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THE EFFECT OF *CANNABIS SATIVA* ON CERTAIN ENZYMES OF CLINICAL SIGNIFICANCE IN RATS AND MEN

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Abstract -This study was designed to estimate changes in some enzymes of clinical importance in rats exposed to canabinoids and in men addicted to *C. sativa* smoking. Injection of rats with different doses of petroleum ether extract of *Cannabis sativa* resulted in a marked change in these plasma enzymes activities. Alkaline phosphatase (ALP) activity was increased in rats with the increase of dose and time and the difference between the level in the high and the low dose groups was significant (P< 0.05) during and after the experimental period. Alanine aminotransferase (ALT) activity showed remarkable increase after the second dose in the high dose group compared to the control group but returned to a lower level after the last dose, and Aspartate amino transferase (AST) activity revealed also significant (P<0.05) elevation after the second dose, but after the last dose the activity decreased significantly (P<0.05) compared to the control group. Adult addicted men smoked *C sativa* for different periods showed slight numerical increase in (ALP) activity with the increase of the length of the period of *C. sativa* smoking. In contrast the activity of the (ALT) and the (AST) reported significantly lower levels compared to the non smokers group. This study suggests that canabinoids increase the (ALP) activity in both injected rats and human smokers and this will increase with the increase of dose and time but the (ALT) and the (AST) increase at the beginning of consumption then will decrease with time.

Introduction

Hashish is one of the oldest of psychoactive drugs in use, yet the literature concerning many of its pharmacological and toxicological effects is inconclusive. One critical area has been the effect of hashish on hepatic function. Some authors reported various degrees of liver dysfunction in experimental animals or humans consuming hashish (Kew *et al* 1969) Also Poddar *et al* {1972) exposed guinea-pigs to delta 9-terahydrocannabinol (THC) for six month at a rate of five route injection⁄week, an exotic effect as a result of drug accumulation in the liver was observed, which provoked an inhibition of certain liver enzymes used to digest the hepatic glycogen. Huy *et al* (1975) ; Cohen and Peterson, (1971) showed that a single dose of delta 9-THC succeeded in inhibiting hepatic microsomal enzymes. Serum glutamate oxaloacetate transaminase (SGOT) activity was increased significantly in adult male rats exposed to hashish smoke for 50 min, whereas serum glutamate pyruvate transaminase (SGPT) activity was unaffected. This assumption is based on the fact that SGPT, which is reported by many authors to be a more sensitive measure in evaluating hepatocelluar damage than SGOT. Furthermore, trauma to skeletal muscle is among the doctors known to increase SGOT activity. On the other hand, it is possible that if the SGPT measurement were performed after a longer period of time, a rise of the enzyme activity might have been detected. Hashish and its constituents have affected certain hepatic enzymes and causing a certain degree of liver dysfunction (Mahfouz *et al* 1975).

The objectives of this study were to investigate and compare the effect of different doses of *Cannabis sativa* extract via intramuscular injection as (0.2, 0.4, 0.6, mg⁄g. B.W.) in rats and to evaluate the effect of smoked *C sativa* on addict men, using the biochemical parameters represented by some plasma enzymes, of clinical significance, namely the Alanine aminotransferase (ALT, SGPT), Aspartate aminotransferase (AST, SGOT) activities and the alkaline phosphatase (ALP) activity.

Materials and Methods

Twenty healthy albino rats (males and females), 6-9 months old, weighing between 70-200 gm, were divided randomly into four groups, the control and 3 treatment groups, five animals in each group. The treated groups were injected four times with Cannabis petroleum extract intramuscularly for 10 days in two days intervals as (0.2, 0.4 and 0.6mg ⁄gm body weight as low, medium and high doses respectively). The dose was prepared by weighing *Cannabis sativa* extract and calculated according to

the animal body weight (Mahfouz *et al* 1975; Rosenkrantz and Esber, 1980). Blood samples (2.5 to 3ml) were collected twice, from the ocular vein of the rats after the second dose and the last dose, at day 5 and 11 from the start of the experiment respectively.

Also eighteen to 60 years old men were selected as (users and addicts) and divided into three groups as mild, medium, and heavy users. The duration of usage was (3-8 years, for the mild users), (11-17 years for the medium users), (18-40 years for the heavy users). Six apparently healthy non-users from the same age group were used as control group. Three ml blood samples were collected from the medium cephalic vein of the arm and used to determine the plasma enzymes, alkaline phosphatase (ALP), alanine aminotransferace (ALT) and aspartate aminotransferase (AST).

(ALP) activity was determined using test kit EC: 3, 1, 3, 1 cataloge No. Qpsol. according to the colormetric optimized standard method by the recommendation of Deatsche and Gesells-Chaft (1972). Plasma (GOT= AST) activity was determined by colorimetric method kit test cataloge No. as 101 as described by Reitman and Frankel, (1957) and Schmidf (1963). Enzyme (GPT= ALT) catalyzed was determined by using test kit cataloge No. AL 100, according to the method described by Reitman and Frankel (1959), and Schmidt and Schmidt (1963).

Statistical analysis was conducted by using completely randomized design. The data were tabulated and subjected to analysis of variance (one way ANOVA) using the Microsoft computer program as described by Steal and Torrie, (1960).

Results

The result of alkaline phosphatase (ALP) activity in rats groups after the second and the last dose and in addict men were presented in Table (1) and (2). The ALP activity showed significantly (P<0.0) higher values in the rats after the second dose administration and by the end of the period of treatment when the groups injected with (0.4 mg/g; and 0.6 mg/g b w) were compared. However, there was no significant deference between the treated groups and the control group. The results of addict men groups showed no significant changes only the levels were numerically higher compared to the control group and increased with the increase of the usage period.

Table (3) and (2) presented the plasma ALT activity for rats and men groups respectively. Plasma ALT activity showed a remarkable increase in all treated rats after the second dose and reached a significant (P < o.o5) level in the groups received (0.4 and o.6 mg/g b w) respectively when compared with the control group. But the level in the group received the highest dose was significantly reduced by the end of the experimental period compared to the one received the medium dose and even lower than the earlier sample taken from the same dose.

Plasma AST activity in rats and men groups are presented in Table (4) and (2) respectively. After the second dose, the treated rat groups showed elevated levels in all groups and the difference was significant in the group treated with medium dose (0.4 mg/g), but after the last dose, the activity was decreased in all groups and reached significantly (P<0.05) lower levels in the medium and high groups compared to the control group. The levels were even lower than the previous dose from the same group. AST activity in addict men groups was significantly lower compared to the levels in the nonusers group. Numerically lower levels were reported in the mild and heavy users compared to the group of medium users.

Discussion

Results from previous studies pointed towards the occurrence of possible marijuana hepatotoxicity. This should be taken into consideration along with other organ affections resulting from chronic marijuana usage, in discussions on its legalization and therapeutic uses (Mahfouz *et al* 1975, Hoffman *et al* 1977, Borini *et al* 2004). In the present study, plasma alkaline phosphatase activity both in rats and men showed non-significant increase after application of *Cannabis sativa* compared to the control group. The rats received 0.6 mg /g body weight *C sativa* extract reported ALP level significantly (P<0.05) higher than the group received 0.2 mg /g body weight after the second dose also the levels were increased, keeping the same pattern, by the end of the experimental period. Hoffman *et al* (1977), discovered that serum ALP activity had an important role in characterizing bone and hepatic disorders, when obstruction of the duct system occurred at any level and hepatic fibrosis was increased, hepatic ALP activity in serum increases, but more common occurrence was its increase in association with hepatic lipidosis and severe starvation. The results of addict men groups in the present study, showed only numerically higher levels compared to the control group, and was increased with the increase of the usage period. Findings in the present work could suggest that a mild liver fibrosis or lipidosis can result from using *C sativa*. However subjects in the present work were prisoners addicted and not well nourished.

Borini *et al* (2004) studied three groups of marijuana users, 26 (21%) using only marijuana, 83 (67.5%) using marijuana and crack, and 14 (11.4%) consuming marijuana and alcohol they found that among users of only marijuana, hepatomegaly was observed in 57.7% and splenomegaly in 73.1%, and slightly elevated AST (42.3%), ALT (34.6%) and ALP (53.8%). The three groups did not differ significantly in the prevalence of hepatomegaly,

splenomegaly and hepatosplenomegaly. The group using both marijuana and alcohol showed the highest prevalence of alterations and highest levels of aminotransferases. Mean ALP levels were above normal in all groups. Boutwell, (1961) recorded that enzymes are to be the more sensitive measure for evaluating hepatocellular damage. Results obtained in rat groups in the present study, showed significantly increased activity in the plasma ALT and AST. This can go in line with the fact reported by Poddar *et al* (1972) and Kew *et al* (1969) that hashish and its constituents are reported to affect certain enzymes causing certain degree of liver dysfunction. But later with time in the present study, these levels were highly decreased in treated rats for both enzymes compared to previous doses. Also in the addict men groups, the activities of ALT and AST were significantly lower than the control group. This was observed in all addict men groups compared to non user group. In previous study, Hegde *et al* (2008) reported that natural cannabinoids such as Δ^9-tetrahydrocannabinol (THC) have shown therapeutic potential in treating inflammatory diseases. Their study investigated the effects of THC in a murine model of concanavalin A (ConA)-induced hepatitis. Intraperitoneal administration of THC after ConA challenge inhibited hepatitis as shown by significant decrease in liver enzymes and reduced liver tissue injury. Also Mahfouz *et al* (1975) suggested that under the effect of hashish, changes in the measured enzymes might suggest that the muscles rather than the liver were the major leakage source of these enzymes. These observations together with our findings in men and rats could suggest that hashish usage can affect liver by causing mild fibrosis or ducts obstruction with transient hepatocellular involvement.

However, Poddar *et al* (1972) and Kew *et al* (1969) suggested that hashish and its constituents affect the levels of certain hepatic enzymes due to liver dysfunction. Also Borini *et al* (2004) concluded that chronic marijuana usage, on its own or in association with other drugs, was associated with hepatic morphologic and enzymatic alterations.

The finding in the present work concluded that further studies are needed to support the idea that values found in all treated rats and the levels in addict men, indicates that cannabinoids are possible hepatotoxic substances but this affects more permanently the obstruction of the bile duct system but the influence to the liver cells could be transient or will be overcome. In addition, Patel and O'Gorman (1975) assayed the serum gamma glutamyl transpeptidase (GGTP), alanine aminotransferase (AlT), aspartate aminotransferasen (AsT), and alkaline phosphatase (ALP) activities in 40 drug dependent patients. GGTP elevation was observed in 50% of drug dependents. The incidences of elevated levels of other enzymes were as follows: AlT 33%; AsT 21.7% and ALP 5% respectively. They cocluded that measurement of GGTP is thus more useful, as a screening test, for involvement of the liver in drug dependent patients than that of the other enzymes. So future study including the GGTP and other liver enzymes for longer experimental periods will give more confirmatory findings

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Table 1- The effect of C. sativa extract on alkaline phosphatase (ALP) activity in rats (mean ±SE):

Treatment	ALP activity U/L After second doses	ALP activity U/L After last dose
Control	163.12 ± 3.48^{ab}	187.37 ± 4.7^{ab}
Low dose 0.2 mg/g	159.1 ±3.11^{b}	180.37 ± 27.9^{b}
Medium dose 0.4 gm/g	168.17 ±2.79^{a}	194.82 ±5.95^{ab}
High dose o.6 gm/g	170.72 ±2.94^{a}	201.29 ±4.93^{a}

*Table 2-The effect of C. sativa on alkaline phosphates (ALP) glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in men (Mean ± S*E):*

Treatment	ALP activity U/L	ALT activity U/L	AST activity U/L
Control	145.75 ±2.58^{a}	21.67 ± 1.91^{a}	45.17 ±12.59^{a}
Mild users	150.72 ±7.71^{a}	8.00 ± 12.79^{b}	19.00 ± 1.88^{b}
Medium users	159.76 ± 10.41^{a}	11.29 ± 1.85^{b}	37.14 ±8.59^{b}
Heavy users	160.64 ± 3.69^{a}	9.29 ± 1.70^{b}	20.00 ± 3.40^{b}

Table 3- The effect of C. sativa extract on Alanine amino transferase activity in rats (Mean ± SE) :

Treatment	ALT activity U/L after the second dose	ALT activity U/L after the last dose
Control	4.80 ± 0.80^{b}	5.6 ± 0.98^{a}
Low dose 0.2 mg/g	6.40 ± 0.97^{ab}	9.8 ± 2.20^{ba}
Medium dose 0.4 mg/g	9.80 ± 3.17^{a}	9.8 ± 3.17^{b}
High dose 0.6 mg /g	12.6 ± 2.73^{a}	8.2 ± 2.37^{a}

Table 4-The effect of C. sativa extract on Aspartate amino transferase aminase ASTactivity in rats (mean ± SE):

Treatment	AST concentration U/L after second dose	AST concentration U/L after last dose
Control	10.0 ± 1.34^{b}	18.2 ± 2.03^{a}
Low dose 0.2 mg/g	16.6 ± 3.79^{b}	15.6 ± 1.94^{a}
Medium dose 0.4 mg/g	28.0 ± 4.70^{a}	8.2 ± 0.73^{b}
High dose 0.6 mg/g	26.8 ± 5.93a^{b}	8.2 ± 0.73^{b}

Means with different letters in the same column are significantly different (P<0.50).