The Effect of Ethanolic Extract of *Cannabis sativa* Leaves from Nigeria on the Antioxidants Markers in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study. Author IW performed statistical analysis. Author EBU wrote the first draft and final manuscript. Authors OGA and UAE proof read the final work. All authors read and approved the final manuscript.

Short Communication

ABSTRACT

Background: *Cannabis sativa* is an annual herbaceous plant in the Cannabis genus. The cannabis plant is widely regarded as a potent psychoactive, medicinal plant. Reportedly used for recreation and as intoxicant. The medical uses of the plant include effective control and management of chronic health problems such as HIV/AIDs, cancer, cachexia, nausea and vomiting, asthma and hypertension. *C. sativa* is known to possess antioxidative properties. This study therefore investigated the effects of *C. sativa* on antioxidant concentrations in albino Wistar rats.
Materials and Methods: Thirty (30) rats used for this study were divided into three groups of 10 rats each. Group 1 received distilled water and served as control. Group 2 received *C. sativa* extract (100 ml/Kg body weight) by gavage and served as low dose group. Group 3 received *C. sativa* (250 ml/Kg body weight) by gavage and served as high dose group once daily for 28 days.
Results: Catalase (CAT) concentration was significantly lower ($P<0.05$) in the low dose group as compared with control. In the high dose group, CAT concentration was significantly lower ($P<0.05$)

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when compared with the low and control groups respectively. Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) concentrations were significantly higher ($P<0.05$) in the low dose group as compared with their respective control. GPx and SOD concentrations were significantly higher ($P<0.05$) in the high dose groups as compared to low dose and control groups respectively.
Conclusion: CAT concentration decreased dose-dependently, while GPx and SOD concentrations increased dose-dependently among treated groups. Treatment with *C. sativa* revealed a paradoxical effect on CAT concentration with respect to GPx and SOD concentrations. Therefore, oral ingestion of ethanolic extract of *C. sativa* may not have significant effect on the body's antioxidant stores due to the balance created for CAT deficiency by increased GPx and SOD concentrations.

Keywords: Cannabis sativa; catalase; glutathione peroxidase; superoxide dismutase; albino rats.

ABBREVIATIONS

ANOVA	: *Analysis of variance*
CAT	: *Catalase*
CAT(U/mgHb)	: *Catalase status in erythrocytes*
CB_1	: *Cannabinoid receptor 1*
CB_2	: *Cannabinoid receptor 2*
CBD	: *Cannabinoid*
DNA	: *Deoxyribonucleic acid*
EDTA	: *Ethylene diamine tetra acetic acid*
G-6-PD	: *Glucose-6-phosphate*
GPx	: *Glutathione peroxidase*
GPx/mgHb	: *Glutathione status in: erythrocytes*
GR	: *Glutathione reductase*
GSH	: *Glutathione*
GSSG	: *Glutathione disulfide*
H_2O_2	: *Hydrogen peroxide*
HIV/AIDS	: *Human immunodeficiency virus, Acquired immune deficiency syndrome*
LSD	: *Least square division*
Nrf2	: *Nuclear erythroid 2- related factor*
O_2^-	: *Superoxide*
ROS	: *Reactive oxygen specie*
SOD	: *Superoxide dismutase*
SOD/mgHb	: *Superoxide dismutase status in erythrocytes*
THC	: *Tetrahydrocannabinol*

1. INTRODUCTION

Cannabis sativa is an annual herbaceous flowering plant indigenous to eastern Asia but now of cosmopolitan distribution due to widespread cultivation [1]. It has been cultivated throughout recorded history, used as a source of industrial fiber [2] seed oil [3], food [4] recreation [5], religious and spiritual moods and medicine [6-8]. Each part of the plant is harvested differently, depending on the purpose of its use.

Cannabis preparation is known by various names worldwide. It is commonly called pot, cannabis, grass, weed, hemp, joint and medically referred to as tetrahydrocannabinol (THC), cannabinoid (CBD). It is known as marijuana in America and hashish in the Middle East [9,10].

1.1 Mechanism of Action of Cannabinoid (CBD) on Antioxidants

As documented [11] cannabinoid (CBD) modifies the redox balance by changing the level and activity of antioxidants [12,13]. CBD antioxidant activity begins at the level of protein transcription by activating the redox-sensitive transcription factor known as the nuclear erythroid 2-related factor (Nrf2) [14] which is responsible for the transcription of cytoprotective genes, including antioxidant genes [15].

Tetrahydrocannabinol (THC) is the primary psychoactive component of the *C. sativa* plant [16]. It mimics the actions of anandamide and neurotransmitters produced naturally in the body [17]. It binds to the cannabinoid receptors (CB_1) in the brain to mediate the effects associated with cannabis and the CB_2 receptor mainly expressed in cells of the immune system [18,19]. CB_1 receptors are also found on myenteric and submucosal nerves; the activation of these receptors by CBD inhibit gastrointestinal motility, intestinal secretion and gastric acid secretion [20]. According to Adami et al. [21] the gastric antisecretory effects of CBD in rat is mediated by suppression of vagal drive to the stomach through activation of CB_1 receptors by THC; and also, by the inhibition of histamine, a process that is helpful in ulcer control [22]. According to Pertwee [18] the regions of the nervous system where CBD receptors are moderately concentrated are the hypothalamus, amygdala, spinal cord, brain stem and the nucleus solitaries. This may explain a possible mechanism through which CBD of *C. sativa*

serves as an effective therapy in the treatment of neuronal defects; and is in consonance with the work of Siegfried et al. [23] who reported a potential therapeutic role of CBD in neuroprotection, as evidenced in rat cortical neuronal cultures. According to Welch et al. [24], CBD possess therapeutic properties including analgesia, alleviating nausea, ocular hypotension and antiemesis.

In the early 1970s, research conducted confirmed that cannabis use increases both the desire to eat and the palatability of food [25]. A 2009 study examining the prevalence of obesity as a function of cannabis use, however, found lower rates of obesity among regular cannabis users than the general population [26]. In a recent study reported in the American Journal of Medicine, marijuana use is associated with 16% lower fasting insulin levels and smaller waist circumferences, which makes it a useful tool in treating diabetes and obesity [27].

C. sativa is known to possess antioxidative properties [28]. Different antioxidants are present at wide range of concentrations in the body fluids and tissues. GSH is an important cellular antioxidant with a central role of maintaining the cell's redox state [29]; a cysteine-containing peptide found in most forms of aerobic life [30]. GSH is capable of preventing damage to important cellular components caused by ROS such as free radicals, peroxides, lipid peroxides, and heavy metals [31]. In cells, GSH is maintained in the reduced form by the enzyme glutathione reductase (GR) which in turn reduces other metabolites and enzyme systems (ascorbate, glutathione peroxidases and glutaredoxins) and as well react directly with oxidants [32]. According to Lu [33] GSH plays a role in progression of cell cycle as well as cell death. GSH levels regulate redox changes to nuclear proteins necessary for the initiation of cell differentiation [29]. Differences in GSH levels also determine the expressed mode of cell death [34]. Interestingly, manageably low levels of GSH result in the systemic breakage of cells, while excessively low levels result in rapid cell death [35].

Oxidative stress from oxidative metabolism cause base damage, as well as strand breaks in DNA [36]. Oxidative stress can cause disruptions in normal mechanisms of cellular signaling [37]. Oxidative stress is 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 [38]. Oxidative stress is also linked to certain cardiovascular diseases, chronic fatigue syndrome and hyperoxia [39]. To balance the oxidative stress, plants and animals have evolved complex systems of overlapping antioxidants, such as GPx, CAT and SOD produced endogenously, or the dietary antioxidants vitamin A, C and vitamin E [40].

Research studies on the effects of *C. sativa* on different body systems and diverse physiological parameters have been carried out in the past but this research problem has not been painstakingly investigated. It is to this end that this study attempted to fill in the existing gap and enhance the understanding of the effect of exogenous CBD in *C. sativa* on endogenous antioxidant concentrations in albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Dried leaves of *C. sativa* were purchased from the office of the National Drug Law Enforcement Agent (NDLEA) and homogenized using the manual blender. 720 gm of the blended leaves was weighed and soaked in 4500 ml of ethanol and allowed for 48 hours. The homogenate was filtered using Whatman's No.1 filter paper as previously described by [41,42] with little modification. The filtrate was stored in clean glass containers and refrigerated. The extract was brought out of the refrigerator 2 hours to oral administration. Different concentration of the extract was administered orally according to body weight.

2.2 Experimental Animals

Approval for the study was obtained from the Animal Ethical Committee of the College of Medical Sciences with the ethical approval number: FAREC-FBMS/25/2016. Strict adherence and compliance to the guide for the care and use of laboratory animals as stipulated by the National Research Council [43] was maintained. Thirty (30) male albino Wistar rats of mean weight 170 gm were used for this study. They were maintained in the animal facility of the Department of Physiology, University of Calabar, Nigeria, at a temperature of 30 ± 2°C and 12 h light/dark cycles with free access to food and water.

2.3 Experimental Design

Thirty (30) rats used for this study were divided into three groups of 10 rats each. Group 1 received distilled water and served as control. Group 2 received *C. sativa* (100 ml/kg, by gavage (low dose group)). Group 3 received *C. sativa* (250 ml/kg, by gavage (high dose group)) once daily for 28 days after which blood samples were collected for estimation of antioxidant concentrations.

2.4 Collection of Blood Samples

Blood samples were collected using cardiac puncture through a modified procedure described by [44] and used by Umoren et al. [45] into 30 EDTA bottles.

2.5 Measurement of Antioxidant Enzymes

The methodologies used in determining antioxidant enzymes SOD, CAT and GPx are standard in histology laboratories.

SOD activity was determined using diagnostic kit RANSOD produced by RANDOX (Randox Laboratories Ltd., Crumlin, County Antrim, UK) according to Arthur and Boyne [46] with little modifications as described by [11] and expressed in U of SOD/mgHb.

GPx activity was determined using diagnostic kit RANSEL produced by RANDOX (Randox Laboratories Ltd., Crumlin, County Antrim, UK) according to Paglia and Valentine [47] with little modifications as described by [11] and expressed in (nmol/min/mL) of GPx/mgHb.

CAT activity was determined by the method of Beers and Sizer [48] as modified and described by [11].

2.6 Statistical Analysis

Results were expressed on the mean ± standard deviation. Statistical analysis was carried out using SPSS software, version 16.0 (SPSS Inc. Chicago II, USA). Differences among the groups were investigated using one-way analysis of variance (ANOVA) followed by a Post Hoc test (Least Square Deviation, LSD). $P<0.05$ was considered statistically significant. Variant means were separated using multiple range Duncan's post hoc test.

3. RESULTS

3.1 Effect of *C. sativa* on CAT Concentration among the Experimental Groups

The effect of *C. sativa* on CAT concentration among the experimental groups is shown in Table 1. There was a significant decrease ($P<0.05$) in CAT concentration in the low dose group following *C. sativa* treatment when compared with control. In the high dose group, CAT concentration was significantly lower ($P<0.05$) when compared to the control and low dose groups.

Table 1. Effect of *C. sativa* on CAT concentration among the experimental groups

Group	CAT(U/mgHb)
Control (distilled water)	0.37± 0.01
Extract of C. sativa (100 ml/kg b.w.)	0.31± 0.01
Extract of C. sativa (250 ml/kg b.w.)	0.21 ± 0.00

3.2 Effect of *C. sativa* on GPx Concentration among the Experimental Groups

The effect of *C. sativa* on GPx concentration among the experimental groups is shown in Table 2. There was a significant increase ($P<0.05$) in GPx concentration in the low dose group following *C. sativa* treatment when compared with control. In the high dose group, GPx concentration was significantly higher ($P<0.05$) as compared to the control and low dose groups.

Table 2. Effect of *C. sativa* on GPx Concentration among the experimental groups

Group	GPx/mgHb
Control (distilled water)	113.80 ± 6.67
Extract of C. sativa (100 ml/kg b.w.)	150.97 ± 5.13
Extract of C. sativa (250 ml/kg b.w.)	282.91± 5.19

3.3 Effect of *C. sativa* on SOD Concentration among the Experimental Groups

The effect of *C. sativa* on SOD concentration among the experimental groups is shown in

Table 3. There was a significant increase (P<0.05) in SOD concentration in the low dose group following *C. sativa* treatment when compared with control. In the high dose group, SOD concentration was significantly higher (P<0.05) when compared to the control and low dose groups.

Table 3. Effect of *C. sativa* on SOD Concentration among the experimental groups

Group	SOD/mgHb
Control (distilled water)	0.67 ± 0.02
Extract of C. sativa (100 ml/kg b.w.)	0.76 ± 0.01
Extract of C. sativa (250 ml/kg b.w.)	0.92 ± 0.02

4. DISCUSSION

The effect of ethanolic extract of *C. sativa* on the antioxidants, CAT, GPx and SOD concentrations in the serum of albino Wistar rats was investigated.

Results of the study showed decreased concentration of CAT in the low dose and high dose groups treated with *C. sativa* extract as compared to control. While SOD and GPx increased dose-dependently among the treated groups, indicating a paradoxical effect of the cannabis extract on CAT concentration with reference to GPx and SOD concentration. SOD is a decisive antioxidant enzyme in aerobic cell. It is responsible for the elimination of superoxide radicals. SOD catalyzes the dismutation of two molecules: hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). GPx on the other hand is a selenocysteine dependent enzyme. In cell, GPx is the most important H_2O_2 scavenging enzyme that converts H_2O_2 to water [49]. SOD and GPx can directly counterbalance the oxidant attack and protect the cells against DNA damage [11]. Since GPx and SOD protect human cells especially neurons from oxidative damage by the active scavenging of H_2O_2 and lipid hydroperoxide, oral ingestion of ethanolic extract of *C. sativa* may not have a significant effect on the body's antioxidant stores as a result of the balance created for CAT deficiency by increased GPx and SOD concentrations.

SOD is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into ordinary molecular oxygen and H_2O_2. CAT and peroxidases convert H_2O_2 into water. If H_2O_2 removal is inhibited, direct toxicity ensues due to H_2O_2-mediated damage. On the other hand, GPx requires several secondary enzymes including glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6-PD) and co-factors including glutathione (GSH), NADPH, and Glucose 6-phosphate to function at high efficiency. If GR is inhibited, cells cannot remove H_2O_2 via the GPx system, thereby increasing the levels of glutathione disulfide (GSSG). Inhibition of glutathione synthesis results in its depletion, and GPx will not be able to remove H_2O_2 [50]. This, therefore implies that intake of CBD from *C. sativa* may improve the levels of these endogenous antioxidants to function maximally.

CAT has one of the highest turnover numbers of all enzymes [51]. Therefore, if CAT levels decline, H_2O_2 cannot be broken down, but will accumulate inside the body. Cells cannot remove H_2O_2 if CAT is inhibited. The accumulated H_2O_2 will then enhance the bleaching of hair from inside out [52]. It has been reported that humans with genetic deficiency or malfunction of CAT suffer age-associated degenerative diseases like diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia [53]. From the result obtained, treatment with *C. sativa* improved GPx level significantly. Since GPx main biological role is to protect human cells especially neurons, from oxidative damage, it therefore implies that many cannabinoids exert a considerable protective antioxidant effect in neuronal cultures.

5. CONCLUSION

CAT concentration following *C. sativa* administration reduced dose-dependently while GPx and SOD increased dose-dependently following *C. sativa* administration among treated groups; indicating a paradoxical effect of the Cannabis extract on CAT concentration with reference to GPx and SOD concentrations. This suggests that oral ingestion of ethanolic extract of *C. sativa* may not have a significant effect on the body's antioxidant stores as a result of the balance created for CAT deficiency by the increased GPx and SOD concentrations. The exogenous administration of CBD of *C. sativa* tends to maintain a relative balance in the antioxidant system. This may be one of the reasons extracts of *C. sativa* is used for the management of degenerative disorders such as Alzheimer's disease and Parkinson's disease.

DISCLAIMER

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.

ETHICAL APPROVAL

All authors hereby declare that strict adherence and compliance to guidance for the care and use of laboratory animals as stipulated by the National Research Council (2011) was maintained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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