Hematological and Immunological Effect of *Cannabis sativa* on Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author GCO managed the literature searches, analyses of the study performed the spectroscopy analysis and authors DEI, UAO and AIO managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

The present study is aimed at ascertaining the effect of oral feeding of *Cannabis sativa* on blood parameters and immunity in rats. Administration of *Cannabis sativa* was done by orogastric feeding. All the groups had free access to water and food. The experiment lasted for 28 days. At the end of the 28 days of feeding, we observed that the mean red blood cell count was $5.68 \pm 0.09 \times 10^6/mm^3$, $5.90 \pm 0.04 \times 10^6 mm^3$ and $6.38 \pm 0.22 \times 10^6 mm^3$ for control, low dose (LD) and high dose (HD) respectively. The red blood cells count in high dose was significantly higher (P<0.05) than low dose and control (P<0.01) respectively. However there was no significant difference between control and low dose. The total white blood cell count for control, LD and HD groups was $2.26 \pm 0.13 \times 10^3/mm^3$, $1.69 \pm 0.04 \times 10^3 mm^3$ and $1.58 \pm 0.09 \times 10^3 mm^3$ respectively. There was however no significant difference between the LD and HD even though both test groups were each

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significantly (P<0.001) lower than control. There was no significant difference in the pack cell volume (PCV) among the groups. The haemoglobin concentration (Hb) did not show any significant difference between LD and HD, but between the control and HD there was significantly higher (P<0.05) Hb concentration in HD. The mean platelet count for control, LD and HD were $252\pm14.94 \times 10^3/mm^3$, $364\pm7.47 \times 10^3/mm^3$ and $424\pm7.47 \times 10^3/mm^3$ respectively. Both experimental group were each significantly higher (P<0.001) than control group. Platelet in the high dose was significantly higher (P<0.01) than in the low dose. In the differential count, the lymphocytes, monocytes and neutrophils were significantly (P<0.001) reduced in high and low dose groups when compared with control. Eosinophils and basophils increased significantly (P<0.001) in both high and low dose groups when compared with control. Mean corpuscular haemoglobin concentration (MCHC) showed a significant increase (P<0.05) in the high dose when compared with control but mean corpuscular Hb was unaffected.

1. INTRODUCTION

Pot, weed, grass, ganja and skunk, are some of the common words used to describe the dried leaves of the plant known as Cannabis. *Cannabis sativa* is one of man's oldest and widely used drugs. It has been consumed in various ways. The response to Cannabis varies according to the form in which it is consumed, the dose and the route of administration. Ingestion of cannabis has a variety of health effects, some beneficial and others not so beneficial. Hemp combines many of the properties of alcohol, tranquillizers, opiates and hallucinogens. It is anxiolytic, sedative, analgesic and psychedelic. It also stimulates appetite [1]. Chronic use of cannabis has been found to have immunosuppressant and endocrine effects. It affects fertility in both males and females and is very harmful to neonates whose mothers are users [2,3]. It leads to psychotic reaction [4], loss of memory and impaired psychomotor coordination [5]. It antagonises the effect of both endogenous and exogenous insulin [6].

The most widely and popular means of using cannabis is in its dry herbal form but more users ingest it in salads, tea additives, ingredient in drugs and vegetable juices [7]. Despite its diminutive size, hemp is increasingly spoken of as one of the most nutritionally complete food sources in the world, second only to soybean in protein content (25% versus 32%) [8,9]. Hemp protein is free of the trypsin inhibitor which blocks protein absorption and free of oligosaccharides found in soy which cause disorders [10]. Aside over 400 compounds and the 60 cannabinoids which are aryl substituted meroterpenes, the most potent ingredient is delta – 9 – tetrahydrocannabinoid THC [11].

The aim of this study is to observe the effect of administration of *Cannabis sativa* on haematological and immunological parameters in albino wistar rats. Blood is a connective tissue, therefore, analysis of haematological indices could provide vital information on what every system is exposed to in the use of *Cannabis sativa*.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Extracts

Cannabis sativa was obtained from a farm in Calabar South Local Government Area of Cross River State, Nigeria. It was certified and classified and recorded by botanists of the University of Calabar Botanical garden. The Cannabis was dried in an oven and blended into snuff-like particles and weighed. The particles were then soaked in 1000 mls of water for 12 hours and then filtered using Whatman's No. 1 filter paper. The filtrate was dried using Astell Hearson oven at 45°C and the dried extracts were collected, weighed and put into an airtight container.

The National Drug Law Enforcement Agency in Cross River State, Nigeria approved the carrying out of the experiment.

2.2 Preparation of *Cannabis sativa* and Administration

One gram (1 g) of the dried extract of *Cannabis sativa* was dissolved in 100 mL distilled water (10 mg/mL). After the LD_{50} was estimated, the low dose (LD) was taken as 0.5 mg/100 g body weight and high dose HD as 0.8 mg/100 g body weight. The rats were divided into three groups

of five [5] rats each. The first group (control) was fed on normal rat chow only and equal volume of distilled water. The second (LD) group was fed on low dose 0.5 mg/100g body weight of Cannabis while the third group (HD) was fed on 0.8 mg/100g body weight Cannabis. The rats were all allowed free access to water and food. The Cannabis was administered orally using an oropharyngeal cannula. The feeding was done daily and for 28 days.

2.3 Determination of the LD$_{50}$ of *Cannabis sativa* in Albino Wistar Rats

In order to determine the LD$_{50}$ of *Cannabis sativa* in albino wistar rats, 30 albino wistar rats, 1ml syringe and needle, 30 cages with each containing water and normal rat chow, *Cannabis sativa* extract, spatula, electronic weighing balance, water, marker and masking tape.

3. METHODS

The 30 albino wistar rats were divided into 6 different groups, with 5 rats each. 500 mg of the *Cannabis sativa* was weighed and diluted in 1ml of distilled water. Different doses of the *Cannabis sativa* extract were administered to the albino rats in their respective groups, by varying the mls of the solution administered (Table 1). The 'control' was allowed free access to food and water. Intraperitoneal route of administration was used. This is shown below:

Table 1. Doses of *Cannabis sativa* administered to determine the LD$_{50}$

Group	Dose (mg/kg) of cannabis administered	Volume in solution (in mls)
1	25	0.05
2	50	0.1
3	75	0.15
4	100	0.2
5	200	0.4
6	0	0

The different groups were put in different cages, which were properly marked and labeled using marker with a masking tape. The set up was left untouched for 48 hours but the rats were allowed free access to food and water. At the expiration of 48 hours, the rats in their respective cages were observed and results recorded for each group.

3.1 Ethical Approval

All authors hereby declare that "Principles of Laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

3.2 Collection of Samples

The animals were anaesthesized with ether and chloroform mixture and the blood collected by cardiac puncture with sterile syringes and needles into an EDTA samples bottle and used for assessment of haematological parameter [12].

3.2.1 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 mm² where N is total number of cells counted in all the 5 corners squares, while CF = correction factor (10,000) [13].

3.2.2 Estimation of PCV (hematocrit)

Packed cell volume was estimated using capillary tube method [13]. The anti-coagulated blood was centrifuged and a microhematocrit reader was used to read the meniscus of the RBC as the PVC in percent.

3.2.3 Estimation of the haemoglobin content

Haemoglobin content of the blood was estimated based on the method by Dacie and Lewis [13]. This method is based on the fact that haemoglobin present in a sample of blood 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:

$$\text{Hb content (g/dl)} = \frac{\text{Reading (\%) x 14}}{100}$$

3.2.4 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 square of ruled areas of the improved Neubauer

counting chamber using x 10 lens. The multiplication factor used was 50n, where n is the number of cells counted in the four corner squares.

3.2.5 Estimation of differential wbc count

Freshly prepared blood was used to determine differential count. The method is as described by [13] 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.

3.2.6 Estimation of platelets

Platelets in the blood were obtained and counted using a light microscope and a haemocytometer [12]. The counting chamber used is the improved Neubauer counting chamber.

3.2.7 Statistical analysis

Results were expressed as mean ± SEM. ANOVA was applied. General group mean were compared by students t-test. Differences were considered significant at $P < 0.05$.

4. RESULTS

4.1 Red Blood Cell Count

Fig. 1 shows a comparison of the mean red blood cell (RBC) count in different experimental groups. The mean red blood cell count for control, low and high doses *Cannabis sativa* were 5.68±0.09, 5.90±0.04 and 6.38±0.22 x $10^6/mm^3$ respectively. The RBC in high dose was significantly higher $P < 0.05$ than low dose and when compared with control ($P < 0.01$). There was however no significant difference between the low dose and control.

4.2 Packed Cell Volume (PVC)

The PCV of the control was 51.31± 0.62% while that of low and high dose groups were 50.21±1.73% and 48.50±0.75% respectively.

There was no significant difference among the groups. This is as shown in Fig. 2.

4.2.1 Haemoglobin concentration

Fig. 3 shows the haemoglobin concentration for the various group studied. The Hb concentrations were 14.74±0.36%, 15.06±0.36% and 15.98±0.44% respectively for control, low and high dose groups. The Hb concentration in high dose was significantly higher ($P < 0.05$) than the control group. However, there was no significant difference between the low and high dose. This is as shown in Fig. 3.

4.2.2 White blood cells count

Fig. 4 shows the white blood cells count in the different groups. The mean WBC count of the control group was 2.26±0.13 x $10^3/mm^3$ while that of the low and high dose were 1.69±0.04 x$10^3/mm^3$ and 1.58±0.09 x $10^3/mm^3$ respectively. The mean values of the experimental groups were significantly lower ($P < 0.001$) when each was compared with control. There was no significant difference between the low and high doses.

4.2.3 Differential white blood cell count

The differential white blood cell count is as illustrated in Fig. 5.

4.2.4 Platelets counts

The mean platelet count for control, low dose and high dose groups were 252±14.94 x $10^3/mm^3$, 364±7.47 x $10^3/mm^3$ and 424±7.47 x $10^3/mm^3$ respectively. The low and high dose groups were each significantly ($P < 0.001$) higher than control. The high dose group was significantly ($P < 0.01$) higher than low dose group. This is as presented in Fig. 6.

4.2.5 Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)

The result of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) is presented in Table 2.
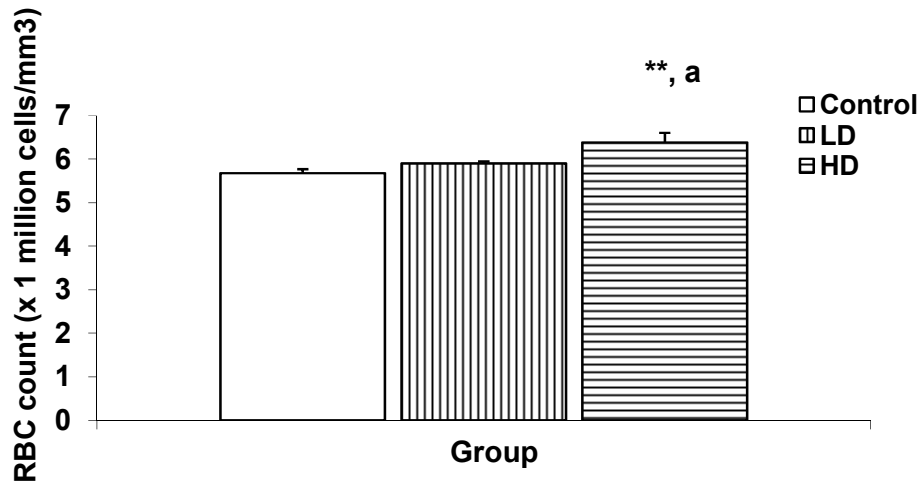
Fig. 1. Effect of administration of *Cannabis sativa* on red blood cell (RBC) count in the different experimental groups
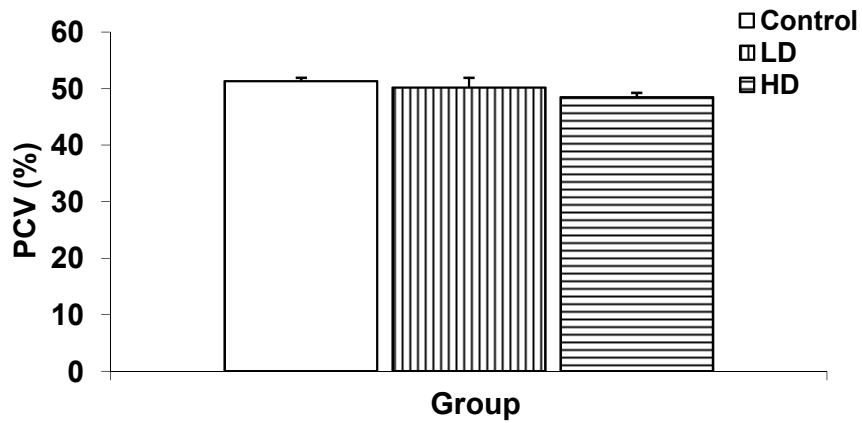*Values are mean + SEM, n = 5*

Fig. 2. Effect of administration of *Cannabis sativa* on packed cell volume (PCV) in the different experimental groups
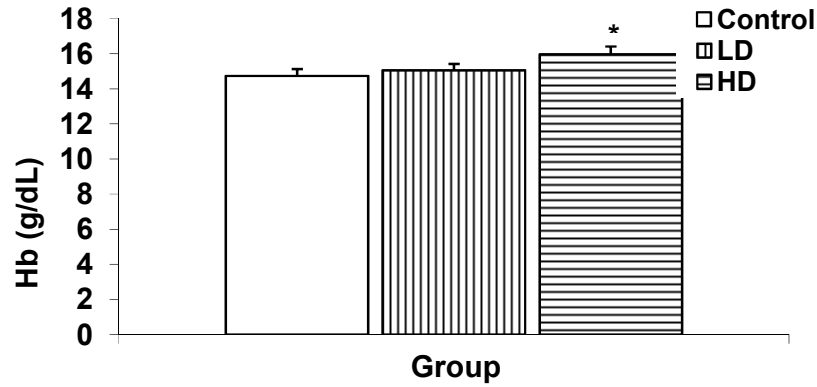*Values are mean + SEM, n = 5*

Fig. 3. Effect of administration of *Cannabis sativa* on haemoglobin (Hb) concentration in the different experimental groups. Values are mean + SEM, n = 5
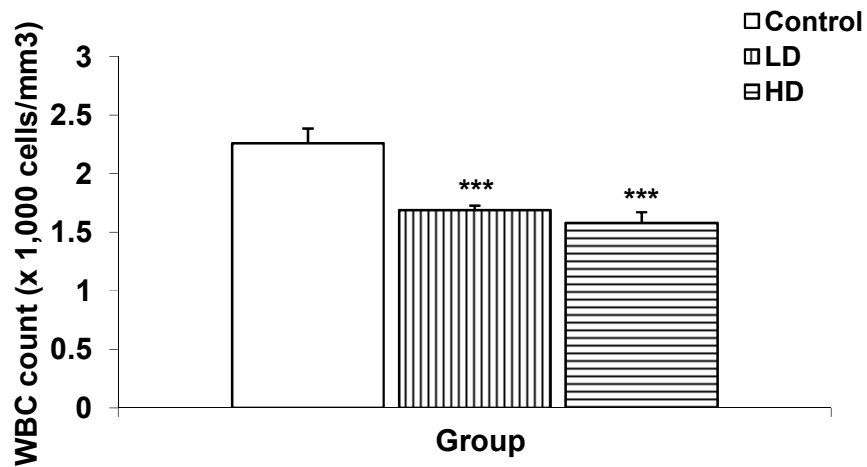**P<0.05 vs control*

Fig. 4. Comparison of white blood cell (WBC) count in the low and high dose groups compared to control. Values are mean + SEM, n = 5
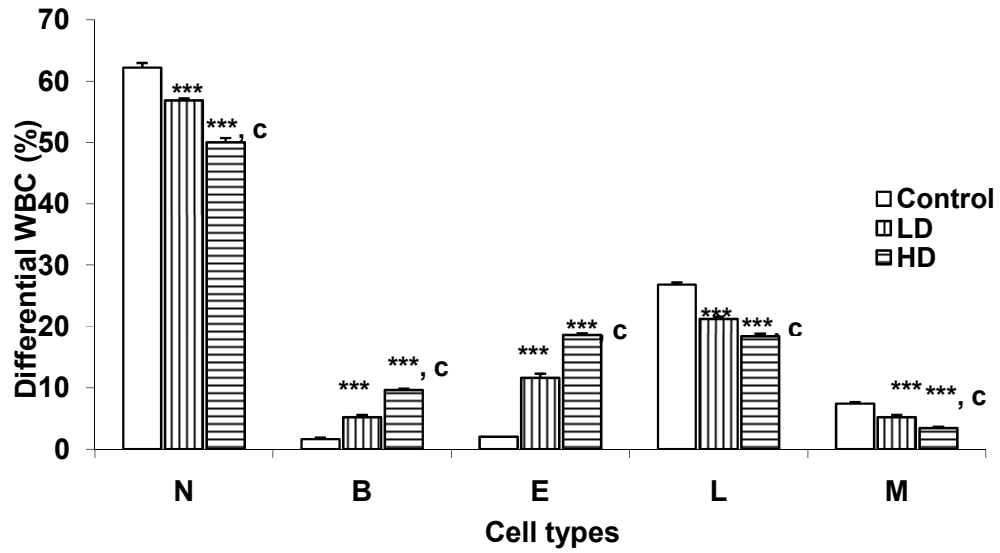****P<0.001 vs control*

Fig. 5. Comparison of differential white blood cell (WBC) count in the different experimental groups. values are mean + SEM, n = 5
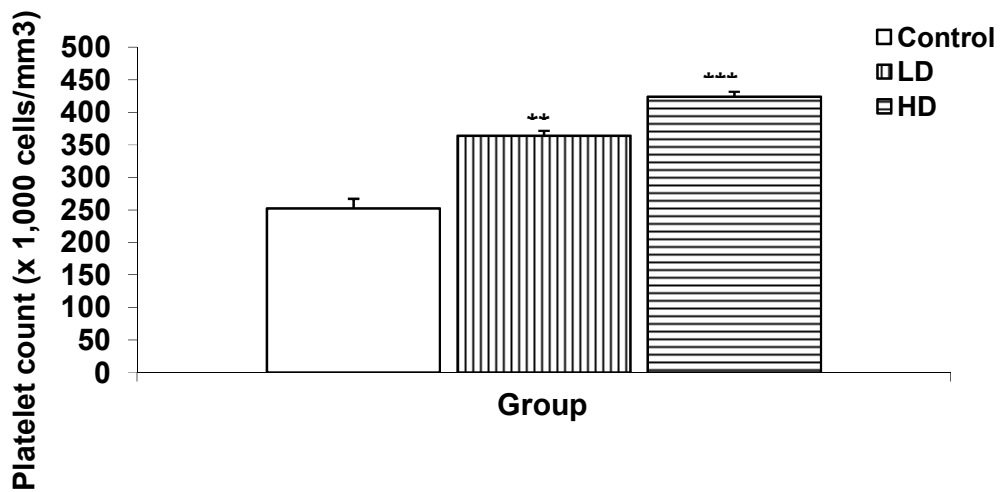*** P<0.001 vs control; c = P<0.001 vs LD*

Fig. 6. Comparison of platelet count in the different experimental groups. Values are mean + SEM, n = 5
*** P<0.001 vs control; b = P<0.01 vs LD*

Table 2. The result of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)

Haematological indices	Unit	Control n=5	Low dose n=5	High dose n=5
Mean corpuscular volume	μ^3	90.45±1.07	85.11±3.07	76.24±1.89***,a
Mean corpuscular haemoglobin	Pg	26.01±0.91	25.52±0.64	25.18±1.22
Mean corpuscular haemoglobin concentration	g/dl	28.77±1.04	30.17±1.49	33.01*±1.28

*** $P<0.001$ vs control; a = $P<0.05$ vs LD, * $P<0.05$ vs control*

5. DISCUSSION

Chronic consumption of *Cannabis sativa* seems to have a variety of health effects, some beneficial and some not so beneficial. The results of the investigation showed that the RBC increased dose dependently with the packed cell volume not showing any considerable difference among the groups. There was increased haemoglobin content which agrees with the findings of [14] and this may indicate that the consumption of high dose of *C. sativa* may improve oxygen carrying capacity in blood. However, the mechanism of action is not known.

There was a decrease in total white blood cells count in the group fed low and high doses of *C. sativa* when compared with control. This may be one of the reasons *C. sativa* is used in anti-inflammatory conditions [15]. The tetrahydrocannabinol THC, a major constituent in the cannabis has long term significant toxicological implications such as bone marrow suppression [14]. This constituent disturbs the maturation of monocytes [16], as well as erythrocytes [14]. These may be responsible for poor immune or defence mechanism as well as anaemic condition in most users.

The result also shows dose dependent increase in platelet count. This is contrary to the report by [17] who reported a decrease in platelets count in human subjects. The disparity in results could be due to difference in species of animals used, duration of test as well as quality and quantity of *Cannabis sativa* used. This result however agrees with the findings of [18,19] who both found that *Cannabis sativa* 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 [20].

Mean corpuscular volume (MCV) which is the average volume of a single RBC showed a significant reduction in the high dose group compared to control and low dose 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) [21]. It is plausible that treatment with high dose of *C. sativa* extract had a microcytic effect, while treatment with low dose of *C. sativa* had a normocytic effect. Another index for diagnosing anemia is mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) [22]. MCH showed no differences between the control and *C. sativa* treated groups. The MCHC also did not show any difference between control and low dose groups, but was significantly raised in high dose group. According to [23], low MCHC is an indicator of hypochromia in early iron deficiency, and MCH level deceases as the hypochromia develops. It is therefore plausible that *C. sativa* contains Hb production enhancing factors. This may therefore prevent the likelihood of hypochromia in the *C. sativa* treated rats.

6. CONCLUSION

In conclusion, consumption of *C. sativa* may lead to thrombo-embolism, production of immature monocytes, reduced white blood cell count and by implication reduced body defence mechanism, and it may improve RBC count with no concommitant increase in PCV but with improved Hb which is also shown in the high MCHC at high volume content and therefore an improved oxygen carrying capacity. It may therefore be recommended that individuals with anaemia or immune complications should not use *C. sativa* in their food preparation on regular basis.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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