THE EFFECT OF KOMBUCHA TEA ON LEARNING AND MEMORY IN RATS

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SUMMARY: Kombucha tea is a health beverage made by incubating the Kombucha mushroom in black tea and sugar. Although various therapeutic benefits have been attributed to the drink, and it is consumed however widely in many populations throughout the world. Neither its beneficial effects nor adverse side effects however have been studied sufficiently. As this beverage contains some alcohol and it may have some effect on central nervous system (CNS), we proposed to study the effect of its chronic consumption on learning and memory which are among the most complex functions in the CNS.

In this study we used a Shuttle Box device to assess active avoidance learning and memory as recently described. Ten Wistar male rats were equally divided into two groups. The animals in control group continued to drink tap water while the animals in the experimental groups had availability of Kombucha tea ad libitum instead, for 2 months until the onset of behavioral studies and continued up to the end of the studies.

Our data showed that although chronic consumption of Kombucha tea during 2 months led to a slight decline in number of shocks receiving by animals in all three stages of the study (learning, short term memory and long term memory). There was no significant difference between animals of the control and the experimental groups ($p=0.539$, $p=0.476$, $p=0.323$ respectively).

Our results indicate that chronic consumption of Kombucha tea had neither significant beneficial nor adverse side effect on learning and memory.

Key Words: Kombucha tea, learning, memory.

INTRODUCTION

Dietary supplements defined as vitamins and minerals, herbal products, tissue extracts proteins and amino acids, and other products are purchased to improve health and to prevent disease. Little has been published, however about their characteristics, benefits or adverse side effects. One of them is Kombucha tea which is a beverage made by incubating the Kombucha mushroom in black tea and sugar.

The Kombucha mushroom which is not a true mushroom but a symbiosis colony of several species of yeast and bacteria including Acetobacter Xylium that are bound together by a surrounding permeable membrane (1), is a traditional remedy that today is being promoted for a variety of conditions, from cancer cure to hair restoration (2). Although various therapeutic benefits have been attributed to this drink, and it is consumed almost widely in many populations throughout the world, its adverse side effects have been reported in some scientific articles (3-6).

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This beverage contains Acetic acid, Gluconic acid, Lactic acid, some antibiotics and some alcohol (1,6). Since alcohol can induce many structural and neurotransmitter abnormalities in the central nervous system (7), it could affect the learning and memory which are among the highest and most complex functions in the central nervous system (8).

MATERIALS AND METHODS

Animals

Wistar male rats, 3–4 months old (200–250 g of body weight), bred and raised in the animal house of Physiology Department in Yazd Medical School were used. Animals were maintained under controlled temperature (23 ± 2°C) and 12:12-h light-dark cycle (lights on at 7:00 a.m.) conditions. Rat chow and tap water were provided *ad libitum* prior to the onset of investigation.

Animals were then randomly divided into two groups (5 animals in each group). While the animals in control group continued on tap water drinking, the animals in case group were placed in cage with availability of Kombucha tea *ad libitum* instead of tap water, for 2 months until the onset of behavioral studies and continued up to the end of the studies. Each behavioral test (learning, short term memory and long term memory) was conducted in separate groups of animals. For behavioral testing permission of the animal ethics committee of Shahid Sadughi Medical University (Yazd, Iran) were obtained.

Apparatus

The active avoidance apparatus consist of two compartments, each room measuring 22 x 21 x 22 cm, connected by a sliding door. The walls of the safe compartment were white, whereas the other compartment, where the animals received foot shock, had black walls. The top of safe compartment was covered with transparent acrylic while the top of the other compartment had an invisible pattern. The floor consisted of a metal grid (0.4 cm-diameter) connected to a shock generator and control module (Ugo Basile model 7551), by which foot shocks of 1 mA and 1 second long could be delivered.

Shuttle box test

After treating the animals in case and control groups with Kombucha tea and tape water respectively for 2 months, the Shuttle Box test was performed basically as described by Urban UA *et al.* (9). The rats were placed in a shuttle box, which is divided into two compartments. Two initial conditioned stimuli (CS) sound and light flash were applied simultaneously followed after 10 sec by an unconditioned stimulus (US), electric foot-shock, 1 mA, whenever animals avoid leaving the dark compartment during this period. Thirty trials were performed each day per animal. The rats with auditory and visual abilities can learn to associate sound and light, respectively, with the unconditional stimulus, and cross into the other compartment within 10 sec from the onset of conditional stimulus without receiving a foot-shock (10–12). The test was performed for 3 successive days in the first week of the testing period to evaluate the learning. Then all animals were trained to reach at least to 70% of full learning (i.e. for each 10 trials at list in 7 trials the animal would leave the dark compartment without receiving any electrical shock) and kept in their assigned conditions for one week. The testing for evaluation of short term memory followed for 3 successive days. After 3 weeks from this, the same experimental procedure was performed for long term memory.

Statistical analysis

The data are presented as means±SEM. The results were analyzed statistically using student's t-test to estimate differences among means. $P<0.05$ has been chosen as the level of significance.

RESULTS

To investigate the effect of chronic consumption of Kombucha tea on learning and memory in rat, we performed a shuttle box active avoidance learning test in which the light and sound stimulations were used as conditioned stimuli (CS) and the foot electrical shock as unconditioned stimulus (US). The animals in both control and case groups performed this test in three different stages i.e. learning, short term memory and long term memory respectively, each for three successive days, and each day in three times with a time interval of 30 minutes and in each time 10 successive trials.

The data were recorded as the number of unsuccessful trials in which foot shock would be received by each animal in each series for 10 trials. The mean±SEM of these records were calculated for both control and experimental groups in each stage.

Our data showed that there was a slight decrease in the total number of trials in which the animals in experimental group unsuccessfully received foot shock, comparing to that of the control group throughout the learning (Table 1), short term memory (Table 2) and long term memory (Table 3) experiments. There was no statistically significant difference between them ($p=0.539$, $p=0.476$, $p=0.323$ respectively, Figure 1).

Table 1: Comparison between the number of shocks received by the control (water consuming group, N=5) and case (Kombucha tea consuming group, N=5) as a learning criterion.

Groups		Mean	SEM	P value
1st day	control	20.6	2.64	0.147
	case	13.5	7.23	
2nd day	control	22	4.24	0.954
	case	21.8	7.182	
3rd day	control	24.2	4.272	0.796
	case	25	3.558	
Sum	control	22.2	3.06	0.539
	case	20.2	5.916	

Table 2: Comparison between the number of shocks received by the control (water consuming group, N=5) and case (Kombucha tea consuming group, N=5) as a short term memory criterion.

Groups		Mean	SEM	P value
1st day	control	27.6	1.731	0.875
	case	27.8	2.499	
2nd day	control	28	0.00	0.215
	case	22.8	6.702	
3rd day	control	26.8	2.061	0.797
	case	27.2	3.096	
Sum	control	27.4	1.032	0.476
	case	26	3.813	

DISCUSSION

The fungus ‘Kombucha’ is a symbiosis of Acetobacter, including Acetobacter Xylium as a characteristic species, and various yeasts. Kombucha is mainly cultivated in sugared black tea to produce a slightly acidulous effervescent beverage that is said to have several curative effects. In addition to sugar, the beverage contains various acids, including acetic acid, Gluconic acid and lactic acid, some antibiotic substances and a small amounts of ethanol (1, 2).

Although advocates of Kombucha tea have attributed many therapeutic effects to the drink, its beneficial and/or adverse effects have not been determined scientifically (1, 5).

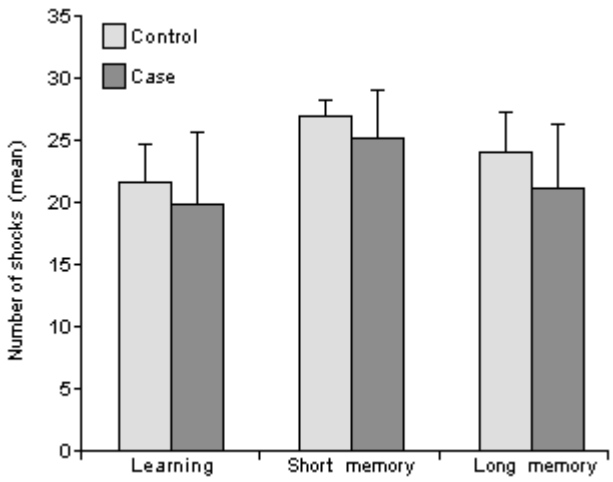
For its effects on the brain it has been suggested that chronic consumption of ethanol, induces tolerance and dependence, as well as cerebral lesions in important brain areas (12–14). These cerebral effects are believed to be the result of compensatory adaptive changes in many signal transduction systems (15–18). Also a number of studies have shown that ethanol had an inhibitory effect on central cholinergic pathways (19–26) and up-regulated the number of Muscarinic acetylcholine receptors (mAChRs) in several brain regions (27, 28). This receptor up-regulation may be the result of adaptive changes to the inhibitory actions of ethanol on this system. Central cholinergic pathways are essential for learning and memory formation, and actions of ethanol upon these pathways may explain the impairment of cognitive functions that can be observed after chronic ethanol intake. In fact, the degree of ethanol-induced impairment of learning and memory seems to be correlated with the degree of ethanol-induced up-regulation of mAChRs (29).

Neuropsychological research revealed that individuals with prenatal alcohol exposure develop wide range of neuropsychological impairments such as verbal learning, memory, academic skills, fine-motor speed, and visual motor integration. (30).

Table 3: Comparison between the number of shocks received by the control (water consuming group, N=5) and case (Kombucha tea consuming group, N=5) as a long term memory criterion.

Groups		Mean	SEM	P value
1st day	control	26.8	2.499	0.173
	case	21.8	5.964	
2nd day	control	22.2	5.619	0.695
	case	20.4	6.402	
3rd day	control	25	3.162	0.436
	case	23.2	2.751	
Sum	control	24.6	2.622	0.323
	case	21.8	4.557	

Figure 1: The effect of Kombucha tea on learning, short and long term memories in control (water consuming, N=5) and case (Kombucha tea consuming, N=5) groups.



Also observations of magnetic resonance images in Clinically Asymptomatic Alcoholic Men are consistent with cognitive studies that commonly report age-related declines in tests assessing prefrontal cortical functions, including cognitive flexibility, working memory, and recall tasks requiring strategic search processes (31).

Alcoholics with alcohol related brain dysfunction had clinical evidence of neurological impairment in at least one domain (cerebellar signs, seizures, frontal lobe dysfunction, memory impairment, altered mental state) (32).

Since acute and chronic alcohol consumption often leads to impairment of recent memory (33), it seems that chronic Kombucha tea consumption which contains some alcohol, can affect the learning and memory, but interestingly our data showed a slight decline in the number of electrical shocks received by animals in case groups in compared to those of control group which is as a criterion for enhancement of learning and memory, although this difference was not statistically significant in learning, short and long term memory ($p=0.539$, $p=0.476$, $p=0.323$ respectively).

In conclusion, our results did not indicate the adverse side effect of this beverage on these higher brain functions. However, it seems that more researches have to be established to investigate the effects of chronic Kombucha tea consumption on various brain functions in details.

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