Disrupted BRAIN OXIDATIVE STRESS MARKERS AND Possible AMNESTIC TENDENCY Traceable to cannabis exposure IN ALBINO WISTAR RATS

ABSTRACT

The uncontrolled repeated use of Cannabis sativa remains a challenge for the potential medical usefulness of the plant. Cannabis being a psychoactive substance with different physiological properties, the onset and extent of its effects are often a factor of the mode of consumption. This present study is aimed at investigating the effects of daily oral ingestion of C. sativa on memory and its amnestic tendencies in wistar rats. Twenty-five albino wistar rats were acclimated to laboratory condition for fourteen days, following which they were separated into 5 groups of 5 animals each. Group I were used as control receiving only distilled water orally, Group II-IV were administered with 0.1ml, 0.2ml and 0.3ml cannabis via oral route respectively for 21days, while group V animals were administered with epinephrine. Cognitive activities were assessed using passive avoidance test, barnes maze test and navigation maze test. The brain oxidative stress markers that were used to determine stress activities include Superoxide dismutase, catalase, reduced glutathione and malondialdehyde'. Results gotten were analyzed and some activities were statically significant ($p< 0.05$) in comparison to the control group. For instance, Cannabis significantly decreased the activity of Superoxide dismutase, reduced glutathione and malondialdehyde but caused an increase in catalase activity when compared to the control. It was also observed that the animals that were administered with cannabis displayed significantly reduced amnesia as the study progressed, increased better stress management and positive enhancement in memory compared to the control. In conclusion, it can be deduced from the result that cannabis demonstrated significantly, ($p<0.05$) cellular re-alignment and rejuvenation in terms of oxidative stress markers activities and potent memory retrieval as the period of administration progressed.

Kewywords: Cannabis sativa, memory, superoxide dismutase, catalase, glutathione, malondehydehde.

Introduction

According to Maurga (2004) [1] some plants have been noted to have medicinal value and has been used worldwide since ancient times in folkloric medicine for treatment of various human ailments and is still prevalent in developing countries till date. This use of medicinal plants in the treatment of diseases and dysfunctions which goes back to several millennia, has contributed immensely to the development of pharmaceuticals since about 25% of modern drugs are derived from plants [2] .As stated by the World Health Organisation (WHO), about 80% of the world

populations (over 4 billion) today depend on plant-based medicine for their health care needs [3]. as several plants contain active principles that have been proven to be beneficial through extensive laboratory tests and repeated clinical trials [4]. Hence, they have been extensively used in production of synthetic drugs as about 25% of active compounds in synthetic drugs currently prescribed were first identified in plant sources. Some plants have also been said to have some neuroactive effects on human. One of the most widely known plant with a long history of use both as a medicinal agent and intoxicant is the *Cannabis sativa* L. [5]. It is also known as marijuana in America and hashish in the Middle East [6]. It is an annual herbaceous flowering plant indigenous to eastern Asia but now of cosmopolitan distribution due to widespread cultivation [5]. It has been cultivated throughout recorded history, used as a source of industrial fiber, seed oil, food, recreation, religious and spiritual moods and medicine [7][8] [9][10]. However, the species *C. sativa* is generally consumed for its psychotropic effects and is seen as the most widely used illicit medicinal plant with an estimated number of 119–224 million users worldwide [11][12]). It is majorly abused by adolescence and young adults all over the globe [5] in their various preparations such as pot, cannabis, grass, weed, hemp and joint [13].

Though Cannabis has several effects on multiple organ systems, its effect is mostly exerted on the CNS as a psychoactive agent due to presence of its main psychoactive ingredient, Tetrahydrocannabinol (THC), which is responsible for most, if not all, of the effects associated with the use of cannabis [14] [15]). In the CNS, the effects of cannabis are directly and irrefutably evident in suppressing neurons in the information-processing system of the hippocampus in the two areas of motor control and memory [16] while its THC constituent have been found to be associated with a number of other neuronal systems, including those involved in motor control, sensorimotor learning and memory filter mechanisms)[17]. Recent studies as

reported by [16] shows that studies in humans and animals have indicated that THC and other marijuana-related cannabinoids (CBD) interfere with the brain's chemical balance by acting on cannabinoid receptors which are found on neurons in many places in the brain.

The brain is one of the most metabolically active tissues and is particularly sensitive to toxicity especially those associated with neurotoxic effects of drugs of abuse which is commonly associated with oxidative stress, mitochondrial dysfunction, apoptosis and inhibition of neurogenesis, in addition to other mechanisms 18]. Oxidative stress from oxidative metabolism cause disruptions in normal mechanisms of cellular signaling [19], base damage, strand breaks in DNA [20] as well as linked to certain cardiovascular diseases, chronic fatigue syndrome and hyperoxia [21], and suspected to be important in neuro-degenerative diseases such as Lou Gehrig's disease, Parkinson's disease, Alzheimer's disease, Huntington's disease, depression, and multiple sclerosis [22]. Hence, as a critical phase for cerebral development, exposure to addictive substances during the adolescence phase of life leads to various alterations in brain functions that can be translated into functional consequences throughout life [23]. In recent years, there has been noticeable increase in cannabis and its products consumption among teenagers and young adults [24]. Though cannabis is broadly supposed to be a safe recreational drug, its use is increasing among adolescents, which upon repeated exposure may develop some unfavourable effects on the brain's functional connectivity, intelligence, and cognitive performance [25]. Hence, the need to investigate the effect of cannabis in inducing oxidative stress and amnestic tendency.

Agents of abuse have been demonstrated to exert detrimental impact upon social, psychological and cognitive behaviour in individual users, thereby affecting their personality [26]. Recently,

Cannabis abuse has been in the increase despite all the convincing neuropsychological effects and has become a source of concern globally affecting almost every nation for decades. The United Nations has found that cannabis is the most used illicit drug in the world and medical use of marijuana is also on the increase being legalized in some countries [27]. Also, the number of illicit drug consumption has unfortunately increased as it has been estimated that 255 million people used illicit drugs, such as cannabis, amphetamines, opioids, and cocaine, in 2015 and this translates into an annual prevalence of illicit drug use of 5.3%.

The regular use of cannabis is of great concern since it is associated with an increased possibility of deleterious consequences [28]. This is coming at the back of several evidences that shows that exposure to *cannabis* can lead to health challenges such as; motor skills impairment [29][26].

2. MATERIALS AND METHODS

Experimental Design

A total of twenty five albino wistar rats were weighed and divided into five groups of five animals in each group

Table 1: Treatment Details

Groups	Number of Animals	Treatment

Group I (control)	5	Feed + water ad libitum
Group II cannabis	5	Feed + water ad libitum + 0.1ml/100g rat
Group III cannabis	5	Feed + water ad libitum + 0.2ml/100g rat
Group IV cannabis	5	Feed + water ad libitum + 0.3ml/100g rat
Group V	5	Drug Epinephrine- treated (0.1ml/100g)

Immediately after which they went through neuro-cognitive behavioural test beginning with passive avoidance test, then to Barnes maze and finally navigational task in no particular order every experimental day. At the end of three weeks, using a random sampling method two rats from each group were sacrificed and the test for the oxidative stress markers (SOD, MDA, GSH and CAT) was done.

Blood sample preparation

After the animals were sacrificed and blood collected from the randomly selected test animals, the blood samples were centrifuged for 10 minutes at 4^{o}c.

Brain Oxidative Stress Marker Determination

Measurement of MDA

Malondialdehyde (MDA) results from degradation of polyunsaturated lipids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to form a 1:2 MDA-TBA adduct, which absorbs at 532 nm. Thus, the quantity of TBARS is proportionate to the amount of MDA. The concentration of TBARS is determined according to a method of Mihara and Uchiyama. The concentration of TBARS was calculated using the MDA standard curve and was expressed as nmol/mg of protein [20].

Estimation of GSH

The GSH level was measured by the method of Beutler et al. (1963) [21]. Briefly, 0.1 ml of sample was added to 0.9 ml distilled water and 1.5 ml of precipitating reagent (3.34 g meta-phosphoric acid, 0.4 g EDTA and 60.0 g sodium chloride). Tubes were shaken and allowed to stand for 5 min at the room temperature (25 ± 1 °C). The mixture was centrifuged for 15 min at 4000 RPM at 4 °C. In the 1.0 ml supernatant, 4.0 ml of phosphate solution (0.3 M disodium hydrogen phosphate) and 0.5 ml 5–50-dithiobis-(2-nitrobenzoic acid) (DTNB) (80 mg in 1% sodium citrate) were added. The development of yellow color complex was read immediately at 412 nm on a spectrophotometer. A standard curve using the GSH level was prepared and GSH concentration in the experimental samples was extrapolated from the standard curve. The GSH concentration was calculated and expressed as μmol of GSH/mg protein.

Measurements of enzymes

The activity of SOD was determined by the method of Marklund and Marklund 1979 [22], using inhibition of pyrogallol autoxidation at pH 8. The specific activity of SOD is expressed as units

per mg protein per minute. The activity of GPx was measured by the method of Paglia and Valentine [23]. GPx catalysis the oxidation of glutathione by Cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance is measured at 340 nm. GR catalyzes the reduction of glutathione in the presence of NADPH, which is oxidized to NADP. The decrease in absorbance is measured at 340 nm. The levels of GPx and GR were expressed as U/mg protein. The CAT activity was assayed by H2O2 consumption, following Aebi's method [24] and modified by Pieper et al. (1995) [25].

Cognitive Tests

Passive avoidance test

The testing apparatus is a trough-shaped alloy divided into two distinct compartments with an opening door. The white, brightly lit compartment is free of aversive stimulation whereas the black, dark compartment is equipped with shock capability. Its measures the basic ability to learn and remember the presence and place of a shock stimulation. In accordance with the guidelines of the American psychological association, the shock intensity used in this task should be the minimal amount needed to motivate the animal. However, no aversive stimulus applied to animals upon re-entry into the dark compartment during testing.

Barnes maze test

The Barnes maze is a behavioural test that was originally developed to study spatial learning and memory in rats (Barnes, 1979). It is a hippocampal-dependent task where animals learn the relationship between distal cues (place learning) in the surrounding environment and a fixed

escape location (Williams, 2003). The typical Barnes maze setup consists of an elevated circular platform with evenly-spaced holes around the perimeter. An escape tunnel is mounted underneath one hole while the remaining holes are left empty. Rats find bright light and open spaces aversive and would therefore want to escape to somewhere dark. The escape tunnel is maintained at a fixed location for the duration of training, which involves multiple daily trials spread over several days. The time it takes to escape into the dark hole is then recorded.

Navigation maze test

The navigation maze test is used to examine spatial learning and memory. It is used in assessment of exploration, path planning and navigation which depends on learning and memory capacities to form cognitive maps. It is used to test the effects of lesion to the brain in areas concerned with memory.

Statistical Analysis

The quantitative data were presented in charts while qualitative data were represented in tables. Data obtained for the different sets of tests were analysed using Analysis of Variance (ANOVA) and Hoc test, $P<0.05$ was considered to be statistically significant. The analysis was performed using statistical package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

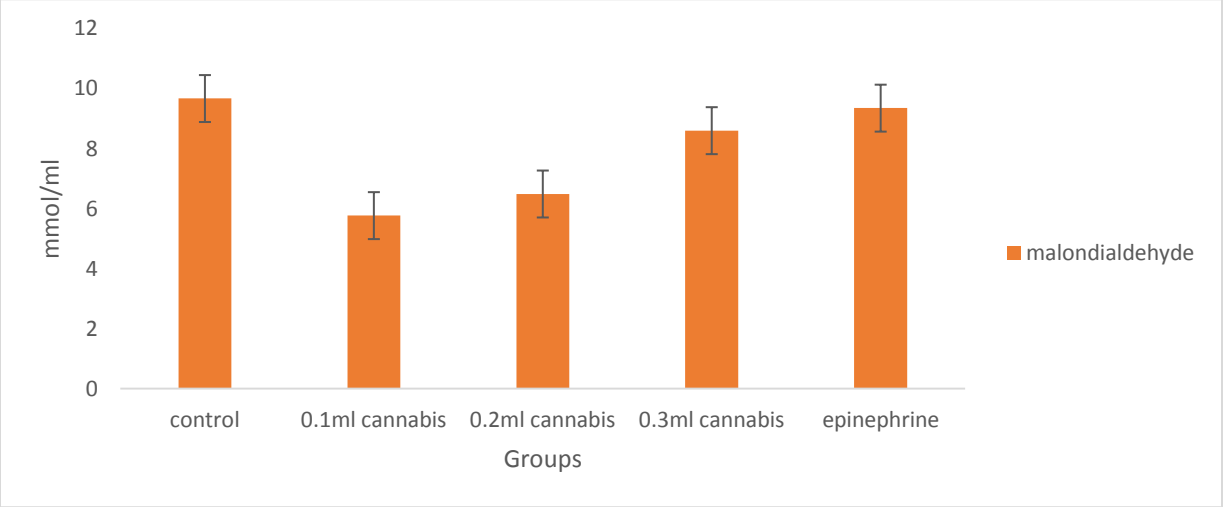

Figure 1 Pattern of response of malondialdehyde after 21 days treatment with cannabis sativa

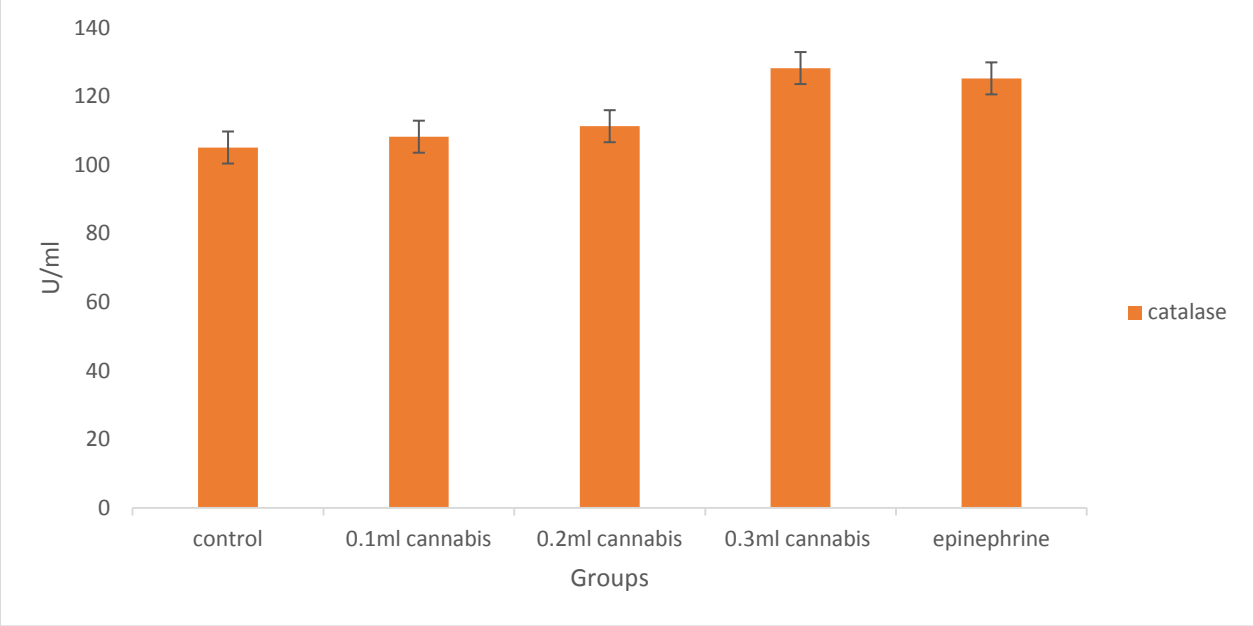

Figure 2 Pattern of response of catalase after 21 days treatment with cannabis sativa.

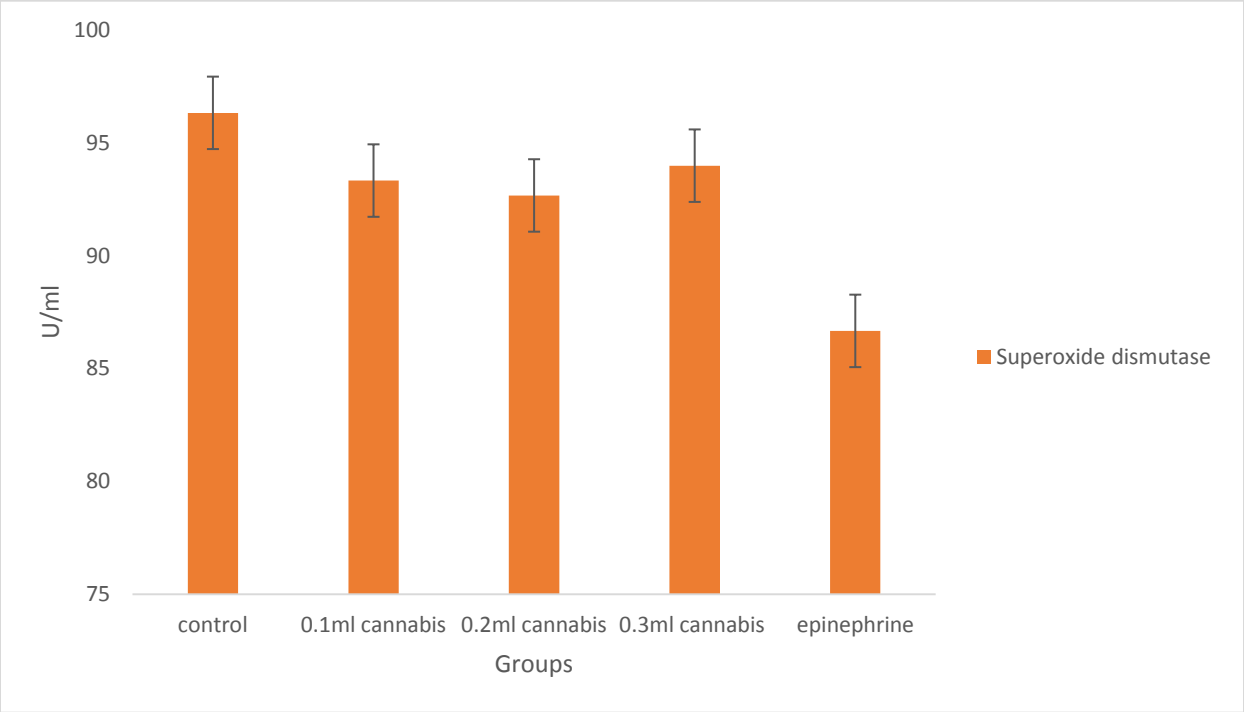

Figure 3 Pattern of response of superoxide dismutase after 21 days treatment with cannabis sativa.

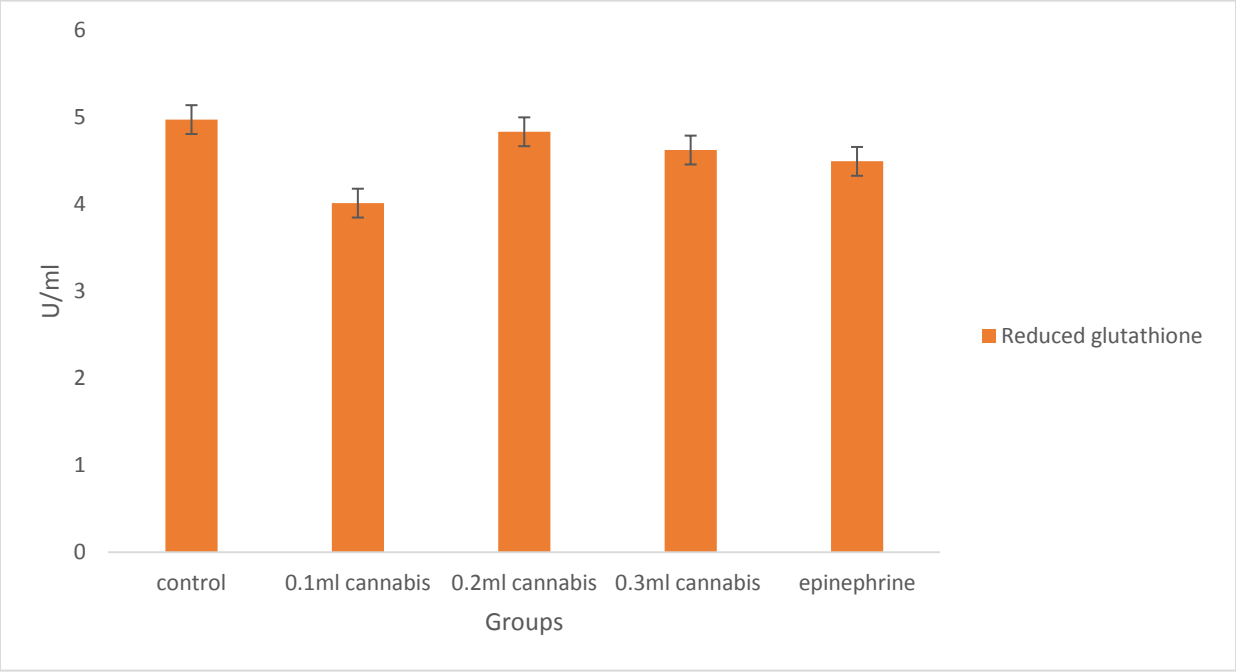

Figure 4 Pattern of response of reduced glutathione after 21 days treatment with cannabis sativa.

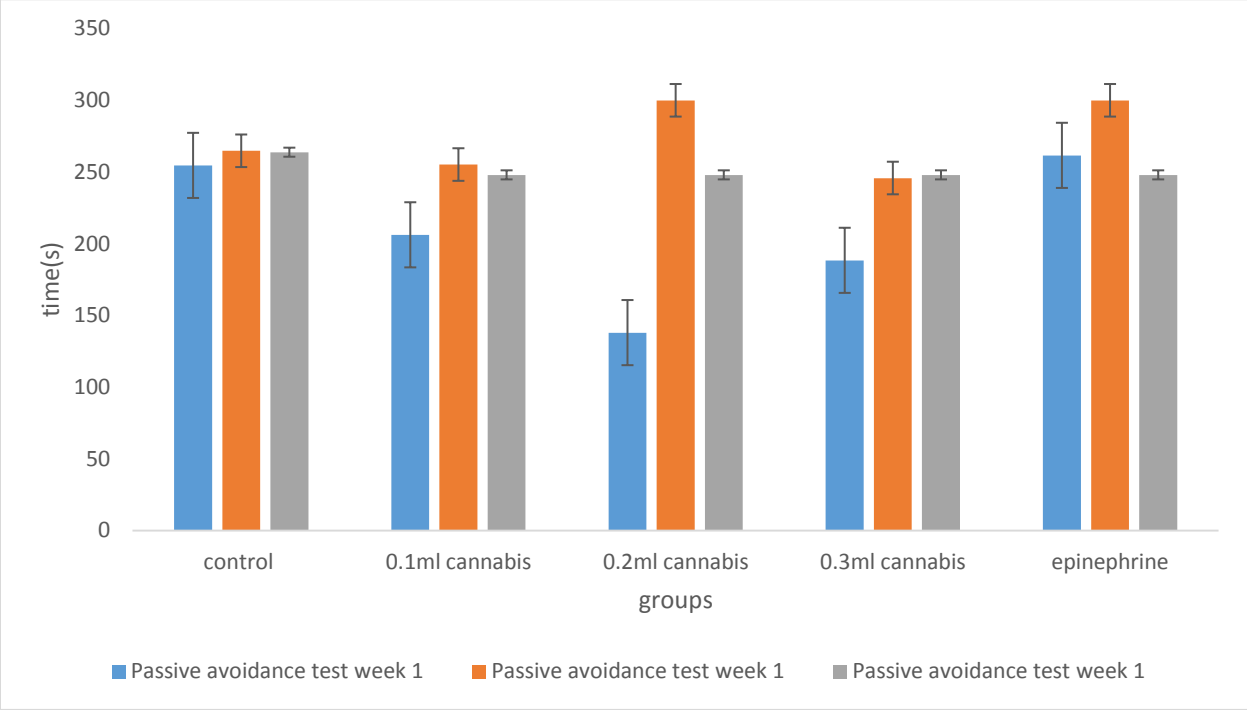

Figure 5 Pattern of Amnestic expression in the test and control groups in week 1

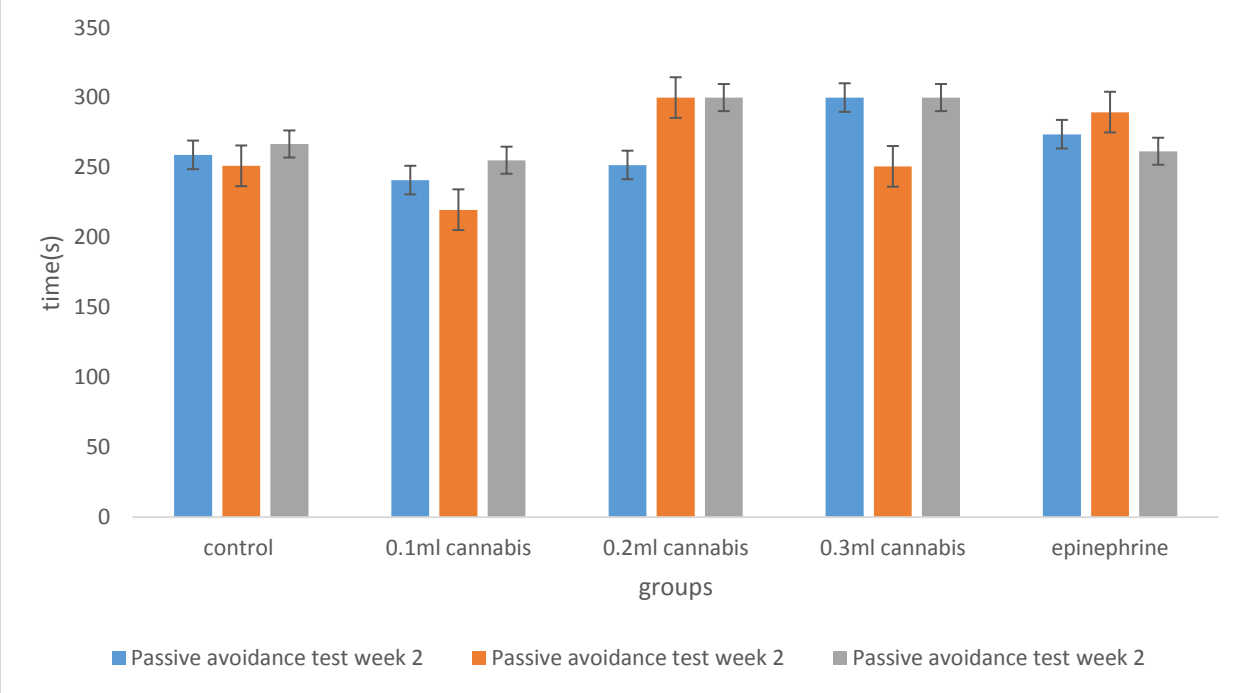

Figure 6. Pattern of Amnestic expression in the test and control groups in week 2

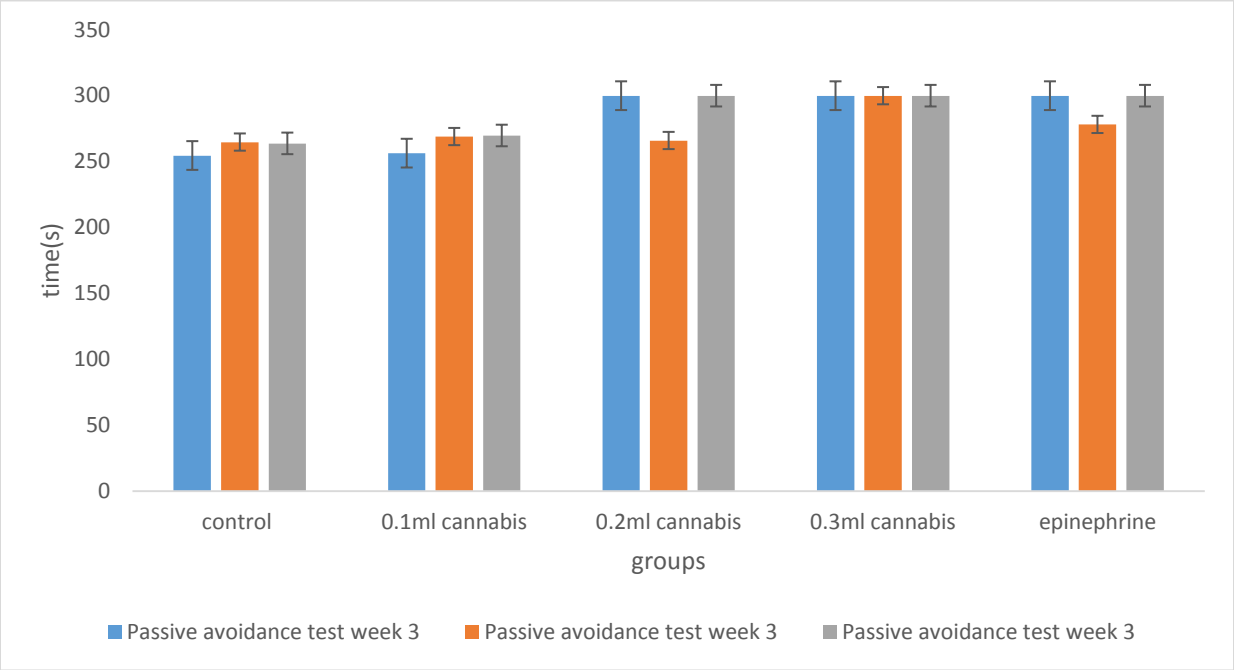

Figure 7. Pattern of Amnestic expression in the test and control groups in week 3

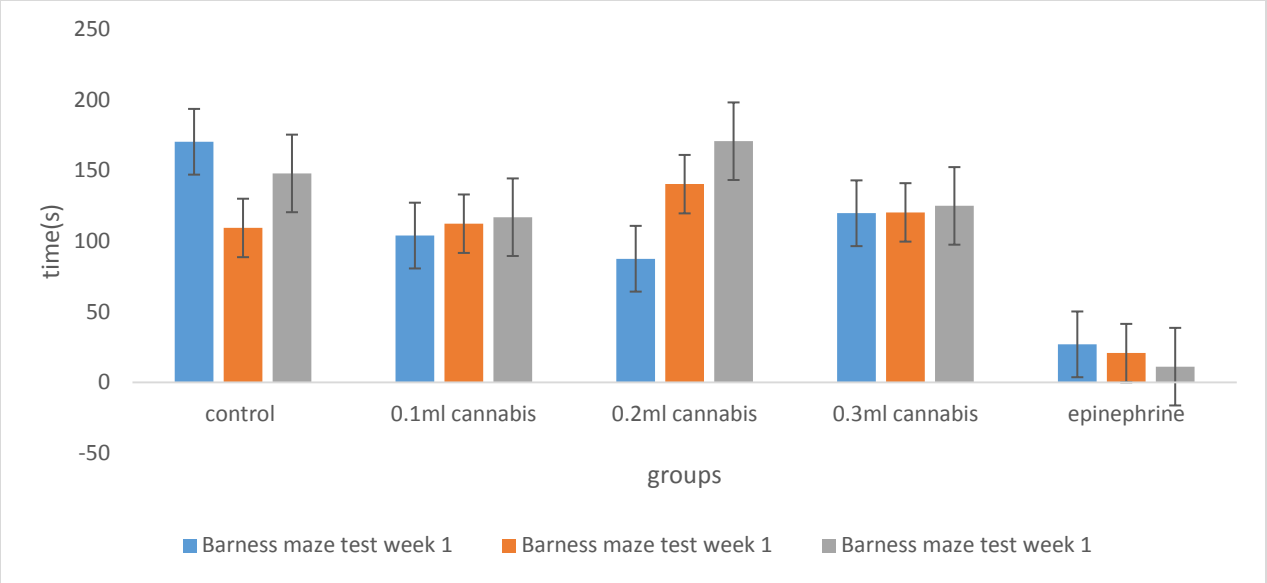

Figure 8. Pattern of Amnestic expression in the test and control groups in week 1

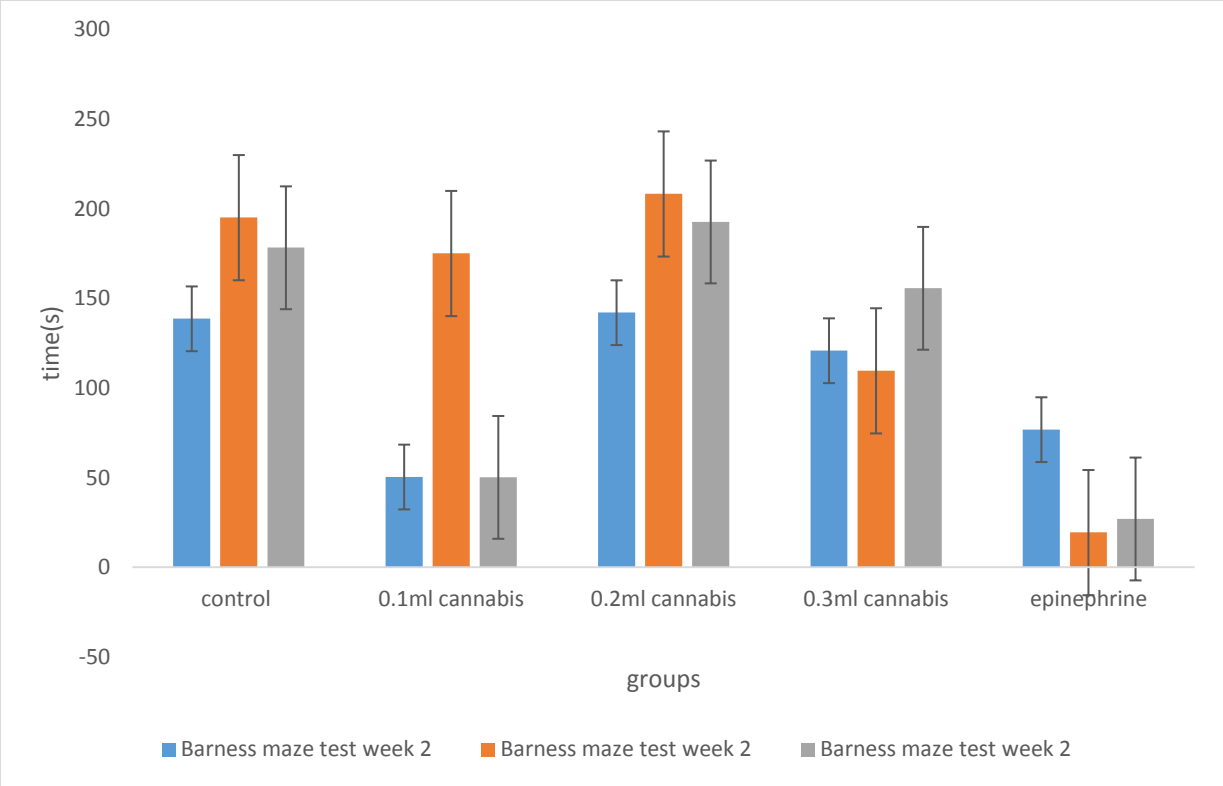

Figure 9. Pattern of Amnestic expression in the test and control groups in week 2

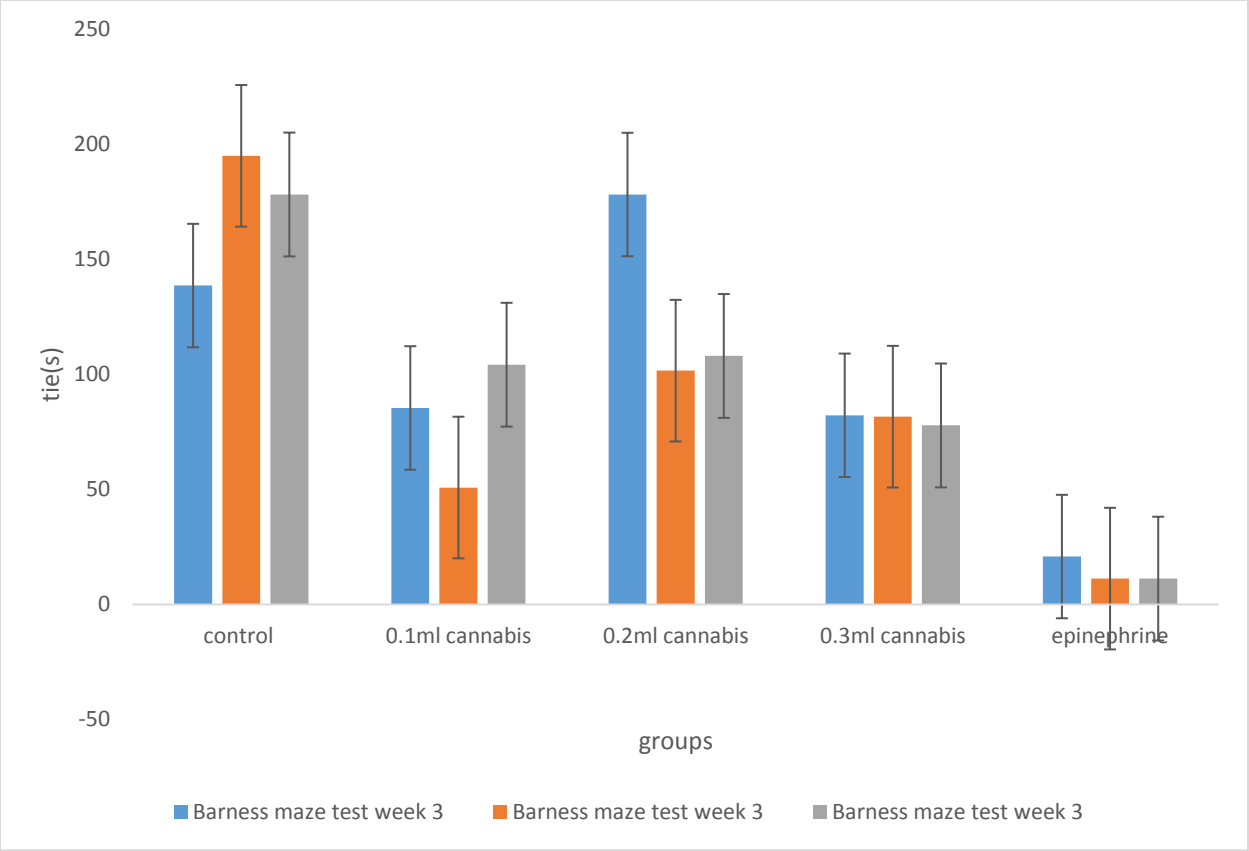

Figure 10. Pattern of Amnestic expression in the test and control groups in week 3

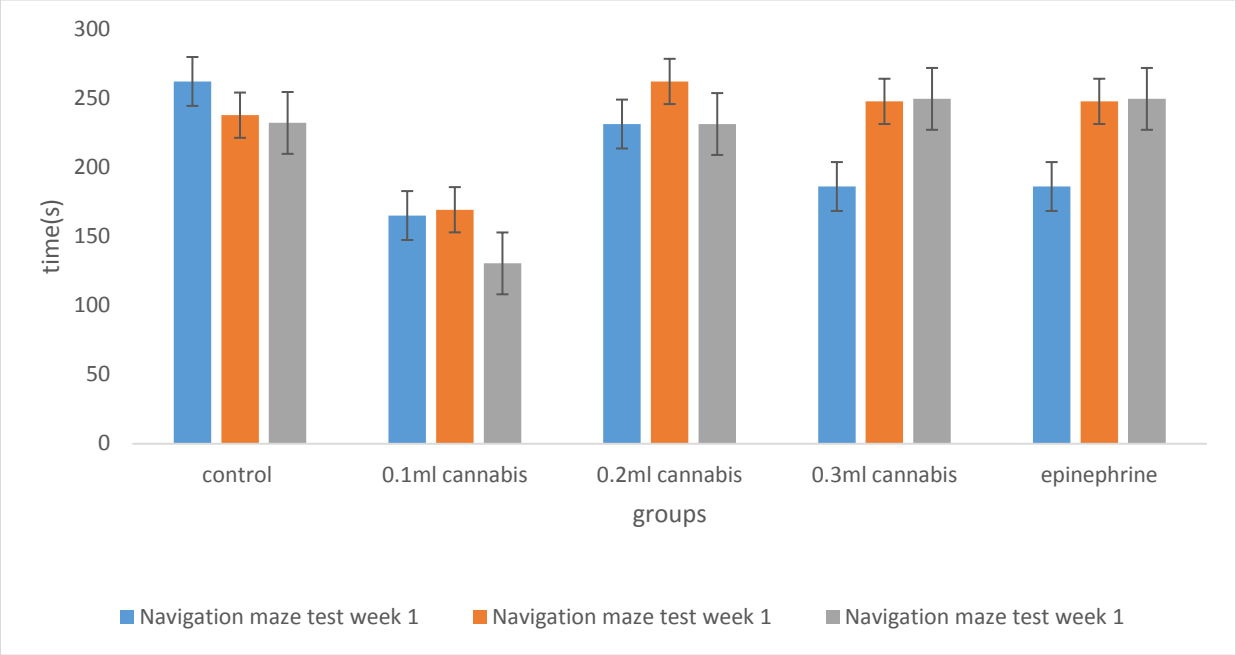

Figure 11. Pattern of Amnestic expression in the test and control groups in week 1

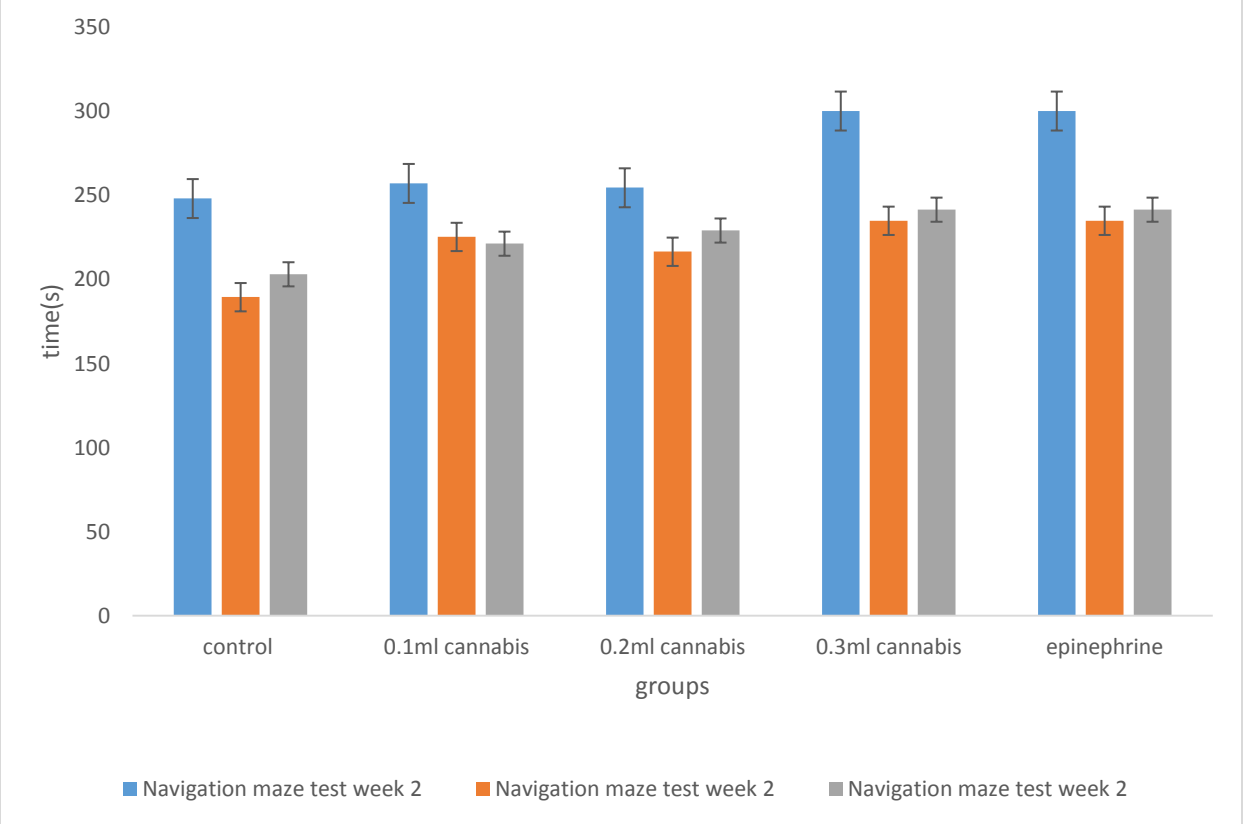

Figure 12. Pattern of Amnestic expression in the test and control groups in week 2

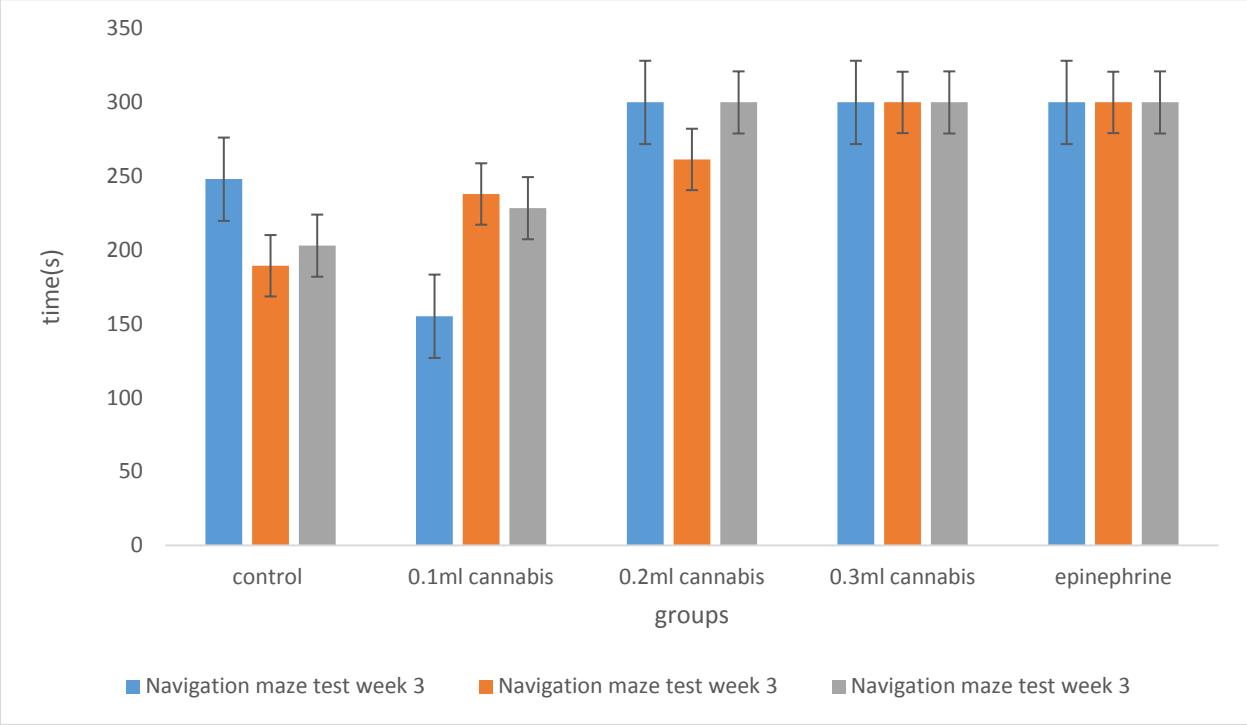

Figure 13. Pattern of Amnestic expression in the test and control groups in week 3

Discussion

Cannabis sativa is a known psychoactive compound used to improve a wide variety of health conditions [30]. Cannabis consumption has a variety of health effects, some are beneficial while other effects are not. The present study was designed to investigate brain oxidative stress markers and Memory in albino wistar rats administered with cannabis sativa. The patterns of expression of oxidative stress markers in the brain such as Superoxide dismutase, catalase, malondialdehyde and reduced glutathione were estimated upon exposure to varying quantities of the drug showed that cannabis could affect in no particular order. Neurobehavioral activities measured using standard procedures such as Navigational maze test, passive avoidance test, Barnes maze test for memory activities showed that Cannabis sativa could inflict substantial

interference on adaptive locomotion and some aspects of memory both at the cerebral and hippocampal circuits and areas of memory consolidation, cognitive learning and motor response. Observed increased pacing and exploratory activities in passive avoidance test, Barnes maze and navigation maze test suggested intact motor system and low anxiety. The malondialdehyde levels in blood (plasma) from test animals that were fed with 0.1ml, 0.2ml and 0.3ml cannabis was significantly reduced compared to the control group. However, for the epinephrine group there was no significant difference in MDA level when compared to the control. The results from figure 1 showed a decrease in SOD activity from the test animals that received cannabis when compared to the control. The epinephrine group revealed a much significant decrease in SOD levels when compared to the mean.The result obtained on the activity of catalase as shown in figure 2 showed significant increase in CAT activity in all groups (0.1ml, 0.2ml, 0.3ml and epinephrine) when compared to the control group. The increase in catalase activity in plasma shows that cannabis sativa is capable of regulating oxidative stress and this is in accordance with [31] who observed in other studies the ability of hemp seed to increase CAT levels in plasma. The results obtained revealed that GSH level was lower in the plasma of test animals that were exposed to 0.2ml, 0.3ml and epinephrine when compared to the control group. 0.1ml group experienced much lower decrease in GSH level in comparison to the control.

The navigation maze test is used to examine spatial learning and memory. It is used in assessment of exploration, path planning and navigation which depends on learning and memory capacities to form cognitive maps. It is used to test the effects of lesion to the brain in areas concerned with memory is also capable of accessing damages to cortical regions of the brain. From the current study the navigational test involving three trials for the total period of three weeks. For week one, (figure 3) there is a significant difference in test animals treated with 0.1ml

cannabis in comparison with the control group. It took the animals exposed to 0.1ml cannabis lower time to navigate its way from the entrance to the exit door therefore showing heightened memory. However, there is no significant difference in test animals treated with 0.2ml, 0.3ml and epinephrine when compared to different shock levels and control. The time taken to perform the navigational is a clear reflection of how alert the animal in challenging situations.

For week two from (figure 4); there is no significant difference in 0.1ml and 0.2mlcannabis treated animals when compared to control group. For the group treated with 0.3ml cannabis and epinephrine, there was a slight increase in time spent when compared to the control group. For week three from (figure 4); there is a statistically significant increase in the time spent by 0.2ml, 0.3ml and epinephrine compared to control. The group treated with 0.1ml however experienced no significant difference.

The passive avoidance test is used to teach subjects to avoid an environment in which an aversive stimulus was previously delivered. For week one, results obtained from (figure 5) there is a significant increase in test animals treated with 0.2ml cannabis and epinephrine at trial 2 in comparison with the control group. However, there is no significant difference in test animals treated with 0.1ml when compared to control. For week two from (figure 6); there is a slight increase in time spent in 0.2ml and 0.3ml cannabis treated animals when compared to control group. For week three from (figure 7); there is a significant increase in the time spent by 0.2ml, 0.3ml and epinephrine compared to control. The group treated with 0.1ml however experienced no significant difference. This result suggests increased spatial learning and memory at week 3 compared to week 1 and 2.

The Barnes maze is a behavioural test that was developed to study spatial learning and memory in rats (Barnes, 1979). It is a hippocampal-dependent task where animals learn the relationship between distal cues (place learning) in the surrounding environment and a fixed escape location (Williams, 2003). Different trails of this test allow to measure spatial learning, response preservation and memory. Week 1 results obtained from (figure 8) showed that there was a lower time to locate the escape box for all cannabis treated rats compared to the control group. The epinephrine group showed significantly lower time to locate the escape box compared to the control. This significantly lower time to locate the escape box in the cannabis treated rats compared to the control rats indicates increased spatial learning and memory. Heavy marijuana use has been associated altered spatial learning and memory [32]. Results obtained from week two (figure 12), a significant decrease was noticed in trial 1 and 2 of 0.1ml cannabis treated group when compared to control. Epinephrine group showed statistically significantly lower time to locate the escape box. In week three (figure 13) it was observed that 0.1ml, 0.3ml cannabis group and epinephrine group had the least time to locate the escape box in comparison to control group.

Conclusions

The administration of cannabis sativa for 21 days resulted in decrease in activity of Superoxide dismutase, reduced glutathione and malondialdehyde while an increase in the activity of catalase was observed showing the presence of oxidative stress and anxiety- like effect. Ingestion of cannabis sativa may temporarily lead to impairment of short-term memory and long term exposure to cannabis can produce long lasting cognitive impairment. , it can be deduced from the result that cannabis demonstrated significantly, ($p<0.05$) cellular re-alignment and rejuvenation

in terms of oxidative stress markers activities and potent memory retrieval as the period of administration progressed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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