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IMPLICATIONS OF *CANNABIS SATIVA* EXPOSURE ON PATTERN OF ADVANCED OXIDATIVE PROTEIN PRODUCTS (AOPP), PAIN HYPERSENSITIVITY AND MEMORY IN ADULT WISTAR RATS

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ABSTRACT
The present study was carried out to investigate the implications of *cannabis sativa* exposure on pattern of advanced oxidative protein products (AOPP), pain hypersensitivity and memory in adult Wistar rats. This was achieved by determining the time of retraction of the experimental animals over a period of three weeks, analysis of oxidative parameters and advanced oxidative protein products. Twenty-five (25) healthy Wistar rats weighing 180-240g were used for this study. They were divided into five (5) groups of five (5) animals each. Group one serve as the normal control group, and was given distilled water, group two was given 0.5ml of cannabis, group three was given 0.7ml of cannabis, group four was given 1ml of cannabis and group 5 was given epinephrine. All administrations lasted for three weeks, during which time, the experimental animals were subjected several tests. Pain sensitivity threshold was determined using the analgesy-meter device with each group undergoing three trials. Passive avoidance test was used to determine the effect of cannabis on memory and spatial recognition in light and darkness. At the end of the administration period, brain specimen of experimental animals were harvested and homogenized and the quantification of oxidative stress parameters such as malondialdehyde, catalase, superoxide dismutase, glutathione reductase and AOPP were carried out. The data obtained was analyzed using Statistical Package for Social Sciences (SPSS version 23). Results obtained showed that sub-acute exposure of cannabis led to reduction in the levels of AOPP in brain tissues. It also revealed in a decrease in the level of oxidative stress parameters such as glutathione reductase, superoxide dismutase and catalase while an increase was seen in malondialdehyde. It also led to some level of reduction in the retraction time indicating an increased sensitivity to pain.

KEYWORDS: Cannabis, OAPP, memory, pain hypersensitivity, oxidative stress parameters.

1. INTRODUCTION
In Nigeria, the burden of drug abuse is on the rise and becoming a public health concern. Nigeria, which is the most populous country in Africa, has developed a reputation as a center for drug trafficking and usage mostly among the youth population.[1] According to the 2018 UNODC report "Drug use in Nigeria"—The first large-scale, nationwide national drug use survey in Nigeria, one in seven persons (aged 15–64 years) had used a drug in the past year. Also, one in five individuals who had used drug in the past year is suffering from drug-related disorders. Drug abuse has been a cause of many criminal offences such as theft, burglary, sex work, and shoplifting. Nigeria is an enormously diverse country with over 400 ethnicities and many religious groups.[2] Drug abuse is therefore viewed within a broader context in Nigeria, due to its multicultural nature. For instance, most societies do not consider the use of some drugs which do not produce overt behavioral changes as drug abuse. However, despite this multicultural nature of the Nigerian population, there is a consistent outcry from both the public, police, preacher's health professionals, teachers, regulatory agencies and parents on the growing burden of drug abuse (abuse of drugs which affect behavior) in the country. The recent call was that of the President of the Pharmaceutical Society of Nigeria.[3] Efforts to prevent the growing incidence of drug abuse in Nigeria involve the identification of evidence-based information on the extent of the problem, from epidemiological studies. To date, most of the information on drug abuse in Nigeria is reported by the media (print, electronic and online).

However, scientific evidence from epidemiological studies has started emerging in recent years. The most frequently implicated drugs, consistently reported by the majority of the studies were; cannabis.[4] Codeine.[5] amphetamine/ dexamphetamine, heroin, cocaine.[6] diazepam, and cough syrup, Reactivan (fencamfamine), Mandrax, and tramadol. Cannabis was the most abused drug reported across the different study populations. The prevalence of cannabis abuse among members of the general public was 10.8% and 22.7% among adolescents of 25 years and younger.[7] The frequency of abuse among secondary school students was between 0.6 and 34%, with a pooled prevalence of 12.5%. The abuse of cannabis among undergraduate students was also common, with a prevalence of 8–11%.[8,9]

Several studies have revealed the unending usage of cannabis and synthetic cannabinoids by youths, undergraduates, adults and sometimes minors. In most cases, it is being used in an unmediated manner and as such may result in several unwanted long-term effects. Since cannabis mostly acts on the central nervous system, especially the brain, there is need to understand the correct pathway by which it exacts its effect. Very few studies have detailed the relationship between cannabis exposure and oxidative protein products, as well as its association with pain hypersensitivity. Most of the previous works conducted on the area, have shown the metabolism of cannabis, its prevalence and addiction. Therefore, this study tends to gear towards understanding how the exposure to cannabis can affect brain pattern and level of hypersensitivity.

MATERIALS AND METHODS

Animal

All Procedure involving the use of animals in this study followed the guiding Principles for research involving animals as recommended by the declaration of research ethics committee of the University of Port Harcourt, Rivers State on the guiding Principles in the care and use of animals and the use and care of animals was in conformity with International acceptable standards.

Experimental design

Twenty (20) Healthy experimental animals (wistar rats) weighing (180-240g) were obtained from the experimental research work from the animal house of the department of pharmacology, university of port Harcourt, Choba rivers state. They were fed with rat diet (palletized poultry feeds) and tap water throughout the course of the experimental research work. The rats were kept in a favourable housing environment, which was a clean plastic cage with wooden shavings to enable daily cleaning of the cage. The cage was constructed to be well Ventilated as wire gauze was used to create the Roof of the cage. The area was devoid of noise and foul smells from the surrounding environment. The cages were regularly cleaned every morning, as their wooden shavings were changed, feeding and drinking trough washed and replaced with new feed and water which were not contaminated. After identification, the animals were weighed and housed in a plastic cage with 4 compartments, under standard conditions (Temperature 25-29ºC, 12 Hours light/darkness cycle), for (2) weeks so as to acclimatize with the environmental condition of where it was being kept.

This experiment was aimed at investigating the effect of pure cannabis administration on advance oxidative protein products and pain sensitivity of Wistar rats. A total of 25 Wistar rat weighing 180 - 240 was used for the study. The rats were grouped into 5 groups with 4 rats in each group. Among the groups, group 1 served as the control, group 2 was given 0.5 ml of cannabis (low dose), group 3 was given 0.7ml of cannabis (medium dose), group 4 was given 1ml of cannabis (high dose) and group 5 was given epinephrine.

Experimental procedure

The study was carried out on the duration of 3 weeks, the animals followed these procedures;

Pain sensitivity test (Use of analgylsmeter)

The Administration of drugs and experimentation was performed for about during the light cycles of the day. The Rats were kept for about 30 minutes after administration for the drugs to be fully effective on the rats.

The force is applied to the animal's paw, which is placed on a small plinth under a cone-shaped pusher with a rounded tip. The operator depressed a pedal switch to start the mechanism which exerts the force: The force increases at a constant rate, thus enabling perfect reproducible measurements to be made. The motor stops immediately the pedal is released. The reading is recorded.

Passive avoidance test

Passive –avoidance test – (Modified method of Kameyama et al, was employed (9). it is a useful task for evaluating the effects of novel chemical entities on learning and memory as well as studying the mechanism involved in cognition.

Principle – The testing apparatus is a trough-shaped alloy divided into two distinct compartment 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.

Procedures

- The animals were placed on the white, brightly lit compartment facing the door such that it was allowed access into the dark compartment through the door.
- When the animal steps into the dark, black compartment with all four paws, a 1-2 seconds foot shock was delivered (0.2 – 0.5 mA shock, minimum required to elicit flinching, running, jumping and /or vocalization).
- After the termination of the aversive stimulus in the dark compartment, some animals ran out of the dark compartment into the bright lit compartment.
- The latency to re-enter the dark compartment was recorded for a maximum time of 300 seconds. However there is no aversive stimulus applied to animal upon re-entry into the dark compartment during testing,
- The procedure was repeated for all the animals and the test were performed for 3 consecutive days with 3trials carried out on each of the days.

Electroconvulsive therapy test

The electroconvulsive therapy test was used to administer shock in the rat, intentionally triggering a brief seizure after small electric current was passed. Each wister rat received ECT once daily for the experiment via placement of the electrode ear clips on each of the wister rat and electric current switched on. The rats were monitored after treatment to ensure that it had brief seizure indicating that it received the shock. The testing was performed in 3 trials per session and was done for a period of three weeks.

Determination of Oxidative Stress Markers

Procurement and Preparation of tissue sample

After sacrificing of the animals, collection of tissue and blood samples were done. The tissue of concern being the brain tissue at negative °C. The tissues were separately weighed and homogenized in 10 volumes of cold phosphate buffer (pH 7.4), using an automatic homogenizer. The homogenates were then centrifuged with a cold centrifuge at 4966rpm for 10 min at 4 °C. Clear supernatants were used for the MDA, Catalase, SOD, GHS, and AOPP assays. Tissue protein levels were also measured at this step according to the method used by Lowry et al.

Test for MDA/ Lipid peroxidation

Materials used in the test included Trichloroacetic acid 15% (TCA), Tiobabuturic acid 0.65% (TBA) and Hydrochloric acid 0.25N (HCL). Preparation of reagents. 15g of Trichloroacetic acid was weighed alongside 0.65g of TBA into a beaker, 2.03 ml of HCL was measured also to give a molar mass of 100ml. 300 ml of sample (homogenized tissue and saline buffer) was mixed with 3ml of working reagent and boiled in the water bath for 15 minutes. The absolvent was measured at a wavelength of 532nm with a spectrometer.

Test for SOD

SOD an enzyme that catalyzes, converts superoxide anion to cingulate O^2 and H^2O^2. The materials used in the test included carbonate buffer with the pH of 10.2, 10mg of Adrenaline. Preparation for carbonate buffer (Na^2CO^3 and Na^2HCO^3) and solution. 100mm of solution (homogenized tissue and saline buffer) and 4ML of carbonate buffer was incubated at 37°C in 5 minutes. The reaction was then supplied with 100mm of Adrenaline and read with a spectrometer at 30 seconds and at 1 minute 30 seconds respectively at 480nm.

Test for Catalase

Catalase is an oxidative enzyme that converts H_2O_2 to H_2O and O_2 The materials used in the test included for the Buffer solution of 0.05M and pH of 7.2 and H_2O_2 0.036M. Preparation of Catalase and H_2O_2. Two solutions are prepared for Catalase, Solution; A 0.8g of Na_2HPO_4 was weighed with 50ml of H_2O Solution B; 0.7g of K_2HPO_3 was weighed with the same 50ml of water. Solution B was further added to A to get a pH of 7.3. The above buffer was then added to 0.3ml of 6% of H_2O_2 to get 50ml together with the supernatant. The solution was read at 480nm or 240nm at 30 seconds and 3 minutes respectively.

Test for GHS

Materials used in this test includes sulfosalicylic acid, phosphate buffer, DTNB and the supernatant

Preparation; 750Nl tissue supernatant and 750Nl 4% sulfosalicylic acid was incubated for 20 minutes and centrifuged at 5000pm for 10 minutes at 4°C, 500ml of DTNB and 500ml of phosphate buffer of 0.1M and pH of 7.4 was added. The resultant was read at 412nm.

Test for AOPP

Materials used in this test includes PBS, Potassium iodide, Acetic Acid and Chloramine T.

Preparation; 400Nl of sample (supernatant) was diluted with 1600Nl of PBS, 200Nl of 1.16 potassium iodide was added and left to react, at 5 minutes the reaction was stopped with acetic acid of 100Nl. Standards where prepared with Chloramine T. The samples were read at 340nm.

Data analysis

The data obtained from this study was analysed using Statistical Packages for Social Sciences (SPSS) version 23 and Microsoft office excel 2016. The results were expressed in tables containing means and standard error means, as well as charts for easy pictorial representation. LSD post hoc multiple comparison was used as inferential statistical measure to access the significance of the data. P value of 0.05 and below were considered statistically significant.

RESULTS AND GRAPHS

Table 1: Pattern of noxious sensitivity and response in the test and control rats in the three weeks study using Analgesylmeter.

GROUPS	DRUGS	ANALGESIMETER RECORDING WEEK1			ANALGESIMETER RECORDING WEEK2			ANALGESIMETER RECORDING WEEK3		
		TRIAL1	TRIAL2	TRIAL3	TRIAL1	TRIAL2	TRIAL3	TRIAL1	TRIAL2	TRIAL3
1	NORMAL SALINE	18.02 ± 4.38	21.36 ± 3.64	25.00 ± 0.00	22.62 ± 2.28	18.30 ± 4.55	13.64 ± 3.01	20.80 ± 2.80	21.80 ± 1.45	21.72 ± 2.08
2	0.5ml CANNABIS	22.12 ± 2.43	24.82 ± 0.18	17.04 ± 4.88	9.96 ± 4.49*	9.44 ± 4.20	17.30 ± 4.83	21.38 ± 2.27	14.32 ± 4.83	25.00 ± 0.00
3	0.7ml CANNABIS	21.92 ± 3.08	20.60 ± 3.58	17.04 ± 4.88	18.32 ± 3.94	20.08 ± 2.02	19.00 ± 4.37	14.30 ± 3.11	15.68 ± 4.27	15.34 ± 1.81
4	1ml CANNABIS	17.04 ± 4.07	16.30 ± 3.70	11.66 ± 5.45*	21.38 ± 2.27	14.32 ± 4.83	25.00 ± 0.00*	11.92 ± 3.49*	7.42 ± 2.74*	18.92 ± 3.91
5	EPINEPHRINE	25.00 ± 0.00	24.00± 2.24	25.00± 0.00	25.00± 0.00	24.00± 1.00	25.00± 0.00	25.00± 0.00	24.00± 1.00	25.00

Values are presented in mean ± sem. N = 5. * means values are statistically significant when compared to the control values. All readings were in time(s).

Table 2: Pattern of noxious sensitivity and response in the test and control rats in the three weeks study using Passive Avoidance Test.

GROUPS	DRUGS	PASSIVE AVOIDANCE RECORDING WEEK1			PASSIVE AVOIDANCE RECORDING WEEK2			PASSIVE AVOIDANCE RECORDING WEEK3		
		TRIAL1	TRIAL2	TRIAL3	TRIAL1	TRIAL2	TRIAL3	TRIAL1	TRIAL2	TRIAL3
1	NORMAL SALINE	180.001 ± 73.49	177.20 ±64.31	180.00 ±73.49	300.00 ± 0.00	130.00 ±69.57	189.00 ±68.35	180.00 ± 73.49	180.00 ±73.49	18.00 ±73.49
2	0.5ml CANNABIS	10.80 ± 4.87*	30.40 ±15.16*	46.40 ±46.40	158.20 ±67.58*	84.00 ±58.79	46.40 ±46.40	92.00 ± 59.28	97.60 ±62.34	192.40 ±61.16
3	0.7ml CANNABIS	78.00 ± 52.46	166.00 ±43.01	46.40 ±46.40	240.60 ±36.89	240.00 ±60.00	206.00 ±61.12	130.60 ± 60.77	184.00 ±71.10	192.00 ±62.21
4	1ml CANNABIS	127.80 ± 39.56	180.00 ±51.86	110.20 ±54.54	85.80 ±54.90	205.60 ±46.19	249.20 ±50.80	133.20 ±107.19	222.60 ±58.14	287.40 ±12.60
5	EPINEPHRINE	120.00 ±73.49	60.00 ±60.00	60.00 ±60.00	120.00 ±73.00	60.00 ±60.00	60.00 ±60.00	120.00 ± 73.49	60.00 ±60.00	60.00 ±60.00

Values are presented in mean ± sem. N = 5. * means values are statistically significant when compared to the control values.

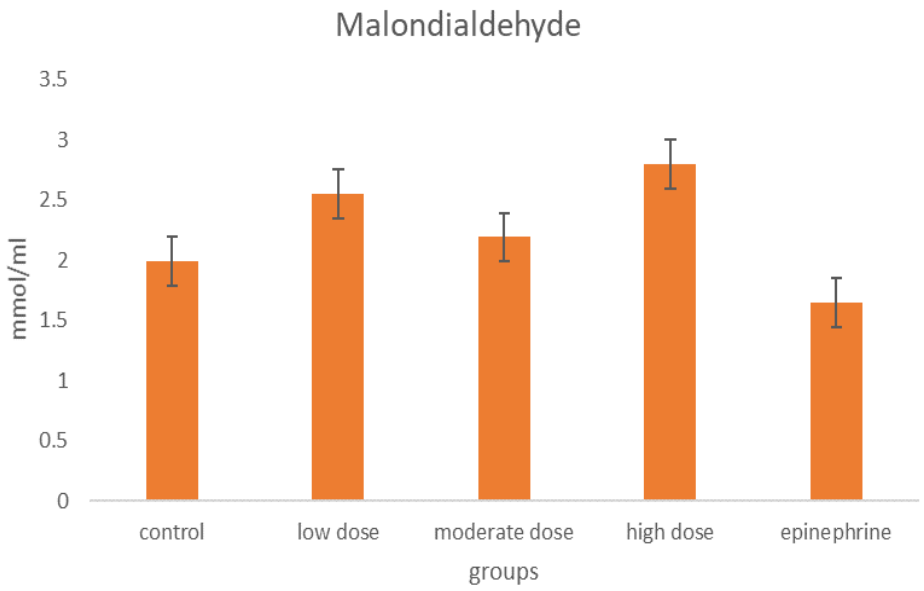

Figure 1. Response pattern of malondialdehyde after sub-acute administration of different concentration of cannabis sativa at the end of 3 weeks.

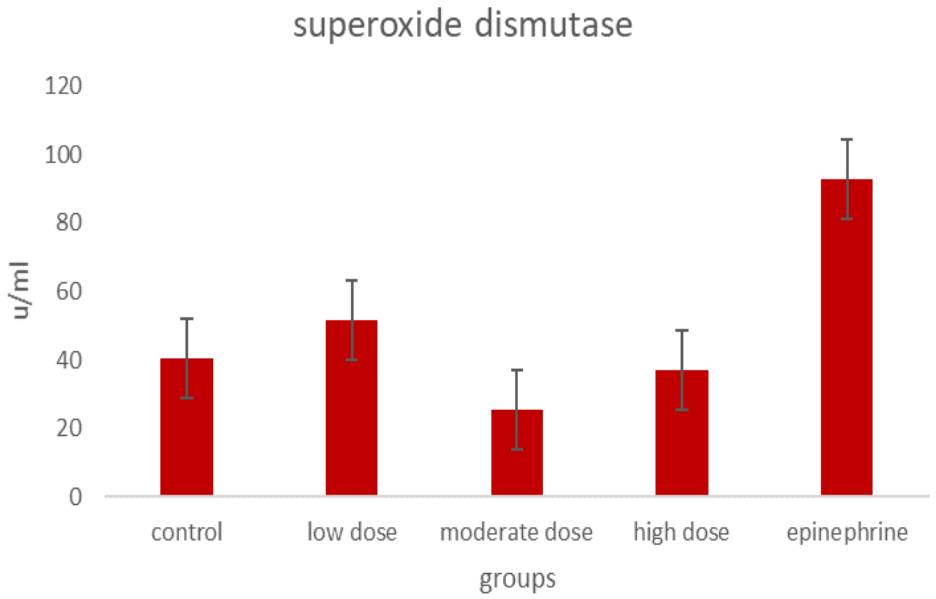

Figure 2. Response pattern of superoxide dismutase after sub-acute administration of different concentration of cannabis sativa at the end of week.

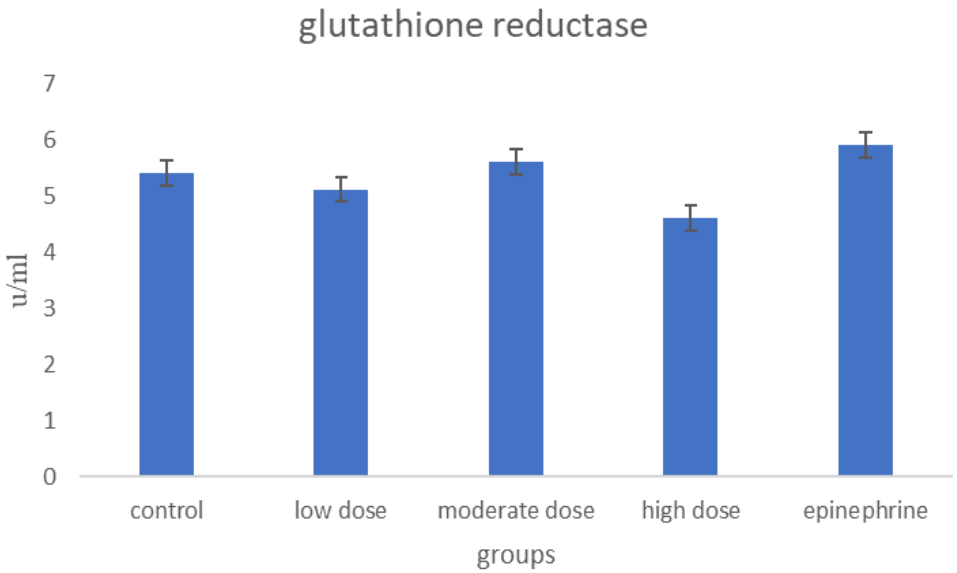

Figure 3. Response pattern of glutathione reductase after sub-acute administration of different concentration of cannabis sativa at the end of week 3.

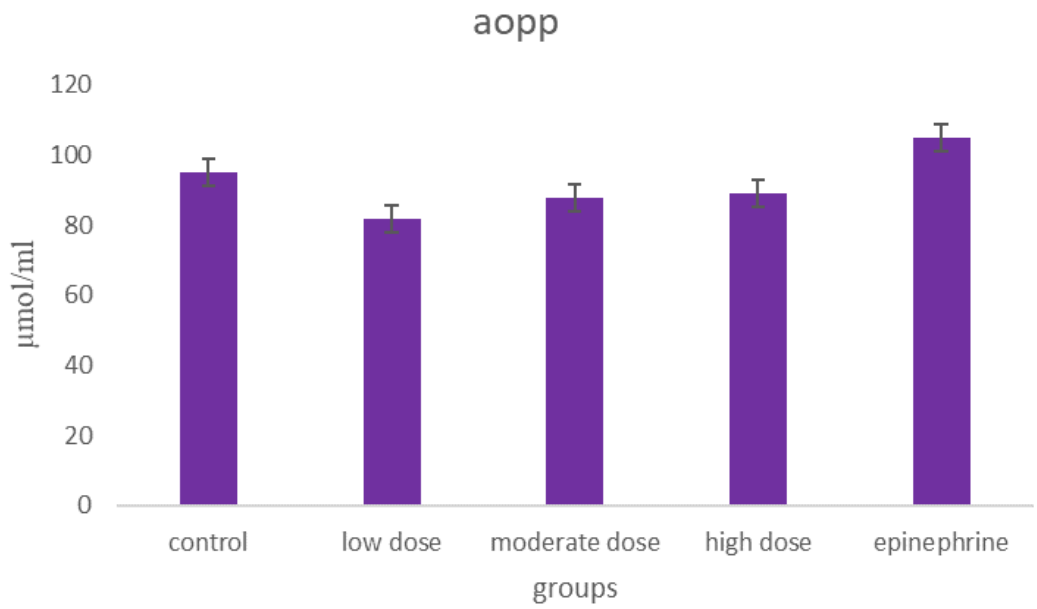

Figure 4. Response pattern of advanced oxidative protein products after sub-acute administration of different concentration of cannabis sativa at the end of week 3.

DISCUSSION

This study was carried out to investigate the effect of sub-acute exposure of cannabis on pattern of advanced oxidative protein products and pain hypersensitivity in adult Wistar rats. It also accessed some cognitive functions such as memory in animals exposed to cannabis.

The data in table 1 displays the pain sensitivity changes during the three weeks of administration of cannabis. During week one of the study, it was observed that the control groups had an increase in retraction time when the first, second and third trial was compared. Contrary to this, the retraction time of the experimental groups (groups administered cannabis), all showed a decrease in retraction with respect to trials. This denotes that at first week of exposure, cannabis served more as a central nervous system stimulant, making the animals more active and more conscious to the environment unlike what was noticed in the control groups. No remarkable changes were noticed in the group administered epinephrine in the different trials of first week of study. From the results, it was noticed that the higher the dose of the cannabis administered, the quicker the retraction time when the paws of the experimental animals were placed in the analgesimeter.

By the second week of administration of cannabis to the experimental animals, reductions in time of paw retraction was seen in the control group which was administered only distilled water. This is an indication that the animals in the control group were becoming aware of the surrounding using their natural CNS response. In the experimental (groups administered cannabis), they relatively responded faster compared to the value obtained in the week before. The group administered 0.5ml of cannabis had the fastest retraction time when compared to other groups administered cannabis. Some previous studies has shown that cannabis at lower doses could serve as central nervous system stimulant while at largest doses, it serves as CNS depressant. However, not all studies accepted this stipulation.[10]

At the end of the third week of administration, there was a reverse response of the experimental animals to cannabis administration when compared with the response obtained from the first week. The group administered 1ml of cannabis has the quickest retraction time, followed by the group administered 0.7ml of cannabis and lastly by the group administered 0.5ml. This would indicate that the experimental animals are becoming dependent on the intake of cannabis to function. This has enabled them increase the response thereby reduction in their retraction time, this was similarly the response obtained by a work done by Fried & Watkinson, (2000). In other studies, a combination of cannabis and morphine led to a significant reduction in pain, making a good combination for the management of pain associative disorders.[11]

Passive avoidance test was used to ascertain the level of awareness and memory retention after sub-acute administration of cannabis to Wistar rats. The result displayed in table 2 showed a decrease in the time taken to complete the test in all the experimental groups when compared with the control. Among the experimental groups, the group administered 0.5ml of cannabis used the shortest amount of time to complete the test during the first week of administration. It was sequentially

followed by the group administered 0.7ml and lastly by 1.0ml. The group administered epinephrine used a much lesser time when compared to the normal control group. The findings are still in line with the notion that lesser acute administration is likely to cause a more stimulating effect than when administered in higher doses. In the second week of administration, the relative time taken to accomplish the passive avoidance test was similar in group administered 0.5ml and 1.0ml of cannabis daily (low dose group and high dose group respectively. This entails a little transition from active stimulant to active depressant. However, these values were lower than what was obtained in the control group. Apparently, the changes caused by the intake of cannabis can significantly alter memory and spatial recognition as seen in the passive avoidance test (table 4.2). After the three weeks of administration, the experimental animals displayed a negative effect of cannabis with the middle and high dose group spending more time to complete the test. 0.5ml group showed a level of positive response and memory potentiation. At the higher doses, the cannabis impeded the memory functions of the animals which made the animals had more difficulty performing the test. A work done by[12] on sub-acute effect of cannabis revealed positives on various body systems especially when administered at lower doses, just as seen in this present study. The previous study also revealed positive effect of cannabis and several other body systems.

Following extraction of brain specimen after three weeks of cannabis sub-acute administration, various oxidative stress enzymes and advanced oxidative protein products were analyzed. Data from table 4.3 revealed a significant increase in malondialdehyde concentration in the group given 1ml of cannabis when compared with the control group, epinephrine group and other experimental groups. In this case, the cannabis has exerted a substantiable amount of stress on the brain tissue when given in high doses. Catalase showed a decrease in concentration in the group administered 1ml of cannabis when compared with other experimental groups and the normal control group, although, the epinephrine group showed the most decrease. However, these differences were seen to be statistically insignificant ($p<0.05$). Catalase is known for its conversion of hydrogen peroxide to water and oxygen, giving the brain more access to oxygen especially during stressed states. More results from table 4.3 revealed a statistically significant decrease in levels of superoxide dismutase in the group administered 0.7ml of cannabis with respect to other groups. The reason for this is likely due to its role in the elimination waste products of metabolism. Glutathione reductase, a very important antioxidant was significantly reduced in the high dose group (1ml group) when compared with the control group, reference group, and other test groups, this was because the brain consumed more glutathione reductase while trying to process the high content of cannabis present in the brain cells. Advanced oxidative protein products levels in the study was found to be significant when administered in low quantities, highest values of AOPP was noticed in the epinephrine group. Generally, AOPP was reduced in all tests when compared with the control groups, proposing a positive relationship between cannabis and advanced oxidative protein products, which means cannabis may lead to reduction in damage of proteins.[13]

CONCLUSION

This study was carried out to examine the effect of sub-acute cannabis exposure on advanced oxidative protein product (AOPP) and pain hypersensitivity in Wistar rats. From the study, sub-acute exposure of cannabis led to reduction in the levels of AOPP in brain tissues. It also revealed in a decrease in the level of oxidative stress parameters such as glutathione reductase, superoxide dismutase and catalase while an increase was seen in malondialdehyde. It also led to some level of reduction in the retraction time indicating an increased sensitivity to pain.

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