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

ABSTRACT

The uncontrolled, repetitive use of Cannabis sativa continues to pose a problem for the plant's potential medical benefits. Because cannabis is a psychoactive chemical with a variety of physiological qualities, the start and intensity of its effects are frequently influenced by the way it is consumed. 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. The use of medicinal plants in the treatment of diseases and dysfunctions, which dates back millennia, has made a significant contribution to the creation of medicines, with plants accounting for around 25% of modern therapeutics [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 in the body, making it particularly vulnerable to toxicity, particularly those associated with the neurotoxic effects of drugs of abuse, which are linked to oxidative stress, mitochondrial dysfunction, apoptosis, and inhibition of neurogenesis, among other things [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]. Cannabis and its products have been increasingly popular among teenagers and young people in recent years [24]. Though cannabis is widely considered to be a harmless recreational substance, its use is rising among adolescents, who may have negative effects on the brain's functional connectivity, IQ, and cognitive performance as a result of repeated exposure [25]. As a result, research into the role of cannabis in generating oxidative stress and amnesia is required.

Agents of abuse have been demonstrated to exert detrimental impact upon social, psychological and cognitive behaviour in individual users, thereby affecting their personality [26]. Despite all of the compelling neuropsychological effects, cannabis abuse has recently increased and has

become a global cause of concern, affecting practically every nation for decades. Cannabis is the most widely used illicit substance in the world, according to the United Nations, and medical marijuana use is also on the rise, with some governments legalising it [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) is produced when polyunsaturated lipids degrade. This substance's synthesis is utilised as a biomarker to determine the level of lipid peroxidation. MDA forms a 1:2 MDA-TBA adduct with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS), which absorbs at 532 nm. As a result, the amount of TBARS produced is proportional to the amount of MDA produced. The concentration of TBARS is determined using Mihara and Uchiyama's technique. 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

SOD activity was measured using the Marklund and Marklund 1979 [22] technique, which used the suppression of pyrogallol autoxidation at pH 8. SOD's specific activity is measured in units

per milligramme of protein per minute. The activity of GPx was assessed using Paglia and Valentine's technique [23]. The oxidation of glutathione by Cumene hydroperoxide is catalysed by GPx. The oxidised glutathione is quickly transformed to the reduced form in the presence of glutathione reductase (GR) and NADPH, while NADPH is simultaneously oxidised to NADP. At 340 nm, the decrease in absorbance is measured. In the presence of NADPH, which is oxidised to NADP, GR catalyses the reduction of glutathione. 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 device is a trough-shaped metal with two chambers separated by an opening door. Aversive stimulation is not present in the white, brightly lighted container, however shock is present in the black, gloomy chamber. It assesses the capacity to recognise and remember the presence and location of a shock stimulus. According to the American psychological association's rules, the shock level employed in this task should be the bare minimum required to encourage the animal. During testing, no unpleasant stimulus was delivered to the animals upon re-entry into the dark chamber.

Barnes maze test

The Barnes maze is a rat behavioural test that was created to investigate spatial learning and memory (Barnes, 1979). Animals learn the association between distal signals (place learning) in

the surrounding environment and a fixed escape location in this hippocampal-dependent task (Williams, 2003). An raised circular platform with uniformly distributed holes around the outside is the standard Barnes maze setup. One hole has an escape tube installed beneath it, while the others are left vacant. Bright light and open places are repulsive to rats, thus they would seek refuge in the shadows. 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

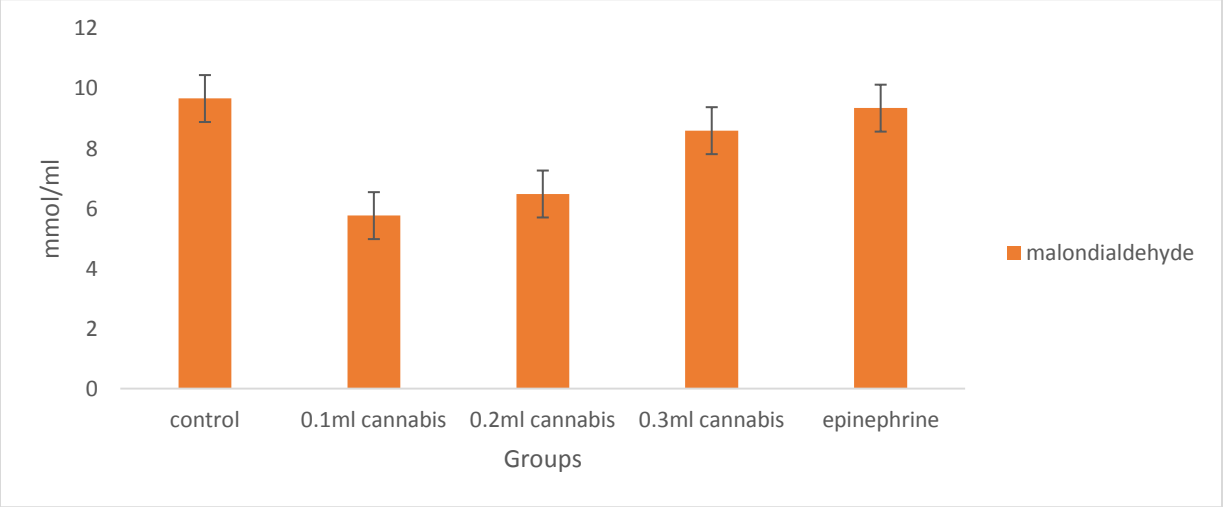

Figure 1 Pattern of response of malondialdehyde after 21 days treatment with cannabis sativa

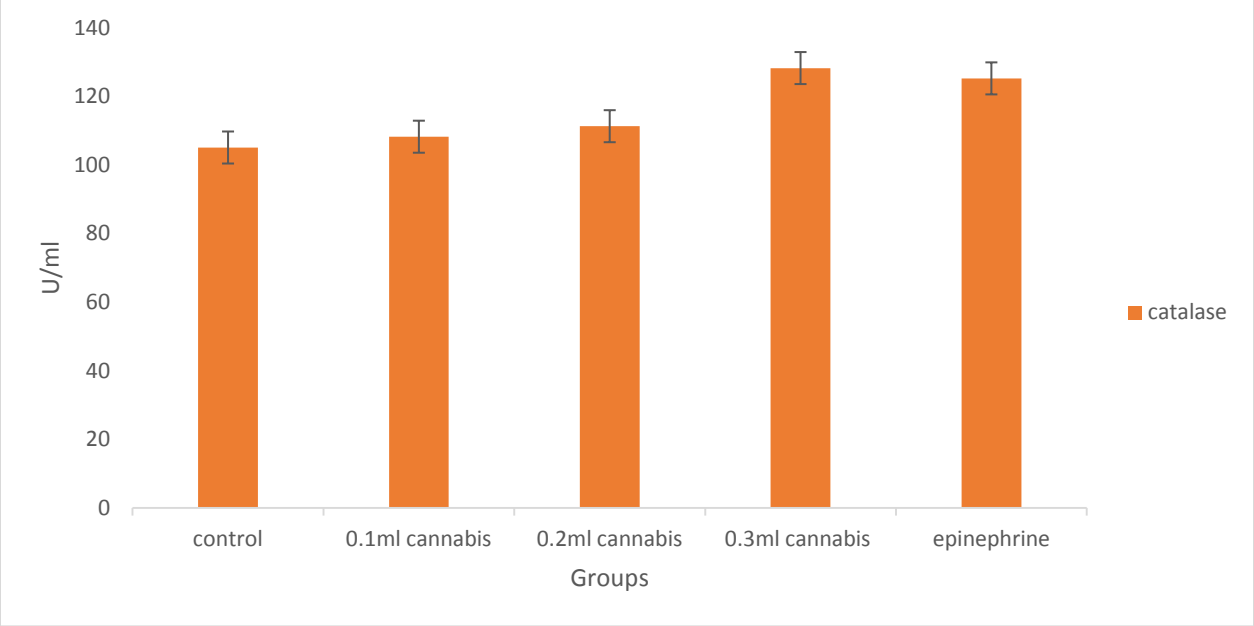

Figure 2 Pattern of response of catalase after 21 days treatment with cannabis sativa.

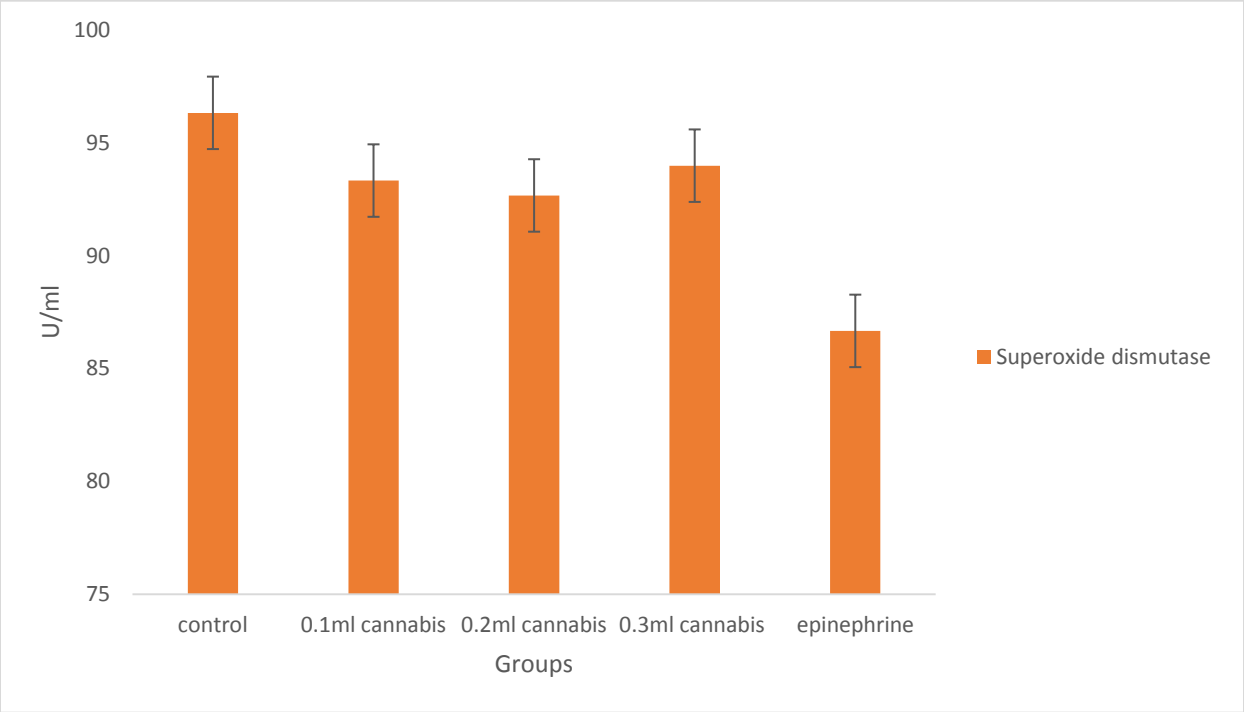

Figure 3 Pattern of response of superoxide dismutase after 21 days treatment with cannabis sativa.

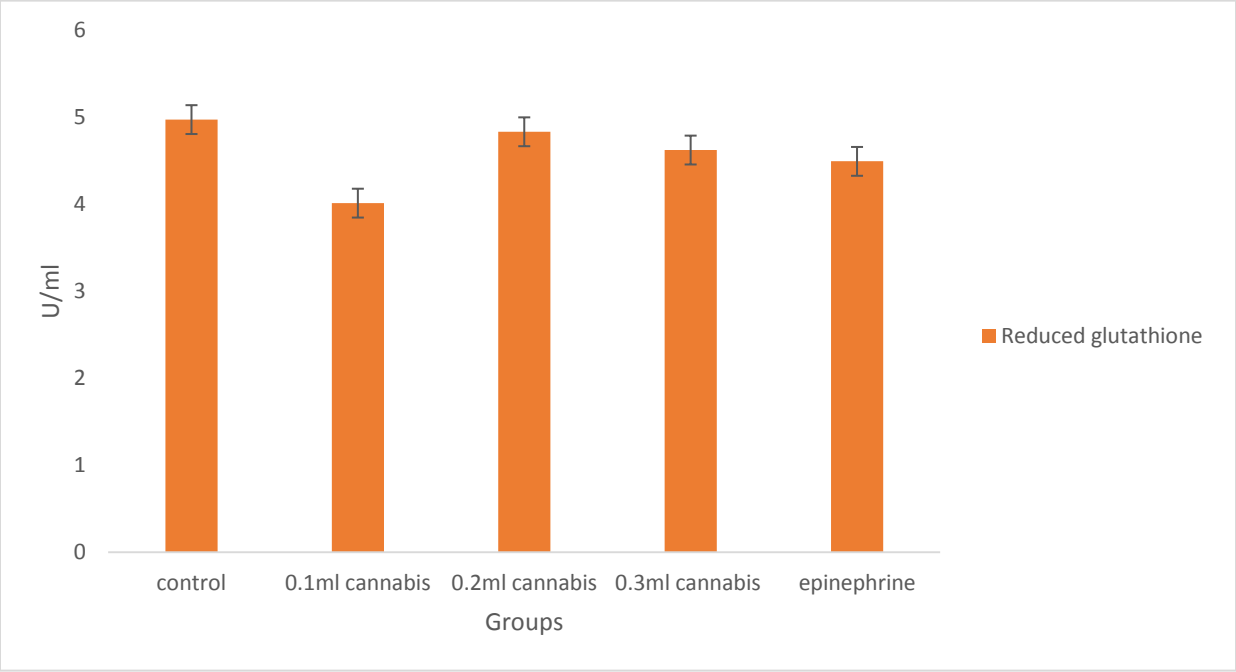

Figure 4 Pattern of response of reduced glutathione after 21 days treatment with cannabis sativa.

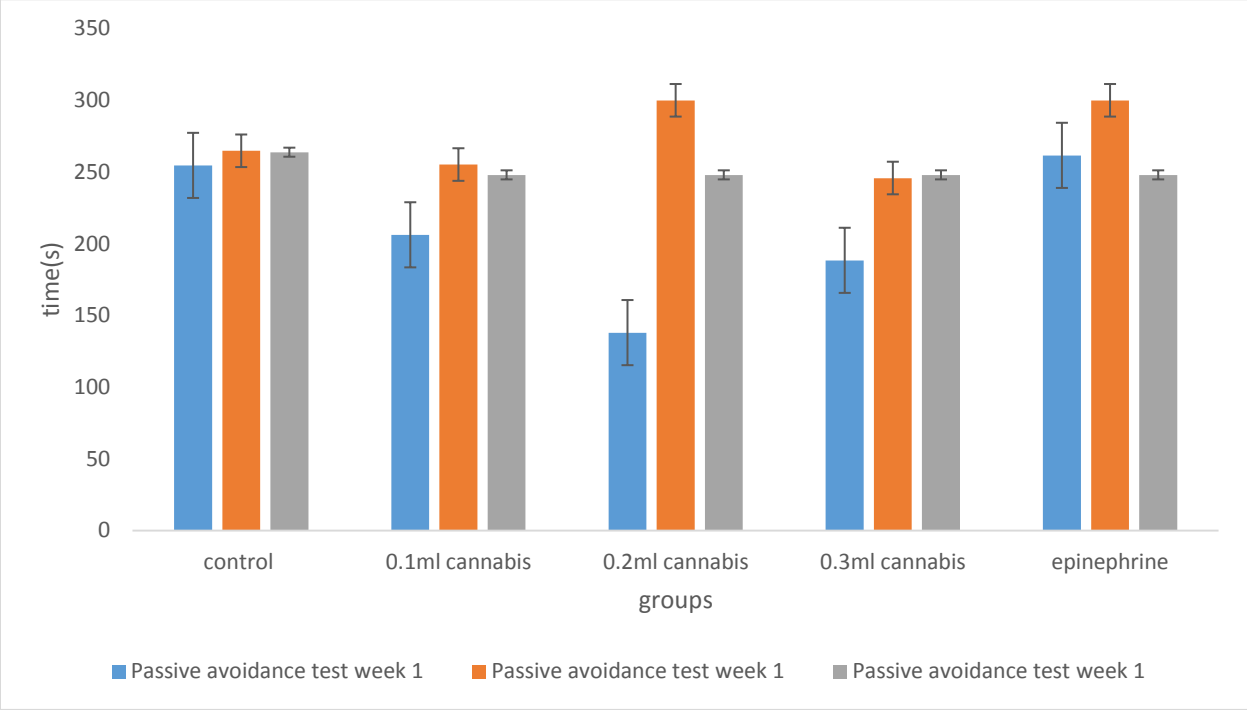

Figure 5 Pattern of Amnestic expression in the test and control groups in week 1

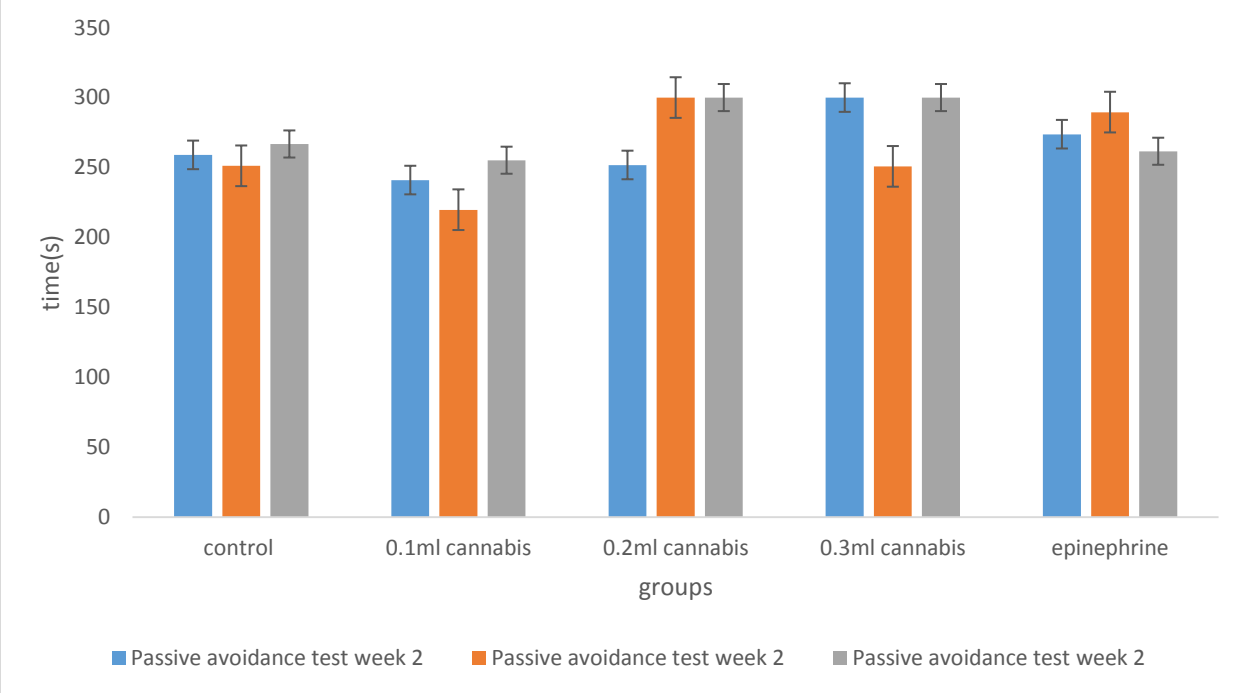

Figure 6. Pattern of Amnestic expression in the test and control groups in week 2

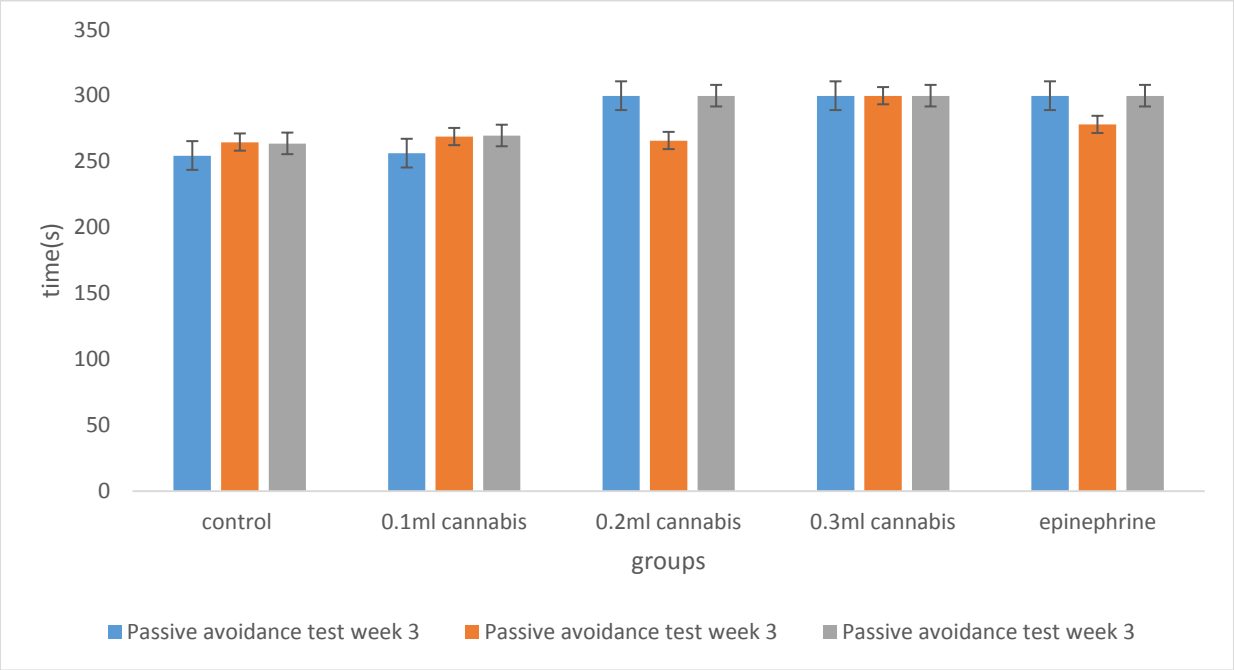

Figure 7. Pattern of Amnestic expression in the test and control groups in week 3

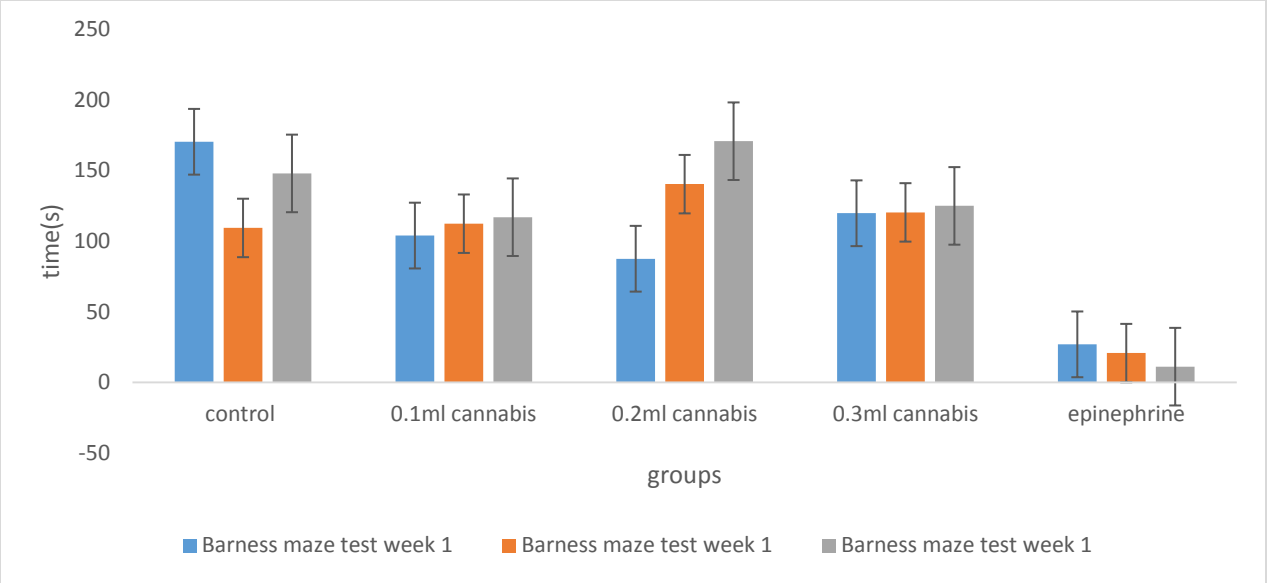

Figure 8. Pattern of Amnestic expression in the test and control groups in week 1

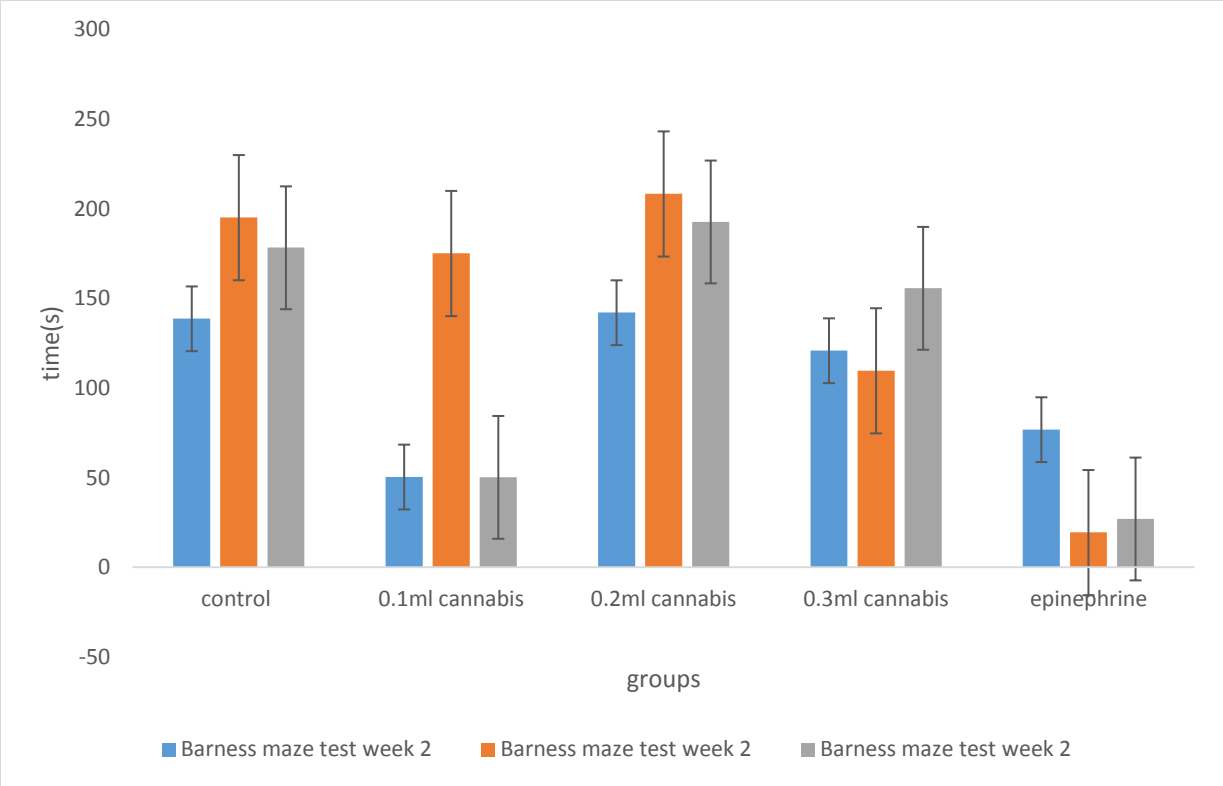

Figure 9. Pattern of Amnestic expression in the test and control groups in week 2

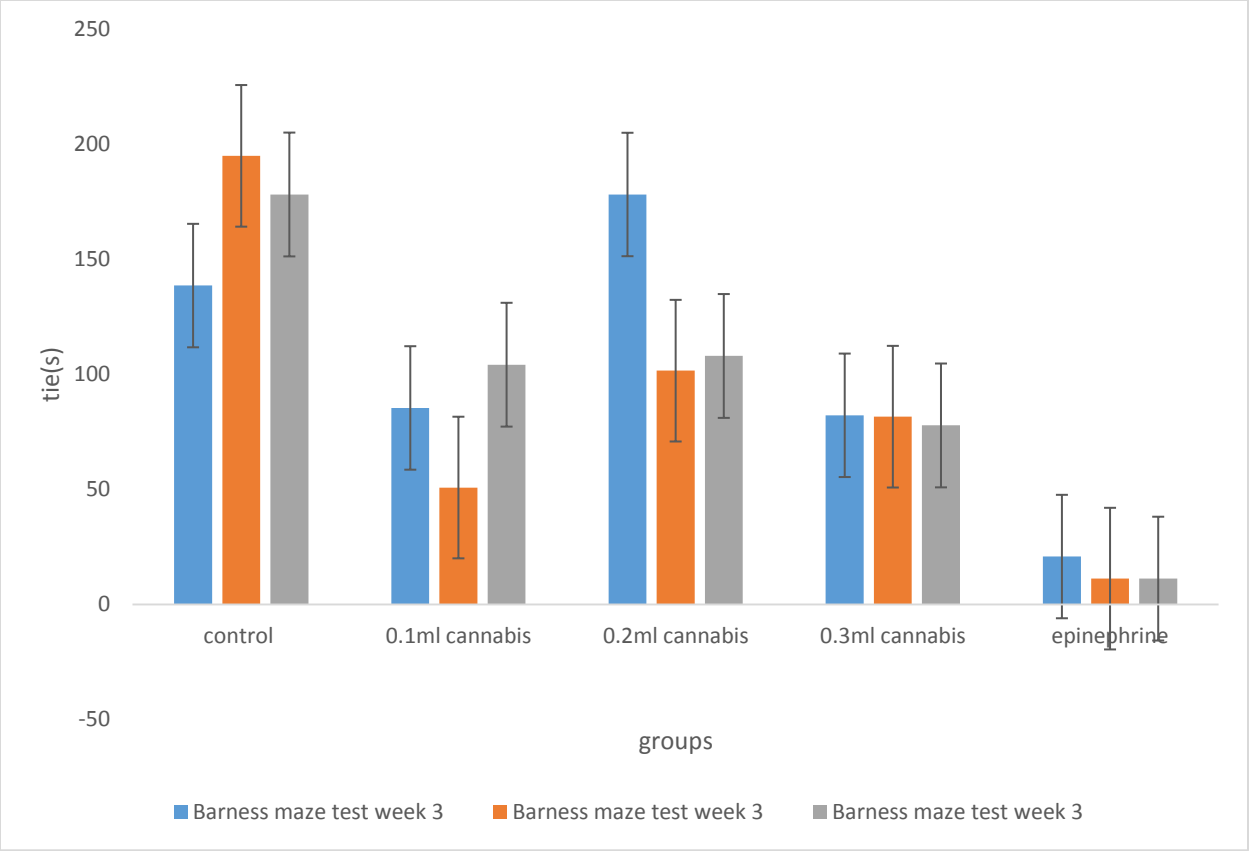

Figure 10. Pattern of Amnestic expression in the test and control groups in week 3

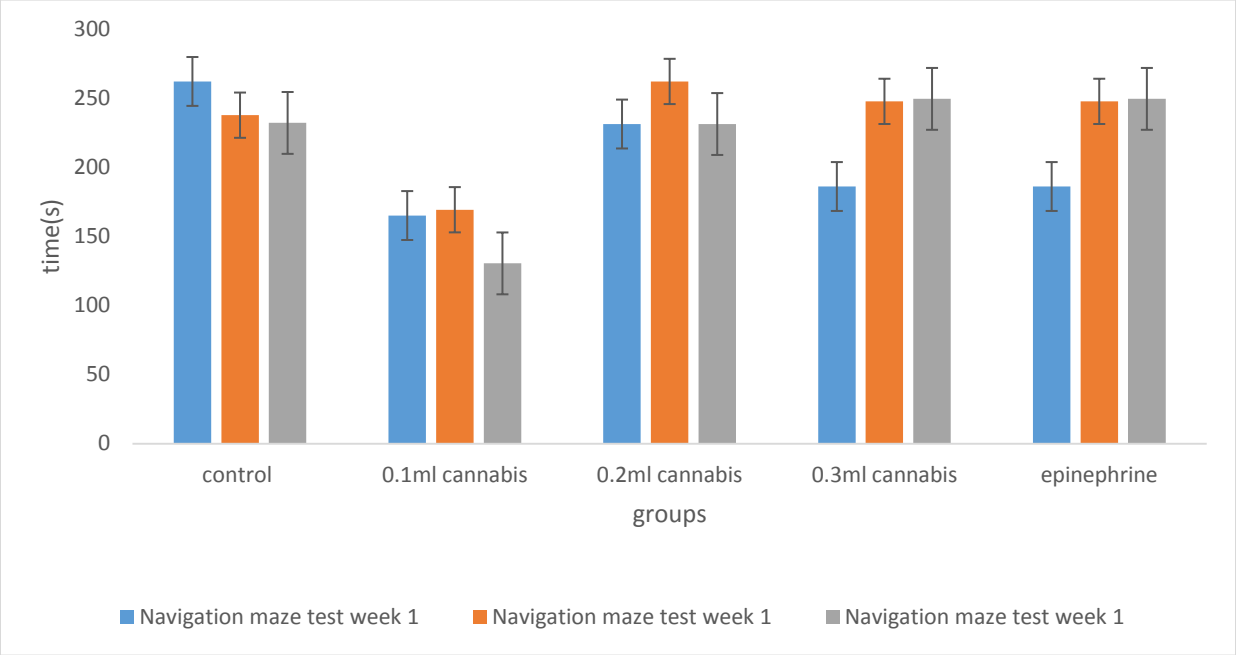

Figure 11. Pattern of Amnestic expression in the test and control groups in week 1

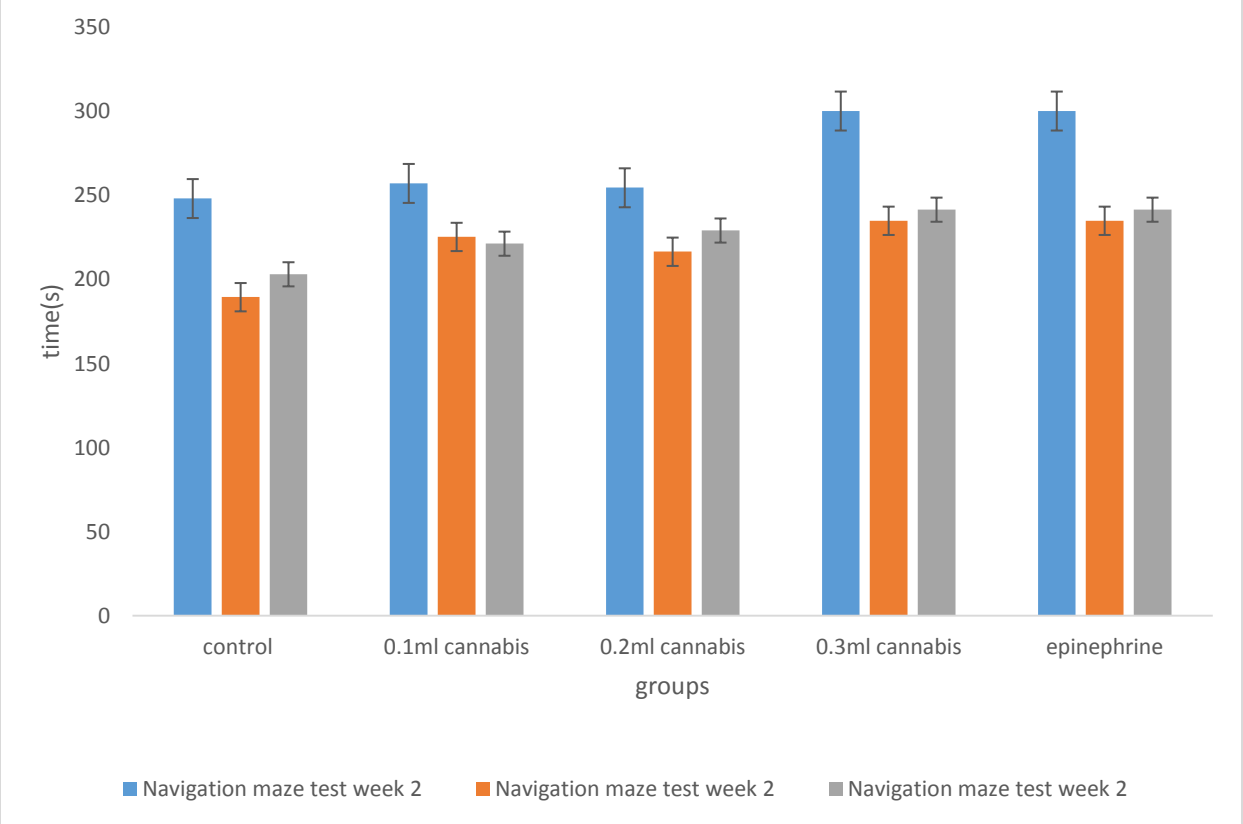

Figure 12. Pattern of Amnestic expression in the test and control groups in week 2

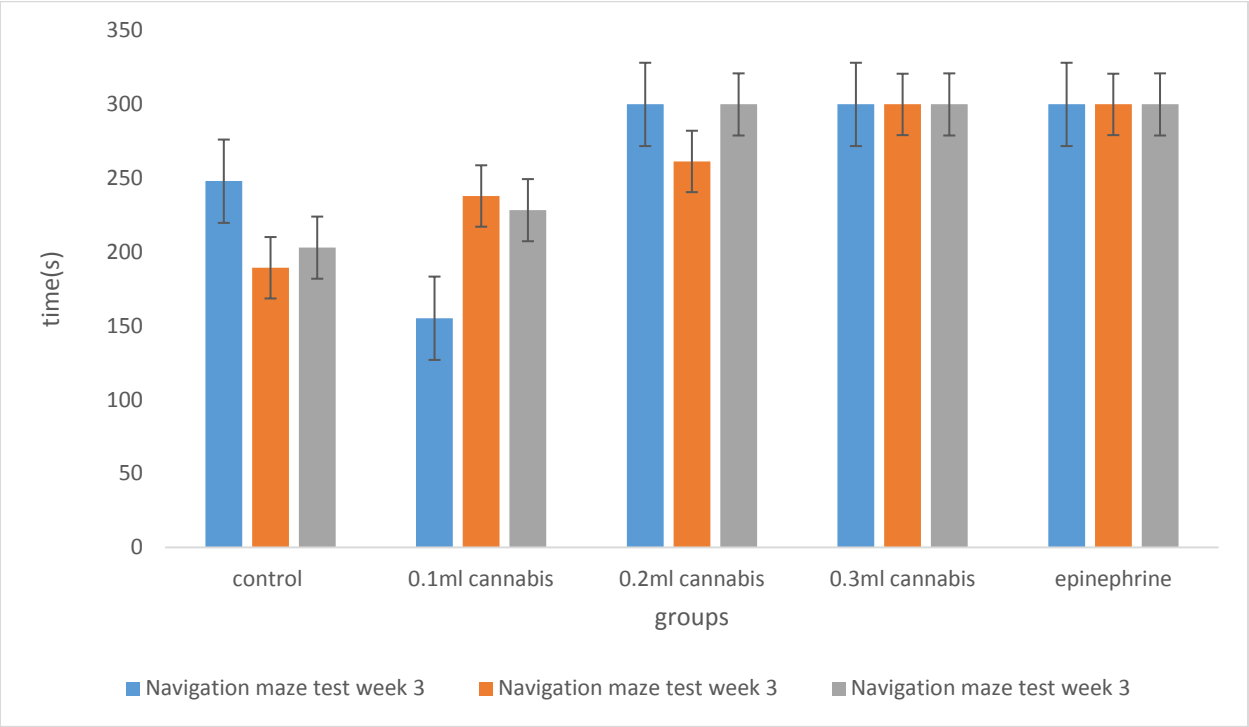

Figure 13. Pattern of Amnestic expression in the test and control groups in week 3

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.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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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