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RESEARCH ARTICLE

EFFECT OF AQUEOUS LEAF EXTRACT OF CANNABIS SATIVA ON LACTATE DEHYDROGENASE ACTIVITY AND CREATININE LEVEL IN MALE WISTAR ALBINO RATS

[1,*]Onuoha, S. C. and [2]Okari, K. A.

[1]Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B 5323, Choba, Rivers State, Nigeria
[2]Deparment of Medical Biochemistry, Faculty of Basic Medical Sciences, University of PortHarcourt, Nigeria

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ABSTRACT

This study investigated the effect of aqueous extract of cannabis *sativa* on lactate dehydrogenase activity (LDH) and creatinine level which are cardiac markers in male Wistar rats. The extract was administered orally at a single dose of 100, 200 and 400 mgkg-1 body weight to the experimental animals daily for 3 weeks. The increase in creatinine level and LDH activity was dose dependent. This present study showes that administration of C. sativa leaf extract at 100, 200 and 400 mgkg-1 increase the activity of LDH and creatinine level which may be deleterious to the heart.

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INTRODUCTION

Cannabis sativa (*C. sativa)* has become one of the widely and wonderful plant grown and used worldwide. It is reverend in most part of the world especially in India (Andrich, 1971). *Cannabis sativa* (*C. sativa*) is used medicinally, commercially, agriculturally and socially. All its parts are useful, the leaves, the stems including the outer covering of the back. Even though the leaves are smoked recreationally, the stem has been known to be used for producing the strongest rope and pot (Maisto *et al.,* 1999) ship builders used marijuana ropes in ship sails. Evidence have also shown that the hemp fibers were employed in the production of clothes and pots. According to Maisto *et al.,* (1999), Hemp fibres were also used for house building in Southern Africa. Although there does not seem to be much question that the clustering of these characteristics occurs in some marijuana users, the causal influence of Cannabis is not clear (Brick, 1990). Also, there are some debate, about just how commonly the syndrome occurs, with some citing it as a fairly infrequent occurrence (NIDA, 1982). In addition, anthropological investigations of heavy Cannabis users in other countries generally have not found the presence of the amotivational syndrome (Carter, 1980, Carter and Doughty, 1976, Comitas 1976), and laboratory studies on Cannabis use in human have not supported the hypothesized syndrome (Foltin *et al.,* 1989, 1990). The therapeutic uses of marijuana today are much more circumscribed. For thep most part, synthetic products such as Levontradol, Nabilone, and Marinol, that chemically resemble the Cannabinoids have been used in current treatment efforts (Sussman *et al.,* 1996, Ungerleider and Andrysiak, 1985). Nevertheless the leaves of this plant is dried and smoked randomly by both the young and aged ones. The prevalence of this necessitated this research.

Corresponding author: **Onuoha, S.C.,**
Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B 5323, Choba, Rivers State, Nigeria.

MATERIALS AND METHODS

Experimental Animals

A total number of thirty-six (36) female male Wister rats weighing between (100-130g) were used for the study. The animals were purchased from the animal house of Department of Biochemistry and kept in the animal house for two weeks to acclimatize. The animals were grouped into experimental and control groups as shown on Table 1 and housed in sanitized wooden cages containing saw dust as bedding. They were also fed with standard rat chow pellet as diet and clean water *ad-libitum* was supplied and maintained under standard laboratory conditions (temperature of 25 ± 3°C and 12 h light/12 h dark

cycle), in accordance with the guidelines for the care and use of laboratory animals by National Academy of Science (1996).

Table 1. Experimental groups

Groups	Treatments	Durations	Number of Rats
I	Normal feed + water	21Days	9
II	100 $mlkg^{-1}$ *C. sativa*	21 Days	9
III	200 $mlkg^{-1}$ *C. sativa*	21Days	9
IV	400 $mlkg^{-1}$ *C. sativa*	21Days	9

Preparation of Extract (*C. sativa*)

Fresh *C. sativa* were purchased from Port Harcourt local market. The leaves were separated, washed and dried for 5 days. The dried leaves were there after ground into powder with the aid of a Corona grinding machine in our Laboratory. About 1.348kg of the grounded leaves was measured out and poured into a neat white transparent bucket with a lid and then soaked in 4 liters of hydro-methanol (80% methanol and 20% distilled water). The mixture was properly stirred to make sure it was properly soaked and allowed to stand for a total period of 24 hours. The soaked grounded leaves were squeezed so as to separate the chaff from the liquid. The liquid obtained was filtered using Whitman's no. 1 filter paper placed in a funnel and held on a clamp stand. The brownish-yellow filtrate was collected in a beaker. This filtrate was then placed in a water bath set at 45ºC, covered with aluminum foil (to prevent reduction of its potency), and left to dry for 7 days (1week). This method of drying was in order to concentrate the extract. After a week the concentrated methanolic extract of *C. sativa* leaves were dissolved in 2 litters of water and poured into a white bucket. A dose of 100, 200 and 400 $mgkg^{-1}$ body weight of rats was used.

Sample Collection and Analysis

Blood samples were collected at intervals of 7days for 21days.. The animals were anaesthetized using chloroform and then sacrificed. Blood was collected via cardiac punctured and put in a labeled Ethylenediaminetetraacetic acid bottle (EDTA) for lactate dehydrogenase (LDH) enzyme and creatinine level, and thereafter centrifuged at 7000rpm for ten minutes(Onyeso et al. 2015). The serum was then collected and stored at -15^0C. Plasma lactate dehydrogenase activity was assayed by formation of NADH at 340 nm in a reaction involving the oxidation of lactate to pyruvate according to the method of Wahlefied (1983). While plasma creatinine was measured according to the method of Jaffe *et al.,* 1987. The two parameter were analyzed using RANDOX kits.

Statistical Analysis

All statistical analysis was performed using the statistical package for social sciences (SPSS version 20.0). The results were analyzed using the one-way analysis of variance (ANOVA), with a statistical significant difference at $p<0.05$. Turkey's multiple comparison was used to test for statistically significant difference between control and experimental groups. The results were presented as Mean $^{\pm}$ Standard Error of Mean (S.E.M).

RESULTS

The results of the effect of *C. sativa* leaf extract administration on creatinine level in Wistar rats are presented on Table 2. The creatinine level in the experimental animal after aqueous extract administration in group II, III and IV (33.2±9.62, 132.6±10.9 and 166.5±13.5 $mgdL^{-1}$) are significantly different ($p<0.05$) when compared to the control group (74.8±13.3 mg dL^{-1}) after one week of *C. sativa* treatment. Also, the increase in creatinine level were dose dependent.

Table 2. The effect of *C. sativa* leaf extract administration on creatinine level in Wistar rats

Groups	Treatment	Week 1	Week 2	Week 3
I	Normal feed + water	74.8 ± 13.3^b	43.2 ± 6.25^b	33.3 ± 5.59^b
II	100 $mlkg^{-1}$ *C. sativa*	33.2 ± 9.62^c	62.1 ± 2.14^c	81.8 ± 12.9^d
III	200 $mlkg^{-1}$ *C. sativa*	132.6 ± 10.9^a	71.7 ± 9.81^d	80.1 ± 12.5^c
IV	400 $mlkg^{-1}$ *C. sativa*	166.5 ± 13.5^d	131.3 ± 14.0^a	361.5 ± 77.7^a

Data represented in mean ± S.E.M respectively of triplicate samples. Mean with the same Superscript are not significantly different while mean with different superscript are significantly different.

The results of the effect of *C. sativa* leaf extract administration on lactate dehydrogenase (LDH) activity in Wistar rats are presented on Table 3. LDH activity of experimental animals in group II, III and V were significantly different ($p<0.05$) when compared to the control group. However, group IV was significantly lower ($p<0.05$) when compared to group II and III. Notwithstanding, group IV statistically showed significant difference ($p>0.05$) with the control group.

Table 3. The effect of *C. sativa* leaf extract administration on lactate dehydrogenase activity in Wistar rats

Groups	Treatment	Week 1	Week 2	Week 3
I	Normal feed + water	178.0 ± 20.9^c	377.9 ± 170.2^d	18.4 ± 2.69^b
II	100 $mlkg^{-1}$ *C. sativa*	311.7 ± 52.7^a	192.9 ± 191.1^c	61.3 ± 4.29^d
III	200 $mlkg^{-1}$ *C. sativa*	336.0 ± 64.9^d	249.6 ± 160.7^b	89.9 ± 22.4^a
IV	400 $mlkg^{-1}$ *C. sativa*	219.7 ± 36.7^c	87.8 ± 16.2^a	45.9 ± 2.20^c

Data represented in mean ± S.E.M respectively of triplicate samples Mean with the same Superscript are not significantly different while mean with different superscript are significantly different.

It was observed that experimental animals in group II, III, and IV exhibited aggressive and restless behavior's when hungry and sluggish behavior when fed as compared to the control group after Week 1 to 3.

Conclusion

There are difficulties in making causal inferences about the acute and chronic adverse health and psychological effects of *C. sativa* use. In this present study, we investigated the effect of *C. sativa* on creatinine level and LDH activity with regards to its health effect. Creatinine being the chemical waste generated by muscle metabolism is not utilized but is excreted out of the body in the urine via the kidney. A high level of creatinine and LDH shows that acute consumption of *C.Sativa* is dentrimental to the heart.

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