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ABSTRACT:
Consumption of *Cannabis sativa* (CS) (Marijuana) has been known to be a psychoactive substance which has deleterious effects on the body cells. This study was conducted to investigate the inflammatory responses in male and female Wistar rats following administration of CS. Twenty male (m) and twenty female (f) rats were separately assigned into four groups of five animals each. The rats in groups 1m & If, 2m & 2f, 3m & 3f and 4m & 4f received orally 1mL of distilled water (control), 2mg/kg body weight (bw) of CS, 4mg/kgbw of CS and 6mg/kgbw of CS respectively for twenty-one (21) days. Inflammatory markers (C-reactive protein (CRP), Tumor necrosis factor (TNF), Interleukin-6 (IL-6), Myeloperoxidase (MPO), and Nitric oxide (NO)) were quantified using standard procedures. There was no significant ($p>0.05$) difference in CRP, TNF, IL-6, MPO, and NO in the groups treated with low dose of CS (2mg) but with significant ($p<0.05$) increase in high doses (4mg and 6mg) groups when compared with the control in both male and female rats. This study showed that CS stimulated inflammatory responses due to increase in CRP, TNF, IL-6, MPO, and NO levels compared to that of the control. However, this effect was dose-dependent, and it was more in male than female rats.

Keywords: *Cannabis sativa*, Inflammatory markers, Dose-dependent, Wistar rat
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INTRODUCTION:

Cannabis sativa (CS) is commonly referred to as Marijuana, dope, pot, grass, weed, head, MaryJane, doobie, bud, ganja, hashish, hash, and bhang and has long been used in folk medicine. It is anxiolytic, sedative, analgesic and psychedelic. About 3.9% of the world's population used CS, according to the World Drug Report [1]. Cannabis derivatives, such as delta-9-tetrahydrocannabinol (delta-9-THC) and cannabidiol (CBD), are involved in several neurotransmitter systems, such as glutamatergic, serotonergic, noradrenergic, and dopaminergic neurons [2]. These neurotransmitters are responsible for the therapeutic and recreational effects of cannabis. Inflammation is the body's innate response to injury or insult, including infection, trauma, surgery, burns, and cancer. Cytokines are a small group of proteins released into the bloodstream during inflammation. If their concentrations increase or decrease by at least 25%, they can be used as systemic inflammatory markers [3-6]. The inflammatory markers, also known as acute phase reactants, that are most measured in clinical practice are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), interleukin-10, interleukin-6, and tumor necrosis factor (TNF). Several research works have been done on effects of cannabis on inflammatory markers both in human and animals [3-6]. However, none of these research works has explored gender differences as well as variations in the dosages of CS. This research work, therefore, aims to bridge these lacunae by investigating the effects of CS on both male and female Wistar rats to identify potential sex differences and examine specific inflammatory markers (CRP, ESR, IL-6, TNT and NO), including their dose-dependent effects.

METHODOLOGY:

Sample collection:

Cannabis sativa (CS) leaves were donated by the National Drug and 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 600 grams of CS in 98% ethanol for 48 hours. It was filtered and the filtrate was poured into a round-boom conical flask it was fixed with a rotary evaporator. The filtrate was then evaporated and cooled. The dried yield of the extract was 60g (weight of the extract obtained after drying).

Experimental animals

Twenty male rats with mean weight of 160 ± 1.12g and twenty female rats with mean weight of 125 ± 1.35gused in the present study were obtained from Temilade Animal Venture,

Ogbomoso, Oyo State, Nigeria. The animals 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 the Jimoh Babalola University, Ilorin, Kwara State, Nigeria with approval number (JBU/ERC/2025/05).

Experimental protocol:

After 2 weeks of acclimatisation, the animals (male (m) and female (f)) were separately and randomly assigned into four groups of five animals each for male and female.

The rats in groups 1m & 1f, 2m & 2f, 3m & 3f and 4m & 4f received orally (in the morning) 1.0 mL of distilled water (control), 2.0 mg/kg body weight (bw) of CS, 4.0 mg/kg bw of CS and 6.0 mg/kg bw of CS respectively, for twenty-one days. The animals had access to food and water ad-libitum. They were sacrificed after day 21.

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 using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). 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.

Assay kits:

The Inflammatory markers (TNF, IL-6, NO, MPO, AND CRP) were quantified according to the instruction provided by assay kit manufacturers, using microplate immunoenzymometric (EMA/ELISA) assays [7]. The serum inflammatory markers concentrations were then interpolated from their respective calibration curves. The analyzer was calibrated and validated for use with rat sera. All the assay kits used were products of Monobind Inc., Lake Forest, California, USA. All other chemicals used were products of Sigma Aldrich Company, Mannheim, Germany.

Statistical analysis:

Results were expressed as the mean ± standard error of mean (S.E.M).

Data was analyzed using a two-way Analysis of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with the aid of graph pad, version 9.0. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS:

Table 1: Showing inflammatory markers of rats (male and female) for control and treated (2.0 mg/kg bw, 4.0 mg/kg bw and 6.0 mg/kg bw) groups.

	Interleukin-6 (pg/ml)	Tumour necrosis factor (ng/ml)	C-reactive protein	Nitric oxide (Um)	Myeloperoxidase (U/l)
Control (m)	0.3±0.012	14.0±0.321	18.0±0.010	3.0±0.112	0.40±0.041
Control (f)	0.1±0.005	9.0±0.270	10±0.341	1.0±.0.051	0.22±0.070
2mg/kg bw CS(m)	0.7±0.021	35.0±1.042	45.0±1.123	5.0±0.250	0.58±0.023
2mg/kg bw CS (f)	0.3±0.012	18.0±0.385	28±1.014	2.0±0.030	0.42±0.050
4mg/kg bw CS (m)	1.2±0.041 [a]	48.0±1.230 [a]	55.0±0.980[a]	9.0±0.741 [a]	0.81±0.037 [a]
4mg/kg bw CS (f)	0.6±0.032[a b]	30.0±0.973 [a b]	40.0±0.778 [a b]	5.0±0.055 [a b]	0.55±0.043 [a b]
6mg/kg bw CS (m)	1.8±0.022 a	60.0±1.721a	75.0±1.312a	12.0±1.027a	0.97±0.008 a
6mg/kg bw CS (f)	1.4±0.017 [a b]	42.0±1.069 [a b]	58.0±1.070 [a b]	7.0±0.084 [a b]	0.73±0.016 [a b]

Note: aP<0.05 vs control and 2.0 mg/kg bw (m and f); bP<0.05 vs (4.0 and 6.0) mg/kg bw male.

The results obtained are presented in Table 1. There was no significant (p>0.05) difference in CRP, TNF, IL-6, MPO, and NO levels in the groups treated with low dose (2.0 mg) of CS when compared with the control. However, there were significant (p<0.05) increases at high doses (4.0 mg and 6.0 mg) of CS when compared with the control. In addition, there were significance (p<0.05) differences in CRP, TNF, IL-6, MPO, and NO levels in the groups treated with high doses between male and female rats.

DISCUSSION:

There exists a tight integration between the immune and nervous systems, the so-called inflammatory reflex, capable of influencing both systems in response to inflammatory and infectious agitation of homeostasis [8]. Essentially, the autonomic nervous system is implicated in a bidirectional inflammatory reflex with the vagal nerve being the main neuronal substrate of an immunoregulatory role, providing a fast and subconscious anti-inflammatory response [9]. Given their important role, many experts have speculated that CS plays a role on inflammatory markers. Several studies have shown that the effects of CS on various physiological processes are dose-dependent [10-18]. Although research implicating the role of inflammatory markers is still evolving, there is some evidence for the role of Inflammatory markers, such as interleukin-6, Nitric oxide, tumour necrosis factor, myeloperoxidase and C-Reactive protein in the body. These inflammatory markers are implicated in

inflammation of the body more broadly and may be activated through injury, burn or infection. For example, a physical condition in which part of the body becomes reddened, swollen, hot and often painful.

Our findings revealed that CS (at high doses) stimulated inflammatory responses by increasing the levels of TNF, IL-6, NO, MPO, AND CRP in the animals. This could be due to tissue injury caused by CS which was consistent with the finding of Wang et al [20]. However, no changes in the levels of these inflammatory markers were observed at low doses, suggesting that the mechanisms of action of CS-receptors are dose-dependent. Additionally, the effect of CS on inflammatory markers were more in male than in female. This may be attributed to sex-dependent differences in cannabis metabolism and interactions between the endocannabinoid system and sex hormones which could prone females to be more sensitive to the behavioral and physiological effects of cannabis than males [18]. Another factor could be through cannabinoid receptor expression and signaling pathways.

Cannabinoid receptors are more widely distributed in males than females [21].

CONCLUSION:

This study showed that CS could cause toxicity on inflammatory markers which could be mediated by causing tissue injuries. However, these effects were dose dependent. This study concluded that consumption of CS at high doses could impose serious threat on inflammatory markers in both sexes.

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