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Effect Of Chronic Consumption of Cannabis Sativa on Bleeding Time, Prothrombin Time and Platelet Count In Albino Rats

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Abstract: ***The effects of chronic consumption of Cannabis sativa on bleeding time and prothrombin time was studied. Fifteen albino Wistar rats weighing 200-220g were used for the study. They were randomly assigned into 3 groups (control, low dose and high dose), n=5. The control group was administered normal saline (0.1ml/kg) while low dose and high dose groups were administered with 0.1mg/kg and 0.2mg/kg of extracts of Cannabis sativa respectively. Orogastric mode of feeding was used for the three groups, and the feeding lasted for 28 days. All the animals were allowed free access tofeed (normal rat chow) and water. After 28 days, the animals were sacrificed and blood samples were collected for analysis using standard methods. The result showed that the bleeding time and prothrombin time were each significantly (P<0.05) higher in the high dose treated group compared to low dose treated group and control group. Also, the mean platelet count showed that the cannabis sativa treated groups were each significantly higher (P<0.001) compared with the control group. Platelet count in the high dose treated group was significantly higher (P<0.01) compared with the low dose treated group. Cannabis sativa increases bleeding time, prothrombin time and platelet count. Cannabis sativa resulted in a dose dependent increase in bleeding time, prothrombin time and platelet counts. as a result, the indiscriminate use of Cannabis sativa should be discouraged as it could be detrimental to the body's hemostatic mechanisms.***

Keywords: *Cannabis sativa*, bleeding time, prothrombin time, platelet count

1. Introduction

Together with coffee and tobacco, Cannabis is one of the most commonly used psychoactive drug worldwide, and it is the single most popular illegal drug. Over 160 million people around the world are implicated in the regular use of cannabis and these number still increases with time [1]. Cannabis, also known as marijuana is a greenish or brownish material consisting of the dried flowering, fruiting tops and leaves of the Cannabis plant. Street terms for Cannabis include bhang, charas, pot, dope, ganja, hemp, igbo, weed, blow. The response to Cannabis varies according to the form in which it is consumed, the dose and the route of administration. The most widely and popular means of the use of cannabis is in its dry herbal form but more users ingest it in salads, tea additives, ingredient in drugs and vegetable juices [2].

More than 61 chemicals called cannabinoids have been identified as specific to the Cannabis plant. One of its constituents, tetrahydrocannabinol (THC) is the main psychoactive cannabinoid, most responsible for the 'high' associated with marijuana use [3]. The pharmacological actions of THC results from its partial agonist activity at the cannabinoid receptor (CB1), which in mammals are found in the CNS, gastro-intestinal organs, and adipose tissue. The other cannabinoid receptor (CB2) are found almost exclusively in peripheral immune cells and are not thought to be involved in energy homeostasis [4].

Cannabis sativa, an annual herbaceous plant in the Cannabaceae family has long been used for religious, medicinal purposes and as a recreational drug (due to its psychoactive effects). Some therapeutic uses of *Cannabis sativa* include; alleviation of symptoms suffered both by AIDS patients and by cancer patients undergoing chemotherapy;reduction of food and water intake, resulting to a decrease in body weight in mice [5]. It has also been shown to reduce intraocular pressure in patients with glaucoma, alleviate neuropathic pain and spasticity in multiple sclerosis [6]. Apart from these positive effects, Cannabis has been associated with increased risk of myocardial infarction [7], psychosis [8], impaired locomotor and exploratory behavior [9]. It also negatively affects sperm production [10] and delay in conception in women following smoking of *Cannabis sativa* [11].

The consumption of cannabis therefore seems to have systemic effect Blood is a connective tissue, therefore, analysis of some hemostatic indices like prothrombin time, bleeding time and platelet count could provide vital information on the implication of chronic consumption of *Cannabis sativa* on the body's hemostatic mechanism.

2. Methodology

2.1 Preparation of *Cannabissativa* extract

Cannabis sativa was obtained from the botanical garden in Calabar South Local Government Area of Cross River State, Nigeria. It was certified and classified 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 1000mls 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 Animal care

Fifteen adult albino Wistar rats were housed singly in metabolic cages under standard laboratory conditions in the animal house of the department of Physiology, University Of Calabar, Calabar. They were fed with normal rat chow and given access to water freely. The experimental animals were allowed to acclimatize for a period of one week before the commencemet of the feeding regimen.

2.3 Animal Treatment

According to toxicity studies reported by Obembe et al, [12], the LD_{50} of *Cannabis sativa* was 34mg/kg. Therefore, the low dose and high dose groups were administered with 0.1mg/kg and 0.2mg/kg body weight of *Cannabis sativa* respectively. Control was administered 0.1ml/kg body weight of normal saline. Administartion of extract was done with the aid of an orogastric cannula for a period of 28 days.

2.4 Collection of samples

At the end of the feeding regimen, 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 bleeding time and prothrombin time [13].

2.5 Determination of bleeding time

This was done using the method as described by Obembe et al, [14]. The tail of the rat was cleaned with methylated spirit and allowed to dry. It was then pricked with a sterile lancet for the blood to ooze out. The time was noted with a stop watch. With a filter paper, the blood oozing out was touched every 30 seconds using a fresh part of the filter paper without pressing or squeezing the wound. Note that the blood spots got smaller till it disappeared when bleeding stopped. The number of blood spotswere counted and divided by two which gave the bleeding time in minutes.

2.6 Determination of prothrombin time

Prothrombin time was determined using the quick-one stage method by [15] which fresh prothrombin free plasma was used as the diluents.The rats were put in a dessicator containing chloroform (which served as the anaesthesia). Blood samples were collected via cardiac puncture into sample bottles containing 0.4ml sodium citrate. The blood sample was then centrifuged immediately to remove Ca^{2+} thereby preventing the convertion of prothrombin to thrombin. The supernatant plasma was then removed and placed in a clean glass tube. This was placed in a water bath at 37^0C and 0.1ml of thromboplastin was added to it. After 1 minute, 0.1ml of warmed 0.25 ml calcium chloride was added and the contents in the tube were carefully mixed. The tube was continuously but gently inclined from the vertical to just short of the horizontal so that its contents were observed for the first signs of clotting. A fibrin clot developed within a second which marked the end point. This was repeated 3 times and the average reading was taken. The results were recorded in seconds.

2.7 Determination of platelet count

Platelets count was determined using the manual methods as described by [16][17].

2.8 Statistical analysis

Results are represented as mean ± standard error of mean (SEM). The data were computed using Microsoft excel analyzer (Microsoft office version 2010). The One-way Analysis of Variance (ANOVA) was used to analyze the data. Values of $P<0.05$ was considered significant.

3. Results

3.1 Effect of *Cannabis sativa* on bleeding time

The mean bleeding time for control, low dose and high dose was 5.4± 0.37, 6.3 ± 1.07 and 6.8 ± 0.58 respectively. The results showed that the high dose group had a significantly ($P<0.05$) higher bleeding time compared to control and low dose (Fig. 1).

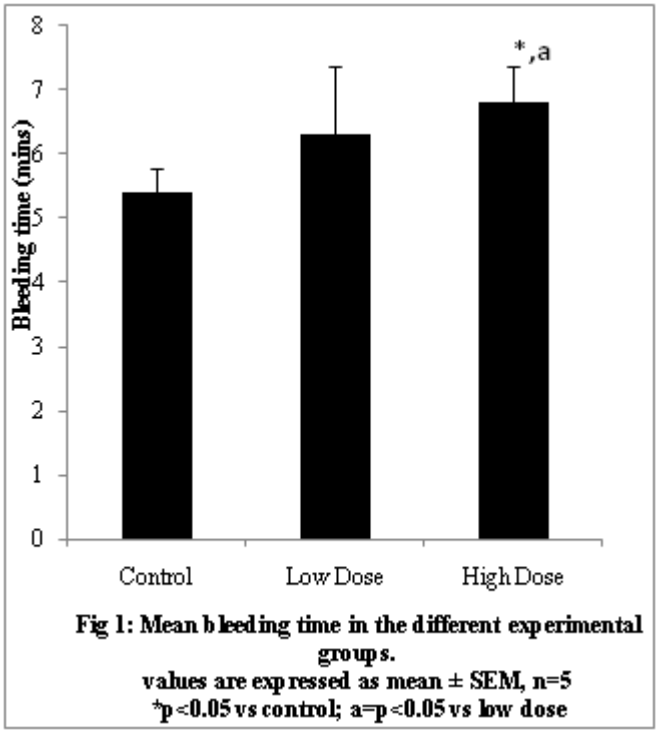

Fig 1: Mean bleeding time in the different experimental groups.
values are expressed as mean ± SEM, n=5
***p<0.05 vs control; a=p<0.05 vs low dose**

3.2 Effect of *Cannabis sativa* on prothrombin time

The results showed that the mean prothrombin time for the experimental groups was 26 ± 0.71, 26.6 ± 0.51 and 29 ± 0.71 for control, low dose and high dose respectively. The high dose group showed a significant increase p(<0.05) in prothrombin time compared to control and low dose (Fig. 2).

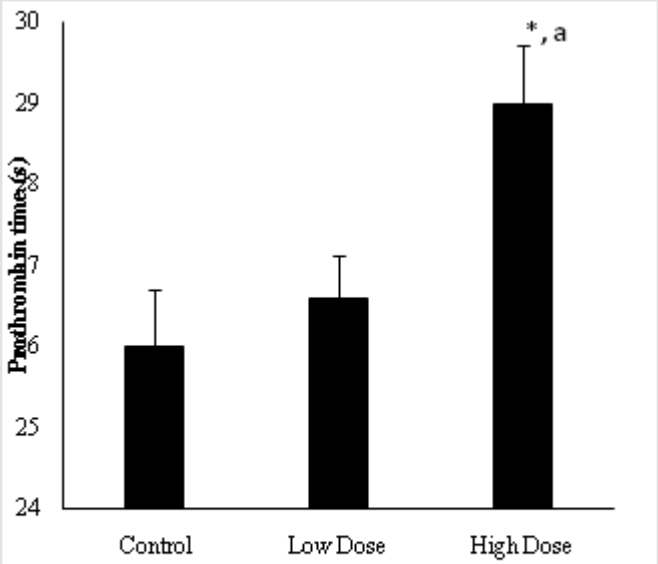

**Figure 2: Comparison of prothrombin time in the different experimental groups.
Values are mean ± SEM, n = 5.
*p<0.05 vs control; a = p<0.05 vs low dose.**

3.3 Effect of *Cannabis sativa* on platelet count

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 had significant increase (P<0.001) in platelet count compared to control. The high dose in treated group inturn was significantly higher (P<0.01) platelet count compared with low dose treated group. (Fig 3).

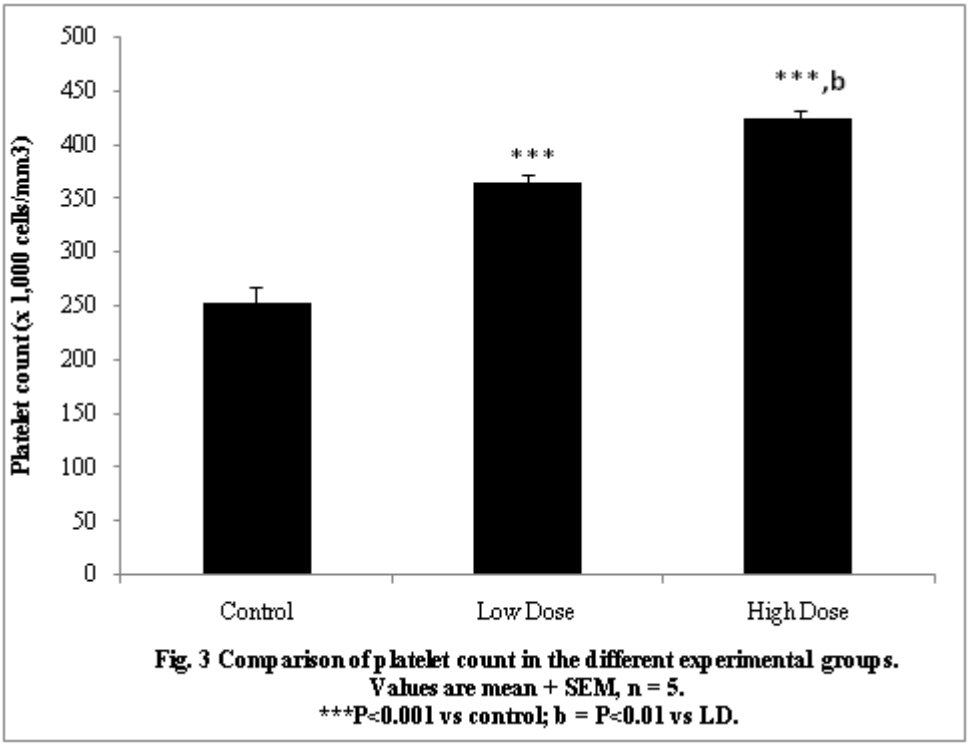

**Fig. 3 Comparison of platelet count in the different experimental groups.
Values are mean + SEM, n = 5.
***P<0.001 vs control; b = P<0.01 vs LD.**

4. Discussion

This study was done to investigate the effects of chronic consumption of *Cannabis sativa* on bleeding time, prothrombin time and platelet count in albino Wistar rats. Bleeding time, prothrombin time and platelet count are measures of hemosatatic disorders [18]. Prothrombin time gives an indication of the concerntration of prothrombin in blood and measures the time required for blood to clot.

The results of this study, showed that the high dose group had a significantly higher bleeding time and prothrombin time compared to control and low dose group. The results of our study is at variance with previous report by William *et al*, [19] that *Cannabis sativa* may not have effect on haematological indices. However, it is in conformity with Mayo Foundation and Medical Education Research [20] where *Cannabis sativa has* been reported to increase the risk of bleeding.

The increased bleeding time and prothrombin time observed especially in the high dose group compared to control suggests a problem in the platelet clotting mechanism. Also in this study, *Cannabis sativa* increased platelet count in the treated groups compared with control. This is contrary to the report by Oseni *et al*, [21] where auhors reported a decrease in platelets count in human subjects. The disparity in results could be due to differences in species of animals used,

duration of test as well as quality and quantity of *cannabis sativa* used. The results of this study, implies that there could be a problem in the clotting mechanisms of the body. The liver is responsible for the production of blood clotting factors including prothrombin and fibrinogen [22]. Most of these clotting factors require the presence of Vitamin K. Vitamin K as a fat-soluble vitamin in turn requires bile salts to be absorbed properly by the body. An impaired liver function in variably alters the production of majority of blood clotting factors leading impaired clotting mechanism and an increase risk of bleeding. Following a report by Paulo *et al*, [23] where authors reported that chronic marijuana usage is associated with hepatic morphologic and enzymatic alterations in humans. It could therefore be deduced that *Cannabis sativa* may possibly contain some hepatotoxic substances which could have probably lead to altered hepatic morphology leading to hepatic malfunctioning and these may account for the observed increased in bleeding and prothrombin time in our study.

5. Conclusion

Chronic consumption of *Cannabis sativa* resulted in a dose dependent increase in bleeding time, prothrombin time and platelet counts. Therefore, the indiscriminate use of *Cannabis sativa* should be discouraged as it could be detrimental to the body's hemostatic mechanisms.

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