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Effect of Cannabis hemp (hashish) on normal and rats subjected to psychological stress

N A KHAN and S S HASAN
Zoology Department, Garhwal University Campus, Pauri-Garhwal 246001, India

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Abstract. The effect of hashish plus cigarette smoke on normal rats and rats undergoing psychological trauma was studied.

Keywords. Cannabis hemp (hashish); psychological stress.

1. Introduction

Effect of cigarette smoking on glycemia was investigated by Bornemisza and Scicin (1980) in diabetic patients, normal controls and in smokers. They found an increase of glycemia following smoking in all the subjects but the effect was more pronounced in the group of diabetics. It was assumed that the increase of glycemia after smoking was due to the mobilization of catecholamine and stimulation of 5-hydroxytryptamine and cortisol production. An investigation by Steer *et al* (1980) revealed the correlates of self-reported and clinically-assessed depression in male heroin addicts. Similarly, Marcovitz and Mayers (1969) observed an extremely poor and irregular work record in marijuana users while Wilson and Linkon (1968) showed the impair of intellectual and social performance in cannabis users particularly among students. In spite of ample documentary data available on the effect of cigarette and nicotine smoking, there is a lack of information on the use of cannabis hemp (hashish) smoking in combination with cigarette on normal and the rats undergoing psychological trauma. Thus, this study was undertaken to investigate the levels of protein and nucleic acids (DNA and RNA) in the brain; cholesterol and alkaline phosphatase contents in testes, 5-hydroxy indol acetic acid (5-HIAA) and 3-methoxy, 4-hydroxy mandelic acid (vinyl mandelic acid, VMA) concentrations in the urine. It was also apt at studying the changes made by testes either in normal or rats subjected to psychological stress in response to inhalation of hashish plus cigarette fumes.

2. Materials and methods

One hundred and twenty healthy adult male albino rats of Holtzman strain (100 to 120 g) were maintained on a supply of standard diet (Hindustan Levers, Bombay) and water *ad libitum*. Rats were divided into equal batch of the following control and experimental groups.

Group I: subjected to psychological stress by keeping the rats in small wire netting cages which were placed in a big cage with a cat for 56 days.

Table 1. Brain-protein, DNA and RNA (mg/g) (Mean ± SD).

Days of sacrifice	Control *A*	Stress *B*	Cigarette *C*	Cigarette + hashish *D*	Cigarette + hashish + stress *E*
7 days of post-stress/ cigarette + hashish inhalation	Protein – 174·99 ± 9·13	170·92 ± 16·51 *A*:*B* $P > 0{\cdot}01$	171·15 ± 8·38 *A*:*C* $P > 0{\cdot}01$	259·61 ± 10·05 *A*:*D* $P < 0{\cdot}01$ *C*:*D* $P < 0{\cdot}01$	188·46 ± 3·84 *A*:*E* $P > 0{\cdot}01$, *D*:*E* $P > 0{\cdot}01$ *C*:*E* $P < 0{\cdot}01$, *D*:*E* $P < 0{\cdot}01$
	DNA – 1·51 ± 0·23	1·35 ± 0·10 *A*:*B* $P < 0{\cdot}05$	1·19 ± 0·08 *A*:*C* $P < 0{\cdot}05$	1·71 ± 0·15 *A*:*D* $P < 0{\cdot}05$ *C*:*D* $P < 0{\cdot}01$	1·56 ± 0·10 *A*:*E* $P > 0{\cdot}05$, *B*:*E* $P < 0{\cdot}02$ *C*:*E* $P < 0{\cdot}01$, *D*:*E* $P < 0{\cdot}01$
	RNA – 1·66 ± 0·08	1·44 ± 0·11 *A*:*B* $P < 0{\cdot}01$	1·36 ± 0·16 *A*:*C* $P < 0{\cdot}01$	3·08 ± 0·05 *A*:*D* $P < 0{\cdot}01$ *C*:*D* $P < 0{\cdot}01$	1·99 ± 0·25 *A*:*E* $P < 0{\cdot}05$, *B*:*E* $P < 0{\cdot}01$ *C*:*E* $P < 0{\cdot}01$, *D*:*E* $P < 0{\cdot}01$
28 days of post-stress/ cigarette + hashish inhalation	Protein – 165·38 ± 8·62	152·88 ± 3·83 *A*:*B* $P < 0{\cdot}02$	144·23 ± 3·60 *A*:*C* $P < 0{\cdot}01$	138·46 ± 2·84 *A*:*D* $P < 0{\cdot}01$ *C*:*D* $P < 0{\cdot}05$	129·80 ± 3·31 *A*:*EP* $< 0{\cdot}01$, *B*:*EP* $< 0{\cdot}01$ *C*:*E* $P < 0{\cdot}01$, *D*:*E* $P < 0{\cdot}01$

	DNA –				
	1·24 ± 0·10	1·15 ± 0·08 *A*:*B P* < 0·1	1·02 ± 0·11 *A*:*C P* < 0·01	0·96 ± 0·07 *A*:*D P* < 0·01 *C*:*D P* < 0·05	0·91 ± 0·05 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·05
	RNA –				
	1·50 ± 0·07	1·36 ± 0·04 *A*:*B P* < 0·01	1·30 ± 0·07 *A*:*C P* < 0·01	1·22 ± 0·03 *A*:*D P* < 0·01 *C*:*D P* < 0·1	1·13 ± 0·04 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01
56 days of post-stress/ cigarette + hashish inhalation	Protein –				
	166·34 ± 12·88	144·23 ± 2·00 *A*:*B P* < 0·01	147·69 ± 1·80 *A*:*C P* < 0·02	134·61 ± 4·0 *A*:*D P* < 0·01 *C*:*D P* < 0·01	124·99 ± 11·76 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01
	DNA –				
	1·30 ± 0·16	1·04 ± 0·03 *A*:*B P* < 0·01	1·26 ± 0·03 *A*:*C P* > 0·01	0·89 ± 0·05 *A*:*D P* < 0·01 *C*:*D P* < 0·01	0·79 ± 0·04 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01
	RNA –				
	1·42 ± 0·05	1·28 ± 0·03 *A*:*B P* < 0·01	1·36 ± 0·03 *A*:*C P* < 0·05	1·19 ± 0·09 *A*:*D P* < 0·01 *C*:*D P* < 0·01	1·95 ± 0·08 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01

Group II: subjected to fumes inhalation of Capstan plain cigarettes (W. D. Wills, Ltd., India) for 56 days. Rats were placed in a box having an inlet for inhalation and an outlet for exhalation of cigarette fumes. Tobacco fumes of Capstan cigarette were blown inside the box through the upper inlet and allowed to passout through the lower outlet.

Group III: subjected to fumes inhalation of cigarette plus cannabis hemp (hashish). In this group the equal quantity of Capstan tobacco and hashish were mixed (1 g) and the fumes were blown into the box containing the rats according to the method mentioned in the group II for 56 days.

Group IV: subjected to psychological stress and received the treatment of cigarette plus hashish fumes according to the method described in groups I and III for 56 days.

Group V: consisting of normal rats to serve as control for groups I, II and III.

Eight rats from each group were sacrificed at intervals of 7, 28 and 56 days. Brains were dissected and 20% homogenates of these brain tissues prepared in sucrose solution, were used to estimate protein (Lowry *et al* 1951), DNA (Burton 1956) and RNA (Dische 1955). Testes were utilized to estimate cholesterol (Saekett 1960) and alkaline phosphatase (King and Wootton 1959). Urine (24 hr) of each rat from each group, before sacrifice, was collected to estimate 5-HIAA (Subramaniyam and Narayanan 1973) and VMA (Armstrong *et al* 1957). Testes were dissected and fixed in Bouin's fluid for histological study. Paraffin sections (5μ thick) were cut and stained in haematoxylin using eosin as counterstain.

3. Results

3.1 *Biochemical studies*

3.1a *Brain-protein* (*table* 1): The protein level decreased gradually in the group subjected to psychological stress and the group receiving cigarette fumes as compared to the normal control. The group having received cigarette plus hashish fumes showed an increase in protein content on the 7th day of treatment and thereafter the level dropped significantly in comparison with the normal control. In the group subjected to stress and receiving cigarette plus hemp fumes, the level of protein was higher than the normal control but lesser than the cigarette plus hemp treated group on day 7 after stress and the 28th day onwards the level remained lower than the rest of the groups.

3.1b *Brain-deoxyribose nucleic acid* (*table* 1): There was less DNA content in the brain of rats subjected to stress and the group receiving cigarette smoke than the normal control. In the group receiving the combined treatment of cigarette plus hashish, there was an increase in the concentration of DNA on the 7th day of treatment and this was followed by a decrease on days 28 and 56, whereas the group subjected to stress and received cigarette plus hashish showed a reduction in the DNA content in comparison with the cigarette plus hemp fumes inhaled rats on day 7, while a significant decrease in the level was noted on days 28 and 56 in comparison with the other groups.

3.1c *Brain-ribose nucleic acid* (*table* 1): Level of RNA content decreased in stress administered rats and rats treated with cigarette fumes as compared to normal control. In the cigarette plus hashish-treated groups and the group treated with cigarette plus

hashish in addition to psychological stress, the level rose on day 7 and declined on days 28 and 56 as compared to normal control. A significant reduction was observed in the RNA level on days 28 and 56 as compared to the other groups including normal control.

3.1d *Testes-cholesterol* (*table* 2): The stress-administered group exhibited rising levels of cholesterol in the testes whereas the group receiving cigarette showed a significant increase only on the 56th day of inhalation. In the group subjected to administration of cigarette plus hashish, there was a reduction in the cholesterol level on the 7th day of treatment and the level rose on days 28 and 56. Rats having received the treatment of cigarette plus hashish together with stress showed an increase in the level of cholesterol on 28th and 56th day when compared with normal control, the group receiving cigarette and the group treated with cigarette plus hashish.

3.1e *Testes-alkalinephosphatase* (*table* 2): In comparison with the normal controls, there was a diminution in the level of alkaline phosphatase in the group receiving psychological stress, the group inhaling cigarette fumes and the group administered with the cigarette plus hashish. The stress-administered group treated with cigarette plus hashish showed a marked decrease in the alkaline phosphatase content on days 28 and 56 as compared to the group treated with the cigarette plus hashish and the normal control.

3.1f *Urine-5-hydroxy indol acetic acid* (*table* 2): Following stress the urinary content of 5-HIAA increased on days 7 and 28 in the group subjected to psychological stress, whereas no significant change in the level was noted on day 56 as compared to the normal control. In cigarette-administered group, no significant change in the level was observed. The group receiving cigarette plus hashish and the group being administered with cigarette plus hashish together with stress showed an increased excretion of 5-HIAA but the level of concentration was higher in the latter group as compared to the former.

3.1g *Urine-3-methoxy, 4-hydroxy mandelic acid* (*table* 2): In stress-administered group, the concentration of VMA was higher in urine than the normal control. The cigarette-treated group showed no significant change in VMA content of the urine. In the group receiving cigarette plus hashish in combination with the stress, there was a rising level of VMA in the urine than the normal control but the concentration was much higher in the latter group than the former on days 7 and 28 after the treatment.

3.2 *Histological studies*

3.2a *Testes*: The testes histology of the control rat showed the sexually active reproductive phase characterized by the reduced leydig tissue, the increased size of seminiferous tubules and the active transformation of seminiferous epithelium into sperms which were present in large numbers in the lumen of testes. Unlike those of cigarette-administered testes, the treatment of cigarette plus hashish affected the testes conspicuously; the testes histology was characterized by the proliferation of leydig tissue, disappearance of sperms, degenerative changes and the loss of spermatogonia and spermatocytes. Large vacant spaces were present among the existing spermatocytes, indicating thereby that a sizeable number of them were destroyed. On the other hand in the testes of cigarette plus hashish-administered rats when exposed to stress, the histological changes appeared to be more deleterious and disastrous when compared with the rats receiving cigarette plus hashish. The spermatogonia were

Table 2. Urine-5-HIAA and VMA (mg/24 hrs) (Mean ± SD).

Days of sacrifice	Control *A*	Stress *B*	Cigarette *C*	Cigarette + hashish *D*	Cigarette + hashish + Stress *E*
7 days of post-stress/ cigarette + hashish inhalation	5-HIAA –				
	0·90 ± 0·17	1·11 ± 0·06 *A*:*B P* < 0·05	0·94 ± 0·02 *A*:*C P* > 0·05	1·11 ± 0·08 *A*:*D P* < 0·05 *C*:*D P* < 0·01	1·65 ± 0·19 *A*:*E P* < 0·1, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01
	VMA –				
	0·67 ± 0·03	1·21 ± 0·06 *A*:*B P* < 0·01	0·75 ± 0·09 *A*:*C P* > 0·01	1·07 ± 0·10 *A*:*D P* < 0·01 *C*:*DP* > 0·01	1·68 ± 1·00 *A*:*E P* < 0·5, *B*:*E P* < 0·05 *C*:*E P* < 0·01, *D*:*E P* < 0·05
28 days of post-stress/ cigarette + hashish inhalation	5-HIAA –				
	0·85 ± 0·25				
		1·02 ± 0·12 *A*:*B P* < 0·05	0·87 ± 0·02 *A*:*C P* > 0·05	1·07 ± 0·12 *A*:*D P* < 0·05 *C*:*D P* < 0·02	1·30 ± 0·13 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*E P* < 0·01, *D*:*E P* < 0·01
	VMA –				
	0·64 ± 0·05	0·91 ± 0·03 *A*:*B P* < 0·01	0·69 ± 0·03 *A*:*C P* > 0·01	0·91 ± 0·09 *A*:*D P* < 0·01 *C*:*D P* < 0·01	1·13 ± 0·09 *A*:*E P* < 0·01, *B*:*E P* < 0·1 *C*:*E P* < 0·01, *D*:*E P* < 0·01
56 days of post-stress/. cigarette + hashish inhalation	5-HIAA –				
	0·83 ± 0·05	0·83 ± 0·02 *A*:*B P* > 0·01	0·86 ± 0·01 *A*:*C P* > 0·01	0·95 ± 0·03 *A*:*D P* < 0·01 *C*:*D P* < 0·01	1·03 ± 0·12 *A*:*E P* < 0·02, *B*:*E P* < 0·01 *C*:*E P* < 0·02, *D*:*E P* < 0·05
	VMA –				
	0·65 ± 0·06	0·80 ± 0·03 *A*:*B P* < 0·01	0·68 ± 0·02 *A*:*C P* > 0·01	0·89 ± 0·06 *A*:*D P* < 0·01 *C*:*D P* < 0·01	0·88 ± 0·08 *A*:*E P* < 0·01, *B*:*E P* < 0·01 *C*:*EP* < 0·01, *D*:*EP* > 0·1

unaffected but the sperm population was altogether absent. The continuation of stress until day 56 brought about very little changes in the testes; degenerative changes were seen in a few spermatogonia only and a large number of them were little affected.

4. Discussion

The inhalation of hashish fumes may increase initially concentration of protein in the brain but its chronic-administration causes depletion of the same. This may attribute to the inhibitory effects of hashish treatment. The protein curtailment is more marked in hashish fumes-administered group than in that receiving cigarette fumes alone. Results further tend to suggest that the chronic treatment of cigarette plus hashish smoke to the rats undergoing psychological stress may prove to be more deleterious as shown by an increased reduction in the level of brain protein of this group. A similar inhibition of protein synthesis in rat brain was observed after the administration of morphine to rats (Clouet and Ratner 1968; Clouet 1968). It was demonstrated by Greene and Magasnik (1967) that when the concentration of the narcotic drug was increased, the inhibition in protein synthesis was apparent 2 min after exposure of cells to the drug. The rise and fall of DNA concentration in the brain of rats receiving the inhalation of cigarette plus hashish fumes seems to stimulate the biosynthetic process of protein but the follow-up of the treatment proves to be inhibitory to the synthesis as indicated by the presence of reduced level of DNA content on days 28 and 56. The rise in RNA contents on day 7 in the cigarette plus hashish fumes administered rats coincides with an increase in the total protein content of the brain. This indicates that inhalation of cigarette plus hashish smoke probably evokes in the beginning an increased mobilization and utilization of protein in the brain.

Protracted inhibition in the protein content by the use of hashish probably leads to diminished synthesis of the RNA on days 28 and 56 in the group being treated with the cigarette plus hashish. Clouet (1968) suggested that inhibition in the protein and nucleic acid (DNA and RNA) synthesis is due to an increased destruction of ATP in the narcotic treated group. Here, it is quite likely that chronic administration of hashish causes an increased annihilation of ATP and thereby inhibits the biosynthetic process of DNA, RNA and protein in the brain of stressed rats.

Our observations on urinary metabolites of 5-hydroxytryptamine and catecholamine show an increased excretion of 5-HIAA and VMA in the urine of cigarette plus hashish smoke-administered rats, which may be due to hyperactivity of 5-HT and nor-adrenalin caused by cigarette plus hashish smoke. Singh *et al* (1980) have also reported an increase in brain serotonin level after prolong use of marijuana fumes while Taylor and Fennessy (1982) have shown reduced levels of homovanillic acid (HVA), 5-HT and nor-adrenalin after chronic treatment of Δ^9-THC. Further rise in the excretion of 5-HIAA and VMA, following cigarette plus hashish inhalation in the urine of rats subjected to psychological stress, may be owing to additional amount of stimulus caused by the psychological stress. Many other workers (Welch and Welch 1968; Bliss 1973; Sarkar *et al* 1977; Hasan *et al* 1979) have also reported an increase in the levels of 5-HT and catecholamine in the animals following stress.

Inhalation of cigarette plus hashish smoke affects adversely the testicular metabolism by bringing about a decrease in androgen sensitive enzyme, *i.e.* alkaline phosphatase.

Similar effects of decreased androgenicity are reported (Vyas and Singh 1976; Husain and Lame 1981; Hong *et al* 1981; Futonoto *et al* 1982).

The increase in the cholesterol accumulation in the testes of rats receiving the treatment of cigarette plus hashish may be due to anti-androgenicity effect of the latter, for the formers are not utilized in the biosynthesis of steroid hormones. The anti-androgenicity effect of cigarette plus hashish smoke is further supported by the histological studies on the testes. This observation bears significance that the chronic inhalation of cigarette plus hashish smoke causes, in the long run, more severe degenerative changes in the testes of rats undergoing psychological trauma.

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