ORIGINAL ARTICLE

Effect of *Cannabis sativa* on Haematological Parameters in Male and Female Wistar Rats

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Abstract

Background: Consumption of *Cannabis sativa* (CS) (Marijuana), a well-known psychoactive substance may impose serious side effects on the body cells. This study aims to investigate the effects of CS on both male and female Wistar rats to identify the potential sex differences and examine specific blood parameters to understand cannabis's impact (taking into consideration, dose-dependent) on blood health.
Method: Administration of CS was done by oral cannula daily for 21 days. All the groups have free access to food and water. At the end of 21 days, all the animals were sacrificed and haematological parameters were measured using microplateimmunoenzymometric (EMA/ELISA) assays.
Results: We observed that pack cell volume (PCV), Red blood cell (RBC), haemoglobin, basophil, eosinophil and mean corpuscular volume (MCV) of the groups treated with high doses (4 and 6mg/kgbw) of CS were each significantly ($p<0.05$) decrease in both male and female groups than the control and low dose (2mg/kgbw) respectively. However, platelet, white blood cell (WBC), neutrophil, lymphocyte, monocyte and mean corpuscular haemoglobin concentration (MCHC) were each increased significantly ($p<0.05$) in both male and female groups treated with high doses of CS than the control and low dose respectively. There was no significant difference in mean corpuscular haemoglobin among the groups in both males and females. Additionally, there was no significant difference in all the haematological parameters between the control and 2mg/kg bw for both male and female groups.
Conclusion: This study showed that CS seriously affected dose-dependent haematological parameters. However, these effects were more pronounced in males than in female rats.

Keywords: Dose-dependent, Short-term, Sex differences, Blood health

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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. It also stimulates appetite (Ontario Hemp Alliance, 2010) (1). CS has been known for its medicinal uses since ancient times, because of its rich supply of phytochemicals (2), hence the quest for harnessing its pharmacological potential by scientists. It is one of the most widely abused illegal substances in the world (Abdel-Salam, 2016). Due to the psychotropic properties of a particular cannabinoid (9-tetrahydrocannabinol; $C_{12}H_{30}O_2$), even though tetrahydrocannabinol (THC) is the primary psychoactive component of cannabis, the plant is known to contain more than 500 different substances, including at least 113 cannabinoids (3). CS has been shown to contain several phytochemicals, primarily cannabinoids such as THC and cannabidiol which affect immune responses by potentially benefiting autoimmune diseases (4). Several studies have shown that CS imposed dangerous treatment on male and female reproductive systems by inhibiting both oogenesis and spermatogenesis (5-10), suggested in their findings that chronic use of cannabinoids can lead to deterioration of hematopoietic cells which was consistent with sub threshold/subclinical megaloblastic anemia with iron deficiency. However, they used human subjects in their study and they didn't consider gender factors. Also, their findings showed that injection of CS petroleum extract into the rats resulted in different effects in some parameters most especially the percentage of lymphocytes compared to those in addicted men who smoked CS for long periods but they failed to consider gender differences (11). Numerous studies have been conducted on the short and long-term effects of CS on haematological parameters in both humans and animals (5, 12-14).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 blood parameters (Total white cell count, Differential

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white count, haemoglobin concentration, platelet count, packed cell volume, red cell count, MCV, MCH and MCHC) to understand cannabis's impact (taking into consideration, dose-dependent) on blood health. The blood described earlier parameters were considered because they provide valuable information about the body's overall health. The objectives of this study are: to investigate the effects of CS on haematological parameters in male Wistar rats; to investigate the effects of CS on haematological parameters in female Wistar rats; to compare the effects of CS on haematological parameters between male and female Wistar rats; and to explore gender-specific differences in haematological responses to CS exposure. The hypotheses of our research work are: CS will not affect haematological parameters in male and female Wistar rats (null), and CS will affect haematological parameters in male and female Wistar rats (alternative).

METHODS

Sample collection

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

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. It was filtered and the filtrate was poured into a round bottom conical flask it was fixed with a rotary evaporator. It was then evaporated and cooled. The dried yield of the extract was 35.5g (weight of the extract obtained after drying).

Experimental animals

Twenty male rats (170g ± 1.24) and twenty female rats (150g ± 1.05) that were used for this research were obtained from Temilade Animal Venture, Ogbomoso, Oyo State, and 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 the Ethical Committee of the Al-Hikmah University, Ilorin, Nigeria.

Experimental protocol

After 2 weeks of acclimatization, the animals (male (m) and female (f)) were separately and randomly assigned into four groups of five animals each for male and female, such that the rats in groups 1m & 1f, 2m & 2f, 3m & 3f and 4m & 4f received orally (in the morning) 1mL of distilled water (control), 2mg/kg body weight (bw) of CS, 4mg/kg bw of CS and 6mg/kg bw of CS respectively, for twenty-one (21) days. The animals were sacrificed after the 21st daily dose with access to food and water.

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 ℃ and used within 12 hours of preparation.

Estimation of RBC

The principle involved diluting a small amount of blood (1:200) with Hayem solution and the red cells were counted in an improved Neubauer counting chamber using a microscope. The red cell count was given as N X CF per mm^2 where N is the total number of cells counted in all the 5 corners squares, while CF = correction factor (10,000) (13).

Estimation of PCV

Packed cell volume was estimated using the capillary tube method (15). The anti-coagulated blood was centrifuged and a micro hematocrit reader was used to read the meniscus of the RBC as the PVC in percent.

Estimation of the haemoglobin content

Using Dacie and Lewis' approach, the blood's haemoglobin content was estimated (15). This method is based on the fact that haemoglobin present in a blood sample is converted to acid haematin when 0.1 N HCl is added. The Hb content is determined by matching this solution against a non-fading brown colour standard and calculated as follows: Hb content (g/dl) = Reading (%) x 14 100.

Estimation of WBC

The estimation of the WBC was as described for RBC. White blood cells were identified by their nuclei. The nuclei of the WBC are stained by gentian violet. WBC was counted in the 4 corner squares of ruled areas of the improved Neubauer counting chamber using an x 10 lens. The multiplication factor used was 50n, where n is the number of cells counted in the four corner squares.

Estimation of differential WBC count

Freshly prepared blood was used to determine the differential count. The method is as described by (15) using Leishman stain (0.15% methylene azure in pure methanol). A drop of blood was placed on one slide while a second smooth-edged slide held at an angle of 45° between the thumb and forefinger was used to spread it. The smear was quickly air-dried. An equal volume of buffer solution was added to the slide and mixed with the stain. The different white blood cells granulocytes (basophils, eosinophils and neutrophils) and agranulocytes (lymphocytes and monocytes) were identified using different stains.

Estimation of platelets

Platelets in the blood were obtained and counted using a light microscope and a haemocytometer (16). The counting chamber used is the improved Neubauer counting chamber.

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 all the parameters graph pad, version 9.0. Differences with values of $P<0.05$ were considered statistically significant

RESULTS

There were significant ($p<0.05$) decreases in PCV, RBC, haemoglobin, basophil, eosinophil and MCV (figures 1- 3 and table 1) of the groups treated with 4 and 6 mg/kg bw in both male and female groups than the control and 2mg/kg bw respectively. However, platelet, white blood cell (WBC), neutrophil, lymphocyte, monocyte and mean corpuscular

Figure 1. PCV of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean ± mean ± standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.

Figure 2. RBC of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean±standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.

Figure 3. HB of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean ± standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.

haemoglobin concentration (MCHC) were each increased significantly ($p<0.05$) (figure 3 and table 1). There was no significant difference in MCH in all the groups for both males and females. Additionally, there was no significant difference in all the haematological parameters between the control and 2mg/kg bw for both male and female groups. However, the increase in the differential of WBC percentage with a reduction in total WBC could be due to the cytotoxic effects of CS which might have damaged some of the WBC.

DISCUSSION

This study has demonstrated the effect of cannabis consumption on some haematological parameters of rats and this cannot be over-emphasized as there were significant differences in most of the parameters between male and female cannabis consumers. There is abundant evidence that haematological values vary considerably between these two study groups; therefore it becomes

Figure 4. Platelet of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean ± standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.

Figure 5. WBC of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean±standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.

necessary to carry out this study.

Consumption of CS seems to have an assortment of health effects, which could be beneficial or detrimental. Our results revealed that the RBC, haemoglobin and PCV decreased dose-dependently in both males and females with no considerable difference among the groups treated with low doses (2mg/kg bw). This finding was in accord with the studies of (17) who recorded lower RBC, haemoglobin and PCV levels in human subjects. This, however, disagreed with the results of (18), who found that rats had increased RBC, haemoglobin, and PCV levels. They explained this rise by suggesting that CS may boost blood's ability to carry oxygen. The results also showed a dose-dependent decrease in basophil and eosinophil which are majorly responsible for fighting against allergens and parasites (19). Thus, suggesting that CS is not allergic or parasitic to the body (20). This study also observed significantly higher values of platelet which was dose dependent. This runs counter to the findings of (17), which showed that the platelet count decreased in human individuals. Differences in the kind of animals could be the reason for the disparity in the results used, the length of the test, and the kind and amount of CS applied. This result however agrees with the findings of (21) who both found that CS consumption leads to platelet activation. The increased platelet count is probably a contributory factor in the thrombogenic potential and its implication in cardiovascular shock and myocardial infarction. There was a decrease in WBC count in the groups treated with high dosages of CS in contrast to control and low doses. This agrees with the findings of (22), (17). There were significant increases in neutrophils, lymphocytes and monocytes in a dose-dependent manner. These agree with the findings of (23) which observed significant increase in all these parameters in human subjects.

Mean corpuscular volume (MCV) which is the average volume of a single RBC revealed a significant decrease in the high doses groups compared to control and low dosage groups. In patients with anaemia, MCV measurement allows for classification as microcytic (MCV below normal range), normocytic (MCV within normal range) or macrocytic (MCV above normal range) (24). It is feasible that treatment with high doses of CS had a microcytic effect, while treatment with low doses of CS had a normocytic effect. Another index for diagnosing anaemia is mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (25). MCH showed no differences between the control and CS-treated groups. The MCHC also did not show any difference between the control and low-dose groups but was significantly increased in the high-dose group. According to (26), low MCHC is an indicator of hypothermia in early iron deficiency, and the MCH level decreases as the hypothermia develops. It is therefore feasible that CS contains Hbproduction-enhancing factors. This may therefore prevent the likelihood of hypothermia in the CS treated high doses groups. Our study revealed that there were gender differences in response to CS which could be due to various factors. One of them is hormonal influences. According to (27), estrogen enhances immune responses, potentially affecting cannabis-induced immunomodulation through hormone-cannabinoid interactions. Another factor could be through cannabinoid receptor expression and signaling pathways. Cannabinoid receptors are more widely distributed in males than females (28). Additionally, via changing global deoxyribonucleic acid (DNA) methylation, CS may modify epigenetic changes and perhaps contribute to cannabis-induced immunosuppression, particularly on white blood cells (29).

Figure 6. Deffrential WBC of rats (male (m) and female (f)) for control, 2mg/kgbw CS, 4mg/kgbw CS and 6mg/kgbw CS respectively. Values are expressed as mean ± standard error of mean. Number of experiments (n=40, 20 males and 20 females). Data were analyzed using a two-way Analysis Of Variance, followed by the LSD post-hoc test to determine significant differences in all the parameters, graph pad, version 9.0.
Note: *P<0.05 vs control; [a]P<0.05 vs 4mg/kg bw male and female; [b]P<0.05 vs 6mg/kg bw male and female

Table 1. Hematological indices of rats (male (m) and female (f)) for control, 2mg/kg BW CS, 4mg/kg BW CS and 6mg/kg BW CS respectively. Values are expressed as mean ± standard error of mean; P<0.05

S/N	Heamatological indices	Unit	Control	2mg/kg BW C	4mg/kg BW C	6mg/kg BW C
1	Mean corpuscular volume (MCV) (m)	µ3	75.12±1.04	72.45±0.95	64.95±1.63*a	50.10±2.10*b
	MCV (f)	µ3	61.94±1.21	56.63±1.23	45.75±1.03*	38.21±1.89*
2	Mean corpuscular haemoglobin (MCH) (m)	Pg	31.12±1.21	33.73±0.73	34.21±1.02	34.98±0.89
	(MCH) (f)	Pg	20.32±1.87	24.11±0.94	26.65±1.37	27.53±1.01
3	Mean corpuscular hemoglobin concentration (MCHC) (m)	g/dl	36.23±1.76	37.45±1.78	43.79±1.34* a	48.98±1.26*b
	(MCHC) (f)	ng/dl	23.11±1.12	25.42±0.98	32.12±1.09*	40.31±1.07*

CONCLUSION

This study showed that CS affects haematological parameters in both male and female Wistar rats. As it is evident in this study, cannabis consumption could cause immune system dysregulation, chronic inflammation, increased risk of infections, and cardiovascular risk due to increased lymphocytes, monocytes and MCV. Also, cannabis consumption may increase the risk of infection and bleeding, impaired wound healing, fatigue, shortness of breath, and decreased quality of life due to decreased total white blood cell count, granulocytes, RBC, haemoglobin, packed cell volume and platelet count. It may be recommended that people should abstain from the consumption of CS due to its detrimental effects on the haematological parameters.

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