefulness of the plant in medical practice (Akinola *et al.,* 2018).

This study was designed to evaluate the neuro-behavioural effects of prolonged ingestion of dried whole *C. sativa* in a murine model using the exploratory and locomotory behaviour in an attempt to decipher the short/medium term physiological effect of *Cannabis* use.

MATERIALS AND METHODS

Ethical Approval: This study was conducted at the Pharmacology and Therapeutics Laboratory, University of Ilorin, Ilorin. Ethical approval for the study was obtained from the University of Ilorin Ethical Review Committee (UERC/ASN/ 2017/857).

Animal and Experimental Design: Twenty-five male Swiss albino mice (average weight of 24 ± 1.05 g) obtained from the animal facility of the Malaria Research Laboratories, Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Ibadan, were used for the experiment. The experimental design used was complete randomized block design comprising five treatments replicated five times with five animals in each group and each animal representing a replicate. The Groups I – V, were fed with 40, 20, 10, 1 and 0 % cannabis-diet *ad libitum* for 14 days. Group V animals were fed the standard mice diet without cannabis for 14 days and serve as control group. The mice were used in accordance with the Institute for Laboratory Animal Research (ILAR) guide for the care and use of laboratory animals (Clark *et al*., 1997).

Plant: Dried leaves, twigs and seeds of the *C. sativa* plant were obtained from the National Drug Law Enforcement Agency (NDLEA), Kwara State Command, Nigeria. Characterization and validation of the plant was done by evidence specialist at NDLEA, Kwara State Command.

Cannabis-Diet: The cannabis-diets were prepared based on weighted percentages of dried *C. sativa* (leaves, twigs and seeds) and standard mice feed. A percentage weighed portion of milled *C. sativa* was mixed with milled mice feed at 40, 20, 10, 1 and 0 % levels of cannabis contents corresponding to Groups I – V respectively. The cannabis plus standard mice feed was pelletized to cylindrical shape sized pellet for mice and labelled cannabis-diet. The diets were presented as substitutes for food. All animals were fed *ad libitum* with corresponding diet and allowed unrestricted access to drinking water. Daily food intake of animals was monitored to evaluate consumption rates across the groups.

Animals Body Weight: The body weight of the 25 animals were weighed at the beginning of the study, and then weighed on day 7, 14 and 21 using Mettler Top Loading Electronic Balance to the nearest 0.01 g.

Determination of Blood Glucose Levels: As the major energy biofuel in the body, the fasting blood glucose level was measured in all study animals on the morning of day 15 after overnight fasting, using a glucometer.

Behavioural Test

All experimental animals were allowed to feed *ad libitum* for 14 days, after which the different behavioural tests were conducted. The neuro-behavioural tests conducted were hole board maze test, open field maze test and light and dark box test using the methods of Rice *et al.* (1981) and Okon *et al*. (2014).

Hole board maze test: The animals were placed into the hole board maze one after the other and allowed to explore for 5 minutes each. The behaviours of the animals in the maze were observed and video recorded. Each animal was placed carefully in the centre of the box, and after the time elapsed, the animal was removed from the cage and the hole board maze cleaned with 70 % ethanol and allowed to dry before another animal was placed in it. This was done to eliminate olfactory stimuli. The Hole Board Maze measures neophilia, anxiety and general locomotory behaviour of test

animals. The behavioural scores observed were head dipping, rearing and grooming (Okon *et al.,* 2014).

Open field maze test: The animals were placed one after the other in the open field maze and allowed to explore for 5 minutes each. Each animal was taken from its home cage and placed in the center square of the maze. The mouse behaviour was observed and video recorded and the animal returned to its home cage. The open field maze is used to measure locomotory, exploratory and anxiety behaviour in animals due to its large center arena using the following behavioural scores; centre freezing time, ambulation and rearing. A high frequency of this behaviour indicates a higher level of anxiety (Okon *et al.,* 2014).

Light and dark box test: The animals were placed one after the other into the transition box and allowed to explore for 5 minutes each. Each animal was placed carefully in the centre of the white compartment facing away from the door. At the end of the 5 minutes, the animal was returned to its home cage and the box was wiped down using cotton wool dipped in 70 % ethanol, and left to dry before another animal was introduced into the box. The behavioural scores used were as follows: dark box duration, light box duration, rearing and stretch (Rice *et al.,* 1981).

Brian Tissues Antioxidant Enzymes Activity Determination

The right hemispheres of the brain tissues was excised and preserved in 30 % sucrose and stored on ice. 10 % homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogenizer at 40 ºC. The homogenate was centrifuged at 5,000 rpm for 20 minutes to remove the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of superoxide dismutase, glutathione peroxidase, Glutathione S-transferase and malonaldehyde.

Superoxide dismutase (SOD): Superoxide dismutase activity was determined according to the method of McCord and Fridovich (1969). Briefly, 0.01 ml of the brain homogenate was mixed with 0.2 ml of 0.1 M EDTA containing 0.0015 % NaCN, 0.1 ml of 1.5 mM NBT and phosphate buffer with pH 7.8 to a total volume of 2.6 ml. On adding 0.05 ml of riboflavin, the absorbance of the solution was measured against distilled water at 560 nm. All the tubes were illuminated uniformly for 15 minutes and absorbance of the blue color formed was measured again. Percent of inhibition was calculated after comparing absorbance of sample to the absorbance of control (the tube containing no enzyme activity). The volume of the sample required to scavenge 50 % of the generated superoxide anion was considered as 1 unit of enzyme activity and expressed in U/L protein.

Glutathione peroxidase (GPx): Glutathione peroxidase activity was determined according to the method of Hafeman *et al.* (1974). The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 minutes at 37 ^{o}C against distilled water.

Glutathione S-transferase (GST): Glutathione S-transferase activity was assessed by the method of Habig *et al.* (1974) activity of the enzyme was determined by observing the change in absorbance at 340 nm. The reaction mixture contained 0.1 ml of GSH, 0.1 ml of CDNB and phosphate buffer in a total volume of 2.9 ml. The reaction was initiated by the addition of 0.1 ml of the enzyme extract. GST activity was calculated using the extinction co-efficient of the product formed (9.6 mM−1 cm−1) and was expressed as n moles of CDNB conjugated/minute.

Lipid peroxidation: The level of lipid peroxidation in the tissue was measured as malondialdehyde (MDA) according to the method of Ohkawa *et al.* (1979). Absorbance of the clear supernatant was measured at 532 nm against butanol: pyridine mixture. The MDA level was calculated and is expressed in µmol/L.

Statistical Analysis: Data obtained for the different sets of tests were analysed with

GraphPad Prism 6.05, using Analysis of Variance (ANOVA). P<0.05 was considered to be statistically significant.

RESULTS

Body Weight: There was a quantity dependent decline in the body weight of animals fed with 40, 20, 10 1 and 0 % cannabis-diet respectively over a period of 14 days. There were significant (p≤0.05) decreases in body weight of animals in the 40, 20 and 10 % cannabis-diet groups relative to animals in the control group. There was no significant difference in body weight of animals fed with 1 % cannabis-diet relative to the control group animals which ate standard mice diet with no cannabis content. Figure 1 presents the progressive decline in body weight of animals in all experimental groups.

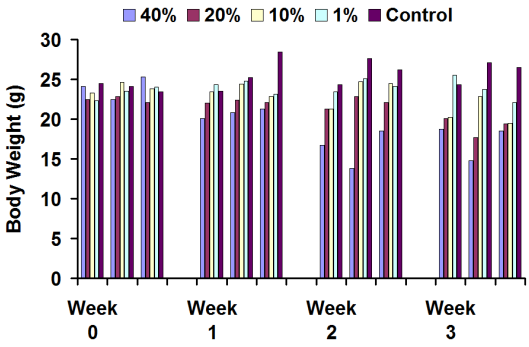

Figure 1: Body weight of animals that consumed 40, 20, 10, 1 and 0 % cannabis-diet for 3 weeks *ad libitum*

Blood Glucose Levels: Data obtained showed sequence of increase in blood glucose level in the mice fed cannabis-diet compared to the control group. Animals fed with 20 and 1% cannabis-diet had significantly increased blood glucose levels when compared to the control animals. The pattern of changes in blood glucose levels between day 0 and day 15 is presented (Figure 2).

Behavioural Test

Hole board maze: There was no difference in the number of times of head dipping across the cannabis-diet groups in comparison to the control group.

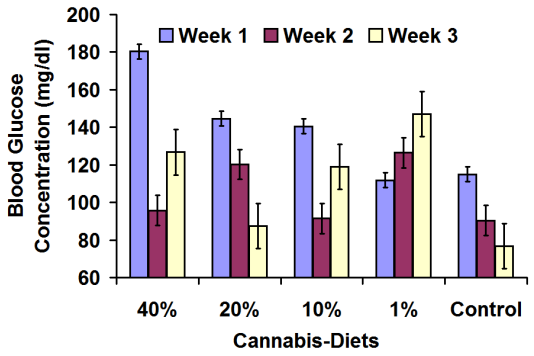

Figure 2: Mean blood glucose levels of animals that fed 40, 20, 10, 1 and 0 % cannabis-diet *ad libitum* for 14 days

There was no statistically significant difference (p≥0.05) in the number of head dipping for the test groups compared to the control group. The number of rearing times for animals in the 40, 20 and 10 % cannabis-diet fed groups increased progressively, though there were no significant differences (p≥0.05) when compared to the control group. However, the number of rearing times for animals in the 1 % cannabis-diet was significantly increased (p<0.05) compared to the control animals with a mean value of 16.75 ± 2.17 compared with 27.75 ± 3.71. The number of times animals groomed themselves in the 40, 10, 20, 1 and 0 % cannabis-diet fed groups were not statistically significant (p≥0.05) compared to the control group. The mean values of number of times of animal grooming, rearing and head dipping in the different groups is presented in Table 1.

Open field maze: The central freezing time, number of line crossing, rearing, and grooming of animals that were fed 40, 20, 10, 1 and 0 % cannabis-diet showed no statistical significance (p≥0.05) when compared to the control (Table 2).

Light and dark box: There was a regressive decrease in the total time spent in the black box for all the animals in the test groups as the percentage cannabis-diet consumed increases from 1 to 40 %. Though only 40 % (103.00 ± 27.08) and 10 % (114.75 ± 14.50) cannabis-diet fed groups showed statistical significance difference (p≤0.05) relative to the control group (179.25 ± 12.79).

Table 1: Behavioural responses from the hole board maze test mice that consumed cannabis-diet *ad libitum* for 14 days

Groups	Head Dipping (Number of times/5 min.)	Rearing (Frequency/5 min.)	Grooming (Frequency/5 min.)
40 %*C. sativa*	28.75 ± 6.54^{c}	19.50 ± 5.62^{b}	2.75 ± 0.48^{c}
20 % *C. sativa*	24.25 ± 5.84^{b}	20.75 ± 4.37^{c}	3.75 ± 0.63^{e}
10 %*C. sativa*	24.00 ± 3.83^{b}	27.00 ± 2.38^{d}	2.50 ± 0.65^{b}
1 %*C. sativa*	17.75 ± 2.02^{a}	16.75 ± 2.17^{a}	2.00 ± 0.00^{a}
Control	23.50 ± 1.76^{b}	27.75 ± 3.71^{e}	3.00 ± 1.47^{d}

Mean ± standard deviation, C. sativa - Cannabis sativa, Means with different letters along a column are significantly different (p<0.05), Min – minutes, Sec. - Seconds

Table 2: **Behavioural responses from the open field maze test mice that consumed cannabis-diet *ad libitum* for 14 days**

Groups	Central freezing time (Sec.)	Ambulation (Number of times of line crossing /5 min.)	Rearing (Frequency /5 min.)	Grooming (Frequency /5 min.)
40 % *C. sativa*	1.00 ± 0.41^{d}	104.75 ± 9.23^{c}	13.00 ± 3.24^{b}	1.00 ± 0.41^{c}
20 % *C. sativa*	1.50 ± 0.96^{d}	89.75 ± 5.50^{a}	12.25 ± 1.11^{a}	0.00 ± 0.00^{a}
10 % *C. sativa*	0.00 ± 0.00^{a}	130.00 ± 16.79^{e}	24.00 ± 1.23^{e}	0.00 ± 0.00^{a}
1 % *C. sativa*	0.50 ± 0.50^{c}	109.00 ± 11.42^{d}	18.25 ± 3.97^{c}	1.00 ± 0.41^{c}
Control	0.25 ± 0.25^{b}	90.25 ± 3.54^{b}	20.75 ± 4.63^{d}	0.75 ± 0.48^{b}

Mean ± standard deviation, C. sativa - Cannabis sativa, Means with different letters along a column are significantly different (p<0.05), Min – minutes, Sec. - Seconds

Table 3: Behavioural responses from the light and dark transition box of mice that consumed cannabis-diet *ad libitum* for 14 days

Groups	Total time spent in black box (Sec.)	Total time spent in white box (Sec.)	Total entry into black box (Number of times/5 min.)	Total entry into white box (Number of times/5 min.)	Stretch (Frequency/5 min.)
40 % *C. sativa*	103.00 ± 27.08^{a}	196.50 ± 31.15^{e}	7.75 ± 1.11^{c}	6.75 ± 1.49^{b}	6.75 ± 0.63^{d}
20 % *C. sativa*	107.75 ± 49.39^{b}	192.50 ± 46.63^{d}	6.75 ± 1.70^{a}	5.75 ± 1.60^{a}	6.00 ± 2.12^{c}
10 % *C. sativa*	114.75 ± 14.50^{c}	183.00 ± 15.29^{c}	7.75 ± 0.95^{b}	7.250 ± 0.85^{c}	5.75 ± 1.75^{b}
1 % *C. sativa*	146.00 ± 16.99^{d}	152.00 ± 24.50^{b}	11.25 ± 2.14^{d}	10.25 ± 2.13^{d}	8.25 ± 2.90^{e}
Control	179.25 ± 12.79^{e}	109.00 ± 10.12^{a}	13.25 ± 2.21^{e}	10.75 ± 2.63^{e}	5.75 ± 0.48^{a}

Mean ± standard deviation, C. sativa - Cannabis sativa, Means with different letters along a column are significantly different (p<0.05), Min. – minutes, Sec. - Seconds

The 40 % (196.50 ± 31.15) and 10 % (183.00 ± 15.29) cannabis-diet fed groups also showed significant increase (p≤0.05) in the total time spent in the white box compared to the control group (109.00 ± 10.12) (Table 3). The entry times into the black and white boxes for the animals that consumed 40, 20, 10 and 1 % cannabis-diet showed no statistical significance (p≥0.05) when compared to the control group. Also, the number of times the test animals stretched into the white box and retracted back into the black box was used as a behavioural mark for fear; stretch in test animals showed no statistical significance (p≥0.05) when compared to the control group. The mean of behavioural observations for the light and dark transition box is presented in Table 3.

Brain Tissues Antioxidant Enzymes

Glutathione peroxidase (GPX): The glutathione peroxidase activities of brain homogenate in the groups that consumed 40, 20 and 10 % cannabis-diet fed groups were not

significantly different ($p \geq 0.05$) when compared to the control. The 1 % cannabis-diet fed group showed a significant increase when compared to the control group, with a mean value of 12.50 ± 0.0 and 5.556 ± 1.389 for 1 % cannabis-diet and control respectively. Figure 3 shows the GPX activities for all experimental groups.

Superoxide dismutase (SOD): The superoxide dismutase activity in the brain homogenates of the groups of animals that consumed 40, 20, 10 and 1 % cannabis-diet showed statistically significant decreases ($p \leq 0.05$) when compared with the control group. Figure 4 presents the differences in SOD activity in studied animals.

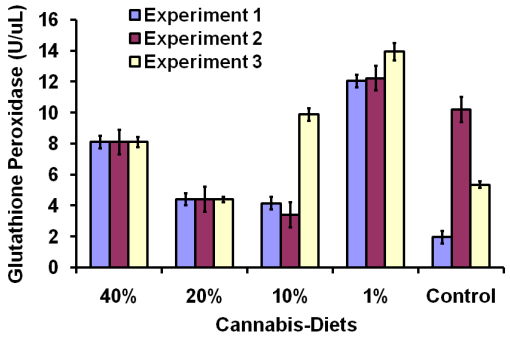

Figure 3: Glutathione peroxidase activities in mice that consumed cannabis-diet a*d libitum* for 14 days

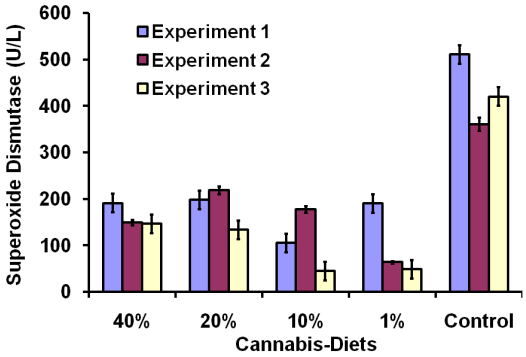

Figure 4: Superoxide dismutase activity of mice fed cannabis-diet feed *ad libitum* for 14 days

Malondialdehyde (MDA): The malondialdehyde levels in brain tissue homogenate from animals that were fed 40, 20, 10 and 1 % cannabis-diet were significantly reduced compared to the control animals ($p < 0.05$). Figure 5 shows the levels of MDA in experimental animals.

DISCUSSION

Cannabis sativa is a known psychoactive compound which has been used to ameliorate a wide variety of ailments (Welty *et al.,* 2014). In the present study, functional changes in mood, explorative behaviours and motor function pertinent to movement were investigated in

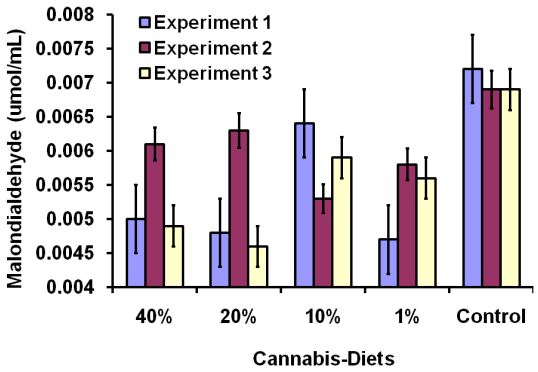

Figure 5: **Malondialdehyde levels in mice fed cannabis-diet *ad libitum* for 14 days**

varied percentages of cannabis-diet fed mice using different paradigms. Increased ambulation and exploratory activities as well as decreased immobilisation on paradigms such as open field maze, hole board maze and white and black box indicated intact motor system and low anxiety. While decrease in activities suggest an expression of anxiogenic state (Rice *et al.,* 1981). Rodents display rearing behaviour by standing on their hind limbs with the forelimbs raised into the air or on the wall of the maze. This behaviour allows the animals to evaluate the potential danger of the immediate space and how to find an escape-route. Increase in this behaviour indicated fear, agitation and anxiety to leave the maze. cannabis-diet decreased rearing behaviour in all groups fed except for group fed low quantity cannabis-diet (1 % cannabis-diet). This may suggest that increased cannabis consumption may reduce anxiety, even though the association between rearing behaviour and anxiety remains controvertible (Seibenhener and Wooten, 2015).

Cannabis-diet fed groups displayed slightly higher locomotor/exploratory activity which is consistent with low levels of anxiety. Our observation was in contrast with the findings of Okon *et al.* (2014) which reported a dose-dependent decrease in locomotion and exploratory behaviour.

Findings in the light/dark transition box showed that cannabis-diet fed mice demonstrated a striking and strong affinity for well illuminated open spaces suggesting that the *C. sativa* has anxiolytic effect. Rodents naturally associate more with dark spaces as it offers hiding spots and safety. Increased attraction to dark space than light space indicates anxiogenic condition. In this study, the cannabis-treated mice had exploratory activity not significantly different from the control, which is in contrast with a previous report (Harte-Hargrove and Dow-Edwards 2012). Although this difference may be as a result of metabolic processes and constituents of *Cannabis* released *in vivo*, as animals in the study were fed whole *C. sativa* and not extracts of the plant. It may also be as a result of the duration of administration of the *C. sativa*. The observation from this study is however in tandem with previous reports (D'Souza *et al.,* 2004), that tetrahydrocannabinol (THC), a psychoactive constituent of *C. sativa* caused significant dose-dependent locomotor, depression and tetrahydrocannabinol (THC) increased anxiety (Genn *et al.,* 2004). The low level of anxiety exhibited by the test groups agreed with the report of Grotenhermen (2004) that *Cannabis* or THC consumption causes a relaxing experience with occasional anxiety, and adversely affects psychomotor and cognitive functions. Also, cannabidiol components of *Cannabis* have been reported to have little or no effect on locomotor activity nor induce motor changes (Riedel *et al.,* 2009).

Locomotion or activity in behavioural paradigms is influenced by the physiological function of the animal's brain. Glucose is important in this brain function and its level is about 15 – 20 % of blood glucose level (Levin, 2000; De Vries *et al.,* 2003). Thus, rise or fall in blood glucose levels alters glucose availability in the brain. Further studies revealed that certain behaviours and conditions are glucose-dependent. Glucose-derived brain energy metabolism is critical or perturbed in apoptosis (Danial *et al.,* 2003), cognition (Scholey *et al.,* 2009), self-control (Baumeister, 2012), neurodegenerative (Lee *et al.,* 2012) disease and other conditions. Interestingly, behavioural indices in the present study did not show any remarkable association with fasting blood glucose levels even in 1 and 20 % cannabis-diet fed mice where significant levels of fasting blood glucose were recorded relative to the control. This study however showed that whole *cannabis* consumption may increase fasting blood glucose as against smoking that has no significant effects on fasting blood glucose (Muniyappa *et al.,* 2013) or *C. sativa* extract that lowers blood glucose level (Levendal and Frost, 2006).

Though there were no significant changes in the levels of activity of glutathione peroxidase in the treatment groups except in the 1 % cannabis-diet, where the increases observed may mediate a reduction in oxidative stress in mice brain due to the effects of *Cannabis* or its constituents. This may be possible by the initiation of repair to traumatically injured brain tissues as previously demonstrated in transgenic mice (Tsuru-Aoyagi *et al.,* 2009). In contrast, the levels of superoxide dismutase in animals that consumed *Cannabis* were significantly lower relative to control mice. The significant loss of superoxide dismutase activity is a manifestation of oxidative damage of the brain (Wang *et al.,* 2018). Likewise, there was a dose-dependent decline in the levels of malondialdehyde in the cannabis-diet groups, another indication of the oxidative damage potential of *Cannabis* consumed orally in this study. Interestingly, the changes recorded in the different antioxidant biomarkers assayed in brain tissues of the study animals did not alter to any noticeable extent the behaviour of the mice. The locomotor function and aggressive behaviour of the test animals were moderate relative to the controls in this study. We hypothesize that this may be as a result of the trade-offs often associated with the use of whole *Cannabis*, where the different constituents of *Cannabis* may mediate different phenotypes by binding to the CB1 and CB2

receptors in the brain and the periphery. This assertion is also driven by the regulatory actions of the cannabinoid receptors in autonomic signalling.

Conclusion: Ingestion of whole *Cannabis* plants may not adversely influence neuro-behavioural patterns in mice. A trade-off between the generation of oxidative radicals and oxidative defence mechanisms in the brain may have been elicited by different constituents of *Cannabis*. There were no correlations between the mild changes in behavioural patterns and oxidative stress differentials in mice that consumed *Cannabis* within study period.

ACKNOWLEDGEMENTS

The authors acknowledge the National Drug Law Enforcement Agency (NDLEA) Headquarters, Abuja and NDLEA, Kwara State Command, for the provision and validation of dried *Cannabis sativa* plant. Special thanks to Mrs. S. Braimoh, Mrs. F. O. Oni and Mr. A. O. Adesua for technical assistance.

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