Can Propolis Be Protective against to Ethanol-impaired Anxiety and Unconditional Fear?

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Abstract

Alcohol metabolism produces acetaldehyde and free radicals which are source of an oxidative stress. The oxidative stress causes oxidative damage on area of learning and memory thus depresses learning and memory processes. Propolis is a component of honeybee that has been used extensively for folk medicine for centuries due to its several biological and pharmacological properties. This work is carried out in order to investigate whether propolis treatment could reverse metabolic alterations on anxiety induced by alcohol.

Key Words: Alcohol, propolis, T-maze

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Introduction

Anxiety and fear is a substantive common health problem and impose large economic and social burdens [1,2]. Life stress and genetic predisposition contribute to development of many different behavioral responses including anxiety. Anxiety may be characterized by restlessness, fatigue, irritability, poor concentration and some other physiological changes [3]. Stress may be as a real or interpreted threat to the physiological or psychological integrity of an individual that result in physiological and/or behavioral responses. Therefore we aim to understand animal behaviors under abnormal conditions, such as rats' innate fear of height and openness [4].

Alcohol abuse and dependence is still a big problem among the human. Alcohol is a short chain lipid soluble compound and has a low molecular weight. Therefore, it crosses the cell membrane very easily. There is evidence for a dose-related response between level of alcohol consumption and risk of harm for various disease and behavior [5]. The relation- ship between alcohol and anxiety is controversial [6,7]. It is believed that alcohol decreased the anxiety for a long time. Hippocrates said that wine removes anxiety and horror from the environment. There is an evidence that chronic alcohol exposure cause brain damage or/and impairment of learning and recent memory [8]. This damage and impairment of learning may be greatly depending on alcohol effects on brain. Because, ethanol alters brain neurobiology only in specific brain regions [8,9]. This specificity of ethanol's action is most likely due to the drug interacting with subsets of specific neurotransmitter systems, such as cholinergic, glutamate, GABAA, serotonergic and NMDA-preferring glutamate systems [10]. Recently, it was reported that anxiolytic disorder was due to alcohol's effect on GABAergic system and that anxiety disorders seen in alcoholics were related to the disorders of the system [11].

Propolis has been employed extensively since ancient times at least to 300 BC and it is a resinous material collected by bees from exudates and bud of the plants and mixed with wax and other substances including bee enzymes [12,13].Chemical composition of propolis includes many different chemical substances such as flavonoids, aromatic acids and esters, terpenes, steroids, amino acids, polysaccharides, hydrocarbons, alcohols, hydroxybenzene and several other compounds in trace amounts[14,15]. Many researchers make a sign for features

of propolis such as immunomodulatory, antitumor, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, antiparasite activities among others [13,15].

The aim of this study was to explain the effect of propolis on anxiety of rats chronically consuming alcohol.

Materials and Methods

Thirty-two Wistar albino rats (150–220 g) were used in this study. The rats were bred in animal laboratory at Inonu University. The rats were quarantined for 8 days at 22–24 ° C with a 12-hour light/12-hour dark cycle before beginning the experimental process for adaptation. The animals were fed a special diet in which caloric intake was strictly controlled and water was available ad libitum. Briefly, the composition of the liquid diet with ethanol was as follows: milk, 925 ml (Pınar, Turkey); ethanol, 25–75 ml (96.5% ethyl alcohol; Tekel Turkish State Monopoly),and sucrose, 17 g [12]. The experimental protocol was conducted after obtaining permission from the local ethics committee. The propolis used in the present study was collected from hives located in the rural area of Anatolia and prepared according to Alves de Lima et al [15].

Experimental Procedure

In group 1 (n = 8), the rats did not undergo any operation and were fed a special diet which has been described previously [15, 16]. This special diet was liquid and calorically equal to those of the other groups which included alcohol or propolis separately or in combination, we applied elevated T maze test 1, 6, 11 and 30 days before the administration of liquid diet and just before the end of experimental procedure. Group 2 (n = 8) was fed the forementioned diet which also included propolis (150 mg/kg) as described previously [15,16]. Elevated T maze was also applied same as the control group. Group 3 (n = 8) underwent a special diet schedule which was described by Saglam et al [16]. Containing alcohol the concentration of which was increased every 6th day of the experiment. Following the first 5 days, Elevated T maze was conducted. Then, we added 75 ml of 2.4% alcohol and decreased the glucose content and volume of the liquid diet to maintain the isocaloric intake of the animals. Between days 6 and 10, the alcohol concentration was increased to 4.8%. We applied elevated T maze again. Between days 11 and 30, the alcohol concentration was 7.2%. At every step of this alcohol percent increase, we decreased the appropriate amount of liquid

diet in order to limit the caloric intake. Application of elevated Tmaze was repeated before and at the end of the experiment. Group 4(n = 8) received the same diet as group 3, but it also contained propolis at 150 mg/kg as described for group 2.We applied elevated T maze test as described in control and other experimental groups.

The apparatus was made in accordance with Zagrossi and Graeff's description[4]. Shortly, elevated T –maze was made of wood and had three arms of equal dimension (50×12) one arm the stem of T arm, was closed by 40 cm high walls and was perpendicular to two opposed open arms to avoid the rats falling down, open arms were surronded by a Pleksiglass rim 1 cm high. The whole apparatus was elevated 50 cm above high walls. The experiment were performed with an observer inside the room

We determined the animal responses under anxiety condition using Zangrossi and Graeff's protocol [4]. Elevated T maze was cleaned with 20% alcohol after each trial. Liquid diet was given to all experimental animal groups and the final T maze was carried out.

Statistical Analysis

Statistical analysis was carried out using the SPSS 13.0 statistical program (SPSS Inc., Chicago IL,USA). Results were expressed as arithmetic mean ±standard deviation. We used Shapiro Wilk normality test for quantitative variable and we determined that variables did not exhibit normal distribution. Therefore we used non parametric test. Variations in times spent by subjects in T labirent were evaluated by Wilcoxon test. We used Mann-Whitney U test to make a comparison between alcohol, propolis, alcohol+propolis and the control groups. The differences between the groups as pairs were evaluated by Benferoni Mann-Whitney U test. Results were statistically analyzed by the Kruskal Wallis H test and were considered significant when $p<0.05$.

Results

Table 1 shows that there was no significant difference at the elevated Tmaze value for baseline latency, avoidance 1 and avoidance 2 and unconditional fear among groups before application of experimental protocol.

On the other hand we determined a significant difference at the elevated T maze value for baseline between the control and fourth group on day 6 of the experiment (Table 2).

Also, we determined a statistically significant difference among the control and the third group for avoidance 1 response. Same is also true for the control and the fourth group for avoidance 1 response on day 11 of the experiment (Table 3).

When compared the baseline among groups at 30th day of the experiment, we found that there were significant differences among the control and the third and the fourth group. This was also valid for the second and fourth group (Table 4).

Interestingly, we could not determine any significant difference to unconditioned fear value in repeated trials of elevated T maze among groups during experiment.

Table 1. Baseline latency, avoidance response 1 and 2 and unconditional fear values of elevated T maze before the application of exprimental protocol. (mean value ± standart deviation)

	Groups (n=8)	**Baseline (sn)**	**Avoidance 1 (sn)**	**Avoidance 2 (sn)**	**Uncontiditional fear (sn)**
1	Control	7,88 ± 1,28	75,25 ± 13,19	273,75±26,25	73,50 ± 7,94
2	Propolis	19,25 ± 7,92	95,00 ± 16,00	293,13±6,87	80,38 ±11,65
3	Ethanol	13,00 ± 2,81	58,88 ± 5,44	288,75±11,25	90,25 ± 9,09
4	Ethanol+Propolis	15,00 ± 4,38	89,13 ± 16,17	300 ± 0	103,88 ± 13,28

Table 2. Baseline latency, avoidance response 1 and 2 and unconditional fear values of elevated T maze at 6th day of experiment. (mean value ± standart deviation)

	Groups (n=8)	**Baseline (sn)**	**Avoidance 1 (sn)**	**Avoidance 2 (sn)**	**Uncontiditional fear (sn)**
1	Control	59,38 ± 5,29	237,5 ± 30,53	300 ± 0	41,75 ± 5,95
2	Propolis	71,88 ± 9,06	244,38 ±27,29	285,63 ± 9,7	80,88 ± 7,02
3	Ethanol	22,88 ± 7,3	180,38 ±12,35	282,13±11,72	86,63 ± 8,70
4	Ethanol+Propolis	29,72 ± 5,91[a]	193,25 ±25,50	300 ± 0	115,88 ± 13,16

a ; p< 0,05 (when compared group 1)

Table 3. Baseline latency, avoidance responce 1 and 2 and unconditional fear values of elevated T maze at 11th day of experiment (mean value ± standart deviation)

	Groups (n=8)	Baseline (sn)	Avoidance 1 (sn)	Avoidance 2 (sn)	Uncontiditional fear (sn)
1	Control	116,13±11,12	244,75 ± 27,79	290 ± 10	64,50 ± 6,49
2	Propolis	107,0 ± 10,06	189,38 ±22,01	300 ± 0	55,88 ± 12,11
3	Ethanol	30,13 ± 7,47	123 ± 19,36[a]	270,13±29,87	130,88 ±10,04
4	Ethanol+Propolis	30,0 ± 8,27	135,13 ± 6,3[a]	259,38±27,29	114,38 ±11,11

a ; p< 0,05 (when compared group 1)

Table 4. Baseline latency, avoidance responce 1 and 2 and unconditional fear values of elevated T maze at 30th day of experiment (mean value ± standart deviation)

	Groups (n=8)	Baseline (sn)	Avoidance 1 (sn)	Avoidance 2 (sn)	Uncontiditional fear (sn)
1	Control	267,5 ± 22,28	300 ± 0	300 ± 0	37,75 ± 10,7
2	Propolis	263,13±19,36	290 ± 9,37	298,75±1,25	56,75 ± 6,36
3	Ethanol	55 ± 18,22[a]	93,75 ± 27	155,63 ± 31	124,38 ±12,58
4	Ethanol+Propolis	162,75±28,53 [a,b]	239,38 ±23,80	300 ± 0	46 ± 4,8

a ; p< 0,05 (when compared group 1)

b ; p< 0,05 (when compared group 2)

Discussion

This study investigated the relationship between alcohol and propolis in the modulation of anxiety and memory in rats observed in the elevated T maze. Our result showed that alcohol supplemented reduced in a significant the latencies to leave the enclosed arm of elevated Tmaze. Before the experiment, we could not determine any significant difference among parameters investigated.

On day 6 of the experiment, we determined significant differences in baseline latency between the first and fourth group. Rats of fourth group stayed for a shorter time period in the enclosed arm of elevated T maze than the first group. This means that alcohol may affect inherent fear behavior. On the other hand, there were some differences for baseline between the control and other experimental groups but these differences were not statistically

significant. There are much evidence indicating the effect of alcohol consumption, either chronically or acutely, on the learning and memory [9, 17].But, effect mechanism of alcohol on nervous system is still unclear. Because there are different types of learning and memory. The learning and memory is a both complex processes and different parts of nervous system take part in these processes. Results of many researchers indicate that alcohol impairs hippocampus dependent learning however it does not alter hippocampus independent learning. The learning is a receptor mediated process e.g. NMDA (10), and it also involves a balance among various neurotransmitter systems. It was reported that intraperitonal alcohol administration can stimulate dopamine release through 5–HT3 subtype and play an important role in modulating the central effect of alcohol. Dorsal raphe nucleus and median raphe nucleus are the main sources of 5–HT innervation to the forebrain [18]. Our results show that propolis might antagonize ethanol-induced changes on behavior performance. However it is unclear at present whether or not propolis is able to reverse ethanol induced deficits in learning. Propolis consists of different organic compounds. It has been reported to have various biological activities such as antioxidant, antimicrobial, anti-inflammatory and anti-cancer. Recent studies show that propolis has neuroprotective effect on neuronal death [1, 19, 20]. However, when baselines of third and fourth group were compared it can be seen that baseline of third group was shorter than that of forth group but, the difference was insignificant. İt is tempting to speculate that propolis may correct the ethanol induced changes to some extent. Caffeic acid phenethyl ester and, terpenoids which are components of propolis take part in this correction. caffeic acid phenetyl ester is one of antioxidant flavanoids, and terpenoids is essential oil of propolis. Both of them are active components of the propolis. Researcher reported that caffeic acid and terpenoids act through multiple mechanism involving free radical scavenging, metalic ion chelation and inhibitory actions on specific enzymes that induce free radical and lipid hydroperoxide formation, anti-inflammatory activity, inhibition of excitotoxicity, inhibition of cell progression [1,19,21].

Elevated T-maze is derived from elevated plus-maze or elevated X –maze [22,23] and it has been developed to generate both conditioned or unconditioned fear response in the same rat. the former being related to generalized anxiety and the latter to panic disorders. On day 11 of the experiment we determined statistically significant differences in inhibitory avoidance latency among the first and third and fourth groups. Latencies for the animals to leave the enclosed arm of the elevated T maze were decreased. Inhibitory avoidance latency reflects

learned fear after consecutive three trials. This means that the animal can learn inhibitory avoidance if repeatedly placed inside the enclosed arm to explore the maze. Animals feel varies degrees of fear and different parts of brain are related to different kinds of fear(Ayşegül et al. 2008[24].Results of other researchers indicate that anxiety model with the elevated T maze increased Fos-like immunureactivity in different parts of limbic system and median raphe nucleus [24]. When we put the animal into closed arm for the first time it went out of this arm to explore its environment slowly. Decline of avoidance latency upon alcohol consumption may depend on effect of alcohol on regulating behavior center. which blocks NMDA-evoked neural activity [10]. Our results are not concordant with some researchers' result [24, 25]. However, this discrepancy may have been caused by differences in species and age of animals used. When we compared avoidance latencies between third and fourth groups, the fourth group had longer latencies than the third group. This follows that propolis can make a corrective effect on behavior regulating center. This effect might depend on terpenoids and flavonoids in propolis [26].

Before, termination of experiment we applied elevated T maze to the experimental groups for the last time and we then determined significant differences for baseline among the control and third and fourth groups. Rats of third group left the closed arm of elevated T maze in shorter latency than control group. This means that alcohol may disrupt regulating behavior center depending on effect mechanisms mentioned above. Rats of fourth group left in a longer latency than control and second experimental group. It follows that propolis may correct regulating behavior center effect of which was explained above.

In conclusion propolis corrected ethanol-induced impairment on regulating behavior of center. But we can suggest that mechanism effect of propolis need still to be detailed by investigation in terms of administration time and route.

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