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OXIDATIVE STRESS MARKERS OF MALE WISTAR RATS FOLLOWING RECOVERY PERIOD FROM EXPOSURE TO *CANNABIS SATIVA*

AMUDA OLUWASOLA & LAILAT. N. USMAN

Department of Human Physiology, Faculty of Health Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria

Correspondence: droluwasola@alhikmah.edu.ng.

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ABSTRACT

Smoking of *Cannabis sativa* (Marijuana), a known psychoactive substance may result in side effect on living cells of the system. The aim of this study was to investigate the effect of *Cannabis sativa* (CS) on oxidative stress and the recovery period in male rats. Forty rats with mean weight of (170 g ± 1.24) were separately assigned into four groups of ten animals each. The rats in groups 1, 2, 3 and 4 received orally 1.0 ml of distilled water (control), 2.0mg, 4.0mg and 6.0mg of CS respectively for two weeks. Five animals from each group were sacrificed on the 15th day and the remaining animals were left for additional two weeks without treatment but with access to water and food before sacrifice. Oxidative parameters such ascatalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH), malondialdehyde (MDA), total antioxidant capacity (TAC) and lactate dehydrogenase (LDH) were determined using standard methods. Data were analyzed using a two-way Analysis of Variance. The groups treated with high dose (6.0 mg) and low doses (2.0mg and 4.0mg) of CS significantly ($p<0.05$) decrease catalase, SOD, GPx, GSH and TAC levels respectively when compared with the control. However, groups treated with CS showed significant ($p<0.05$) increase in MDA and LDH levels which were dose dependent when compared with the control. In addition, all the groups treated with low doses showed no significant difference in all the parameters but those treated with high dose showed significant ($p<0.05$) different when compared with the control after treatment. In conclusion, it could be deduced that these alterations in oxidative stress biomarkers were dependent on the doses of CS consumed. However, groups treated with low doses were able to recover from the damages caused by CS after treatment. This study recommends that people should abstain from the consumption of CS due to its detrimental effect in the body. Further research studies are needed to show if increase in the dose of melatonin may prevent CS consumption at high dose (6mg) since the same dose of melatonin was given at both low and high doses in this study.

Keywords: *Cannabis sativa,* Oxidative stress, Dose dependent, Recovery period
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INTRODUCTION

Cannabis sativa (CS) has been utilized for medicinal purposes since ancient times due to its abundant phytochemical content [1], hence the quest for harnessing its pharmacological potential by scientists. This substance is widely used as an illicit drug globally [2].The normal redox state in cells can be disrupted, leading to the production of peroxides and free radicals, damaging some of the cell components, including proteins, lipids, and DNA [3]. Oxidative stress (OS) in cells and tissues is caused by a disruption in the balance between pro-oxidants and antioxidants [4]. The ratio can be influenced by elevated levels of reactive oxygen species (ROS) or a decrease in antioxidant defense mechanisms [5]. OS can arise from an imbalance in the body's oxidizing system, primarily composed of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) [6].Antioxidant systems are essential in neutralizing free radicals that can have numerous harmful effects [7, 8]. OS is involved in aging [9], and is present in certain chronic diseases like diabetes, cancers, hypertension, and coronary heart disease [10], and also certain infections, particularly by the RNA viruses [11], a family to which belong corona viruses [12]. However few researches have been done on the effect of different doses of CS Wistar rats.

This research work examined the short-term effect of different concentrations of CS on oxidative stress in male Wistar rats and their recovery period after exposure to CS.

MATERIALS AND METHODS

Sample collection:

Cannabis sativa (CS) leaves were donated by National Drug Law Enforcement Agency (NDLEA), Nigeria, for research purpose only.

Extraction of *Cannabis sativa* leaves:

Extraction of *Cannabis sativa* (CS),was done with Soxhlet apparatus by soaking 800 grams of CS in 98% ethanol for 48 hours. The extract was filtered and the filtrate was poured into a round bottom conical flask which was fixed with a rotary evaporator. It was then evaporated and cooled. The dried yield of the extract was 55g.

Experimental animals:

Forty male rats with mean weight 170 g ± 1.24g used for this research were obtained from Temilade Animal Venture, Ogbomoso, Oyo State. They were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. The experimental protocol was approved by Ethical Committee of Al-Hikmah University, Ilorin, Nigeria.

Experimental protocol:

After 2 weeks of acclimatization, the animals were separately assigned into four groups of ten animals each, such that the rats in groups 1, 2, 3 and 4 respectively received orally 1 ml of distilled water (control), 2 mg/kg body weight (bw) of CS, 4 mg/kg bw of CS and 6 mg/kg bw of CS respectively for two weeks. Five animals from each group were sacrificed on the 15th day and the remaining animals were left for additional two weeks without treatment but with access to water and food before sacrifice.

Preparation of serum:

The male and female rats were sacrificed under ketamine anesthesia and blood was collected by cardiac puncture into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×*g* for 10 min. The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Drug and assay kits:

Lactate dehydrogenase (LDH) activity was assayed spectrophotometrically following the kit manufacturer's procedures (product code BXC0243; Fortress Diagnostics, UK). The determination of serum Superoxide Dismutase (SOD) concentration was done with SOD colorimetric assay kit (Fortress Diagnostics Ltd., Antrim, UK; Product code: BXC0531), following the manufacturer's protocols.

The determination of serum Glutathione Peroxidase (GPx) activity was done with GPx colorimetric assay kit (BioVision Inc., Milpitas, CA, USA), following the manufacturers protocols.

Based on the manufacturer's protocol, total Anti-oxidant capacity (TAC) measurement in the serum was done with a spectrophotometric microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA, USA) using Oxi-Select TAC assay kit that uses the single electron transfer mechanism (Cell Biolabs, Inc. San Diego, CA. cat no: STA-360).

The continuous Catalase activity was determined through spectrophotometric reading[1].

Reduced Glutathione (GSH) was measured according to the method of [2].

The assay method of [3], modified by [4] was adopted for Malondiadehyde (MDA).

Statistical analysis:

Results were expressed as the mean ± standard error of mean. Data were analyzed using a two-way Analysis of Variance, followed by the LSD post-hoc test to determine significant differences in the entire parameters graph pad, Version 9.0. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS:

Table 1 shows the LDH levels of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) increase LDH level when compared with the control (table 1). However, all the groups treated with low doses showed no significant difference in LDH but the group treated with high dose showed significant ($p<0.05$) increase in LDH when compared with the control after treatment (table 1).

Table 2 shows the Catalase levels of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) decrease catalase level when compared with the control (table 2). However, all the groups treated with low doses showed no significant difference in catalase but the group treated with high dose showed significant ($p<0.05$) decrease in catalase when compared with the control after treatment (table 2).

Table 1: Lactate Dehydrogenase levels in serum of rats

Groups	**LDH (u/l)**
Control	440.63±13.9
2mg/kg bw of CS (DT)	513.2±5.3[a]
2mg/kg bw of CS (AT)	469.8±13.9
4mg/kg bw of CS (DT)	609.9±3.9[a]
4mg/kg bw of CS (AT)	517.5±2.3
6mg/kg bw of CS (DT)	726.5±8.5[a]
6mg/kg bw of CS (AT)	712±3.8[b]

Values are expressed as mean±SED; aP<0.05 vs control ; bP<0.05 vs control and other treatment groups. During treatment (DT), after treatment (AT)

Table 2: Catalase levels of rats

Groups	Catalase (u/l)
Control	29±0.6
2mg/kg bw of CS (DT)	25±1.5[c]
2mg/kg bw of CS (AT)	28±0.7
4mg/kg bw of CS (DT)	20±1[c]
4mg/kg bw of CS (AT)	24±0.6
6mg/kg bw of CS (DT)	13±1.9[c]
6mg/kg bw of CS (AT)	14±1.3[v]

Values are expressed as mean±SED; cP<0.05 vs control ; vP<0.05 vs control and other treatment groups. During treatment (DT), after treatment (AT).

Table 3: GSH levels of rats

Group	GSH (u/l)
Control	84.7±1.3
2mg/kg bw of CS (DT)	65.7±0.8[k]
2mg/kg bw of CS (AT)	72.6±0.9
4mg/kg bw of CS (DT)	52.6±1.4[k]
4mg/kg bw of CS (AT)	62.1±0.7
6mg/kg bw of CS (DT)	41.1±0.8[k]
6mg/kg bw of CS (AT)	42.4±1.1[j]

Values are expressed as mean±SED; kP<0.05 vs control ; jP<0.05 vs control and other treatment groups. During treatment (DT), after treatment (AT).

Table 3 shows the GSH of rats for control, 2.0 mg/kgbw CS, 4.0 mg/kgbw CS and 6.0 mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) decrease GSH level when compared with the control (table 3). However, all the groups treated with low doses showed no significant difference in GSH but the group treated with high dose showed significant ($p<0.05$) decrease in GSH when compared with the control after treatment (table 3).

Table 4 shows the SOD of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) decrease SOD level when compared with the control (table 4). However, all the groups treated with low doses showed no significant difference in SOD but the group treated with high dose showed significant ($p<0.05$) decrease in SOD when compared with the control after treatment (table 4).

Table 5 shows GPx of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) decrease GPx level when compared with the control (table 5). However, all the groups treated with low doses showed no significant difference in GPx but the group treated with high dose showed significant ($p<0.05$) decrease in GSH when compared with the control after treatment (table 5).

Table 6 shows MDA of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbw CS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) increase MDA level when compared with the control (table 6). However, all the groups

treated with low doses showed no significant difference in MDA but the group treated with high dose showed significant ($p<0.05$) increase in MDA when compared with the control after treatment (table 6).

Table 7 shows TAC of rats for control, 2.0mg/kgbw CS, 4.0mg/kgbw CS and 6.0mg/kgbwCS during and after treatment respectively. Administration of high dose (6 mg) and low doses (2mg and 4 mg) of CS significantly ($p<0.05$) decrease TAC level when compared with the control (table 7). However, all the groups treated with low doses showed no significant difference in TAC but the group treated with high dose showed significant ($p<0.05$) decrease in TAC when compared with the control after treatment (table 7).

Table 4: SOD level of rats

Group	**SOD (g/l)**
Control	8.2±0.2
2mg/kg bw of CS (DT)	5.8±0.04^{e}
2mg/kg bw of CS (AT)	6.8±0.1
4mg/kg bw of CS (DT)	4.6±0.1^{e}
4mg/kg bw of CS (AT)	5.7±0.1
6mg/kg bw of CS (DT)	3.7±0.1^{e}
6mg/kg bw of CS (AT)	3.8±0.1^{r}

Values are expressed as mean±SED; $^{e}P<0.05$ vs control ; $^{r}P<0.05$ vs control and other treatment groups. During treatment (DT), after treatment (AT).

Table 5: GPx level of rats

Group	**GPx (mmol/l)**
Control	1.4±0.2
2mg/kg bw of CS (DT)	0.7±0.3^{z}
2mg/kg bw of CS (AT)	1.2±0.2
4mg/kg bw of CS (DT)	0.3±0.02^{z}
4mg/kg bw of CS (AT)	0.7±0.02
6mg/kg bw of CS (DT)	0.2±0.02^{z}
6mg/kg bw of CS (AT)	0.3±0.01^{f}

Values are expressed as mean±SED; $^{z}P<0.05$ vs control ; $^{f}P<0.05$ vs control and other treatment groups. During treatment (DT), after treatment (AT).

Table 6: MDA levels of rats

Group	MDA (mmol/l)
Control	35.6±1.4
2mg/kg bw of CS (DT)	37.4±0.9[t]
2mg/kg bw of CS (AT)	36.4±0.9
4mg/kg bw of CS (DT)	38.6±1.5[t]
4mg/kg bw of CS (AT)	37.5±1.0
6mg/kg bw of CS (DT)	40.4±1.1[t]
6mg/kg bw of CS (AT)	40.1±0.9[d]

Values are expressed as mean±SED; tP<0.05 vs control ; dP<0.05 vs control and other treatment groups. During treatment (DT), after treatment (AT).

Table 7: TAC level of rats

Group	TAC (umol/l)
Control	69.9±1.2
2mg/kg bw of CS (DT)	50.5±1.6[i]
2mg/kg bw of CS (AT)	66.9±1.3
4mg/kg bw of CS (DT)	42.7±1.4[i]
4mg/kg bw of CS (AT)	54.8±2.6
6mg/kg bw of CS (DT)	34.8±1.7[i]
6mg/kg bw of CS (AT)	32.0±1.8[j]

Values are expressed as mean±SED; iP<0.05 vs control; jP<0.05 vs control and other treatment groups. During treatment (DT), after treatment (AT).

DISCUSSION

When the quantity of reactive oxygen species (ROS) exceeds the amount of antioxidants that can scavenge them, oxidative stress takes place and might have harmful consequences. Reactive oxygen species' systemic manifestation and a biological system's capacity to quickly detoxify the reactive intermediates or repair the harm they cause are out of balance, which is what is known as oxidative stress [17]. When a cell's normal redox state is disturbed, peroxides and free radicals are produced, which harm various parts of the cell, including DNA, lipids, and proteins, and can have toxic effects [18]. In addition to base damage, oxidative stress resulting from oxidative metabolism also damages DNA strands [19]. The majority of base damage is indirect and results from the generation of reactive oxygen species, such as superoxide radical (O_2 −), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) [20]. According to study [21], oxidative stress is thought to play a role in the development of attention deficit

hyperactivity disorder, cancer, Parkinson's disease, Lafora disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, sickle-cell disease, lichen planus, vitiligo, autism, infection, chronic fatigue syndrome, and depression.

This oxidative stress can be prevented by the body system through the production of antioxidant enzymes [22].

According to our findings, CS significantly increased LDH dose-dependently when compared with the control. This is in agreement with the studies of [8, 24] who observed increase in LDH following the administration of CS. The work also confirmed a previous study [25] which reported that LDH plays a significant role in the generation of NADPH, which could be due to nicotinamide adenine dinucleotide phosphate (NADPH), a fuel for ROS generation. The reduction in TAC observed in cannabis-treated rats when juxtaposed with the control indicated a role for oxidative stress in their gonadotoxic consequences. This is also in line with the study of [8] that showed significant decrease in TAC following administration of different doses of CS in rats. This could have contributed to the build-up of ROS because it is consistent with the increase in lactate dehydrogenase activity that was previously seen in the cannabis-treated rats.

Additionally, it was shown that CS increased MDA level which was dose dependent. This finding corresponds to that of [26] who observed increase in MDA level following administration of CS in rats. This finding suggests that CS may cause lipid peroxidation of polyunsaturated fatty acids, which ROS break down [8]. More so, the results indicated a decrease in glutathione reductase (GSH), superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These were consistent with the increase in lactate dehydrogenase activity in these animals, which led to accumulation of ROS. These are in line with the findings of [8, 27] who observed decrease in antioxidant enzymes following administration of CS in rats.

However, when compared to the control group following treatment after two weeks, the groups treated with high dose exhibited significant differences, while the other groups that were treated with low doses did not demonstrate any significant difference in any of the parameters. This could mean that it might take the body longer period of time to recover from oxidative damage brought on by the high dose of CS before withdrawal.

CONCLUSION

This study revealed that CS could stimulate oxidative stress which was dose dependent. However, withdrawal from its consumption could ameliorate the damage caused. It may be recommended that people should stay away from

the consumption of CS because of its detrimental effect most especially on the oxidative stress in the body.

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