Effects of chronic consumption of *Cannabis sativa* extracts on haematological parameters in male rats

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ABSTRACT

Introduction: There are reports on how *Cannabis* sativa (CS) affect most of the body organs and systems.

Objectives: This study was designed to investigate the effects of consumption of CS on haematological parameters.

Materials & Methods: Fifteen male rats weighing 200-220g were divided into 3 groups. Control group, administered 0.1ml/kg normal saline; Low dose (LD) group and High Dose (HD) group, administered 0.1mg/kg and 0.2mg/kg aqueous extract of CS respectively. The oral administration lasted for 28 days and blood samples were obtained via cardiac puncture for haematological parameter analysis.

Results: There was significant increase in the level of erythrocyte (RBC), packed cell volume (PCV), Haemoglobin concentration (Hb) and Granulocytes count in LD and HD compared to the control. Total leukocytes (TLC), Monocytes and Lymphocytes counts in LD were significantly increased compared to control and HD groups. Platelet count decreased significantly in LD when compared to HD and control. Mean corpuscular volume (MCV) decreased significantly in the LD compared to the control. There was no significant difference in Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC).

Conclusion: This study revealed that low dose of CS enhanced RBC and TLC productions. However, at high dose, CS increased susceptibility to infections due to immature blood cells production.

Keywords: Cannabis, Haematological Parameters, Rats, Consumption, Marijuana, India Hemp

INTRODUCTION

Cannabis Sativa (CS), commonly known as Marijuana, is a hemp plant that grows freely throughout the world especially in tropical climate[1]. In Nigeria, Cannabis is known to be cultivated in many rural areas and consumed mostly by youths especially for its psychoactive properties. C. sativa contains over 400 compounds including 60 cannabinoids[2]. Delta-9-tetrahydocannabinol (Δ^9-THC or THC) and Cannabidiol (CBD) are the two most abundant cannabinoids usually produced, but only THC is psychoactive[3].

CBD constitute 40% of the plant extract, with highly variable concentrations that depends on the growing conditions, the different phenotypes and parts analyzed[4, 5]. Regardless of the route of Cannabis consumption, the active component (THC) is carried from the bloodstream to the different organs of the body where its adverse effect is experienced[6]. Although synthetic form of THC is used as a treatment for a wide range of medical conditions[7], Chronic administration of high dose of THC have however been reported to lower testosterone secretion, impair sperm production, motility, and viability, and disrupts the ovulatory cycle[8]. Chronic Cannabis smoking is associated with bronchitis and emphysema, and affects the immune and endocrine system[2]. Marijuana affects blood chemistry and may also affects neural functions[9]. It has been reported to reduce blood glucose levels to insignificant level in rats[10]. Also, researchers identified that substance evolved during smoking may influence blood functions and levels[11]. They reported that cigarette smoking caused increased Pack Cell Volume (PCV) in male smokers and Haemoglobin (Hb) levels in female smokers. This was explained by increased carbon monoxide level in the blood of smokers which induced erythrocytosis[12]. Some researchers described that Cannabinoids have the ability to decrease inflammation, and produce neuroprotective effects on gastric mucosa[13, 14].

MATERIALS AND METHODS

Experimental design

Fifteen healthy male rats weighing between 200-220g were used. The rats were purchased from the animal house of the Faculty of Basic Medical Sciences, Cross River University of Technology, Okuku Campus. The rats were divided into three groups and acclimatized for 2 weeks. The control group was administered 0.1 ml kg^{-1} normal saline while low dose (LD) and high dose (HD) groups were administered 0.1 mg and 0.2mg kg^{-1} aqueous extract of C. sativa respectively.

The administration was done orally for 28 days. All groups were allowed free access to feed and water. The animals were sacrificed by cervical dislocation and blood samples were collected from the animals via cardiac puncture into EDTA (Ethylene Diamine Tetra Acetic Acid) sample bottles. All animal handling and experiment protocols conformed to the

international guidelines for laboratory animals as supported by the Cross River University of Technology, Faculty of Basic Medical Sciences ethical committee.

Blood analysis

The blood samples were analysed by automated haematology analyser (Mindary BC-3200 Haematology Analyser) to determine the haematological parameters such as: Red blood cell (RBC) count, Packed cell volume (PCV), Haemoglobin (Hb) concentration, Platelet, Total leucocytes count (TLC), Granulocytes, Monocytes and Lymphocytes counts, Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). The automated haematology analyser reading correlated with standard manual methods[15].

RESULTS

The results obtained were presented as the mean ± standard error of mean (SEM) and analyzed using analysis of variance (ANOVA) with post-hoc test (Least Significant Differences) through Graphpad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The results were considered significant at $P < 0.05$.

Table 1 shows the effects of consumption of CS extracts on RBC, PCV, Platelet count, Hb conc., TLC, Monocytes, Granulocytes, Lymphocytes, and MCV, MCH and MCHC of albino wistar rats. Aqueous extract of CS caused a significant increase in RBC count in LD ($P < 0.001$) and HD ($P < 0.05$) compared to the control. TLC in LD rats was significantly higher compared to the control ($P < 0.001$) and High dose ($P < 0.001$) groups. PCV and Hb conc. increased significantly in the LD ($P < 0.01$) and HD ($P < 0.01$) compared to control. Platelet count decreased significantly in the LD ($P < 0.001$) and HD ($P < 0.001$) compared to the control; Platelet count in LD was however significantly lower than the HD. Granulocytes count was significantly increased ($P < 0.05$) in the LD and HD compare to control. Monocytes count was also significantly higher in LD than the control ($P < 0.01$) and HD ($P < 0.05$). Lymphocytes count in LD rats was significantly higher compared to the control ($P < 0.01$) and HD ($P < 0.001$). Mean corpuscular volume (MCV) was significantly decreased ($P < 0.001$) in the LD rats compared to the control and no significant difference was observed in the Mean corpuscular haemoglobin (MCH) and the Mean corpuscular haemoglobin concentration (MCHC).

Table 1: Haematological parameters in control and C. sativa extracts treated groups (n=5).

Haematological Parameters	Control (n=5)	Low Dose (LD) (0.1mg/kg) (n=5)	High Dose (HD) (0.1mg/kg) (n=5)
RBC(×106/mm 3)	7.60 ± 0.10	8.76 ± 0.18***a	8.20 ± 0.18*[a]
WBC(×103/mm3)	10.29 ± 0.32	20.45 ±0.02***a	9.15 ± 1.53***[b]
PCV (%)	40.37 ± 0.48	43.28 ± 0.60**a	43.32 ± 0.41**[a]
Hb (g/dL)	12.57 ± 0.22	13.60 ± 0.25**a	13.57 ± 0.27*[a]
PLT (×103/mm 3)	768.67 ± 9.94	595.67 ±7.47***a	633.33 ± 9.71***A, *[b]
Granulocytes (%)	1.53 ± 0.16	2.10 ± 0.10*a	2.09 ± 0.13*[a]
Monocytes (%)	0.99 ± 0.31	2.51 ± 0.25**a	1.29 ± 0.25*[b]
Lymphocytes (%)	7.78 ± 0.48	15.85 ± 0.44**a	5.77 ± 2.08***[B]
MCV (fL)	53.12 ± 0.67	49.41 ±0.33***a	52.83 ± 0.53
MCH (Pg)	16.50 ± 0.31	15.53 ± 0.07	16.55 ± 0.47
MCHC (g/dL)	31.14 ± 0.51	32.42 ± 0.20	31.33 ± 0.41

DISCUSSION

Controversial findings have been reported in many researches concerning the effects of CS on haematological parameters and immunity. It was reported that comparison of blood chemistry and haematological values before, during and after exposure to Marijuana indicated no differences for most parameters[16]. This report contradicts the findings of this research which observed significant differences in haematological indices between CS treated rats and controls group, except in the values of MCH and MCHC. In addition, it has also been reported that Marijuana affects blood chemistry and may also affect neural functions[9].

In this study, the investigation of the blood parameters showed that RBC count was significantly increased in the low and high dose groups compared to control group. There was however no significant difference between low dose and high dose groups. This agrees with the findings of some researchers who reported that cannabinoid acted through cannabinoid CB_2 to synergize with colony-stimulating factor (CSFS), interleukin-3 and erythropoietin in order to stimulate haematopoiesis[17]. Thus, this may probably be the reason for increased level of RBC in the CS treated rats. TLC was significantly increased in the low dose group compared to the control and high dose groups.

On the other hand, no significant difference was noted between high dose and control groups. The increase in TLC in the low dose may be associated with the ability of CS to reduce or prevent infection. However, previous research explained that the benefit of Cannabinoids include the suppression of inflammation and various cell-mediated immunity[18].

Moreover, previous researchers described that Cannabinoids has the ability to decrease inflammation, and produce neuroprotective effects on gastric mucosa[13, 14]. Myrcene and Caryophyllene contained in Cannabis have also been reported to have anti-inflammatory property which can decrease or prevent inflammation[19, 20]. The decrease in TLC in

the high dose may indicate that following CS chronic exposure, the constituents of *C*. sativa weakened or destroyed the White blood cells due to excessive stimulation of the haematopoietic cells.

PCV and Hb were significantly increased in the treated rats when compared to the control. There was however no significant difference between the low dose and high dose groups. This result agreed with the findings of Oseni[21], who reported marginal increase in PCV level in CS smokers compared to non-smokers. Also, the result was in line with a research which reported that blood haemoglobin concentration was not altered in both addicted men and treated rats[18]. MCV was decreased in the experimental groups compared to the control. The MCV of low dose group decreased significantly when compared to the control. It is unclear why the MCV of low dose group decreased but the decrease may indicate the presence of true polycythaemia. True polycythaemia may be associated with high affinity of haemoglobin or abnormal haemoglobin without oxygen-carrying capacity. Platelet count decreased significantly in the low and high dose groups compared to the control. The low dose group was significantly lower than the high dose group. Thus, this agreed with the finding which reported decrease in platelet count[21].

Granulocytes and Monocytes counts were increased significantly in treated rats compared to the control group. This result was not in line with the Oseni[21] who reported that monocytes and granulocytes were marginally lowered in CS smokers compared to non-smokers. However, research has revealed that the concentrations of Cannabidiol are highly variable and depend on the growing conditions, the different phenotypes of illicit Cannabis and the part of the plant analysed[5]. This could be the possible reason for the difference between this study and the Oseni report[21]. Lymphocyte level in the low dose group was significantly increased when compared to the control and the high dose groups. The lymphocytes of high dose group decreased within normal range compared to the control. This result was not in agreement with a research which reported a significant decreased in lymphocytes level in all rats treated with Cannabis sativa[18]. Also, the result was not in agreement with the findings of Oseni[21], who reported a decreased in lymphocyte level in Cannabis treated rats.

CONCLUSION

This study indicates that CS intake based on medical grounds may be useful. The study thus suggests that, CS at a low regulated dose can be of medical benefits especially in stimulating RBC and WBC production. On the other hand, at a high or unregulated dose, CS intake may be toxic to the body as it may lead to true polycythaemia and increased susceptibility to infection. CS intake for recreational purposes should however be discouraged because of its overwhelming adverse effects.

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