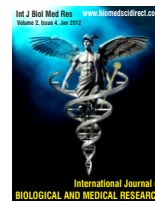


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Original article

A study of effect of Stress on Voluntary alcohol Intake in Wistar rats on Behavioral changes

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ABSTRACT

Intake of alcohol in many animals' species depends on numerous genetic and environmental factors. One of the major factors that can affect alcohol intake is stress. Stress has long been thought to influence the initiation and maintenance of alcohol drinking in humans. Alcohol drinking procedure in Wistar rats was used and was compared with different ethanol concentration along with behavioral assessments. The experiment was carried out with 24 healthy Wistar rats. The animals were randomly divided into four groups of 6 animals each. Group – I (non alcoholic): Control Group, which consists of 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given only tap water, then after 3 weeks, behavioral assessment was done by open field apparatus for central Peripheral ambulation, grooming, rearing and immobilization. Group – II alcoholic group consists of alcohol fed 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% and 40%, ethyl alcohol solution about 3 weeks. Group – III it is also alcoholic group consists of alcohol fed 6 animals were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% and 40%, ethyl alcohol solution about 3 weeks. Stress was applied after deprivation and the consumption of alcohol was assessed. Group – IV it is a Preference group consists of 6 animals, were randomly divided into 3 sub groups of 2 animals. Each sub group was given tap water and 5%, 20% and 40%, ethyl alcohol solution for about 3 weeks. The behavior was assessed before and after stress effect. Stress led to a significant increase ($p < 0.05$) in alcohol intake in Wistar rats. After stress, however, their preference for the 20% alcohol solution was increased. Repeated swim stress caused an increase in alcohol intake and they responded to behavioral changes before and after stress. This study reveals the effect of alcohol deprivation and stress on the drinking behavior at different concentration with specific or non specific preference groups with its behavioral modulations. Wistar rats differentially respond to and stress. In these animals alcohol drinking is an attempt to cope with stress.

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1. Introduction

Alcohol intake depends on numerous genetic and environmental factors in several animals including man [1]. One of the major factors is Stress [2]. Stress "is often used to describe the

ones feeling of pressure or tension. [3]. Studying the link between alcohol consumption and stress is further our understanding of drinking behavior. Stress is of different kinds which influence the behaviour of the individual. Stress causes the body to change in behaviour in a process of continual adaptation, with coping with the stress [4]. Damage can occur, under chronic stressful condition when the body fails to compensate it, such damage resulting in impaired learning and memory. Alcohol drinking in humans was influenced and initiated by Stress. The adolescent brains have higher sensitivity to alcohol consumption which induces brain

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damage and cognitive impairment [5]. However this would lead to a great risk for developing alcohol addiction [6]. Furthermore, alcohol drinking is currently recognized as the most common cause for abnormal human health, in which the liver is the most commonly affected organ [7].

2. Materials and Methods

Animals: The present study was conducted at the Department of Physiology MMC & RI, Enathur and Kanchipuram. The experiment was carried out with 24 healthy Wistar rats. The animals were randomly divided into four groups of 6 animals each. In stress group – forced swim test was performed in special plastic tanks. The Wistar albino rats (220 ± 20 g) were procured from Kings Institute Guindy (Chennai). The animals had free access to standard rat pellet with water supplied ad libitum and strict hygienic conditions. Animals were habituated to laboratory conditions (i.e. room temperature of 25 ± 2 C; relative humidity 45-55% and a 12:12 light/dark cycle) for 48h prior to experimental protocol to minimize non specific stress if any. The approval of the Institutional Animal Ethical Committee (IAEC) of MAHER was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the purchase of control and supervision of experiments on animals (CPCSEA).

2.1. Experimental design

The experiment was carried out for a period of 60 days with 24 healthy albino rats. Before starting the experiment, the animals were made to acclimatize to the laboratory environment for one week before starting the experiment. Then animals were randomly divided into four groups of 6 animals each.

2.2. Alcohol self administration procedure

After 2 weeks of habituation in the animal room, rats were given ad libitum access to tap water with 5%, 20% and 40% ethanol solution along with food. Alcohol drinking solutions were taken from 99.9% ethanol diluted with tap water to the different concentrations. Spilling and evaporations were minimized by the use of special bottle caps. In the present study the first group was taken as a control treated with tap water. Second and third groups were divided into three consecutive subgroups. Each sub group received 5%, 20% and 40% ethanol along with tap water. Fourth group was kept as preference group.

2.3. Stress induced alcohol intake

The stress procedure was performed in 3 consecutive days and for 10mins. Then alcohol consumption was measured, daily before each stress procedure and 2 days after the stress procedure. To perform the forced swim test, rats were placed into cylindrical plastic tanks (55cm height and 35cm diameter). Filled with tap water up to a level of 40 cm. The animals were observed for the latency time until the animal started to float was measured. After the swim stress, animals were dried with a towel and returned to their home cages.

2.4. Stress induced behavioral changes-by Open field test

This test was performed in the control group. The reading for ambulation, rearing, excreting feces and urine was assessed. One week after the completion of the voluntary alcohol drinking, the

rats were subjected to the open field apparatus which consisted of four identical controlled cages. Each cage was transected by two perpendicular, co-planar arrays of 16 infrared photocells which were intended to measure forward locomotion by determining the rats position every 0.1s. The forward locomotion was defined as the distance in 9(inch) travel by the rat during the five minutes test session. Another set of photo cells located 15 cm above the cage floor measured the number of rearing. After initial habituation of 5 min to the test room each rat was introduced to the test cage for another 5 min. The cages were cleaned carefully between the recordings. The test sessions were conducted between 10.00 and 14.00 to avoid errors attributed to the variations in motor activity of the day activity cycle.

2.5. Statistics

The statistical package SPSS (17.0 VERSION) was used. Data obtained from alcohol deprivation and stress experiments behavioral measures were analyzed by using two-way analysis of variance (ANOVA) with repeated measures. $P < 0.05$ had taken as statistically significant.

3. Result

All the three groups (except Group one) showed an increase in total alcohol intake during the first 4 weeks of the acquisition period with the increase consumption mainly from the 5% solution. After, stress all 3 alcohol Group animal lines slightly increased basal total alcohol intake. The following analysis showed that Stress led to a significant increase in alcohol intake and preference in unselected Wistar rats after Stress on day 1 -14. The total alcohol preference increased temporarily in group 2, 3 and 4 animals from 1.0 ± 0.3 before Stress to 1.9 ± 0.2 on the first day after Stress and was still significantly elevated on the fourteenth day 1.9 ± 0.3 but not on the fifth day or later.

Our observations suggest that stimuli associated with the availability or consumption of ethanol can evoke subjective feelings of craving and trigger episodes of relapse in abstinent alcoholics. Changes in alcohol intake caused by forced swim stress for 10 min on 3 consecutive days were significantly different between the four rat groups. The preference values for the selected lines before Stress were higher than normal, thus precluding any increases being caused by stress. Post hoc comparisons revealed a significant increase in alcohol consumption by unselected Wistar rats after swim stress for 5min on 3 consecutive days led to a significant increase in alcohol consumption in Wistar rats. In particular, intake from the 20% alcohol solution was increased after forced swim stress (2.6 ± 0.2). The results shows that the animal behavioral parameters in open field apparatus such as ambulation, rearings and grooming were increased in comparison with normal phase ethanol intake

Table 1. Ethanol intake before Stress and After Stress effect

	Before Stress			After Stress		
	5%	20%	40%	5%	20%	40%
Group II	1.0±0.3	1.9±0.2	2.6±0.2	1.3±0.4	2.6±0.2	2.2±0.2
Group III	0.7±0.1	1.2±0.2	2.1±0.2	1.8±0.4	2.1±0.2	2.0±0.4

Data are presented as mean \pm SEM. P<0.05 was considered as significant. (Table-1).

Table - 2. Stress induced immediate alcohol intake

Groups	Stress induced Ethanol drinking g/kg/day					
	Before stress			After stress		
	5%	20%	40%	5%	20%	40%
Group II	1.8±0.2	2.2±0.2	2.2±0.3	1.2±0.4	4.4±0.3	2.5±0.3
Group III	1.2±0.2	2.5±0.3	2.0±0.2	2.1±0.3	3.6±0.2	2.3±0.2

Data are presented as mean \pm SEM. P<0.05 was considered as significant. (Table-2).

Table. 3 Ethanol preference group

Groups	5%	20%	40%
Groups IV	10.8±0.6	44.0±0.5	14.1±0.2

Data are presented as mean \pm SEM. P<0.05 was considered as significant. (table-3).

Table. 4 Behavioral changes Before Stress and After Stress

Animals	Before Stress phase				After Stress phase			
	A	R	G	I	A	R	G	I
Average	76	12	04	06	56	10	01	12

(Note- A-ambulations, R-rearings, G-grooming and I-immobilization. (Table-4)

4. Discussion

The most commonly used psycho-active substance in the society is alcohol [8]. The effect of A third generation non selective β -blocker acute carvedilol pretreatment causes gastric mucosal injury in male Wistar albino rats induced by 80% ethanol consumption [9]. Ethanol is one of the most commonly used substances which causes a withdrawal syndrome associated with increased sensitivity to pain, hyperalgesia, anxiety, [10]. Stress may become a conditioned withdrawal stimulus which promotes the development of alcohol addiction and consumption [11]. If a person addicted to alcohol, zobo drink is a alcoholic beverage made from different varieties of dried petals and calyces of the flower Hibiscus by boiling and filtration can be used until the problem subsides [12]. The findings show that exposure to stress can influence the ingestion of alcohol, but this effect is stressor specific. This is observed in animals that reflects some aspect of alcohol relapse in humans also [13]. Furthermore, the effects of Stress were examined in all animals, After Stress phase of two weeks, a significant transient increase in voluntary alcohol intake and preference ensued in the Wistar rats was observed [14]. Repeated swim stress caused a slight increase in alcohol intake in Wistar rats and alcohol consumption after short (24 hr) Stressful

phase produced abnormal behaviour [15]. This might be true for stressful life events and maladaptive responses to stress which influence alcohol drinking and relapse behavior. Although the relationship between stress and alcohol drinking in humans and laboratory animals is complex, it might be that in some animal's alcohol drinking is an attempt to cope with stress [16-19].

The different stress group produced different physiologic and hormonal consequences for a given period, it is not surprising that the effect of stress on ingestion of alcohol is stressor specific. After ADE effect of 2 weeks following chronic alcohol drinking, involving certain serotonin (5HT) pathways, develop in individuals at risk for alcoholism.

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