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Research article

Administration of Cannabinol and Alcohol Altered the Liver Enzymes, Lipid Profile and Antioxidant Status of Male Wistar Rats

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ABSTRACT

Background: Cannabis and alcohol increasingly gain legalized status in many countries, the popularity and prevalence of use continues to grow.

Objective: This study was designed to investigate their effects on liver enzymes and lipid profile of rats.

Methods: Twenty five male rats were divided into 5 groups; group 1 was given distilled water; groups 2, 3, 4 and 5 rats were treated with 5% methanol, 25% alcohol, 10 mg/kg of cannabinol, 25% alcohol and 10 mg/kg of cannabinol p.o for 8 weeks respectively. Statistical analysis was done using the ANOVA.

Results: The serum concentrations of AST and ALT were significantly higher in groups 3, 4 and 5 when compared with groups 1 and 2. Groups 1 and 2 had LDL and HDL that were significantly lower than that of groups 3 and 4. Rats in group 5 had HDL that was significantly higher compared to group 1. The total cholesterol level of groups 3, 4 and 5 was significantly higher than that of groups 1 and 2. However, the rats in group 5 had total cholesterol level that was significantly lower when compared with groups 3 and 4. Groups 3, 4 and 5 had triglycerides concentration in the plasma that was significantly higher than that of groups 1 and 2. The photomicrographs of groups 3, 4 showed signs of dilated sinusoids when compared with groups 1 and 2.

Conclusion: It was concluded that the administration of alcohol and cannabinol altered the liver enzymes, lipid profile and antioxidant status of male rats.

Keywords: Cannabinol; Alcohol; Drug abuse; Liver enzymes; Rats

INTRODUCTION

Cannabis is a psychoactive product of the plant *Cannabis sativa* used primarily for recreational and medicinal purposes [1]. Cannabis has various mental and physical effects, which include euphoria, altered states of mind and sense of time, difficulty in concentrating, impaired short term memory, body movement, relaxation, and an increase in appetite [2-4]. When administered at high doses, mental effects can include anxiety, delusions, hallucinations, panic, paranoia, and psychosis.

Cannabinol is one of the major constituents of *Cannabis* popularly known as marijuana or ganja. Marijuana is the most commonly abused illicit drug in most countries. Sweeping legalization reforms in the United States in the last decade have broadly expanded access to marijuana; such that 8 states have legalized marijuana for recreational use while an additional 19 states permit marijuana for medical purposes.

Alcohol is gotten from various alcohol beverages such as beer, gin, whisky and rum. Alcohol belongs to a class of drugs known as sedatives and hypnotics which cause a general lowering of the Central Nervous System (CNS).

In many parts of the world, drinking alcoholic beverages is a common feature of social gatherings. In Nigeria, increasing prevalence of recreational cannabinoids and alcohol use among the young population has been a thing of concern to the

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government. Also, the rate of intake of alcohol by Nigerian youths and adults is really becoming worrisome and has assumed a frightening dimension in both urban and rural areas. Despite several efforts by regulatory agencies, aimed at educating the public on the negative impacts of alcoholism, the habit is still being embraced by many youths [5]. The consumption of alcohol carries a risk of adverse health and social consequences related to its intoxicating, toxic and dependence producing properties.

As cannabis and alcohol increasingly gain legalized status in many countries, the popularity and prevalence of use continues to grow. Medically, cannabis has been reported to demonstrate therapeutic promise in some areas such as multiple sclerosis and chronic neuropathic pain, the potential adverse effects remain widely under studied. Therefore, this study seeks to investigate in a rat model the effect of alcohol and cannabinol on the liver, since the liver is the principal organ in the detoxification harmful substances, that is, change them to less harmful ones.

MATERIALS AND METHODS

Animal care and drugs

25 male wistar rats weighing between 175 and 210 g obtained from National Institute of Pharmaceutical Research and Development (NIPRD) Iju Abuja, Nigeria, were used for the study. The rats were allowed to acclimatize for a period of 2 weeks before the commencement of the experiment. They were housed separately in wire mesh cages and fed standard rodent pellet and water ad libitum. All experimental procedures adopted in this study were in strict accordance with the criteria outlined in the guide for the care and use of laboratory animals prepared by the National Institutes of Health's (NIH) guide for the care and use of laboratory animals and approved by Bingham university's animal research ethics committee [6].

Cannabinol solution (cat no. C6 520) was purchased from sigma (St. Louis., MO, USA) and ethanol was obtained from (BDH laboratories supplies, Poole UK).

Experimental design

The rats were divided into 5 groups with 5 rats in each group. Group 1 (control group) were given distilled water; group 2 was administered with 5% methanol; group 3 was administered with 3 g/kg of alcohol (25% v/v); group 3 was given (10 mg/kg) of cannabinol; group 4 were administered with (10 mg/kg) cannabinol and alcohol once a day for a period of 8 weeks respectively. At the end of the experiment, blood was collected *via* retro orbital sinus into non heparinized capillary tube and put into plain bottles. Blood was allowed to clot and serum was drawn into plain bottles for the assays of liver enzymes and lipid profile test. Thereafter, the rats were sacrificed and their livers were removed and cleared of adherent tissues and weighed with an electronic weighing balance (Model DT 1000).

Biochemical assay: Serum aspartate aminotransferase, alanine aminotransferase, high density lipoprotein, low density lipoprotein, triglyceride, and cholesterol were determined by standard colorimetric method using assay kits purchased from randox laboratories Ltd (Crumlin, Antrim, UK).

Lipid peroxidation product: Lipid peroxidation was measured as Malondialdehyde (MDA) according to the method described by Ohkawa, et al. [7].

Histological studies: The livers of the rats were fixed in 10% formol saline, dehydrated in graded alcohol and embedded in paraffin wax. These were cut into 4 µm-5 µm thick sections and stained with hematoxylin eosin for photo microscopic assessment using Leica DM 750 (Leica microsystems, Wetzlar, Germany) at 400X magnifications.

Statistical analysis

Data analysis was carried out using one-way Analysis of Variance (ANOVA) followed by Newman-keuls' test for multiple comparisons. Results were presented as mean ± SEM and the differences were considered significant at $p<0.05$.

RESULTS

The control and rats treated with methanol had % weight gain that was significantly higher ($p<0.05$) than rats treated with alcohol, cannabinol and alcohol+cannabinol. In comparison, the alcohol+cannabinol group had the lowest % weight gain when compared with the alcohol and cannabinol groups (Table 1).

Table 1: Effect of alcohol and cannabinol on % weight gain of male rats.

Treatment	Initial body weight (kg)	Final body weight (kg)	% weight gain
Control	190 ± 6.33	236.2 ± 4.99	17.8
Methanol	205 ± 12.25	228 ± 9.82	16.8
Alcohol	185 ± 10.00	211 ± 16.8	3.1
Cannabinol	198 ± 4.90	193 ± 8.60	2.6
Alcohol and cannabinol	192 ± 10.68	197 ± 23.11	0.5

The mean weight of the lungs of rats treated with alcohol, cannabinol and cannabinol+alcohol was significantly lower ($p<0.05$) when compared with that of the control and rats treated with methanol. Furthermore, the cannabinol treated rats had mean weight of the lungs that was significantly lower ($p<0.05$) when compared with that of the rats treated with alcohol and alcohol+cannabinol. The rats treated with cannabinol had mean weight of spleen that was significantly lower ($p<0.05$) when compared with that of the control, methanol and alcohol treated groups. However, the group treated with cannabinol and alcohol had mean weight of spleen that was significantly higher ($p<0.05$) than that of the control, methanol and alcohol treated groups. The mean weight of the brain of rats treated with cannabinol was significantly lower ($p<0.05$) when compared with the control, methanol, cannabinol+alcohol groups. The control and rats treated with methanol had mean weight of the liver that was significantly lower ($p<0.05$) than that of the alcohol, cannabinol and cannabinol and alcohol treated groups. The mean weights of the kidney and heart were not significantly different across the group (Table 2).

Table 2: Effect of alcohol and cannabinol on the mean weight of visceral organs in male.

Treatment	Heart (g)	Lungs (g)	Spleen (g)	Kidney (g)	Brain (g)	Liver (g)
Control	0.855 ± 0.01	1.840 ± 0.03	0.808 ± 0.02	0.609 ± 0.09	1.645 ± 0.06	6.670 ± 0.41
Methanol	0.754 ± 0.07	1.912 ± 0.28	0.726 ± 0.08	0.609 ± 0.05	1.540 ± 0.06	6.860 ± 0.99
Alcohol	0.778 ± 0.04	1.720 ± 0.47$^{*\&}$	0.820 ± 0.10	0.610 ± 0.07	1.667 ± 0.13$^{\$}$	5.818 ± 0.58$^{*\&}$
Cannabinol	0.778 ± 0.04	1.060 ± 0.53$^{*\&}$	0.648± 0.09$^{*\&}$	0.691 ± 0.09	1.496 ± 0.08$^{*\&}$	5.665 ± 0.99$^{*\&}$
Cannabinol and alcohol	0.867 ± 0.10	1.480 ± 0.27$^{*\&}$	0.955 ± 0.04$^{*\&\$}$	0.641 ± 0.05	1.668 ± 0.04$^{\$}$	5.730 ± 0.20$^{*\&}$

Note: Data are expressed as mean ± S.E.M. (n=5); $p<0.05$; *=significantly different from the control; $^{\&}$=significantly different from methanol group; $^{\$}$=significantly different from cannabinol

The Lower Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) of the control and rats treated with methanol was significantly lower ($p<0.05$) when compared with that of the rats treated with alcohol and cannabinol. The level of HDL was significantly lower in rats treated with alcohol +cannabinol when compared with the rats treated with alcohol and cannabinol but not significantly different when compared with the control rats ($p<0.05$). On the other hand, the LDL level of the rats that were administered with alcohol+cannabinol was significantly lower than that of rats administered with alcohol; however, it was significantly higher when compared with the control. The total cholesterol level of the groups treated with alcohol, cannabinol and alcohol plus cannabinol was significantly higher ($p<0.05$) than that of the control and methanol treated group. However, the rats treated with alcohol plus cannabinol had total cholesterol level that was significantly lower ($p<0.05$) when compared with the alcohol and cannabinol treated rats (Table 3).

Table 3: Effect of alcohol and cannabinol on lipid profile test and liver enzymes in male wistar rats.

Treatment	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	ALT (IU/L)	AST (IU/L)	TC (mg/dl)
Control	0.02 ± 0.00	0.06 ± 0.01	0.03 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	46.87 ± 2.06
Methanol	0.06 ± 0.01	0.14 ± 0.06	0.11 ± 0.02	0.05 ± 0.01	0.03 ± 0.00	51.41 ± 10.43
Alcohol	1.92 ± 0.08$^{*\&\#}$	1.72 ± 0.13$^{*\&\#}$	0.21 ± 0.05$^{*\&}$	0.57 ± 0.01$^{*\&}$	0.53 ± 0.03$^{*\&}$	85.68 ± 6.79$^{*\&}$
Cannabinol	1.60 ± 0.18$^{*\&}$	1.33 ± 0.37$^{*\&\#}$	0.29 ± 0.07$^{*\&}$	0.61 ± 0.03$^{*\&}$	0.52 ± 0.02$^{*\&}$	82.83 ± 6.62$^{*\&}$
Alcohol +cannabinol	1.63 ± 0.45$^{*\&}$	0.10 ± 0.03	0.20 ± 0.04$^{*\&}$	0.55 ± 0.02$^{*\&}$	0.61 ± 0.02$^{*\&}$	70.21 ± 5.72$^{*\&}$

Note: Data are expressed as mean ± S.E.M (n=5); $p< 0.05$; *=significantly different from the control; $^{\&}$=significantly different from methanol group; $^{\#}$=significantly different from alcohol+cannabinol

The serum concentrations of AST and ALT were significantly higher in the rats that were administered alcohol, cannabinol and alcohol+cannabinol when compared with the control and group treated with methanol. Similarly, the groups treated with

alcohol, cannabinol and alcohol+cannabinol had triglycerides concentration in the serum that was significantly higher than that of the control and rats treated with methanol.

The serum concentration of Malondialdehyde (MDA) was significantly higher in rats treated with alcohol, cannabinol and cannabinol+alcohol when compared with the control and rats treated with methanol (Figure 1).

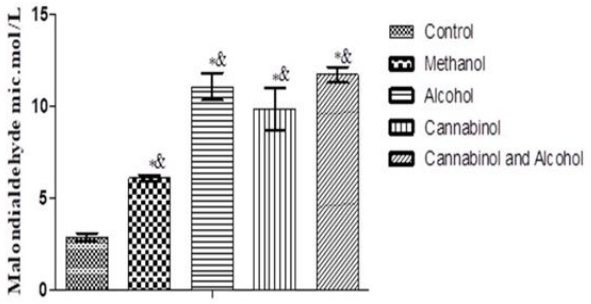

Figure 1: Effect of alcohol and cannabinol on malondialdehyde in male wistar rats.

Data are expressed as mean ± S.E.M (n=5). $p<0.05$.

Note: *=significantly different from the control; &=significantly different from methanol group.

Photomicrographs of the liver tissue of rats treated with alcohol, cannabinol and cannabinol plus alcohol showed signs of dilated sinusoids when compared with the control and rats treated with methanol. Group 4 also reveals the aggregation of inflammatory cells. Hepatocytes appeared normal across the groups (Figure 2).

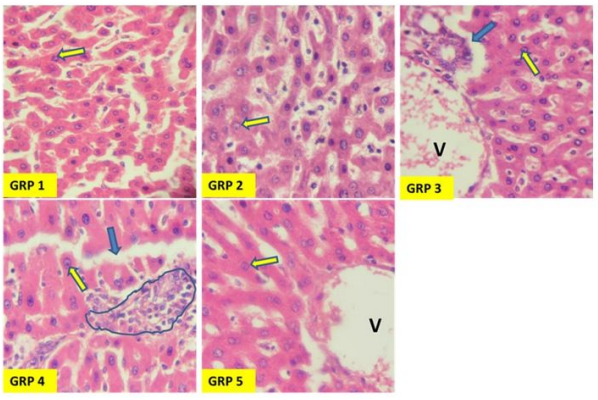

Figure 2: Photomicrographs of the liver of wistar rat treated with alcohol, cannabinol.

Note: Hepatocytes appeared normal across the groups (yellow arrows); Groups 3 and 4 however show signs of dilated sinusoids (blue arrows) when compared with groups 1 and 2; Group 4 also reveals the aggregation of inflammatory cells (outline); V=central Vein; H and E × 400.

DISCUSSION

Therapeutic and deleterious effects of cannabinol and alcohol administration on some organs such as brain, bone, heart and the kidney have been reported in previous studies [8-12]. In this study, the combined effect of alcohol and cannabinol on lipid profile and liver enzymes were carried out in male wistar rats.

Alanine Aminotransferase (ALT) is known to increase in the blood when there is damage to the liver and it has been used as a tool for measuring hepatic necrosis [13]. Aspartate Aminotransferase (AST) on the other hand, is not specific to the liver; it is also found in the cells of the gills, kidney, and muscle [14]. It increases level in the blood may not necessarily indicates liver damage. In this study, the ALT and AST levels of rats treated with alcohol and cannabinol was significantly higher when compared with the control and methanol treated rats. This is consistent with the findings of Ewing, et al., who reported that cannabinol caused dose-dependent increases in both AST and ALT serum levels after 10 days of exposure. The photomicrographs of the liver section of rats treated with cannabinol showed normal central venule and few inflammatory cells infiltrating the liver sinusoids with hepatocytes appearing normal. The onset of inflammation has been reported to cause over production of free radicals that can damage the liver cells and impair their functions [15,16]. Thus, it can be inferred that alcohol and cannabinol induced liver necrosis or damage could require a longer period of treatment to develop in rats.

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. Malondialdehyde is a known marker of oxidative stress [17]. An increase in free radicals' generation in the cell causes overproduction of MDA. From the study carried out, alcohol, cannabinol and alcohol plus cannabinol caused a significant increase in MDA when compared with the control group. This indicates that alcohol and cannabinol cause increased free radicals' generation in the liver tissues resulting from inflammation. This is substantiated by the photomicrograph of the liver tissue of the rats treated with cannabinol which showed inflammatory cells infiltrating the liver sinusoids.

Triglyceride level was significantly increased in the alcohol, cannabinol and alcohol plus cannabinol treatment groups indicating that alcohol and cannabinol do affect triglyceride levels. This result is consistent with the findings of Miller, et al., who reported that alcohol administration has a potent effect in raising triglycerides level in the blood because alcohol is high in calories and sugar. Rats in groups treated with alcohol, cannabinol and alcohol plus cannabinol showed significant increase in serum cholesterol levels when compared with the control and methanol treated rats. Al-Jameel, et al., who reported a significant increase in total serum cholesterol in rats treated with alcohol when compared with the control rats. Alcohol and cannabinol were seen to cause a significant increase in HDL and LDL. The same was noticed in a human study, in which ethanol consumption typically raises HDL cholesterol levels. Alcohol plus cannabinol, however, was seen to produce a significant increase in LDL and a decrease in HDL. Huang, et al., reported that moderate alcohol consumption on a daily basis lowers HDL. This may potentially cause Cardiovascular Disease (CVD) by causing inflammatory disorder of the arteries, initiated by risk factors such as hypercholesterolemia, inflammation and oxidative stress.

Alcohol and cannabinol did cause significant changes in weight of lungs, spleen, brain and the liver when compared with the control group, and there was significant decrease in percentage body weight change also. This is consistent with the findings of Pyun, et al. They reported a significant decrease in food intake and body weight gain after chronic alcohol consumption in rats. However, the percentage body weight gain seen in this study disparate with the report of Justice, et al. They reported no significant differences in body weight gain between the control and alcohol treated rats. The discrepancy observed may have resulted from the concentration of alcohol administered, 20% alcohol was administered in their study while this study used 25% alcohol. A marginal body weight loss was observed in rats administered with cannabinol in a study conducted, which consented with the result obtained in this study. However, the dose of cannabinol used in their study did not result in consistent decrease in organ to body weight ratio which differs from the finding of this study. The difference observed could be attributed to the dose and duration of cannabinol used. The decrease in body weight gain observed in the alcohol treated rats when compared with the control rats could be in part due to their tendency to consume less food. Though, food intake was not measured in this study. Another plausible explanation is that substantial use of alcohol has been reported to have profound effect on nutritional status which may cause primary malnutrition by displacing other nutrients in the diet. Cannabinoid has been reported to have the ability to alter body weight gain, possibly *via* the CB2 receptors. Also, Cannabinoid receptor (CB1) has been associated with the brain regions that control food intake. Thus, the decrease in body weight gain in cannabinol treated rats as observed in this study may have resulted from the stimulation of cannabinoid receptor agonists which inhibit food consumption and thereby causing body weight loss in rats.

CONCLUSION

The administration of alcohol and cannabinol was seen to alter the liver enzymes, lipid profile and antioxidant status of male rats by causing inflammation and generation of free radicals, thereby indicating potential adverse effects of these substances if ingested for longer period.

COMPETING INTERESTS

The authors declare no conflicts of interest for this study.

FINANCIAL DISCLOSURES

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