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ABSTRACT

By critically examining studies ranging from randomized controlled trials to meta-analyses and cross-sectional studies, we seek to elucidate the potential benefits of vitamin C (vit. C) supplementation in mitigating oxidative stress associated with cannabis (CS) consumption.

Twenty female rats with mean weight of (160 g ± 1.09) were separately assigned into four groups of five animals each. The rats in groups 1, 2, 3 and 4 respectively received orally 1.0 ml of distilled water (control), 2.0mg/kg body weight (bw) CS+4mg/kg bw vit. C, 4.0mg/kgbw CS+4.0mg/kg bw vit. C and 6.0mg/kgbw CS+mg/4.0kg bw vit. C respectively for two weeks. All the groups have free access to food and water. All the rats were sacrificed on the 15th day. Lactate dehydrogenase (LDH), Catalase, Glutathione reductase (GSH), Glutathione peroxide (GPx), Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) were determined using standard methods. Data were analyzed using a two-way Analysis of Variance. This study revealed that co-administration of CS and vit. C showed no significant difference in all the parameters across the groups treated with low doses of CS (2.0mg and 4.0mg) when compared with the control. However, co-administration of CS and vit. C showed significant ($p<0.05$) increase in LDH and MDA levels with significant ($p<0.05$) decrease in Catalase, GSH, GPx, and TAC in the groups treated with high dose of CS (6.0mg) when compared with the control and low dose groups. In conclusion, this study suggests that CS could cause oxidative stress at both low and high doses, which could be prevented by vit. C when consume at low doses. Since the same dose of vit. C was co-administered with different doses of CS, this could be the reason why vit. C was unable to prevent the side effect of CS on the animals that were given high dose of CS. This study recommends that people should abstain from the consumption of CS. However, because of its medicinal importance, consumption of vit. C supplement maybe used to prevent its negative effects in the body.

Keywords: *Cannabis sativa,* Vitamin C, Oxidative stress, Co-administration

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INTRODUCTION

Cannabis, scientifically known as Cannabis sativa (CS), is a versatile flowering plant that has been cultivated for various purposes for thousands of years. Belonging to the Cannabaceae family, cannabis is native to Central Asia but is now grown globally in diverse climates [1]. This plant has garnered significant attention due to its unique chemical composition, which includes over 100 different compounds known as cannabinoids, along with terpenes, flavonoids, and other phytochemicals [2].The most well-known cannabinoids found in cannabis are Tetrahydrocannabinol (THC) and Cannabidiol (CBD).

THC is the primary psychoactive compound responsible for the euphoric sensation or "high" associated with cannabis use [3]. On the other hand, CBD is non-intoxicating and is believed to have various therapeutic properties, including anti-inflammatory, analgesic, and anxiolytic effects [4]. Cannabis is cultivated for a wide range of purposes, including medicinal, recreational, industrial, and spiritual uses. In the medical realm, cannabis has gained attention for its potential to alleviate symptoms associated with various health conditions, such as chronic pain, nausea, epilepsy, and certain psychiatric disorders [5]. Some regions have legalized medical cannabis, allowing it to be prescribed or recommended by healthcare professionals for specific indications [6].Recreational cannabis use is also prevalent in many parts of the world, often sought after for its psychoactive effects [7]. Studies have shown that CS has the potent ability to stimulate oxidative stress in humans and animals [8, 9].

Vitamin C (vit. C), also known as ascorbic acid, is often hailed as a powerhouse nutrient due to its antioxidant properties [10]. Antioxidants are like the superheroes of the body, fighting off harmful molecules called free radicals [11]. These free radicals can wreak havoc in our cells, damaging DNA, proteins, and lipids, and contributing to various health issues, including aging and disease [12]. It also helps regenerate other antioxidants in the body, such as vitamin E, further boosting the defense system against oxidative stress [13]. Vit. C acts as a potent antioxidant in plants, including cannabis, helping to neutralize harmful effects of reactive oxygen species (ROS) and protect cellular structures from oxidative damage [14]. By studying the role of vit. C in combating oxidative stress, researchers can develop strategies to enhance the antioxidant defense mechanisms, ultimately improving resilience to environmental stressors [15]. This study investigated the effects of vit. C on oxidative stress caused by the consumption of CS in female wistar rats.

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 500 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 (this was to completely remove ethanol from the final extract so as not to contaminate it). The dried yield of the extract was 30g (weight of the sample obtained after dying).

Experimental animals:

Twenty female rats with mean weight of (160g±1.09) that were 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 five animals each. The rats in groups 1, 2, 3 and 4 respectively received orally, 1.0 ml of distilled water (control), 2.0mg/kg body weight (bw) CS+4mg/kg bw vit. C, 4.0mg/kgbw CS+4.0mg/kg bw vit. C and 6.0mg/kgbw CS+mg/4.0kg bw vit. C respectively for two weeks. All the groups have free access to food and water. All rats were sacrificed on 15th day.

Preparation of serum:

The 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 Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Drug and assay kits:

Vitamin C (Liquid form) was purchased from Mom Rota pharmaceutical company, Ilorin, Nigeria. Lactate dehydrogenase (LDH) activity was assayed spectrophotometrically following the kit manufacturer's procedures (product code BXC0243; Fortress Diagnostics, UK). 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 OxiSelect TAC assay kit that uses the single electron transfer mechanism. The continuous catalase activity was determined through spectrophotometric reading [16].

Reduced glutathione (GSH) was measured according to the method of [17]. The assay method of [18], modified by [19] 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

Tables 1 and 2 show LDH and MDA levels of rats for control; 2.0mg/kg body weight (bw) CS+4mg/kg bw vit. C; 4.0mg/kgbw CS+4.0mg/kg bw vit. C; 6.0mg/kgbw CS+4.0mg/kg bw vit. C, respectively.

Co-administration of CS and vit. C showed no significant difference in the LDH and MDA levels across the groups treated with low doses of CS (2.0mg and 4.0mg) when compared with the controls (tables 1 and 2). However, co-administration of CS and vit. C significantly ($p<0.05$) increase LDH and MDAlevels in the groups treated with high dose of CS (6.0mg/kgbw) when compared with the controls and low dose groups (tables 1 and 2).

Table 1: LDH level in serum of rats

Group	LDH (u/l)
Control	626.13±23.38
2.0mg/kg bw of CS+4mg/kg bw vit. C	641.78±23.08
4.0mg/kg bw of CS+4mg/kg bw vit. C	694.76±23.01
6.0mg/kg bw of CS+4mg/kg bw vit. C	826.11±19.24[q]

Values are expressed as mean ± SEM; qP< 0.05 vs control and low dose groups.

Table 2: MDA level in serum of rats

Group	MDA (umol/l)
Control	23.40±0.52
2.0mg/kg bw of CS+4mg/kg bw vit. C	25.60±0.25
4.0mg/kg bw of CS+4mg/kg bw vit. C	26.00±0.32
6.0mg/kg bw of CS+4mg/kg bw vit. C	40.35±0.49[q]

Values are expressed as mean ± SEM; qP< 0.05 vs control and low dose groups.

Tables 3, 4, 5 and 6 show Catalase, GSH, GPx and TAC levels of rats for control, 2.0mg/kg body weight (bw) CS+4mg/kg bw vit. C, 4.0mg/kgbw CS+4.0mg/kg bw vit. C and 6.0mg/kgbw CS+mg/4.0kg bw vit. C respectively.

Co-administration of CS and vit. C showed no significant difference in the Catalase, GSH, GPx and TAC levels across the groups treated with low doses of CS (2.0mg and 4.0mg) when compared with the control (tables 3, 4, 5 and 6). However, co-administration of CS and vit. C significantly ($p<0.05$) increase the Catalase, GSH, GPx and TAC levels in the group treated with high dose of CS (6.0mg/kgbw) when compared with the control and low dose groups (tables 3, 4, 5 and 6).

Table 3: Catalaselevel in serum of rats

Group	Catalase (u/l)
Control	28.05±0.51
2.0mg/kg bw of CS+4mg/kg bw vit. C	25.00±0.32
4.0mg/kg bw of CS+4mg/kg bw vit. C	24.94±0.42
6.0mg/kg bw of CS+4mg/kg bw vit. C	16.80±0.49[g]

Values are expressed as mean±SEM; gP<0.05 vs control and low dose groups.

Table 4: GSHlevel in serum of rats

Group	GSH (U/L)
Control	110.08±4.27
2.0mg/kg bw of CS+4mg/kg bw vit. C	98.5±1.58
4.0mg/kg bw of CS+4mg/kg bw vit. C	94.28±1.12
6.0mg/kg bw of CS+4mg/kg bw vit. C	69.8±2.02[g]

Values are expressed as mean±SEM; gP<0.05 vs control and low dose groups.

Table 5: GPxlevel in serum of rats

Group	GPx (mmol/l)
Control	3.07 ±0.26
2.0mg/kg bw of CS+4mg/kg bw vit. C	2.80±0.20
4.0mg/kg bw of CS+4mg/kg bw vit. C	2.28±0.09
6.0mg/kg bw of CS+4mg/kg bw vit. C	0.72±0.15[g]

Values are expressed as mean±SEM; gP<0.05 vs control and low dose groups.

Table 6: TAClevel in serum of rats

Group	TAC (umol/l)
Control	87.40±1.50
2.0mg/kg bw of CS+4mg/kg bw vit. C	77.03±0.46
4.0mg/kg bw of CS+4mg/kg bw vit. C	73.39±2.11
6.0mg/kg bw of CS+4mg/kg bw vit. C	55.6±1.23[g]

Values are expressed as mean±SEM; gP<0.05 vs control and low dose groups.

DISCUSSION

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, and has been implicated in the pathogenesis of numerous diseases such as neurodegenerative disorders, cardiovascular diseases, and cancer [20]. Vit. C(ascorbic acid), is a highly potent water-soluble antioxidant that plays a vital role in scavenging ROS, regenerating other antioxidants, and modulating the cellular redox status [21]. Furthermore, vit. C has been reported to possess anti-inflammatory properties and to regulate gene expression associated with oxidative stress pathways [22].

Lactate dehydrogenase (LDH) is an enzyme involved in the conversion of pyruvate to lactate during anaerobic glycolysis [23]. It is found in almost all body tissues and is released into the bloodstream when cells are damagedor under anaerobic conditions [23]. Elevated levels of LDH in the blood can indicate tissue damage or cell death, which can result from conditions like myocardial infarction, liver disease, hemolysis, and certain cancers [24]. Tissue damage often leads to oxidative stress, so high LDH levels can indirectly reflect oxidative stress [25]. The significant increase in LDH levels in the high dose of CS and vit. C when compared to the control indicates tissue damage. However, no effect was observed in low dose of CS and vit. C, suggesting that vitamin C was able to suppress the oxidative stress by regulating LDH levels, thereby preventing tissue damage. This result aligns with the study of [26] who showed that vit. C supplement led to a decrease in lactate dehydrogenase (LDH) activity in rats, indicating its role in preventing cell damage during thermal stress.

Furthermore, catalase is one of the most important antioxidant enzymes which catalyzes the conversion of two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water in a two-step reaction [27]. Additionally, the assessment of glutathione (GSH) as a marker of oxidative stress has drawn more attention in recent years because GSH

plays a part in detoxifying xenobiotics and defending against oxidative stress, its availability in the reduced form (GSH) may be crucial for maintaining health [28]. A decline in GSH concentrations has been linked to aging and the development of numerous diseases, including AIDS, rheumatoid arthritis, muscular dystrophy, amyotrophic lateral sclerosis, Alzheimer's disease, alcoholic liver disease, cataractogenesis, respiratory distress syndrome, progeria, and Werner syndrome, according to research conducted on both humans and various animal models [29]. Catalase, GSH, GPx, and TAC showed no effect in the low doses of CS and vit. C. However, their levels decreased at high dose of CSand vit. Cwhich is an indication of oxidative stress. These findings are consistent with other research showing that oxidative stress can lead to the depletion of antioxidants [8, 9].

Furthermore, Lipid peroxidation produces malondialdehyde (MDA), which has been employed as a biomarker to assess oxidative stress in a variety of biological samples, including blood, urine, and exhaled breath condensate, in patients suffering from a variety of illnesses, such as cancer, cardiovascular, pulmonary, and neurological conditions [30]. Moreover, the identification of these end products in inflammatory diseases implies a major involvement of lipid peroxidation in this kind of illness [31]. The increase in the MDA levelobserved in high dose of CS and vit. C could indicate tissue damage which was ameliorated by vit. C when co-administered with low doses of CS possibly by regulating the MDA level and preventing tissue damage [32].

CONCLUSION

In conclusion, this study suggests that CS could cause oxidative stress at both low and high doses, which could be prevented by co-administration with vit. C when consume in low doses. Since the same dose of vit. C was co-administered with different doses of CS, this could be the reason why vit. C was unable to prevent the oxidative stress caused by CS at high dose.

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