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Decreased biliary secretion, biliary composition and serum electrolytes with chronic administration of *Cannabis sativa* in albino wistar rats

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Abstract

The effect of oral administration of *Cannabis sativa* on biliary secretion and electrolytes was studied using albino Wistar rats.15 rats(200-250g) were randomly assigned into 3 groups; control, low dose and high dose groups and administered 0.5ml/100g body weight of normal saline,0.25ml/100g body weight of *Cannabis sativa, and* 0.4ml/100g body weight of *Cannabis sativa* respectively. The results showed that the rate of bile secretion was significantly (P<0.001) lower in the *Cannabis sativa* groups compared to control with the high dose group being significantly(P<0.05) lower compared to the low dose group. Biliary electrolytes(Na^+, K^+ and Cl^-)were significantly(P<0.001) lower in the test groups dose dependently compared to control. Biliary HCO_3^- however was significantly increased(P<0.001) in the high dose group compared to control and low dose groups. Biliary urea and creatinine levels were significantly(P<0.001) lower in the test groups compared to control. Biliary cholesterol levels was significantly(P<0.05) increased in the low dose group and significantly(P<0.01) decreased in the high dose group compared to control. The serum electrolytes (Na^+, K^+, and Cl^-)were significantly(P<0.001) lower in the test groups dose dependently compared to control. At high dose, serum urea and creatinine levels were significantly(P<0.001) increased compared to control and low dose group, even though they were increased at low doses. The high dose group showed significantly(P<0.001)increased glucose concerntration compared to control and low dose while serum cholesterol levels was significantly(P<0.01) increased in the test groups compared to control. *Cannabis sativa* may reduce bile secretion dose dependently and may alter the concerntration of biliary electrolytes. Therefore, the indiscriminate use of *Cannabis sativa* should be discouraged.

Keywords: *Cannabis sativa*, biliary secretion, serum, electrolytes, concerntration

Introduction

Background of the study

Together with coffee and tobacco, Cannabis is the most commonly used psychoactive drug worldwide, and it is the single most popular illegal drug. Worldwide, over 160 million people are using Cannabis regularly and these numbers are still rising (United Nations Office on Drugs and Crime, 2006). Cannabis, also referred to 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 using cannabis is in its dry herbal form but more users ingest it in salads, tea additives, ingredient in drugs and vegetable juices.

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; reduces food intake, water intake and body weight in mice (Okon et al, 2014) [3]. It has also been shown to assist some glaucoma patients by reducing pressure within the eye, alleviate neuropathic pain and spasticity in multiple sclerosis (Grotenhermen and Muller-Vahl, 2012) [4]. Apart from these positive effects, Cannabis has been associated with increased risk of myocardial infarction (Aryana & Williams, 2007) [6], psychosis, reduced locomotor and exploratory behavior (Okon et al, 2014) [3]. It also negatively affects sperm production and women who smoke it experience delay in getting pregnant (Waldron et al, 2009) [7].

The consumption of cannabis therefore seems to have systemic effect as all organs seem to be affected. Due to the beneficial and harmful effects of *Cannabis sativa* observed in users, it became expedient to carry out this study to ascertain its effects on the rate of bile secretion and biliary electrolytes using albino Wistar rats as a model.

Materials and Methods

1. Preparation of *Cannabis sativa* extract

Cannabis sativa was obtained from the botanical garden in Calabar South Local Government Area of Cross River State, Nigeria, identified and authenticated by Pastor Frank J. Apejoye and Mr. Effa A. Effa in the department of Botany, University of Calabar and given herbarium number 7. 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. Animal care

Fifteen **(**15) adult albino Wistar rats weighing 200-250g were housed singly in metabolic cages under standard laboratory conditions in Physiology Department, University Of Calabar, Calabar with room temperature of 25 ± 2^0C, and where they could observe the dark/light cycle throughout the duration of the experiment. Their beddings were changed regularly. They were fed with normal rat chow and given water freely for one week to allow for acclimatization before commencing the experiment which lasted for 28 days.

3. Ethical approval

All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the appropriate ethics committee.

4. Model of the experiment

The animals were randomly assigned to 3 groups of 5 rats each. The low dose group was administered 0.25ml/100g body weight of *Cannabis sativa* while the high dose group was administered 0.4ml/100g body weight of *Cannabis sativa*. Control was administered 0.5ml/100g body weight of normal saline. Orogastric method of feeding was used.

5. Collection of biliary secretion

Biliary secretion was collected by the method of Vickers et al (1998) [8]. The rats were starved for 12 hours prior to the time of experiment. The animals were weighed and anaesthesized by intraperitoneal administration of sodium thiopentone (6mg/100g body weight), and were quickly pinned to a dissecting board for a tracheostomy performed to clean the airways for easy breathing. The stomach was opened along the linea alba to prevent bleeding. A laparotomy was performed and the liver lobes deflected anterolaterally to expose the common bile duct. The common bile duct was then cannulated with a portex cannula (0.5mm in diameter) after a small incision was made. The cannula was held in place with a thread tied round the bile duct. The bile content was collected at 3hours interval for each group studied.

6. Determination of biliary electrolytes: sodium and potassium

Principle:

Sodium and potassium in the bile were determined using a flame photometer (Model 410C, Petracourt Ltd England). The bile was sprayed into a non-luminous gas flame which became coloured by the characteristic emission of metallic ions in the sample. The wavelength of the metals 598nm and767nm for sodium and potassium respectively, were selected by the light prism system and allowed to fall on a photosensitive detection system. The amount of light emitted is dependent on the concerntration of the metallic ion present by comparing the amount of light emitted from the sample with that from the standard solution. The amounts of sodium and potassium were determined.

7. Determination of bicarbonate in bile

Plasma carbondioxide (CO_2) was measured by the modified method of Obembe et al, 2010) [9].

Principle

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbondioxide (bicarbonate) to form oxatoacetae and phosphate ion with simultaneous oxidation of an aquimolar amount of reduced NADH (Nicotinamide adenosine dinucleotide) to NAD. The reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to CO_2 concerntration in the sample.

The CO_2 in the sample is determined as follows:

$$CO_2 \text{ content} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample x concerntration of standard}}{\text{Absorbance of blank} - \text{Absorbance of standard}}$$

8. Determination of biliary chloride

Principle

The principle is by end point calorimetric titration.

Procedure

2mls of buffer solution was placed in a conical flask and 0.2ml of bile was added and mixed. Four drops of diphenyl carbazene indicator was added. This was titrated with mercuric nitrate from a 2ml micro pipette. The end point was indicated by a violet colour. The standard solution (0.2ml) was added to 2.0ml buffer solution and indicator added and similarly titrated.

Biliary chloride concerntration was obtained from the following calculation and expressed in units of mEq/L.

$$\text{Biliary chloride ion} = \frac{\text{Titrated value of test x 100}}{\text{Titrated value of standard}}$$

9. Statistical analysis

All results were expressed as mean ± SEM. In all cases, the comparison of the different sets of data was done using the student's t-test.

Results

Effects of *Cannabis sativa* on the rate of biliary secretion

The rates of biliary secretion in control, low dose and high dose groups of *Cannabis sativa* were 1.14 ± 0.04, 0.56 ± 0.04, and 0.46 ± 0.01 respectively. The test groups were significantly (P<0.001) lower compared to control with the high dose being significantly (P<0.05) lower compared to the low dose group. Fig. 1

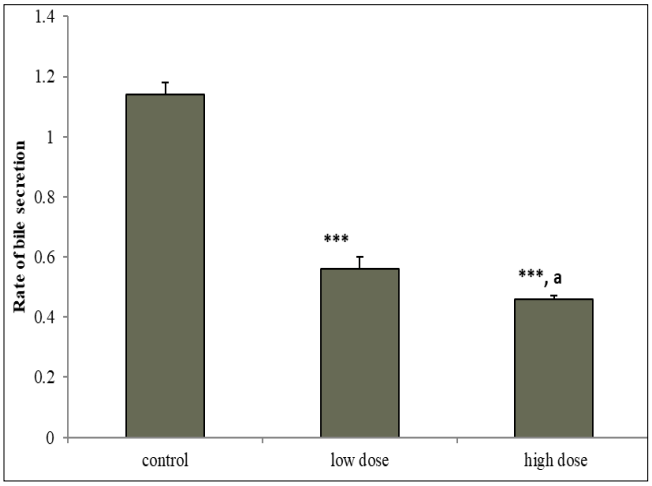

Fig 1: Comparison of rate of bile secretion in the different experimental groups. Values are mean ± SEM, n=5. ***P<0.001 vs control, a= P<0.05 vs LD

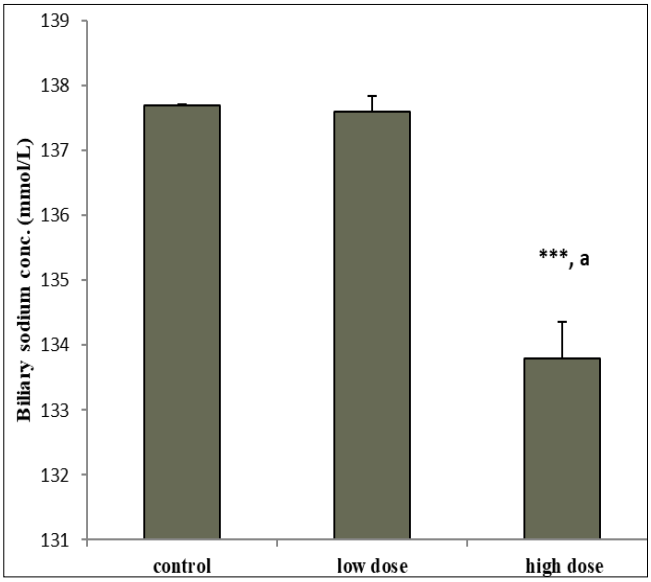

Fig 2: Comparison of biliary sodium concerntration in the different experimental groups. Values are mean ± SEM, n=5. ***P<0.001 vs control, a= P<0.001 vs LD

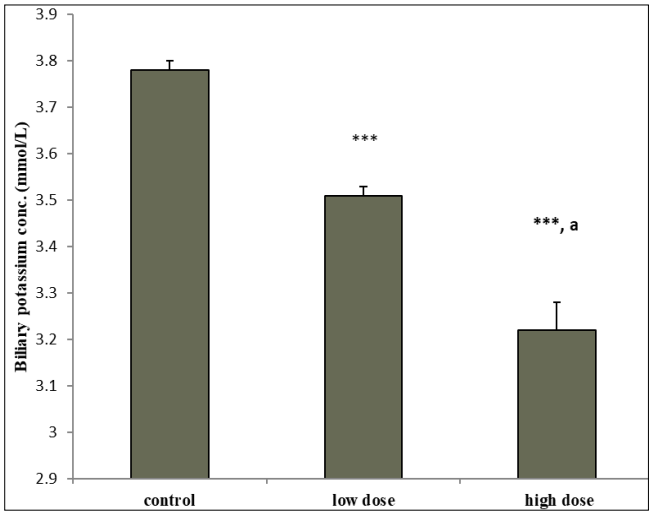

Fig 3: Comparison of biliary potassium concerntration in the different experimental groups. Values are mean ± SEM, n=5. ***P<0.001 vs control, a= P<0.01 vs LD

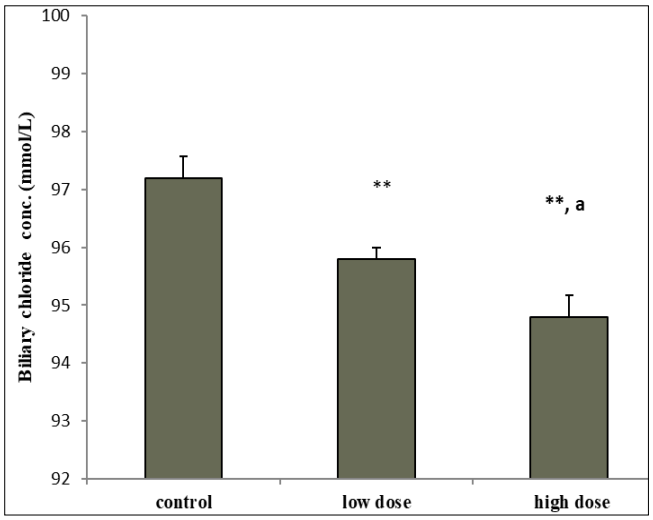

Fig 4: Comparison of biliary chloride concerntration in the different experimental groups. Values are mean ± SEM, n=5. ***P<0.01 vs control, a= P<0.05 vs LD

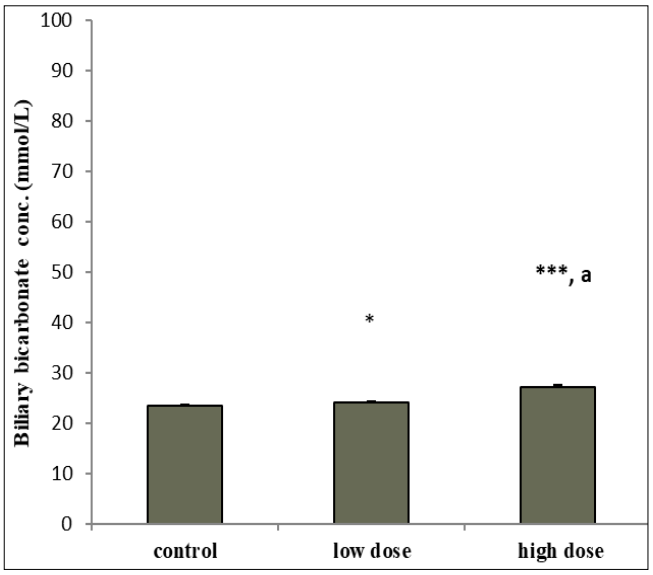

Fig 5: Comparison of biliary bicarbonate concerntration in the different experimental groups. Values are mean ± SEM, n=5. *P<0.05, ***P<0.001 vs control, a= P<0.001 vs LD

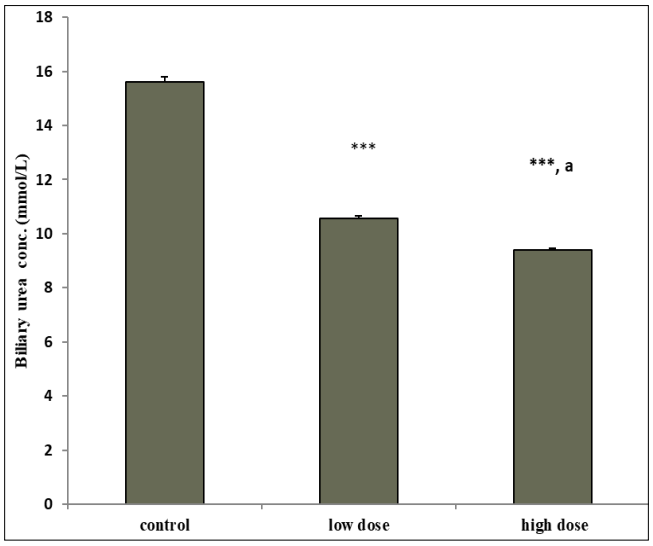

Fig 6: Comparison of biliary urea concerntration in the different experimental groups. Values are mean ± SEM, n=5. ***P<0.001 vs control, a= P<0.001 vs LD

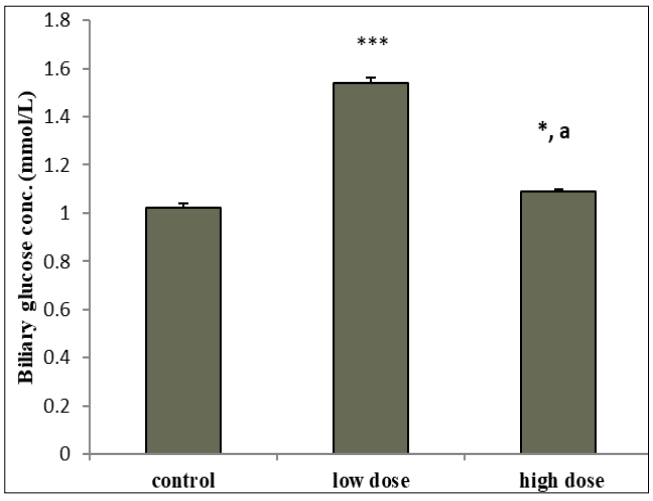

Fig 7: Comparison of biliary glucose concerntration in the different experimental groups. Values are mean ± SEM, n=5. *P<0.05, ***P<0.001 vs control, a= P<0.001 vs LD

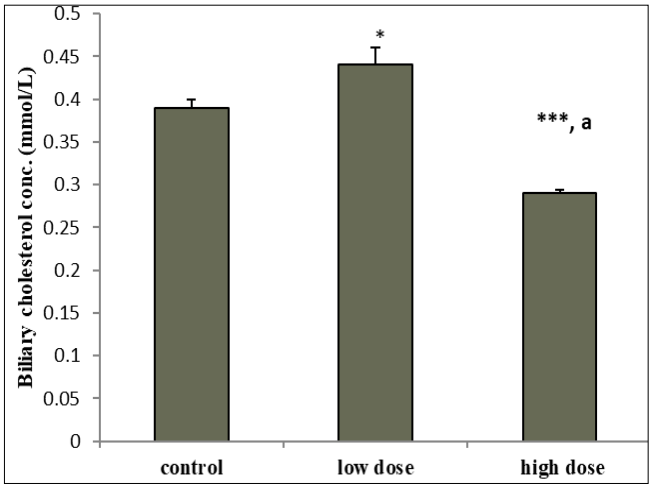

Fig 8: Comparison of biliary cholesterol concerntration in the different experimental groups. Values are mean ± SEM, n=5. *P<0.05, ***P<0.001 vs control, a= P<0.001 vs LD

Biliary sodium levels in the control, low dose and high dose groups were 137.70 ± 0.02, 137.60 ± 0.24, and 133.80 ± 0.56 respectively. The high dose group was significantly (P<0.001) lower than control and low dose groups. Fig 2.

The biliary potassium levels were 3.78 ± 0.02, 3.51 ± 0.02 and 3.22 ± 0.06 in the control, low dose and high dose groups respectively. The test groups were significantly (P<0.001) lower compared to control. High dose group was also significantly (P<0.01) lower compared to the low dose group. Fig. 3.

The mean biliary chloride in the control, low dose and high dose groups were 97.20 ± 0.37, 95.80 ± 0.20 and 94.80 ± 0.37 respectively. The test groups were significantly (P<0.01) lower compared to control. Also, the high dose group was significantly (P<0.05) lower compared to the low dose group. Fig. 4.

The biliary bicarbonate levels in the control, low dose and high dose groups were 23.4 ± 0.24, 24.2 ± 0.19 and 27.2 ± 0.37 respectively. The low dose and high dose groups were significantly (P<0.05 and P<0.001) higher respectively compared to control. The high dose group was significantly (P<0.001) compared to low dose. Fig. 5.

The biliary levels in the control, low dose and high dose groups were 15.60 ± 0.19, 10.55 ± 0.11 and 9.41 ± 0.06 respectively. The test groups were significantly (P<0.001) lower compared to control. The high dose group was significantly (P<0.001) lower compared to the low dose group. Fig. 6.

The biliary mean values of glucose in the control, low dose and high dose groups were 1.02 ± 0.02, 1.54 ± 0.02 and 1.09 ± 0.01 respectively. The low dose and high dose were significantly (P<0.001 and P<0.05) higher respectively compared to control; while the high dose group was significantly (P<0.001) lower compared to the low dose group. Fig. 7

The biliary cholesterol levels for the control, low dose and high dose groups were 0.39 ± 0.01, 0.44 ± 0.02 and 0.29 ± 0.004 respectively. The low dose was significantly (P<0.05) higher compared to control while the high dose groups was (P<0.001) lower compared to low dose and control. Fig. 8

Discussion

The effects of *Cannabis sativa* on biliary secretion and electrolytes in albino Wistar rats were studied. The results observed after 28 days of administration of the extract showed that there was a significant decrease in biliary secretion in the treated groups dose dependently. There was a significant decrease in biliary sodium, chloride, potassium concerntrations. Conversely, bicarbonate concerntration and glucose levels were significantly increased in the *Cannabis sativa* treated animals when compared with control. Urea levels were lower in the test groups dose dependently while cholesterol was decreased in the high dose group compared to control.

The liver's main function is to secrete bile. Bile helps in the digestion and absorption of fats. It also serves as a means for the excretion of several important waste products from the blood like urea and creatinine (Guyton & Hall, 2019) [5]. The reduced biliary secretion, observed in the treated groups of *Cannabis sativa* may be as a result of liver damage resulting in the inability of the liver to secrete bile at normal rates like the control group. Injury in the liver may alter biliary secretion (Obembe et al, 2010 [9], Guyton & Hall, 2019) [5]. Omar et al., 2012 had earlier on reported that *Cannabis sativa* administration alone caused histological liver damage and fibrosis. The decreased production of bile could result in the alteration of the emulsification function of bile on fats thereby impairing fats digestion and absorption.

The increased concerntration of electrolytes, bicarbonate observed in the results at high doses of *Cannabis sativa* could be useful in the neutralization of acids in the duodenum and alleviate ulcerative conditions. Omar et al, 2015 [10] had earlier on reported that *Cannabis sativa* may be beneficial in treating gastric ulcers since it increases gastric mucus output. It is possible that *Cannabis sativa* has a secretin-like effect or stimulates the secretion of secretin. Secretin stimulates the release of bicarbonate rich electrolytes in bile and gastric mucous (Guyton & Hall, 2019) [5]. Further research is however needed.

Biliary sodium, chloride and potassium concerntrations in this study were significantly reduced in the *Cannabis sativa* groups compared to control. The mechanism through which this occurs is still unclear.

The reduced biliary urea and creatinine levels observed in the test doses may have been due to impaired liver function. One of the liver's function is to convert ammonia to urea (deamination) for clearance through bile (Guyton & Hall, 2019) [5]. Also, the liver converts glucose to glycogen but, glucose levels were higher in the test doses implying impaired liver function. Cholesterol produced by the liver for the synthesis of bile acids was decreased in the high dose compared to control and low dose. This could lead to the impairment of the digestion of fats leading to steatorrhoea. Serum electrolytes (Na^+, K^+, and Cl^-, HCO_3^-) were significantly lower in the test groups dose dependently compared to control.

In conclusion, chronic administration of *Cannabis sativa* may reduce bile secretion dose dependently and may alter the concerntration of biliary and serum electrolytes. It may result in hypokalemia and hyponatriemia. Kidney and liver function may be impaired; and digestion and absorption of fats may be disrupted. High dose of Cannabis sativa may result in diabetes mellitus and atherosclerosis. However, the high concerntration of bicarbonates it produces in bile dose dependently, may be beneficial in alleviating the development of ulcers. Therefore, the indiscriminate use of *Cannabis sativa* should be discouraged.

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