Modulation of Disorders in Excitatory and Inhibitory Neurotransmitter Metabolism Related to Ethanol Withdrawal Can Be Achieved with Ginger Extract Administration

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Abstract: The perturbation in excitatory glutamatergic neurotransmission and GABA-mediated synaptic inhibition during ethanol withdrawal was established decades ago. The present study describes the neurochemical changes involved in ethanol administration and withdrawal from ethanol on the glutamine-glutamate–GABA cycle and the influence of ginger on these changes. Aim: The neuroprotective efficacy of the ginger extract has been comprehensively assessed with specific regard to conventional biochemical neurotransmitter amino acid estimations and the principal mechanism of action has been evaluated. In this study, the effectiveness of ginger extract in the regulation of alterations in glutamate-GABA metabolism was evaluated in albino rats during withdrawal from chronic ethanol administration. In Conclusion: This study provides evidence that ginger has a profound influence in the regulation of GABAergic function and neuronal hyperexcitibility during ethanol withdrawal, which may in the future lead to the development of new therapeutic strategies for the treatment of neurological disorders that involve disturbances of GABAergic activity may be treated.

Key words: Brain %Ethanol Withdrawal %Neuronal Excitibility %Glutamate(Glu)-GABA Cycle %Ginger

INTRODUCTION

Sudden discontinuation from chronic ethanol administration, referred to as ethanol withdrawal (EW), results in the development of alcohol tolerance and physical dependence. Repeated episodes of withdrawal symptoms, as may occur in many alcoholics, due to recurring detoxifications, can be detrimental to the Central Nervous System (CNS), a process known as “kindling of withdrawal symptoms” [1]. Many clinical conditions of CNS disorders, including major neurological diseases, appear to involve an imbalance in excitation and inhibition induced by neurotransmitters and therapeutics thus deals with attempts to restore this balance [2]. Although this would be more informative, the significance of the Glutamine(Gln)-Glutamate(Glu)-GABA cycle and its alterations that are due to stress during various pathological conditions, particularly in relation to ethanol, should never be overlooked.

A Gln-Glu-GABA shunt has been proposed [3] where glutamate that is released from neurons is taken up by astrocytes, converted to glutamine with the enzyme glutamine synthetase (GS)(E.C. 6.3.12) and subsequently transported back to neurons. Much of the glutamate and GABA used as neurotransmitter is derived from glial storage pools of glutamine (Gln) [2, 4]. In neurons, glutaminase (GLS) (E.C. 3.5.1.2) has been proposed to be the enzyme that converts glutamine to glutamate and consequently, it is thought that this enzyme is essential for both glutamatergic and GABAergic transmissions. Glutamate dehydrogenase (GHD) (EC 1.4.1.3) catalyzes the reversible deamination of L-glutamate to 2-oxoglutarate. The enzyme glutamate decarboxylase (GAD) (E.C.4.1.1.15) synthesizes GABA from glutamate and hence plays an important role in the balance of glutamate/GABA concentrations in the brain [5,6]. Finally, GABA is catabolized by the action of 4-aminobutyrate transaminase (GABA-T) (EC 2.6.1.19), which deaminates GABA to make
succinic semialdehyde (SSA) and then SSA dehydrogenase (SSADH) converts SSA to succinate, which enters the Kreb’s cycle. SSA can also be converted to (−hydroxybutyrate (GBH) by SSA reductase [7-9].

The alcohol withdrawal syndrome is considered as a manifestation of neuroadaptive responses to chronic alcohol use with a special emphasis on the excitatory and inhibitory neurotransmitters. The glutamate-glutamine cycle in brain glial cells is essential to continue the biosynthesis of glutamate and GABA, which in turn helps in the maintenance of neurotransmitter balance [10]. Studies indicate that during alcohol withdrawal, GABA transmission decreases and glutamate transmission increases [11], reflecting the opposite effects of acute exposure [12]. Earlier reports have demonstrated elevations in glutamate, GABA, alanine and taurine in the hippocampus of fully kindled rats and suggest a shift in the balance between neurotransmitters towards increased production of excitatory amino acids, mediated by group II metabotropic glutamate receptors (mGluRs) during seizure kindling [13]. Syndler et al. [14] reported a fourfold increase in glutamate or GABA ratio, a theoretical marker of the neuronal excitation level in the kindled animals. The unique relationship of GABA activity strongly opposing glutamate function, both in the presence of ethanol and sudden withdrawal from ethanol [15], make the study of these two systems particularly interesting. The discontinuation of chronic administration of ethanol is associated with excitatory withdrawal signs called ethanol withdrawal syndrome (EWS). EWS is the most important evidence indicating the development of physical dependence on ethanol [16]. Although, attenuating the severity of EWS is very important, current treatment choices are very limited, except for the use of benzodiazepines, which comes with various side effects.

Medicinal plants in the form of plant extracts or their active components are used by almost 80% of the world population as a primary health care aid [17]. Ginger has a long history of use as herbal medicine and is the first reported plant with constituents possessing neuroprotective properties [18]. Ginger extract has been found to possess neuroprotective [19] and anxiolytic effects [20]. Earlier reports suggested that ginger also possesses protective efficiency against focal cerebral ischemia; ginger could attenuate memory impairment, neurodegeneration and brain infract volume in this condition [21]. Waggas et al. [19] demonstrated the neuroprotective properties of ginger root extract in monosodium glutamate-induced toxicity in different brain regions in male albino rats. In vitro data has shown that ginger’s active principles protect nerve cells and may have potential in the treatment of Alzheimer’s disease [22] and Parkinson’s disease [23].

This article highlights the effectiveness of ginger in the regulation of glutamine-glutamate-GABA metabolism that can lower glutamate levels and restore GABA synthesis during withdrawal from chronic ethanol consumption and prevent glutamate induced excitotoxicity, a root cause for neurodegeneration and induction of seizures—hence, rendering neuroprotection in ethanol withdrawal syndrome (EWS).

MATERIAL AND METHODS

Drugs and Chemicals: All chemicals used were of analytical grade unless otherwise mentioned.

Collection of Plant Material and Extract Preparation: Aqueous ginger extract was prepared from locally available ginger roots. Ginger rhizomes purchased from the local market were thoroughly washed, sliced, grated and ground to a fine paste. A weighed quantity (30g) of the paste was subjected to continuous hot extraction in a soxhlet apparatus using double distilled water. The extract was evaporated under reduced pressure using a rotary evaporator and then lyophilized until all of the solvent was removed. This aqueous ginger extract (AGE) was stored at 4°C.

Animals: Wistar strain male albino rats (3 months old; 200 - 220g) purchased from Sri Venkateswara Traders Pvt. Limited, Bangalore were maintained in polypropylene cages in the animal house of the department and used for the study. Standard conditions of humidity(50±9% relative humidity), room temperature (25 - 28°C) and 12 h light/dark cycle (6:00 A.M. to 6:00 P.M.) were maintained. A standard rodent diet (M/s Hindustan Lever Ltd., Mumbai) and water was provided ad libitum. All experimental procedures were approved by the CPCSEA on Animal Care, Govt. of India, bearing the CPCSEA No. 438 / 01/a/CPCSEA / IAEC / SVU / KSR-1 (dt: 11.09.2008).

Treatment Protocol: After a two-week adaptation period, animals were divided, six per group. Grouping involved 3 batches. The first batch included the control group, which was given normal saline. The second batch involved two groups of rats that were given 20% Alcohol (p.o.) at a dose of 2g/kg body weight and another group that was set to receive alcohol at same dose along with AGE (200 mg/kg body weight) via intra gastric intubation.
The third batch was treated the same way as the second batch, except that they were allowed to withdraw. All of the treatments were administered for 6 weeks (42 days); however, the withdrawal groups were allowed to withdraw from the drug for 3 days (72h) after the last dose.

In all, there were 5 experimental groups: I) Control group, II) ethanol treated group; III) ethanol plus AGE treated group; IV) ethanol withdrawal group; V) ethanol withdrawal plus AGE treated group.

**Tissue Collection:** After the stipulated period of treatments of the respective groups, the animals were sacrificed by cervical dislocation. The whole brain was removed, washed with ice-cold saline, blotted dry, frozen in liquid nitrogen and immediately transferred to the ice chamber at -80°C. Cerebrum (CC), hippocampus region (HC), pons medulla (PM) and cerebellum (CB) were separated as described by Nayak and Chatterjee [24] and used for the assays.

**Biochemical Procedures:** GDH activity was assayed using the method of Lee and Lardy [25], GS was estimated using the method of Wu [26], GLS was estimated spectrophotometrically using the method of Meister [27], Gln content was estimated by the acid hydrolysis method described by Colowick and Kaplan [28]; GABA-T activity and SSA contents in different areas of brain were estimated spectrophotometrically using the methods described by Nayak and Chatterjee [29].

**Data Analysis:** Statistical analysis was carried out by two-way ANOVA measurements to assess the statistical significance of effects using the Statistical Analysis System (SAS) software 9.0 and multiple comparison Tukey test. Bonferroni corrections were applied wherever necessary. Results are presented as mean ± SD, with P values of <0.01 considered significant.

**RESULTS**

**Effect of AGE on the Estimated Biochemical Parameters in Rat Brain Regions under Withdrawal Stress Induced Due to Chronic Ethanol Administration:**

**GDH Activity:** Figure 1 displays the comparison of activities of GDH between the withdrawal rats treated with ginger and the non-treated rats in the selected brain regions. In the CC and the PM, EtoH was significantly increased (p<0.01) compared to the control group. In the CB, EW was lower compared to the EtOH group and in the HC and the PM, EW was lower (p<0.01) compared to both the control and the EtoH groups.

**GS Activity:** GS was significantly increased (p<0.01) in the CC and the HC regions of the brain compared to the control group during withdrawal and markedly decreased in chronic ethanol intoxication. In the CB and the PM, significant increase was recorded in EW compared to the control and EtOH groups (p<0.01). The changes were insignificant when EtOH+ AGE and EW+ AGE were compared with the controls (Figure 2).

**GLS Activity:** The activity of GLS was found to be significantly less compared to the control group in the CC for the EtoH group, while EW was significantly increased compared to EtoH. In the CB, the only significant increase was observed in EW compared to EtOH group (Figure 3). In the HC and the PM, EW showed a significant increase (p<0.01) in activity compared to control while the enzyme activity was significantly decreased in the EtOH group compared to the control group. The changes were insignificant when EtOH + AGE and EW+ AGE were compared with the controls.

**Gln Content:** Metabolic concentrations of glutamine are compared in Figure 4. It is found that in the CC and HC of EW rats, significantly low Gln levels are recorded compared to control and ethanol groups. In CB and PM ethanol is significantly higher and EW is significantly (p<0.01) lower from control and also EW is less compared to EtOH. The changes are insignificant (p>0.01) when EtOH + AGE and EW+ AGE are compared with the controls.

**GABA-T Activity:** The activity of this enzyme remained significantly higher in the CC and CB regions compared to ethanol (Figure 5), while for other regions and comparison with control the changes remained insignificant (p<0.01). The changes were insignificant when EtOH+AGE and EW+AGE were compared with the controls.

**SSA:** The amount of change in succinic semialdehyde content was insignificant at p<0.01 (Figure 6). The changes were not as striking as for the other biochemical parameters that were referred to above, except for the only significant change in EW compared to EtoH in the CC region.
Fig. 1:

Changes in Glutamate Dehydrogenase (GDH) activity on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC: cerebral cortex, CB: cerebellum, HC: hippocampus, PM: pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from EtOH group.

Fig. 2:

Changes in Glutamine synthetase (GS) activity on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC: cerebral cortex, CB: cerebellum, HC: hippocampus, PM: pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from EtOH group.
Fig. 3:

Changes in Glutaminase (GLS) activity on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC- cerebral cortex, CB- cerebellum, HC- hippocampus, PM- pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from EtOH group.

Fig. 4:

Changes in Glutamine (Gln) content on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC- cerebral cortex, CB- cerebellum, HC- hippocampus, PM- pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from EtOH group.
Fig. 5:

Changes in γ-aminobutyrate transaminase (GABA-T) Activity on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC- cerebral cortex, CB- cerebellum, HC- hippocampus, PM- pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from Ethanol group.

Fig. 6:

Changes in Succinic Semialdehyde (SSA) content on pretreatment with aqueous extract of Ginger (AGE) in different brain regions of control and experimental rats (CC- cerebral cortex, CB- cerebellum, HC- hippocampus, PM- pons medulla). The data represents means ± S.D (n=6). ‘a’ statistically significant at p<0.01 from control group, ‘b’ statistically significant at p<0.01 from Ethanol group.
DISCUSSION

Alcohol dependence syndrome (alcoholism) includes the set of symptoms that appear when prolonged and excessive alcohol consumption is discontinued [30]. Development of acute withdrawal is a diverse and temporally dynamic process [31]. Therefore, it will be crucial to study the neurochemical changes during acute withdrawal, which may reveal larger changes; however, protracted withdrawal can last up to 1 month of abstinence in humans [32]. It is widely believed that the excitatory glutamatergic neurotransmission is responsible for the initiation and spread of seizure activity during ethanol withdrawal and GABA-mediated synaptic inhibition is known to be critical in regulating seizure activity and even a minor disinhibition can trigger neuronal hyperexcitability [33].

Perturbations in glutamate metabolism and glutamatergic neurotoxicity are known to be the characteristic features of the neurodegenerative effects of alcohol use disorders [34]. Changes occurring during EW and also parallel changes observed on pretreatment with AGE in the EW group are represented in Figure a.

Glutamate is the major excitatory neurotransmitter of the mammalian central nervous system and the major source of GABA [35]. Our study revealed increase in glutamate level in withdrawal rats to be significantly higher in the hippocampal region compared to the other regions of rat brain and these findings were consistent with the previously published reports [36]. Mitochondrial enzymes glutamate dehydrogenase, aspartate aminotransferase and glutaminase participate in the breakdown and generation of glutamate [37]. One of the pathways for glutamate formation is via glutamine, which is imported from glia by phosphate-activated glutaminase. Alternatively, glutamine may be formed from glutamate, originating from α-ketoglutarate in glia [38]. In the CNS, Glu is a precursor for both Glu as well as for GABA [39-41]. The glutamine/glutamate cycle participates in cell metabolism and has important relevance in normal and pathological functions. Glutamine synthesis is important in maintaining glutamate homeostasis in the brain, thereby protecting the brain from excitotoxicity [42-44]. When this cycle does not function adequately, CNS functional damage can appear and even cellular death can occur [45]. Glutamine synthetase is one of the key enzymes in glutamate metabolism and its decreased activity leads to augmentation of glutamate in the extracellular space. In the CNS, the conversion of glutamate to glutamine by glutamine synthetase represents a key mechanism in the regulation of excitatory neurotransmission under normal conditions as well as in injured brain [46]. Increased intracellular accumulation of Glutamate may decrease pH in astrocytes due to transporter-mediated inward H+ and outward K+ and OH− transport [47]. This Glutamate-induced acidosis may inhibit the glutamine synthetase pathway [48].

A decreased glutaminase activity during chronic ethanol ingestion might be responsible for decreased glutamate levels; decreased glutamate dehydrogenase and glutamine synthetase activity, during ethanol withdrawal might prevent the reversible deamination of L-glutamate and thereby lead to accumulation of the neurotransmitter, which may play a key role in excitation during withdrawal. There is enough scientific evidence which suggest that reduction in the activity of glutamate dehydrogenase was associated with many heterogeneous neurological disorders, like multisystem atrophy [49], temporal lobe epilepsy [50] contribute to cellular degeneration in PD [51]. Studies have also reported that elevated levels of glutamate are associated with decreased GDH activity in the focal area of human epileptic brain [52-54]. In the present study, during withdrawal, an increase in glutamine synthetase activity was observed which is consistent with the previous findings by other authors [55-57] suggesting an important role of glutamine synthetase in regulating the effects of widely accepted glutamatergic activation in the brain regions of withdrawal rats.

There has long been an association between GABA and ethanol [58, 59]. In another study, we reported the reduced concentration of GABA in brain during EW, which may be because of the altered metabolism of GABA and is in corroboration with the earlier studies [60]. Increasing evidence from the studies suggest that attenuation of GABAergic activity may result from decreased GABA synthesis or its increased catabolism, decreased release of GABA from nerve endings or its increased uptake and decreased efficacy of GABA at the post synaptic membrane or any of these combinations [61]. In neurons, glutamate is the primary source for GABA formation via GAD reaction [35]. Due to EW, increased glutamate level was presumed to cause inhibition of GAD by promoting dissociation of pyridoxal phosphate (PLP) from the GAD apoenzyme, in spite of the tight binding between PLP and GAD [62]. PLP is required
A decrease in GAD results in lesser production of GABA which further elevates excitotoxicity of the glutamate. GABA is metabolized by the enzyme GABA transaminase (GABA-T) to form succinic semialdehyde which is metabolized further to form succinic acid. Ginger is inhibiting the GABA-T activity thus exhibiting the property of GABAergic drugs. As a result the SSA levels decreased with a concurrent increase in the GABA content in the brain regions of EW rats treated with the ginger extract.

Another significant effect observed was the decreased GAD activity which has a key role in maintaining the levels of GABA during EW. A decrease in GAD is responsible for decreased GABA levels and GABA production and thereby an overall reduced inhibitory activity, all of which contribute to the withdrawal induced excitation and may lead to related seizures. Studies showed that brain substrates that trigger EW related seizures are largely in the brain stem and therefore, are distinct from those believed to be responsible for other clinically important seizure types [63]. Interestingly, AGE prevented the formation of substrates that set the stage for the onset of AWS in some of the most sensitive regions of brain with highest activity in the pons medulla. Therefore, it is recommended that ginger is a promising candidate to be developed as a food supplement against EW.

From our results, the antiglutamatergic effect of ginger appeared to show almost the same as the positive controls used in the study. It is apparent from our data that ginger is preventing the interruption of the glutamate-glutamine-GABA cycle that represents an...
important neurotoxic mechanism, which is the main cause for excitotoxicity during EW. Ginger is able to decrease glutamate levels by increasing the activity of GS and GDH and also by increasing the GABA levels by activating GAD. The results are consistent with decreased GABA levels in the brain stem, decreased GABA-T activity in the hemispheres and brain stem and enhanced GABA-T activity in the striatum of ethanol preferring rats compared with the control group following chronic consumption of ethanol [64]. A decrease in the GABA producing enzyme, GAD, was also reported in the behaviorally ethanol dependent model [65]. Decreased activity of SSA-R and SSA-DH was observed in the hemispheres and brain stems of alcohol-treated rats compared to the control rats in accordance with the fact that withdrawal (0.5-7 days) induced numerous profound changes in GABA metabolism [64].

Several studies have reported the use of various drugs against glutamate excitotoxicity in various neuropathological conditions; however, this study is the first of its kind which explored the potential use of ginger in the management and prevention of neuronal hyperexcitability during EW. While acute alcohol withdrawal can be easily managed with benzodiazepines [66], benzodiazepines, unless provided under controlled conditions of a detox unit, will not only be ineffective in preventing return to heavy drinking, but in fact increase the risk for it [67]. In animals, benzodiazepines have yielded variable effects, in some cases slowing withdrawal-induced kindling and in other cases, causing paradoxical worsening [68-70]. Therefore, exploring natural therapeutic agents to be used in the treatment of alcohol withdrawal or prevention of the onset of alcohol withdrawal syndrome (AWS) is the need of the hour.

The limitation of the study is that varied effects of ginger on brain glutamate and GABA composition through increase in GAD activity and changes in GABA-T activities during EW are regulated by distinct cellular and molecular factors that remain yet to be elucidated and requires further detailed study. Although use of ginger in itself cannot be recommended as a therapy for alcohol related brain disorders, its bioactive ingredients interact with neural substrates and developing new versions or analogs of these compounds may prevent the onset of hyperexcitability due to EW. Further exploratory studies allowing more sophisticated and detailed knowledge about the area under discussion are encouraged.

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CONCLUSION

It is concluded that the dietary ginger supplementation plays a significant role in the withdrawal induced altered status of the brain glutamate and GABA systems by influencing the GABA shunt and may suppress seizures due to EW. However, this requires further investigative studies at physiological, molecular, behavioral and genetic levels. In all, our current findings suggest that regulation of neural adaptations to withdrawal from chronic ethanol ingestion by ginger treatment observed in the cerebral regions appear to be mediated by factors regulated by distinct cellular and molecular mechanisms that remain yet to be elucidated. The follow up of this study will help in screening natural plant extracts, especially ginger, as the best alternative to antipsychotic drugs for use in drug withdrawal treatments.

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