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EFFECT OF Δ^9-TETRAHYDROCANNABINOL ADMINISTRATION ON HEPATIC FUNCTIONS

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Abstract—The effects of single (10 and 50 mg/kg) and chronic (10 mg/kg/day for 21 days) i.p. administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) on hepatic functions of rat were studied at cellular and subcellular levels. After chronic administration, (a) protein, RNA, phospholipid, cholesterol contents of liver microsomal fraction and microsomal Gl-6-Pase and Mg^{+2}-ATPase activities increased significantly, (b) microsomal lipid peroxidation value decreased and (c) GOT and GPT activities of liver and serum and hepatic triglyceride contents remained unchanged. Most of the parameters studied were unaffected after single administration of the drug excepting a decrease in microsomal lipid peroxidation and increase in Mg^{+2}-ATPase activity. No apparent harmful effect of the drug on hepatic functions is obvious from the present study.

The relationship between long-term Cannabis use and the possible health hazards has been of interest for many years. Although liver is the organ which is most significantly involved in the metabolism and storage of this drug [1, 2] and liver microsomal enzymes [3, 4] play an important role in the biotransformation of Δ^9-THC and its hydroxylated metabolites, there are controversial reports in the literature regarding the effect of short- and long-term administration of Δ^9-THC on hepatic functions. Recent studies of Kolodney *et al.* [5] and Rosenkrantz *et al.* [6] failed to demonstrate any clinically important hepatic damage even after chronic marihuana consumption, while Kew *et al.* [7] showed some injurious effect of the drug on hepatic functions. In the present study the action of Δ^9-THC on biochemical changes of liver and some related serum enzymes have been studied after acute and chronic administration of Δ^9-THC.

MATERIALS AND METHODS

Adult male albino rats of Charles Foster strain weighing about 100–150 g and maintained on laboratory stock diet were used. The stock diet used has the following composition in g/100 g diet: Wheat flour: 70; fish meal: 15; casein: 10; groundnut oil: 5; shark liver oil: 2; USP XVII salt mixture: 4; A.O.A.C. Vitamin mixture: 1. For acute studies, Δ^9-tetrahydrocannabinol (suspended in saline containing 1% tween 80) was administered i.p. to the animals at dosages of 10 and 50 mg/kg and the animals were sacrificed by decapitation after different time interval. During chronic treatment, animals received Δ^9-THC at a dose of 10 mg/kg/day for 21 consecutive days and were sacrificed 6 hr after the last injection. Control rats corresponding to acute and chronic experiments received the saline-tween 80 vehicle through the same route and were sacrificed under similar conditions.

Blood-free liver tissue was collected weighed and immediately placed in chilled container. Microsomes were prepared from 0.25 M sucrose liver homogenates (1:10, w/v) by differential centrifugation at 0–4° as described by Schenkman *et al.* [8]. Liver homogenate and microsomal suspensions of liver were processed by the method of Schmider for the estimation of nucleic acids [9]. Estimation of RNA was carried out by the orcinol method of Mejbaum [10]. DNA was estimated by Burtons' [11] modification of Disches' diphenylamine method [12]. Protein was estimated by the method of Gornall *et al.* [13]. The lipid materials were extracted and washed by the method of Floch *et al.* [14]. Total lipid content was estimated by evaporating the measured amount of extract. Phospholipid was assayed by the estimation of inorganic phosphorus according to the method of Fiske and Subbarow [15]. The cholesterol was estimated by the method of Sperry and Webb [16]. The triglyceride was separated by thin layer chromatography on silica gel using solvent system, Petroleum ether–diethyl ether–acetic acid (90:10:1) and was estimated according to the method of Amenta [17]. Glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) of liver and serum were measured according to the method of Reitman and Franknel [18]. The supernatant fraction obtained after centrifugation of 10% liver homogenate in 0.25 M sucrose for 30 min at 5000 *g* at 0–4° served as the enzyme source in case of liver GOT and GPT. Alkaline phosphatase activity of liver and serum was estimated by determining the rate of hydrolysis of *p*-nitrophenyl phosphate as advocated by Bessey *et al.* [9]. A 10% homogenate of liver in 0.25 M Sucrose was centrifuged at 10000 *g* for 30 min at 0–4° and the supernatant fraction served as the enzyme source in this case. Microsomal glucose-6-phosphatase (Gl-6-Pase) activity was measured according to the method of Harper [20]. Mg^{+2} ATPase activity of microsomes were determined according to the method of Swanson [21] and inorganic phosphate was estimated by the method of Lowry *et al.* [22]. Lipid peroxidation was determined by measuring the diene conjugate in the microsomal lipid extract according to the method of Klaassen and Plaa [23].

Table 1. Effect of acute and chronic administration of Δ^9-THC on protein, nucleic acids and lipid composition of rat liver

Parameters studied	Acute*			Chronic†	
	Control (Saline-Tween 80)	Δ^9-THC treatment (10 mg/kg)	Δ^9-THC treatment (50 mg/kg)	Control (Saline-Tween 80)	Δ^9-THC treatment (10 mg/kg/day)
		Whole liver			
Liver weight (g/100 g body weight)	3.82 ± 0.32	3.57 ± 0.25	3.70 ± 0.20	4.04 ± 0.24	3.96 ± 0.20
Protein (mg/g)	130.80 ± 5.90	127.90 ± 5.00	122.41 ± 2.80	131.58 ± 1.74	152.30 ± 9.30‡
RNA (mg/g)	4.23 ± 0.14	4.08 ± 0.12	4.38 ± 0.15	4.35 ± 0.10	5.24 ± 0.35‡
DNA (mg/g)	2.49 ± 0.19	2.32 ± 0.29	2.12 ± 0.35	2.39 ± 0.26	2.69 ± 0.14
Total lipid (mg/g)	123.08 ± 4.52	130.12 ± 3.48	128.20 ± 3.50	126.90 ± 3.50	138.11 ± 2.30‡
Phospholipid (mg/g)	81.36 ± 3.20	74.26 ± 2.10	72.90 ± 4.50	88.60 ± 8.90	110.70 ± 4.40§
Cholesterol (mg/g) (mg/g)	5.83 ± 0.48	5.54 ± 0.32	6.80 ± 1.22	5.13 ± 0.33	5.95 ± 0.30
Triglyceride (mg/g)	8.27 ± 0.75	6.88 ± 1.22	8.76 ± 0.92	7.66 ± 0.71	7.12 ± 0.77
		Microsome			
Protein (mg/g)	21.02 ± 0.54	21.25 ± 0.72	22.78 ± 0.68	20.55 ± 0.48	24.14 ± 0.65§
RNA (μg/mg protein)	93.70 ± 3.60	103.66 ± 4.20	104.26 ± 3.50	83.55 ± 1.55	103.10 ± 1.72‖
Total lipid (μg/mg protein)	620.21 ± 30.51	625.10 ± 41.18	681.42 ± 35.38	630.27 ± 27.24	706.81 ± 20.21‡
Phospholipid (μg/mg protein)	464.60 ± 19.05	454.05 ± 19.08	429.16 ± 21.28	450.49 ± 26.70	525.96 ± 10.42‡
Cholesterol (μg/mg protein)	30.02 ± 0.46	29.69 ± 1.25	28.98 ± 0.66	28.60 ± 1.43	37.68 ± 1.96§
Triglyceride -μg/mg protein)	12.85 ± 1.50	12.80 ± 0.81	14.66 ± 1.38	14.23 ± 1.42	13.88 ± 1.01

Values expressed as mean of seven observation ± S.E.M. from seven rats. See Materials and Methods for experimental details.

* Rats were sacrificed six hr after single i.p. administration of Δ^9-THC at the respective doses.

† Treatment was continued for 21 consecutive days.

‡ Significantly different from control $P < 0.05$.

§ $P < 0.005$.

‖ $P < 0.001$.

RESULTS

It is evident from the results of Table 1 that single administration of Δ^9-THC does not produce any significant alteration in protein, nucleic acids and lipid parameters of rat liver observed either at the level of whole liver or microsomal fraction, while after chronic administration of Δ^9-THC moderate and significant increase of protein, RNA and phospholipid contents in whole liver and also in hepatic microsomes is observed, with a slight increase in total lipid level. A significant increase in cholesterol per mg protein ratio has been observed in microsomal fraction and no noticeable alteration in triglyceride content has been observed either in whole liver or microsomes under this condition of treatment. No significant alteration in liver weight/body weight ratio has been observed after chronic Δ^9-THC administration. From the results of Table 2 it appears that under acute condition of treatment with Δ^9-THC, GPT activity of liver shows a significant increase at both the doses within 12–18 hr of drug administration and a slight increase of GOT activity of liver at the higher dose of Δ^9-THC is also apparent at those hours. Activities of both the transaminases reach the normal levels within 24 hr. Δ^9-THC administration appears to have no significant effect on liver alkaline phosphatase activity, under similar conditions. Serum GOT, GPT and alkaline phosphatase activities do not undergo any noticeable alterations up to 24 hr after a single administration of Δ^9-THC (Table 3). No significant

Table 2. Effect of acute Δ^9-THC administration on rat liver glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase activity*

Time after injection (hr)	GOT (μmole pyruvate/mg protein/hr)			GPT (μmole pyruvate/mg protein/hr			Alkaline phosphatase (μg p-nitrophenol/mg protein/hr)		
	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)
3	4.79 ± 0.16	5.07 ± 0.44	5.27 ± 0.23	9.00 ± 0.04	8.62 ± 0.58	10.40 ± 0.60	11.32 ± 0.75	9.88 ± 0.54	10.01 ± 1.04
6	4.70 ± 0.14	5.03 ± 0.34	5.38 ± 0.43	8.92 ± 0.30	9.60 ± 0.76	11.72 ± 0.18§	10.10 ± 0.80	9.52 ± 0.40	8.40 ± 0.85
12	4.87 ± 0.15	5.78 ± 0.43	6.20 ± 0.31‡	9.02 ± 0.02	12.35 ± 0.08‡	13.81 ± 0.64§	9.99 ± 0.75	8.81 ± 0.62	9.33 ± 0.55
18	4.70 ± 0.12	5.07 ± 0.18	5.92 ± 0.29‡	9.24 ± 0.12	11.38 ± 0.02†	13.02 ± 0.12	11.01 ± 0.92	10.48 ± 1.02	11.96 ± 0.81
24	4.82 ± 0.20	4.97 ± 0.42	5.10 ± 0.12	8.76 ± 0.32	8.68 ± 0.47	9.20 ± 0.50	10.23 ± 0.62	11.08 ± 0.75	9.88 ± 1.01

* Values are expressed as mean ± S.E.M. of seven observations from seven rats. See Materials and Methods for experimental details.

Significantly different from control,

† $P < 0.05$

‡ $P < 0.005$

§ $P < 0.001$.

Table 3. Effect of acute Δ^9-THC administration on serum glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) and alkaline phosphatase activities*

Time after injection (hr)	SGOT (i.u./lit serum/min)			SGPT (i.u./lit serum/min)			Alkaline phosphatase (King Angstron units/100 ml serum)		
	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)	Control (Saline-Tween 80)	Δ^9-THC (10 mg/kg)	Δ^9-THC (50 mg/kg)
3	9.10 ± 0.80	9.60 ± 1.21	8.92 ± 0.71	5.15 ± 0.24	4.40 ± 0.24	5.05 ± 0.36	6.16 ± 0.20	5.68 ± 0.24	6.50 ± 0.25
6	9.00 ± 0.75	10.75 ± 1.15	11.40 ± 0.82	5.05 ± 0.36	5.80 ± 0.27	6.22 ± 0.42	5.99 ± 0.30	6.96 ± 0.40	7.02 ± 0.75
12	8.38 ± 0.70	8.32 ± 1.08	8.02 ± 1.05	5.22 ± 0.42	5.88 ± 0.33	5.66 ± 0.48	5.69 ± 0.18	6.55 ± 0.20	4.69 ± 0.50
18	9.05 ± 0.65	11.27 ± 0.82	8.63 ± 0.51	5.20 ± 0.33	4.01 ± 0.30	5.60 ± 0.45	5.77 ± 0.14	6.03 ± 0.25	6.28 ± 0.32
24	10.08 ± 0.75	9.15 ± 0.50	11.32 ± 0.45	5.65 ± 0.24	6.25 ± 0.41	6.75 ± 0.70	5.81 ± 0.10	6.12 ± 0.14	6.22 ± 0.25

* Values are expressed as mean ± S.E.M. of six observations from six rats.

change in the activities of liver and serum GOT, GPT and alkaline phosphatase activities compared to those of saline-tween-80 control have been observed after daily administration of Δ^9-THC for 21 consecutive days (Table 4). Results presented in Table 5 indicate that upon single administration of Δ^9-THC the glucose-6-phosphatase activity of the liver microsome does not change significantly from that of the control as measured after different time interval under this condition, a significant increase in Mg^{+2}-ATPase activity of the microsomes is observed at 3 and 6 hr after Δ^9-THC administration at both the doses. The effect at the higher dose being greater than that observed at the lower one. The results of Table 5 also show that acute administration of Δ^9-THC inhibits lipid peroxidation of rat liver microsomes in a dose dependent manner and the maximum decrease in lipid peroxidation is noted at 6 hr. This inhibitory effect persists even up to 18 hr after the administration of Δ^9-THC. It is evident from the results of Table 6 that chronic administration of Δ^9-THC appears to

Table 4. Effect of chronic Δ^9-THC administration on rat liver and serum glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase activities*

Source	Enzymes	Control (Saline-Tween 80)	Δ^9-THC treatment† (10 mg/kg/day)
Liver	GOT (μmole pyruvate/mg protein/hr)	4.89 ± 0.39	5.77 ± 0.31
	GPT (μmole pyruvate/mg protein/hr)	8.97 ± 0.46	9.20 ± 0.70
	Alkaline phosphatase (μg p-nitrophenol/mg protein/hr)	10.50 ± 1.50	9.47 ± 1.40
Serum	GOT (i.u./litre serum/min)	11.00 ± 1.02	8.77 ± 0.74
	GPT (i.u./litre serum/min)	6.18 ± 0.24	5.60 ± 0.30
	Alkaline Phosphatase (King Angstron Unit/100 ml serum)	6.02 ± 0.14	5.24 ± 0.50

* Values are expressed as mean ± S.E.M. of six observation from six rats.

† Treatment was continued for 21 consecutive days.

Table 6. Effect of chronic administration of Δ^9-THC on rat liver microsomal glucose-6-phosphatase, Mg^{+2}-adenosine triphosphatase (Mg^{+2}-ATPase) and lipid peroxidation.

Enzyme	Control (Saline-Tween 80)	Δ^9-THC treatment* (10 mg/kg/day)
Glucose-6-Phosphatase (μmole Pi/mg protein/20 min)	3.078 ± 0.14	4.65 ± 0.26†
Mg^{+2}-ATPase (μmole Pi/mg protein/hr)	8.21 ± 0.48	12.40 ± 0.91†
Lipid Peroxidation (Δ.O.D./mg protein × 10^3)	68.56 ± 4.08	39.60 ± 2.50†

Values expressed as mean ± S.E.M. of six observations from six rats.

† Significantly different from control, $P < 0.001$.

* Treatment was continued for 21 consecutive days.

Table 5. Effect of acute administration of Δ^9-THC on rat liver microsomal glucose-6-phosphatase (Gl-6-Pase), Mg^{+2}-adenosine triphosphatase (Mg^{+2}-ATPase) and lipid peroxidation: enzyme activity*

Time after injection (hr)	Gl-6-Pase‖			Mg^{+2}-ATPase¶			Lipid peroxidation**		
	Control	Δ^9-THC treatment (10 mg/kg)	Δ^9-THC treatment (50 mg/kg)	Control	Δ^9-THC treatment (10 mg/kg)	Δ^9-THC treatment (50 mg/kg)	Control	Δ^9-THC treatment (10 mg/kg)	Δ^9-THC treatment (50 mg/kg)
3	3.14± 0.15	3.61± 0.59	4.25± 0.68	7.66± 0.33	9.82±† 0.72	10.95±‡ 0.63	65.53± 4.24	60.41± 4.02	58.74± 3.64
6	3.15± 0.37	3.20± 0.36	3.50± 0.30	7.40± 0.25	9.15±‡ 0.31	10.01±‡ 0.49	68.40± 3.85	44.20±§ 2.61	36.83±§ 3.58
12	3.20± 0.60	4.24± 0.72	3.67± 0.58	7.82± 0.42	8.15± 0.39	8.82± 0.48	63.68± 3.92	43.96±‡ 2.74	38.37±‡ 4.21
18	3.14± 0.28	2.88± 0.76	3.04± 0.48	7.44± 0.31	8.03± 0.52	7.37± 0.58	62.50± 4.57	53.10± 3.19	47.97±‡ 5.14

*Values are expressed as means ± S.E.M. of six observations from six rats. See Materials and Methods for experimental details.

† Significantly different from Control, $P < 0.05$.

‡ $P < 0.005$.

§ $P < 0.001$.

‖ μmole of Pi liberated/mg protein/20 min.

¶ μmole of Pi liberated/mg protein/hr.

** Δ.O.D./mg protein × 10 at 243 mμ.

increase significantly, both the Gl-6-Pase and Mg^{+2}-ATPase activities of rat liver microsomes. But under similar condition of treatment, lipid peroxidation of liver microsome decreases.

DISCUSSION

From the results of the present study it appears that repeated administration of Δ^9-THC for 21 consecutive days produces characteristic changes in the protein, RNA and lipid pattern of the whole liver and microsomal fractions of rat liver, while after the acute administration of Δ^9-THC no noticeable alteration in the macromolecular composition of the hepatic cell is evident, which may be due to the insufficient concentration of the drug attained in the liver after single administration. Moderate increase in protein and RNA contents of both the whole liver and microsomal fractions observed after chronic Δ^9-THC administration perhaps indicates increased functional status of the hepatic cells. Lipophilic Δ^9-THC has been known to be associated primarily with the lipoprotein components of the membranes [24] and hence it was expected that this drug would be capable of interacting with the lipid components of the hepatic cell. It has been known that increased proportion of phospholipid enhances the suitability of the membranes to sequester the foreign compounds [25]. So it appears that the increased level of phospholipid in whole liver as well as in microsomal fraction observed after chronic Δ^9-THC treatment perhaps provides a site for storage and for intimate contact between the membrane enzymes and lipophilic Δ^9-THC. Increase of cholesterol content of the microsomes observed under this condition suggests that repeated administration of Δ^9-THC perhaps stabilizes the molecular arrangement of the hepatic microsomal membrane as the presence of cholesterol within the membrane structure has been related to the rigidity of the membrane by its ability to condense with the unsaturated fatty acids of phospholipids [26]. One important point which emerges from these results is that unlike the action of many hepatotoxic agents which cause accumulation of triglycerides in the liver cells [27], Δ^9-THC even after chronic treatment did not produce any change at the level of hepatic triglycerides.

The unchanged activities of GOT, GPT and alkaline phosphatase of liver and serum compared to saline-tween 80 treated controls observed after chronic administration of Δ^9-THC for 21 days indicates the absence of any cytotoxic injury to the liver cells and apparently normal hapatic function. This observation is also in agreement with those of Rosenkrantz *et al.* [6] and Kolodney *et al.* [5]. However the transient rise in activities of liver transaminases specially GPT, observed after acute Δ^9-THC administration may be due to the phenomenon of enzyme induction associated with hypersecretion of ACTH since Δ^9-THC has been reported to be a potent stimulator of ACTH secretion [28].

Liver glucose-6-phosphatase, a constitutive enzyme, firmly bound to endoplasmic reticulum plays a strategic role in the vectorial release of glucose from liver and regulation of blood glucose. Its activity depends on the maintenance of normal lipid-protein interrelations of the reticulum membrane [29]. Marked increase in the microsomal Gl-6-Pase activity after chronic Δ^9-THC treatment may indicate a certain change in membrane conformation. Chronic administration of Δ^9-THC has been reported to cause depletion of liver glycogen content [30] without significant alteration of blood glucose level. Hence it seems probable that increased enzyme activity may be due to the *de novo* synthesis of the enzyme, in favour of increased gluconeogenesis, which thus maintains the normal blood glucose level under this condition.

It has been suggested that liver microsomal Mg^{+2}-ATPase activity is associated with an ATP-dependent reduction of pyridine nucleotides [31] and the increased activity of the enzyme observed at 3 and 6 hr after acute Δ^9-THC administration and also after chronic drug treatment may be correlated with the requirement of reduced pyridine nucleotides by the liver microsomes, possibly for the metabolism of Δ^9-THC by the microsomal hydroxylases.

Our present result of inhibition of lipid peroxidation of rat liver microsomes after both acute and chronic administration of Δ^9-THC tallies well with the formerly reported inhibitory effect of the drug on hepatic microsomal lipid peroxidation under *in vitro* condition [32]. This may be due to the antioxidant properties of either Δ^9-THC or its metabolites or due to the competition for reducing equivalents and perhaps indicates a stabilizing influence of the drug at the liver microsomal membrane.

In the light of the present results it appears that after Δ^9-THC administration no prominent deleterious effect is observed at the level of the functional activities of liver tissue. This may be due to some apparent protective effect of the drug which supports detoxification as well as the working function of the liver by stabilization of the enzyme system. But whether the drug can produce hepatotoxic manifestation on more prolonged treatment and at still higher doses or in the presence of other cannabinoids, particularly Cannabidiol, Cannabinol etc. which are generally present in natural cannabis, remains to be elucidated.

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