EFFECT OF AQUEOUS EXTRACT OF GINGER ON ACETYLCHOLINE IN BRAIN AND ITS POSSIBLE ROLE IN LEARNING AND MEMORY DURING ETHANOL WITHDRAWAL

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ABSTRACT

Objective: Alcohol depletes acetylcholine levels in the brain thus accounting for some of the cognitive impairments and withdrawal symptoms associated with alcohol ingestion. Low acetylcholine levels produced by alcohol intake results in long and short term memory deficits, trouble focusing, concentrating as well as stage 4 (REM) sleep deprivation.

Methods: Measurements of brain acetylcholine levels and acetylcholine metabolizing enzyme AchE were made in rats treated with either ethanol (2g kg^{-1} body wt daily) alone or ethanol with ginger extract supplementation (200 mg kg^{-1} body wt daily for 6 weeks). EtOH with or without ginger extract was administered orally by oral intubation for 42 days. Matched controls were given saline.

Results: ACh synthesis in the brain of rats treated with ethanol alone for 6 weeks was significantly (P less than 0.01) inhibited. Ach concentration in the brain of rats treated with ethanol plus ginger extract for 42 days was three times that of rats treated with ethanol alone. ACh synthesis in rats with ethanol withdrawal and ginger extract supplementation was also significantly (P less than 0.01) higher. Spatial memory and learning of rats were tested by Morris water maze test with or without ginger extract supplementation during withdrawal from chronic ethanol consumption was also tested. The effect of EtOH on ACh synthesis and influence of ginger extract on Ach concentration in the brain was found to be positively correlated.

Conclusion: The current data suggest that chronic ethanol exposure may decrease ACh synthesis while ginger extract restores and exhibits protective capability and cognitive enhancing properties during ethanol withdrawal.

Keywords: Ginger Extract; Alcohol; Cognitive Behaviour; Acetylcholine; Ethanol Withdrawal

INTRODUCTION

Acetylcholine (ACh) is a neurotransmitter that has a crucial role in the central nervous system and its implication in behavioural (including learning and memory) and neuro-degenerative disorders has long been known [1,2]. ACh is also a neurotransmitter of the peripheral parasympathetic or cholinergic system [3]. There are a number of enzymes that can hydrolyse Ach in central or peripheral tissues. The two major types are Achesterase (AChE) and butyryl-cholinesterase or cholinesterase (ChE) [4]. AChE degrades ACh in all effector organs or surrounding fluids and is of importance for cognitive functions and anaesthetic medication in both humans and experimental animals [5]. AChE activity in the cerebrospinal fluid is assumed to be a biochemical marker for clinical diagnosis and prognosis of several central and peripheral nervous system dysfunctions, such as Alzheimer's disease, parkinson's disease, dementia, schizophrenia and chronic alcoholism [6].

Cholinergic deficit is a major neuropathological feature that is associated with memory loss, and is closely correlated with the severity of cognitive dysfunction in AD [7]. On other hand, cholinergic transmission is terminated mainly by ACh hydrolysis through the enzyme AChE, which is responsible for degradation of ACh to acetate and choline in the synaptic cleft [8]. Inhibition of AChE serves as a strategy for the treatment of AD and other cognitive impairments.

The cholinergic neurotransmission system in the basal forebrain plays an important role in learning and memory. It has been suggested that the impairments in learning, memory and behavior observed in patients with dementia are caused, at least in part, by changes within the cholinergic system [9]. A number of studies on rodent brains have explained the reductions in cholinergic activities, such as high-affinity choline uptake, acetylcholine synthesis and

acetyl choline release by ageing [10]. The reduction in acetylcholine release is thought to be directly related to a decreased synaptic transmission in cholinergic neurons. The acetylcholine release per synapse was later shown to be decreased in aged rats in experiments using synaptosomes [11].

A class of compounds known as acetylcholinesterase inhibitors (AChEIs) has been found to help maintain and restore ACh levels, enabling proper memory function to prevail. Acetylcholinesterase, or AChE, is the enzyme that breaks down ACh, so compounds such as galantamine, which inhibit AChE's function, serve to boost ACh levels. These compounds, found mostly in so-called medicinal plants, have been used in traditional Chinese, Indian, and European folk medicines for the relief of cognitive impairment in the elderly. The long-term use of AChEI-containing plant extracts in these traditional medicines has demonstrated an impressive absence of toxicity.

Having high antioxidant effect due to its high of polyphenol content [12], Ginger is well established in the ayurvedic system of medicine for treating a number of disorders. Ginger has antipyretic, anti-inflammatory, antimicrobial, antiparasitic, antiplatelet aggregation, antioxidant and analgesic effect as it is used to ameliorate pain in migraine ,osteoarthritis and dysmenorrheal. It also has several cardiovascular and gastrointestinal effects as its anti-ulcer, antispasmodic and anti-emetic effects. Its effects are due to its various constituents. Most of the therapeutic focus is on the pungent taste compounds, called Gingerols and Shoagols, were thought to be responsible for most effects of ginger particularly its antioxidant and anti-inflammatory effects. Ginger has positive inotropic effect on cardiovascular system [13]. Its content of Gingerols and diarylhepatanoids which are anti-prostaglandin compounds exhibit anti-inflammatory activity [14]. Effects on the gastrointestinal tract

includes carminative, appetite stimulant and amelioration of nausea associated with motion sickness effects [15].

Interestingly, compounds from ginger rhizomes were found to inhibit acetylcholinesterase [AChE] activity in vitro [16]. Moreover, previous studies highlighted that the supplementation of ginger extract significantly reversed the scopolamine, a muscarinic antagonist, induced memory impairment in the elevated plus-maze and passive avoidance paradigm maze models [17,18]. The present experiment is intended to study the effects of aqueous ginger extract on ACh levels and AChE activity in rat brain. The 45 day treatment with AGE showed an elevation in acetylcholine levels, which might be due to a significant reduction of cholinesterase activity in the brain of ethanol withdrawal rats.

MATERIAL AND METHODS

Reagents

All the chemicals and reagents used were of high quality analytical grade reagents. 5, 5'-Dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine esterase, acetylcholine iodide, and all the standards were purchased from Sigma, USA. All other chemicals used were of analytical grade.

Preparation of the extract

Whole rhizome of ginger obtained from local market, Tirupati was thoroughly washed, sliced, grated and grind to fine paste. A weighed quantity (30 gm) of the paste was subjected to continuous Soxhlet extraction using nanopure water as the solvent. The extract was evaporated using rotavapor and then lyophilized. The dried extract was stored at 4°c and used as AGE (aqueous ginger extract) for further studies.

Animals

Wistar strain albino rats weighing (175-200gm) were used for in vivo study. All animals were housed in polypropylene cages in a room at 27 ± 3°C, 68 ± 5% humidity and were provided with a standard laboratory diet and water ad libitum. Animal procedures were performed in accordance with the recommendations of the Ethics Committee for animal protection and welfare bearing the CPCSEA No. 438 / 01/a / CPCSEA / IAEC / SVU / KSR-1 (dt: 11.09.2008).

Experimental Protocol

The animals were divided into 5 groups. Each group comprised of 6 animals.

Group I. Control group. The animals were orally given saline orally

Group II. Ethanol group: This group received 2% ethanol for 6 weeks and served as negative control.

Group III. Ethanol + Extract treated group: received 2% ethanol for 6 weeks while the latter received ginger extract at concentration of 200 mg/kg bw for 6 weeks along with ethanol administration

Group IV. Ethanol withdrawal group: Animals were treated with 2g/kg b wt of ethanol for 6 weeks, ethanol was withdrawn by preventing the administration for the next 72 hrs after the last dose..

Group V.The animals received ethanol as well as ginger orally given at dose of 200 mg/kg body weight for 6 weeks and then subjected to withdrawal for 72 hrs.

Isolation of Tissues

After fixed duration, the animals were sacrificed by cervical dislocation and different brain regions such as Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC)) were immediately isolated, frozen in liquid nitrogen and were stored at -40⁰C until analyses.

Biochemical Analysis

Acetylcholine assay

Acetylcholine content in all the tissues was estimated by the method of Hestrin (19) as given by Augustinson [20]. The tissues were boiled to in activate the enzyme and to release the bound Ach. Which reacts with ferric chloride , a brown colour developed was read at 540 nm against the reagent blank. The acetylcholine content was expressed as μmoles of acetylcholine/gm wet weight of tissue.

Acetylcholinesterase assay

The AChE activity in all the brain regions was estimated by the method of Ellman *et al.*, 1961 [21]. The procedure was based on the production of thiocholine by the action of acetylcholinesterase which forms a yellow color with 5,5'-dithiobis(2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, was proportionate to the enzyme activity in the sample. Enzyme activity is expressed in μ moles of Acetylthiocholine iodide hydrolyzed/mg Protein/hr.

Behavioural Tests

Morris Water Maze Test

The water maze consisted of a metal pool (170 cm in diameter × 58 cm tall) filled with tap water (25°C, 40 cm deep) divided into 4 quadrants. In the center of 1 quadrant was a removable escape platform below the water level and covered with a nontoxic milk powder. The pool was divided into 4 quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. For each animal, the location of invisible platform was placed at the center of one quadrant and remained there throughout training. The rats must remember location of the platform in relation to various environmental cues. Each rat was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it found and climbed onto the platform. During the training sessions, the rat was gently placed on the platform by the experimenter when it could not reach the platform in 60 s. In either case, the rat was left on the platform for 15 s and removed from the pool. The time for the animals to climb on the hidden platform was recorded as escape latency. Retention memory was also determined on the next day. The platform was removed and the animals were placed into the water maze for 60s. The retention of the memory or the time that the animal spent to swim around the location of the platform before it was removed was recorded.

If the spatial memory of the rat for the trained platform location was accurate, the rat will swim to the platform location and search around the exact location. Therefore, the more accurate the spatial memory was, the greater the number of times the rat will swim across precious location of platform. In each trial, the animal was quickly dried with towel before being returned to the cage [22]. All Morris water maze tests were carried out within 30 minutes after the oral administration of the test substances

Passive avoidance test

This test was always conducted 2-3 days after Y-maze task. The apparatus consisted of an illuminated chamber connected to dark chamber by a guillotine door [23]. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber.

After a habituation period (2 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 2 s once) was delivered. In this trial, the initial latency (IL) of entrance into the

dark chamber was recorded and rats with IL greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through

Statistical analysis

All the experiments were performed in triplicate and the values were expressed as mean±SD. The statistical analysis was done by one-way ANOVA followed by a tukey post hoc comparison test. In all calculations, a difference at $p<0.01$ was regarded as significant.

RESULTS

Effect of AGE on acetylcholine assay

Table 1 shows the effect of AGE on the ACh levels in the different regions of rat brain homogenate. Six weeks of administration with AGE resulted in improvement of ACh levels in different regions of rat brain. The results indicate significant increase in levels of Ach in ethanol treated and withdrawal rats that receive the extract were comparable to the effect of the control ($p < 0.01$).

Table 1: Changes in the Acetylcholine (ACh) content in different brain regions of rats during ethanol-induced withdrawal and Pre-treatment with aqueous extract of *Zingiber officinale*

(Values are expressed in μ moles of Acetylcholine/g wet wt of tissue)

Sl.No.	Brain Area	Control	EtOH	Etoh+EXT	EW	EW+ EXT
1	CC	5.739 ±0.158	4.300* ±0.149 (-25.18)	5.122 ±0.448 -6.67 [19.13]	3.851[b] ±0.334 (-15.47) [-10.89]	5.062[b] ±0.470 (-11.79) [-17.65]
2	CB	5.405 ±0.374	4.265[a] ±0.157 (-21.08)	[b]5.295[a] ±0.742 -34.96 [24.25]	3.901[b] ±0.111 (-22.27) [-8.64]	5.759[b] ±0.143 -6.53 [35.84]
3	PM	5.495 ±0.348	3.732[a] ±0.500 (-32.07)	5.934[b] ±0.492 -7.97 [59.25]	2.639[b] ±0.502 -2.61 [-29.28]	4.909[b] ±0.096 (-10.67) [31.51]
4	HC	9.036 ±0.155	5.753[a] ±0.574 -36.27	[b]6.273[a] ±0.090 (-30.57) [9.02]	4.760[b] ±0.113 (-47.88) [-17.46]	7.387[b] ±0.155 (-18.25) [-28.41]

All the values are mean, ±SD of six individual observations.
Values in '( )'parentheses are % change over saline control and values in '[ ]' are % change over EtoH treatment.
Values with * are significant at P<0.01 compared to Control; (a) are significant at P<0.001 compare to SC; and (b) are significant at P<0.001 compare to EtOH in Scheffe test.

Effect of AGE on acetylcholinesterase assay

The effect of AGE on acetylcholinesterase activity in the homogenate of different brain regions was evaluated (Table 2). The AGE (200mg/kg, p.o.) significantly ($p < 0.01$) inhibited the activity of AChE in withdrawal rat brain compared with the control animals.

Table 2: Changes in the activity levels of Acetylcholinesterase (AChE) in different brain regions of rats during ethanol-induced withdrawal and Pre-treatment with aqueous extract of *Zingiber officinale*

(Values are expressed in μ moles of Acetylthiocholine iodide hydrolyzed/mg protein/hr)

Sl.No.	Brain Area	Control	EtOH	Etoh+EXT	EW	EW+ EXT
1	CC	5.43 ±0.219	2.798[a] ±0.142 (-48.46)	3.207[a] ±0.127 (-40.93) [12.76]	2.19[a] ±0.680 (-59.66) [-21.70]	4.346 ±0.084 (-19.97) [35.48]
2	CB	6.546 ±0.250	2.769[a] ±0.638 (-57.69)	3.059[a] ±0.451 (-3.27) [9.46]	2.782[a] ±0.488 (-57.50) [-9.05]	5.430[b] ±0.219 (-17.04) [77.52]
3	PM	5.507 ±0.564	3.748[a] ±0.177 (-31.93)	3.796[a] ±0.125 -31.07 [1.25]	2.654[a] ±0.242 (-51.79) [-30.06]	5.340[b] ±0.099 (-3.03) [40.67]
4	HC	4.936 ±0.666	4.454 ±0.175 (-9.76)	3.560* ±0.547 -27.86 [25.09]	3.54* ±0.007 (-28.26) [-0.55]	[b]7.654[a] ±0.171 -55.07 [114.97]

All the values Values are mean, ±SD of six individual observations.
Values in '( )'parentheses are % change over saline control and values in '[ ]' are % change over PTZ treatment.
Values with * are significant at P<0.01 compared to Control; (a) are significant at P<0.001 compare to SC; and (b) are significant at P<0.001 compare to EtOH in Scheffe test.

Effect of EW on acquisition and retention in passive avoidance test

Figures 1, 2 and 3 show the performance of ethanol and withdrawal rats in passive avoidance paradigm as indicated by initial and STL.

The withdrawal rats developed a significant impairment in acquisition and retention in passive avoidance test. In this respect, the mean IL which is indicative of acquisition in passive avoidance test showed a non-significant increase in withdrawal group after

chronic ethanol consumption. This parameter exhibited a significant increase after ginger extract administration. Therefore, withdrawal from ethanol markedly increased acquisition, and clearly decreased consolidation and recall of a passive avoidance response after chronic ethanol consumption.
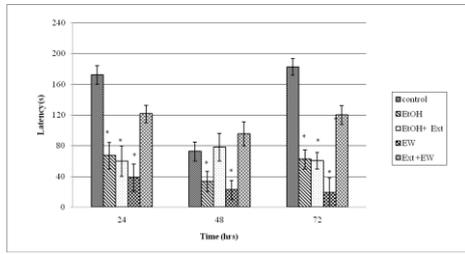

Figure 1: The effects of pretest injection of aqueous ginger extract (AGE) on time latency for entering the dark compartment. The animals of the control group received saline while the animals of ethanol group were treated by 20% ethanol and withdrawal group were subjected to alcohol abstinence for 72 hrs. EtOH + EXT and EW+ EXT groups were treated with 200 mg/kg bw of AGE (orally), respectively. The data were presented as mean ± SEM of the time latency (4 animals in each group). * significant compared with the control group.
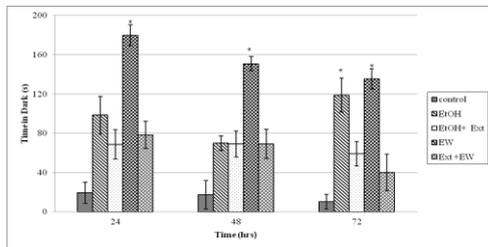

Figure 2: Comparison of the total time spent in the dark compartment 24, 48 and 72h after receiving the treatment between

the groups. The animals of the control group received saline while the animals of ethanol group were treated by 20% ethanol and withdrawal group were subjected to alcohol abstinence for 72 hrs.

EtOH + EXT and EW+ EXT groups were treated with 200 mg/kg bw of AGE (orally), respectively. The data were presented as mean ± SEM of the total time spent in the dark compartment (4 animals in each group). * significant compared with the control group.
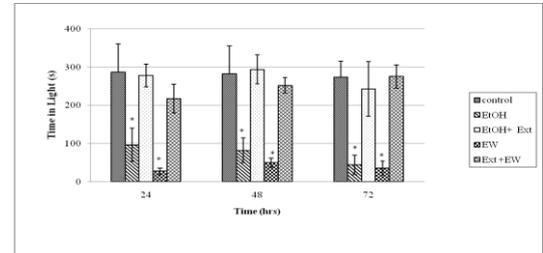

Fig. 3: Comparison of the total time spent in the light compartment 24, 48 and 72h after receiving the treatment between the groups. The animals of the control group received saline while the animals of ethanol group were treated by 20% ethanol and withdrawal group were subjected to alcohol abstinence for 72 hrs. EtOH + EXT and EW+ EXT groups were treated with 200 mg/kg bw of AGE (orally), respectively. The data were presented as mean ± SEM of the total time spent in the light compartment (4 animals in each group). * compared with the control group.

Z. officinale on Spatial Memory

Effect of *Z. officinale* on spatial memory was determined using escape latency and retention time as indices. It was found that all groups treated with *Z. officinale* could significantly decrease escape latency throughout the experimental period as shown in Figure 4 (*P*-value <0 .01 as compared with control).
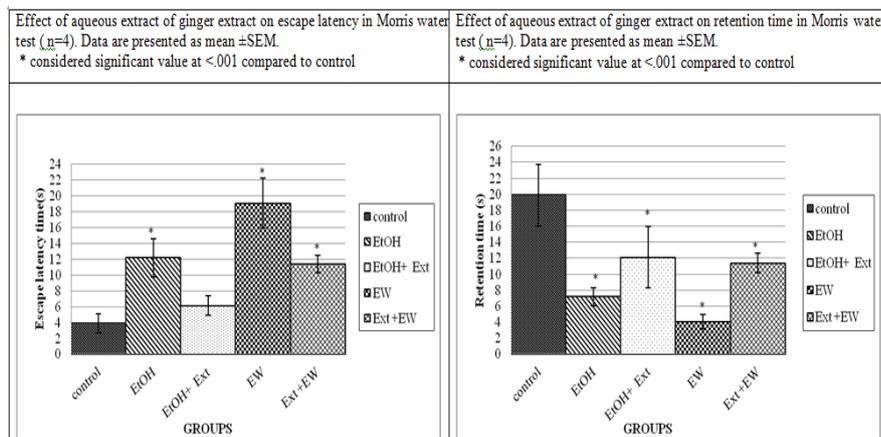

Figure 4: Effect of ginger Extract on Spatial Memory during chronic ethanol intoxication and ethanol withdrawal.

DISCUSSION

The enzymes of the cholinergic system have been investigated in discrete brain areas in alcohol-dependent rats, which were still intoxicated or were undergoing withdrawal. The ethanol intoxication resulted in a slight, but significant decrease in acetylcholine levels in the tested brain regions 72 h after the last dose of ethanol. All the brain regions investigated, e.g., cerebellum, pons-medulla, cortex and hippocampus showed changed AchE activity. Rats were also analyzed immediately following the onset of a withdrawal for the cognitive and learning capabilities. These results show that ethanol intoxication leads to a perturbation in the synthetic capacity of acetylcholine in certain defined brain

structures and that this may have some correlation to the observed behavioral impairments.

In the present study, the results of Morris' water maze showed that reference memory improved in all groups but, in both ethanol and withdrawal animals, the degree of learning was less when compared with the control-operated animals. However, ginger extract pre-treatment in both ethanol and withdrawal animals improved the reference memory component in comparison with their respective control animals. The result of the probe trial, an indication of working memory, showed that the both ethanol and withdrawal animals swam in the quadrant in which the platform was kept earlier for a lesser period of time and ginger pre-treatment had significantly increased the time spent in the same quadrant,

suggesting that ginger pre-treatment prevented the damage caused by both ethanol and withdrawal. The spatial learning, as evidenced by new platform test, was affected by both ethanol and withdrawal and was normalized by ginger pre-treatments in both ethanol and withdrawal cholinergic deprivation. The present study confirms the memory-enhancing effect of this herbal drug.

The results of the present study on passive avoidance paradigm showed marked deficits in the avoidance learning task and retention of the memory in rats subjected to chronic ethanol consumption and ethanol withdrawal, which was in consonant with earlier reports [24,25] The pre-treatment with ginger in both ethanol and withdrawal groups preserved learning and retention of learned behavior.

Studies which assess cognitive functions associate the chronic ingestion of ethanol with the reduction in the brain concentration of acetylcholine, both in humans and mice, caused by the degeneration of brain tissues [26]. Cholinergic mechanisms have also been shown to have a role in the susceptibility to epileptiformic seizures, and are also supposed to be involved in the generation of ethanol withdrawal-induced convulsions [27]. Imperato et al., 1998 [28] have shown a rapid increase in acetylcholine release in the rat hippocampus during ethanol withdrawal. Majchrowicz also showed that dependence and signs of ethanol withdrawal could be produced in rats with 4-day intragastric administration of 9–15 g/kg of ethanol per day [29]. In the present study, the rats were exposed to high doses of ethanol for a longer period. On the other hand, Miller and Rieck [30] suggested that six weeks [42 days] of ethanol [6.7% v/v] administration to rats by the liquid diet technique was sufficient to alter the cholinergic innervations of the cerebral cortex. Accordingly, we gave ethanol to rats for 6 weeks. Thus, the results of the present study clearly indicate that there might be a relationship between cerebral ChE activity and development of physical dependence to ethanol. However, data provided by the present study are not adequate to explain which mechanism could be responsible for the increases of ChE activity during chronic ethanol consumption. Further studies are needed in order to clarify these points.

Many of the previous studies addressing the effects of ethanol on the cholinergic system have been focused on AChE. These studies generally indicated an inhibition of AChE activity in the brain [31, 32] and erythrocyte [33]. In another study, Husain and Somani [34] studied the effects of chronic ethanol ingestion [2 g/kg per day for 6.5 weeks] on ChE activity in plasma and AChE activity in several regions of the brain. They found increased ChE activity in plasma and decreased AChE activity in the hypo-thalamus. Our results indicate significantly increased AChE activities in the chronic ethanol consumption are in line with these results. We confirmed Husain and Somani's findings and, additionally, we showed that the increased AChE activity persisted at the end of the 72 h ethanol-withdrawal period. These findings imply that the effects of ethanol on AChE activity are due to earlier consumption and that the persistently high level of AChE activity after 72h of withdrawal was not related to blood ethanol level. In addition, because no significant differences were observed between the groups ingesting the higher concentrations of ethanol for various periods of time, the increases of AChE activities may not be related to the duration of ethanol consumption. Overall, the data indicate that AChE activity seems to be an indicator that can provide some information about the effects of chronic ethanol consumption and ethanol withdrawal on the cholinergic system. Consistent with our previous findings in the same model of rats [35- 39], the present data demonstrated that daily ethanol consumption above 2g/kg for 42 consecutive days produced physical dependence in male wistar rats.

Surprisingly, *Z. officinale* studies indicate increase the neurons' density in hippocampus and improved the spatial memory. Although the neurodegeneration in hippocampus was reported to be associated with the spatial memory deficit [40, 41], the present findings showed that the improvement of spatial memory was not tightly correlated with the increase of neuronal density in the hippocampus. It was found that all doses of *Z. officinale* could improve spatial memory while *Z. officinale* at dose of 300 mg/kg body weight failed to show neuroprotective effect in this area. This indicates that the cognitive enhancing effect of *Z. officinale* occurred not only due to increasing neuronal density in hippocampus but also due to other mechanisms. *Z. officinale* has previously been reported to induce vasodilation [42]. Therefore, it might be possible that *Z. officinale* could enhance cerebral blood flow resulting in the improvement of spatial memory as Piracetam [17]. A recent study clearly demonstrates that *Zingiber officinale* may enhance both the attention and cognitive processing in middle-aged women. [43]. Choline esterase inhibitors have been reported to be effective in treating memory impairment and other cognitive dysfunctions. Plant derived AchE inhibitors, especially flavonoids could be appropriate in treating this condition because of less side effects and many such compounds have been reported earlier [44, 45]. For the first time, we are reporting here the acetylcholine esterase inhibitory activity of ginger during ethanol withdrawal.

Moreover, it is evidenced that this plant extract and its active component, 6-gingerol, also inhibited the cholinesterase activity which in turn increased acetylcholine [ACh], a neurotransmitter that plays an important role in learning and memory [46]. A recent study demonstrated that ginger extract enhanced the memory performance induced by cerebral ischemia by decreasing infarct volume in both cortical and subcortical areas [17]. Therefore, taking all data together, we suggest that the cognitive enhancing effects of *Zingiber officinale* might be partly associated with the modulation effect of this plant extract on the alteration of the cholinergic system in various brain areas, including the cerebral cortex, hippocampus, medulla and cerebellum. Accumulating lines of evidence show that dietary enrichment with nutritional antioxidants could improve brain damage and cognitive function [47, 48]. Also it was established that antioxidants could also improve cognitive performance in healthy elderly subjects [49, 50]; therefore, the association between the antioxidant effects of *Zingiber officinale* and the cognitive enhancing effects still cannot be excluded [17, 51]. However, the precise underlying mechanism and possible active ingredient responsible for the cognitive enhancing effect of *Zingiber officinale* still require further investigation.

CONCLUSION

The present study demonstrates that ginger extract enhances both learning and cognitive processing capabilities of ethanol withdrawal rats. Therefore, our data reveal that *Zingiber officinale* extract was a potential brain tonic to enhance cognitive function for ethanol related cognitive deficits. However, further study about the precise underlying mechanism especially the effect of the extract on the alteration of acetylcholine and monoamine transmitters should be performed.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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