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Evaluation of Effect of Cannabis Smoking on the Hematological Properties of Selected Adult Male Students Smokers

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Research Article

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Abstract

The study investigated the effect of *cannabis sativa* smoking on some hematological characteristics on the male students consumers. Blood samples were collected in triplicates from twenty (10) randomly selected male voluntary marijuana smokers (test) and ten (10) voluntary male non-smokers (control) in Choba Community, Port Harcourt, Rivers State. The parameters considered were body temperature, pulse rate, Red blood cells (RBC) count, white blood cell (WBC) count, packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and hemoglobin estimation (Hb). The research study revealed significant ($p \le 0.05$) differences in the hematological parameters between the smokers (Test) and non-smoker (control) subjects. Faster pulse rate and lower body temperatures were seen among test subject as compared to control. The values observed for total white blood cell (TWBC), ESR and RBC were lower in test subject (smokers) than the control (non-smokers), while the values observed for ESR, Hb, and RBC were relatively close. The mean values observed for PCV, TWBC and Hb were markedly lower ($p \le 0.05$) in test subject than in control and could be indicative of reduced percentage of blood in the cell, porous immune system and decreased iron group of heme.

Keywords: Cannabis; Hematological indices; Smokers; Non-Smokers; Blood Profile.

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Introduction

Highest populations of psychiatric patients have been associated with drug abuse especially Indian hemp, cocaine, and heroin. The products from the plant, *Cannabis sativa* or marijuana are commonly abused and widely trafficked as illicit drugs in the world. Marijuana is a green leaf or grey mixture of dried shredded flower from the hemp plant. Sinsemilla is the same form of drugs prepared from the unpollinated female plant. The most contentious use of the plant is for its psychoactive substance (*cannabinoid*), a chemical Delta-9-tetrahydriocannabinol (THC) [1]. Cannabis smoke contains various chemicals, including THC, carbon monoxide and carcinogens (substances that cause cancer) [2]. According to Tashkin et al. [3] research showed that with each puff, the components of cannabis smoke become more concentrated, meaning the joint gets stronger and stronger towards the end. So, smoking fewer cannabis cigarettes down to a shorter butt length could mean taking in a greater number of smoke components than smoking the same amount of cannabis in more cigarettes smoked to a longer butt length [4].

In 1990, the discovery of *cannabinoid* receptors located throughout brain and body, along with the endogenous *cannabinoid* neurotransmitter like anandamide, which is a lipid material derived ligand from arachidonic acid, suggested that the use of cannabis affect brain in some manner as a naturally occurring brain chemical [5]. Effect of cannabis on brain follow the standard principle of transduction of signals.

Cannabinoid receptor is important in basic role of human; it helps to decrease adenylcylase activity, inhibits calcium N channels and disinhibits K⁺ A channels. There are two types of *cannabinoid* receptors (C_{B1} and C_{B2}). The C_{B1} receptors are found primarily in brain and mediate psychological effects of THC. The C_{B2} receptor is found abundant in cells of the immune system. *Cannabinoid* acts as immune modulators of C_{B2} receptors i.e. they increase some immune response and decreases some others; for e.g. non-psychotropic *cannabinoids* can be used as a very effective anti-inflammatory [2, 6].

Haematology is simply defined as the study of blood and blood forming tissues and the disorders associated with them. Haematological indices therefore, are index of some component which are present in the blood. These are; red blood cell (RBC), white blood cells (WBC), hemoglobin (Hb), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) etc. Erythrocytes are flattened, bioconcave disc, about 7µm in diameter and 2.24m thick. They lack nucleus (anucleated) and mitochondria. They obtain energy through anaerobic respiration. Packed cell volume (PCV) is simply known as the percentage of blood that is in cell. The average normal value of the packed cell volume is 37% to 48% in females and 45% to 52% in males, when their white blood counts are normal [7, 8]. Erythrocyte sedimentation rate is simply the rate at which red blood cells (erythrocyte) settle and of suspension in the blood plasma measured under standardized conditions.

The erythrocyte sedimentation rate increases if the levels of certain proteins in the plasma rises, as in rheumatic diseases, chronic infections and malignant diseases, and thus provide a simple but valuable screening test for these conditions. WBC contain nuclei and mitochondria and can move in an amoeboid fashion. They can squeeze, through pores in capillary wall and move to side of infection, whereas erythrocytes usually remain confined within blood vessels in the body [8]. Packed cell volume is simply known as the percentage of blood that is in cell. It is also the volume of red blood cell (erythrocytes) in blood, expressed as a fraction of the total volume of the blood. This is also known as haematocrit. The average normal value of the packed cell volume is 37% to 48% in females and 45% to 52% in males, when their white blood counts are normal and a white blood count of over 11,000 cell/ mm³ is termed a leukocytosis, a normal haemeostatic response to bacteria or viral infection [7, 8]. Erythrocyte sedimentation rate is simply the rate at which red blood cells (erythrocyte) settle and of suspension in the blood plasma measured under standardized conditions. The measurement of ESR can help indicate the abnormalities as result of smoking of cannabis sativa.

Medical researchers have isolated substances from plants cannabis that can be used in precise dose or in combination with other medicine to achieve predictable effects. Certain *cannabinoid* drugs have been approved by the United States food and drugs administration (FDA) and in parts of Europe and Canada to relieve nausea and vomiting, increase appetite in people with cancers and AIDS [9, 10]. There is always an increase in body temperature as a result of inhibition of sweat, feeling the hunger sensation, dryness of mouth as well as throat hyperthermia that may also occur [11] (Mooreet al., 2005).

The research work seeks to study the effects of Indian hemp on haematological indices of chronic male Indian hemp smokers. The findings of this work will serve as a guide for future researchers and will give physicians an insight to basic haematological abnormalities which may arise as a result of smoking of Indian hemp and to proffer an informed solution to diseases that may arise thereof.

Materials and Methods

Design and blood collection

The subjects (with consent) used in this research study were drawn from Choba community in Obio/Akpor Local Government Area of Rivers State Nigeria.

A total of twenty (20) male subjects were used in the study, all the subjects were divided into two categories or groups: i) Non –smokers (control), who do not smoke at all and have never smoked in life. ii)Current chronic smokers (test), who smoke Indian hemp on a daily basis say 4-8 wraps of Indian hemp daily. All the subjects used were apparently in a healthy condition.

Subject was asked to make a fist to make it easier to locate the vein. After the collection of blood samples in replicates of three, they were then transferred into the storage bottles i.e. the anticoagulant bottle. 5mls of blood was collected from each of the subject in replicates of three. Necessary precautions were taken to avoid contamination.

Analysis of blood samples

Hb Concentration: Acid Heamatin Method, which involves matching the brown colour of haematin formed from Hb on reacting with HCl, was employed.

PCV: Microheamatocrit Method was used. The capillary tubes were two-third filled with well mixed venous blood. One end of the tube was then sealed with plasticine (modeling clay). The filled tubes were then placed in the micro-haematocrit centrifuge with the sealed end out wards. However balancing of the tubes was not required since the tubes were very light and up to 20 tubes could be centrifuged at the same time. The cover slip/plate was then screwed tightly and the lid closed. The centrifuge was spun at 2.000g for 5 minutes that is about 3,000 rate per min. The centrifuge was then allowed to settle down completely before the lid was opened. The tubes were removed and placed in the grove of the haematocrit reader so that the lowest blood level (at the plasticine plug) was leveled with the lower line of the microhaematocrit reader. Also the instrument is adjusted so that the upper level of the plasma was leveled with the slanting line at the top. The movable knob was also adjusted so that the line running from it coincides with junction between the red cell mass and the plasma. The PCV or haematocrit was then read off from the scale as a percentage.

The calculation is as follows:

RBC: Manual Method was used by diluting (1:200) appropriately using an isotonic diluents to avoid lysis of red cells in a Thoma pipette. The number of red cells in a known volume and of known dilution was counted (40x objective) using an improved Neubauer counting chamber was then charged with the well mixed diluted blood.

WBC (Total): Manual Method was used as whole blood was diluted approximately using a diluent which haemolyses red cells leaving all the nucleated cells intact. The number of white cells in known volume dilutions was counted using counting chambers as in RBC.

Body temperature measurements

Oral thermometer was inserted into the mouth of subjects after 30 minutes of smoking for a more reliable result as well as in control.

Pulse rate determination

A wireless integrated pulse Oximeter system that enables the measurement of blood oxygen saturation and the direct measurement of heart rate was used. It was put on a VLSI chip so it can be attached on a person's ear lobe and finger as well for confirmatory reading. The chip includes a radio transmitter/telemetry system to enable measurement data to be linked to a data acquisition system.

Statistical analysis

Means of ten replicate data were subjected to student's T - test and comparisons made at 95% confidence level for test of significance.

Results and Discussion

The results of the haematological parameters for the non-smokers of cannabis are as given in Tables 1 and 2, while the comparative haematological property for test and control is as given in Figure 1. The body temperature and pulse rate are as given in Figure 2.

From the results obtained, lower body temperature readings (below normal range of 33.2-38.2 °C) obtained for the tests agrees with the findings of Solowij et al. [12], who attributed such observation to Cannabis cofactor compounds modulating immune functioning and cell protection.

Also, observed redness of eye could be due to congestion of conjuctival blood vessel [13]. Increased pulse rate in comparison to control (Figure 1) was well documented throughout the experiment and could denote tachcardia. Average pulse rate of 75 beats/min compares favourably with the finding of Enright and Sherill [14] whose normal male subject in their experiment scored 74 beats/min. Effects of marijuana smoking on the hematological parameters of test subjects and control subjects highlighted by the students 'l' test carried out to analyze the mean results, and indicated that there was no significant difference in the level of RBC (P ≤ 0.05) between the two subject.

Longer term effects on RBC may not have manifested. Other hematological parameters however, were statistically significantly different.

The significant differences in PCV between smokers and nonsmokers of Indian hemp at (p<0.05) level could be as a result of reduction in quantity of oxygen available to the tissues as a result of smoking as the mean value was reduced in test subject in comparison to control. PCV values fell within normal range of 40% - 54% for males, although average value for test was marginal (Figure 2).

Statistical analysis of the level of Hb indicated significant difference between the subjects and control (P < 0.05) although those of the test fell marginally below the recommended limit of 13 to 18g/100ml in male. This may be as a result of reduced Iron group of heme due to reduced oxygen - carrying pigment. This in essence could drastically cause a decrease in the quantity of car-

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|--------------------|----------------|----------------------|---------------|-------------|
| Table 1. Levels of | naematological | parameters for the | non-smokers (| (control). |

| Donors | PCV (%) | TWBC (10 ⁹)(Cell/mm ³) | ESR (mm/hr) | RBC (10¹²) (cell/mm³) | Hb (g/dl) |
|--------|--------------------------|--|-----------------|--|-------------------|
| 1 | 47 ± 1.00a | $9.2 \pm 0.1 \mathrm{x}$ | $23 \pm 0.56e$ | $5.2 \pm 0.09i$ | 16.0 ± 0.29 k |
| 2 | $49 \pm 0.57 \mathrm{b}$ | $5.6 \pm 0.06 y$ | $19 \pm 1.00e$ | $4.98 \pm 0.9i$ | 14.0 ± 0.10 k |
| 3 | $46 \pm 0.00 \mathrm{b}$ | 6.8 ± 0.15 y | $35 \pm 1.15 f$ | 5.21 ± 0.0i1 | 13.0 ± 0.29 k |
| 4 | $45 \pm 1.15b$ | 8.1 ± 0.06 y | $40 \pm 1.15 f$ | 4.18 ± 0.00i | 14.0 ± 0.29 k |
| 5 | $52 \pm 0.58 \mathrm{b}$ | $5.4 \pm 0.00 y$ | 15 ± 1.15e | 5.32 ± 0.01i | 15.0 ± 0.56 k |
| 6 | $47 \pm 0.58b$ | 4.5 ± 0.1y | 29 ± 1.00e | 4.18 ± 0.02i | 13.2 ± 0.00 k |
| 7 | $49 \pm 1.00 \mathrm{b}$ | $7.5 \pm 0.1 y$ | $28 \pm 0.56e$ | $6.74 \pm 0.01i$ | $14.0\pm0.06k$ |
| 8 | $50 \pm 0.58 \mathrm{b}$ | $2.7 \pm 0.15z$ | $18 \pm 0.00e$ | $6.74 \pm 0.00i$ | 14.3 ± 0.00 k |
| 9 | $46 \pm 0.58b$ | $13.7 \pm 0.06 x$ | $21 \pm 0.56e$ | 4.45 ± 0.01i | 12.2 ± 0.06 k |
| 10 | 47 ± 1.00 b | 4.2 ± 0.06 y | $10 \pm 0.56e$ | $3.5 \pm 0.06i$ | 11.4 ± 0.15 k |
| Mean | 47.8 ± 0.69 | 6.77 ± 0.1 | 23.8 ± 0.88 | 5.05 ± 0.04 | 13.71 ± 0.1 |

Values are Mean \pm SE. Means in the same column with different alphabets are significantly different at p < 0.05.

| Table 2. Levels of haematological | parameters for the smokers |
|-----------------------------------|----------------------------|
|-----------------------------------|----------------------------|

| Donors | PCV (%) | TWBC (10 ⁹) (Cell/mm ³) | ESR (mm/hr) | RBC(10¹²) (cell/mm³) | Hb (g/dl) |
|--------|--------------------------|---|-----------------|---|---------------------------|
| 1 | $42 \pm 1.53 \mathrm{x}$ | $7.5 \pm 0.12a$ | 18 ± 0.56 m | $5.50 \pm 0.06 s$ | $13.13 \pm 0.36e$ |
| 2 | $34 \pm 1.15 \mathrm{x}$ | $6.2 \pm 0.15a$ | 16 ± 1.15m | $3.50 \pm 0.15r$ | $10.7 \pm 0.1 \mathrm{f}$ |
| 3 | $39 \pm 0.58 \mathrm{x}$ | $4.2 \pm 0.10a$ | 26 ± 1.00 n | $4.90 \pm 0.12r$ | $13.0 \pm 0.29e$ |
| 4 | $39 \pm 1.52 \mathrm{x}$ | 3.6 ± 0.15a | 8 ± 1.00 m | $4.95 \pm 0.02r$ | $12.3 \pm 0.09e$ |
| 5 | $46 \pm 1.15 \mathrm{x}$ | 2.8 ± 0.06 b | 27 ± 0.56 n | $5.20 \pm 0.11r$ | $14.7 \pm 0.1e$ |
| 6 | $47 \pm 0.58 \mathrm{x}$ | 7.2 ± 0.10 a | 1 ± 0.00 o | 5.00 ± 0.10 r | 15.0 ± 0.56 g |
| 7 | $37 \pm 0.58 \mathrm{x}$ | 3.4 ± 0.10 b | 19 ± 0.56 m | 5.40 ± 0.06 s | 13.0 ±1.53e |
| 8 | $45 \pm 0.58 \mathrm{x}$ | 4.9 ± 0.00 a | 43 ± 0.56n | 4.65± 0.01r | $10.7 \pm 0.15 f$ |
| 9 | $39 \pm 1.00 \mathrm{x}$ | $4.8 \pm 0.15a$ | 37 ± 1.00 n | $4.85 \pm 0.01 r$ | 11.6 ± 0.1 |
| 10 | $39 \pm 0.58 \mathrm{x}$ | $5.3 \pm 0.15a$ | 12 ± 0.56 m | $6.30 \pm 0.12s$ | 14.5 ± 0.06 |
| Mean | 40.7 ± 0.88 | 4.99 ± 0.07 | 20.7 ± 0.67 | 5.03 ± 0.09 | 12.86 ± 0.76 |

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Figure 2. Comparative hematological mean parameters in test and control.



bonic anhydrase, which catalyzes the reversible reaction between carbondioxide and water, decreasing therefore, the rate of this reaction several thousand fold [15, 16].

Observed markedly reduced levels of WBC in smoking subjects could be as a result of the accumulated active ingredient, *cannabinoid* inhibiting both T and B-lymphocytes through blocking of amino acid uptake into the stimulated lymphocytes. This low level of lymphocytes in chronic cannabis smoker could predispose them to overwhelming infection, cold, influenza or cancer. The most likely consequences are easy predisposition of cannabis smoker to preventable infection, consequent upon the possibility of reduced humoral and cell mediated immune responses.

Conclusions

Generally, the hematological characteristics of cannabis smoker differed significantly from those of non-smokers. A decrease in mean value of TWBC of cannabis smokers is a pointer to fact that immune defense in the body of test subjects, which also is regarded as body defense against infection, may have become porous.

The health risk of smoking cannabis has been shown to be so enormous that one can not justify this substance abuse. Depleted levels of Hb and PCV were evident in studied test subjects which may necessitate diet supplementation as well as enlightenment in the short and long term as observed trend may deteriorate with time.

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