

## Ginger Extract Defies Changes In Brain Serotonin Levels and Enzymes of Monoamine Metabolism During Withdrawal Following Chronic Ethanol Ingestion

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**Abstract:** In this study, the ameliorative effects of ginger extract on catecholamine metabolism were investigated in different brain regions of male wistar rats 72h post chronic ethanol (EtOH) ingestion. Main methods: Ethanol (2% w/v) was administered to rats by orogastric intubation. Biochemical investigation was performed for monoamine metabolising enzymes in five groups: (i) Control, (ii) EtOH treated, (iii) EtOH treated along with ginger extract (iv) Ethanol withdrawal (EW) and (v) EtOH withdrawal along with ginger extract groups of rats. Rats in each group were decapitated after six weeks except for the withdrawal groups, which were sacrificed 72h after the last dose of ethanol administration. The brains were removed and cerebral cortex, hippocampus, pons medulla and cerebellum were dissected. The biochemical estimations were done by conventional spectrophotometry. Key findings: Most significant difference in serotonin levels and monoamine metabolizing enzyme activities is observed among normal, ethanol treated and ethanol withdrawal groups ( $p < 0.01$ ). However, the serotonin levels and monoamine metabolizing enzyme activities in both EtOH + ginger extract and EtOH withdrawal + ginger extract group of rats was much lower as compared to the control group ( $p < 0.05$ ). The differences between EtOH intoxicated model and EW experimental group in comparison with their respective ginger extract treated groups, were highly significant ( $p < 0.01$ ). Significance: Current results suggest that monoamine metabolism seems to play a critical role in the brain regions during chronic ethanol consumption and undergoes drastic changes during EtOH withdrawal, the effects of which are modulated by ginger extract administration.

**Key words:** Chronic Ethanol Administration • Ethanol Withdrawal • Monoamines • Brain • Ginger

### INTRODUCTION

Long term ethanol (EtOH) intake affects the release of major neurotransmitters, such as dopamine (DA), gamma amino butyric acid (GABA), serotonin, noradrenaline and opioid peptides [1-3]. Alcohol intoxication results in tolerance and physical dependence. Changes in various neurotransmitter systems contribute to alcohol dependence and craving for alcohol [4, 5]. Several neuroadaptation theories held withdrawal as the result of physiological changes occurring within brain reward circuitries that divulge drug-opposite responses once the drug exposure is terminated [6]. The monoamine neurotransmitters, dopamine and serotonin have been implicated in EtOH abuse, as well as many other forms of

abuse and have been subjected to extensive study [7-9]. The understanding of the principle of modulating toxic effect of EtOH interfering with the neurotransmitters may have a significant impact on the development of successful therapeutic approaches for withdrawal and reversal.

Several classes of medications for managing ethanol withdrawal and relapse exist, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors and atypical antidepressants. All of these, however, have some sedative effects as well as some stimulating activity and, in some cases, adverse consequences. In view of the lesser side effects in comparison with their chemical counterparts, usage of herbal psychotropics is highly

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warranted and of late there has been a relentless search for medicinal plants to treat conditions such as anxiety, depression, seizures, poor memory, dementia, insomnia and drug intoxication [10-16]. Also studies indicated that the aqueous extract of ginger contains potent antioxidants such as polyphenols that may play important role in protecting the body against the hazards caused by alcohol abuse [17].

In this article, the psychotropic or behavior modifying activity of ginger in line with its previously established neuroprotective capability is evaluated. Several authors documented ginger's ability of neuroprotection against many conditions like Alzheimer's disease [18], in treatment of Parkinson's disease [19], against focal cerebral ischemia in attenuating memory impairment, neurodegeneration and brain infarct volume [20] and against monosodium glutamate -induced toxicity [21]. *Zingiber officinale* (Zingiberaceae) rhizome is also known to possess potent memory enhancement in scopolamine - induced memory impairment [22] and exhibits anxiolytic properties too [23, 24]. The neuroprotective effect and cognitive enhancing properties of ginger is partly attributed to its antioxidant activity [25]. The bio-active compounds of ginger, 6-gingerol and 6-shogaol have been shown to have a number of pharmacological activities, including antipyretic, analgesic, antitussive and hypotensive effects [26]. This study predicts prevention in the alteration of the levels of monoamines due to ginger administration against a deficiency in the release of these neurotransmitters and their metabolites in different regions of rat brain during withdrawal from chronic ethanol ingestion.

## MATERIAL AND METHODS

**Collection of Plant Material:** The rhizomes of *Z. officinale* used in this work are purchased from local vegetable market, Tirupati Andhra Pradesh, India and authenticated by qualified botanist at Department of Botany, S.V. University, Tirupati andhra Pradesh, India.

**Preparation of Aqueous Ginger Extract:** Whole rhizome of ginger is thoroughly washed, sliced, grated and grind to fine paste. A weighed quantity (30g) of the paste is subjected to continuous extraction in soxhlet apparatus using nanopure water as the solvent. The extract is evaporated under reduced pressure using rotary evaporator and then lyophilized until all the solvent has been removed to give an aqueous ginger extract (AGE) sample and stored at 4°C for further studies.

**Maintenance of Experimental Animals:** Male wistar albino rats (200-220g) purchased from Sri Venkateswara animal suppliers, Bangalore are used in the study. They are placed in polypropylene cages and maintained in a quiet, temperature and humidity-controlled room (24± 4°C and 50± 9%, respectively) in which 12-24h light-dark cycle is maintained (06:00-18:00 h light). The animals are fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water is given *ad libitum*. The Animal ethical Committee of the Department of Zoology, IAEC (Institutional Animal Ethics Committee), Sri Venkateswara University, Tirupati approved all experiments. Animal experiments are carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and empowerment, Government of India. All efforts are made to minimize animal suffering and reduce the number of animals used.

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

**Treatment Protocol:** Rats are divided into five experimental groups randomly (n=6 for each group): five groups: (i) Normal, (ii) EtOH treated, (iii) EtOH treated along with aqueous ginger extract (AGE) (iv) Ethanol withdrawal (EW) and (v) EtOH withdrawal along with ginger extract (AGE) groups of rats. Except for normal group, other groups were subjected to the administration of ethanol (2%, w/V) for 6 weeks as previously described [27]. Orogastric administration of ethanol is given to the rats at the same time of day (10:00 h). Ginger extract is dissolved in double distilled water at a concentration of 200 mg/ Kg bw and is prepared fresh on the morning of each experiment and is administered via intragastric intubation.

Normal group is given normal physiological saline. Both the ethanol dependence model and the ethanol along with 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. For the EW group, at the end of the exposure to 2% ethanol for 6 weeks, ethanol is withdrawn by preventing the administration for the next 72 hrs (10:00 h) after the last dose; the EW+ GAE group is treated the same way as the EW group except that this group is pretreated with ginger extract at concentration of 200 mg/kg bw before withdrawal.

**Isolation of Tissues:** After the intended treatment duration, the animals are sacrificed by cervical dislocation and the brain tissues (Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC)) were immediately isolated, frozen in liquid nitrogen and were stored at -80°C until analyses.

#### Biochemical Analysis:

**Serotonin (5-Hydroxytryptamine, 5-HT) Estimation:** Serotonin (5-HT) was estimated by the method of Kari *et al.*, [28]. The amount of serotonin was calculated by the method of Ansell and Beeson [29] and expressed in µg/gm wet weight of tissue.

**5-HydroxyIndole Acetic Acid (5-HIAA) Levels:** 5-HIAA was assayed by the method of Haubrich and Denzer [30].

#### Enzymatic Analysis

**Tyrosine hydroxylase Activity (TH):** Tyrosine hydroxylase activity was estimated by the method of Craine *et al.*, 1972 [31] and Shiman *et al.*, [32].

**Dopamine beta hydroxylase (DBH):** The activity of Dopamine β Hydroxylase (DBH) is assayed by the method of Srivastava [33] and Kapoor [34] by a modification on the procedure of Nagatsu and Udenfriend [35].

**Monoamine Oxidase (MAO) Activity:** Monoamine oxidase activity was measured by the method of and Green and Haughton [36].

**Catechol-O-Methyltransferase (COMT) Assay:** The procedure carried out is based upon Arnow's method [37] as described by Dhar and Rosazza [38].

**Statistical Analyses:** All the data presented are as mean ± SD. Comparison of more than two groups is performed by analysis of variance (ANOVA) done using SPSS statistical software, followed by multiple comparison test using Scheffe's *posthoc* analysis.

## RESULTS

**Neurochemical Changes:** The following changes in the serotonin metabolism are observed during chronic ethanol intoxication as well as acute withdrawal from ethanol under the influence of ginger extract administration in the cerebral cortex (CC), cerebellum (CB), hippocampus (HC) and pons-medulla (PM) regions of rat brain

**Serotonin:** An increase in ( $p < 0.001$ ) serotonin levels following chronic ethanol treatment is indicated from the results in the order given below (Table 1):

$$HC > CC > CB > PM$$

Following the ethanol withdrawal, decreased 5-HT levels are observed in all areas of the brain when compared with controls. HC recorded the highest decrease (39.79%), followed by PM (36.02%), CB (30.80%) and CC (30.23%).

$$HC < PM < CB < CC$$

**5-Hydroxy- 3-Indoleacetic Acid (5-HIAA):** Elevated levels of 5-HIAA is seen in all brain regions during chronic EtOH treated group as follows (Table 1):

$$HC > CC > CB > PM$$

Whereas levels of 5-HIAA recorded decrease that is statistically significant at  $p < 0.05$ , in all the brain regions during EW in the following order. The decrease is maximum in CB and the least in PM.

$$CC (-29.09\%) > PM (-16.79\%) > HC (-14.33\%) > CB (-11.82\%)$$

Ginger extract treatment to the animal groups showed no significant changes from the normal controls.

**5-HIAA/5-HT:** A decrease in 5-HIAA/5-HT ratio in the brain regions is observed at chronic ethanol intoxication in the order as follows (Fig A.):

$$HC (-44.18\%) > CC (-34.54\%) > CB (-26.67\%) > PM (-20.54\%)$$

5-HIAA/5-HT ratio recorded a increase that is statistically significant at  $p < 0.05$ , in the ethanol withdrawal group in the following order

$$HC (+37.2\%) > CC (+21.81\%) > CB (+15.55\%) > PM (+12.32\%)$$

However, 5-HIAA/5-HT ratio recorded minor changes that are statistically insignificant in the groups treated with the ginger extract on comparing with the normal controls.

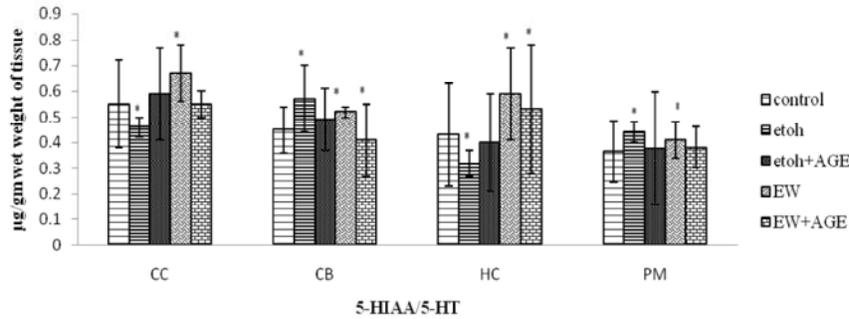


Fig. A: Changes in 5-HIAA/5-HT ratio in different brain regions due to pretreatment of ginger extract prior to ethanol withdrawal. Values are expressed in µg /gm wet weight of tissue. All the values are mean±SD of six individual observations. \* indicates values are significant at  $p < 0.001$  compared to Control in Scheffe's test

Table 1: Serotonin Metabolism during chronic ethanol intoxication as well as acute withdrawal from ethanol under the influence of ginger extract administration. Values are expressed in µg /gm wet weight of tissue. All the values are mean±SD of six individual observations. Values with <sup>a</sup> are significant at  $p < 0.05$  compared to Control; <sup>b</sup> are

GROUPS	5-HT				5-HIAA			
	CC	CB	HC	PM	CC	CB	HC	PM
Control	3.282±0.27	2.935±0.19	3.483 ±0.34	2.149±0.26	1.801 ±0.8	1.311±0.14	1.507±0.53	1.721±0.31
Ethanol	4.891 <sup>a</sup> ±1.48	4.046 <sup>a</sup> ±1.64	5.268 <sup>a</sup> ±1.09	2.631 <sup>a</sup> ±0.42	2.673 <sup>a</sup> ±0.22	1.776 <sup>a</sup> ±0.74	2.394 <sup>a</sup> ±0.89	1.983 <sup>a</sup> ±0.11
Ethanol+AGE	3.057 <sup>b</sup> ±0.483	2.673 <sup>b</sup> ±0.39	3.73 <sup>b</sup> ±0.42	2.241 <sup>b</sup> ±0.64	1.795 <sup>b</sup> ±0.48	1.301 <sup>b</sup> ±0.89	1.49 <sup>b</sup> ±0.45	1.692 <sup>b</sup> ±0.18
EW	2.29 <sup>ab</sup> ±0.34	2.031 <sup>ab</sup> ±0.56	2.097 <sup>ab</sup> ±0.24	1.46 <sup>ab</sup> ±0.27	1.636 <sup>bc</sup> ±0.5	1.178 <sup>bc</sup> ±0.27	1.410 <sup>bc</sup> ±0.19	1.652 <sup>bc</sup> ±0.34
EW + AGE	2.974 <sup>bc</sup> ±1.83	2.898 <sup>bc</sup> ±0.98	3.866 <sup>bc</sup> ±0.27	1.992 <sup>bc</sup> ±0.73	1.277 <sup>ab</sup> ±0.91	1.156 <sup>ab</sup> ±0.172	1.29 <sup>ab</sup> ±0.19	1.65 <sup>ab</sup> ±0.65

Significant at  $p < 0.05$  compared to EtoH; <sup>c</sup> are significant at  $p < 0.05$  compared to EW; <sup>a</sup> are significant at  $p < 0.001$  compared to Control in Scheffe's test

Table 2: Changes in activities of Monoamine Synthesizing Enzymes

GROUPS	TH‡				DBH			
	CC	CB	HC	PM	CC	CB	HC	PM
Control	5.531±0.28	4.564±0.92	4.854±0.17	4.746±0.57	3.471±0.51	5.499±0.43	3.471±0.51	6.081±0.54
Ethanol	7.948 <sup>a</sup> ±.24	6.377 <sup>a</sup> ±0.21	6.323 <sup>a</sup> ±0.35	5.897 <sup>a</sup> ±0.40	1.864 <sup>a</sup> ±0.34	3.787 <sup>a</sup> ±0.3	2.68 <sup>a</sup> ±0.64	4.818 <sup>a</sup> ±0.15
Ethanol+AGE	5.091 <sup>b</sup> ±0.23	4.257 <sup>b</sup> ±0.30	4.566 <sup>b</sup> ±0.19	4.398 <sup>b</sup> ±0.41	3.613 <sup>b</sup> ±0.33	7.074 <sup>b</sup> ±0.54	4.89 <sup>b</sup> ±0.09	7.074 <sup>b</sup> ±0.56
EW	2.512 <sup>ab</sup> ±0.51	2.526 <sup>ab</sup> ±0.18	2.88 <sup>ab</sup> ±0.15	1.998 <sup>ab</sup> ±0.31	5.844 <sup>ab</sup> ±0.24	8.641 <sup>ab</sup> ±0.31	5.206 <sup>ab</sup> ±0.28	8.084 <sup>ab</sup> ±0.44
EW + AGE	4.821 <sup>bc</sup> ±0.34	3.1987 <sup>bc</sup> ±0.15	2.598 <sup>bc</sup> ±0.13	3.473 <sup>bc</sup> ±0.29	3.287 <sup>bc</sup> ±0.19	7.679 <sup>bc</sup> ±0.27	4.803 <sup>bc</sup> ±0.18	6.131 <sup>bc</sup> ±0.51

All the values are mean±SD of six individual observations. Values are expressed as ‡ = µmoles of L-DOPA /mg of protein /hr; ¶ = µmoles of β-hydroxybenzaldehyde/mg of protein /hr.

Values with <sup>a</sup> are significant at  $p < 0.05$  compared to Control; <sup>b</sup> are significant at  $p < 0.05$  compared to EtoH; <sup>c</sup> are significant at  $p < 0.05$  compared to EW; <sup>a</sup> are significant at  $p < 0.001$  compared to SC in Scheffe's test

**Enzyme Analysis**

**Monoamine Synthesizing Enzymes:**

**Tyrosine Hydroxylase (TH) Activity:** During chronic ethanol intoxication the TH activity is found to be increased ( $p < 0.05$ ) in the following order in the brain regions under study (Table 2):

CC(+43.71%) > CB (+39.72%) > HC (+30.27%) > PM(+24.25%)

EW could decrease ( $p < 0.05$ ) the tyrosine hydroxylase activity in the following series:

CC(-54.58%) > CB (-44.65%) > HC (-40.67%) > PM(-26.82%)

Ginger extract administration to the EW rats increased ( $p < 0.001$ ) the enzyme activity in comparison with the ginger extract untreated EW group.

**Dopamine Beta Hydroxylase (DBH) Activity:** DBH activity is found to be increased ( $p < 0.05$ ) during EW and decreased ( $p < 0.05$ ) during chronic ethanol treatment in comparison with the saline control as follows (Table 2):

Table 3: Changes in activities of Monoamine Degrading Enzymes

GROUPS	COMT□				MAO@			
	CC	CB	HC	PM	CC	CB	HC	PM
Control	5.304±1.05	3.958±1.94	5.087±1.72	3.45±1.87	1.921±0.78	1.164±0.75	1.037±0.47	1.159±0.82
Ethanol	3.281 <sup>a</sup> ±1.99	2.933 <sup>a</sup> ±1.62	3.867 <sup>a</sup> ±1.53	2.732 <sup>a</sup> ±1.15	2.622 <sup>a</sup> ±0.75	1.534 <sup>a</sup> ±0.56	1.432 <sup>a</sup> ±0.93	1.513 <sup>a</sup> ±0.56
Ethanol+AGE	5.112 <sup>b</sup> ±1.27	3.824 <sup>b</sup> ±1.91	4.979 <sup>b</sup> ±1.99	3.936 <sup>b</sup> ±1.96	1.834 <sup>b</sup> ±0.677	1.188 <sup>b</sup> ±0.53	1.121 <sup>b</sup> ±0.64	1.097 <sup>b</sup> ±0.69
EW	8.399 <sup>ab</sup> ±1.22	6.035 <sup>ab</sup> ±1.18	7.677 <sup>ab</sup> ±1.57	4.897 <sup>ab</sup> ±1.65	1.758 <sup>ab</sup> ±0.69	1.179 <sup>ab</sup> ±0.82	1.023 <sup>ab</sup> ±0.69	1.196 <sup>ab</sup> ±0.87
EW + AGE	5.394 <sup>bc</sup> ±1.74	4.101 <sup>bc</sup> ±1.15	5.112 <sup>bc</sup> ±1.11	3.873 <sup>bc</sup> ±1.82	1.767 <sup>bc</sup> ±1.48	1.132 <sup>bc</sup> ±0.68	1.089 <sup>bc</sup> ±0.44	1.201 <sup>bc</sup> ±0.99

All the values are mean±SD of six individual observations. Values are expressed as ?= μmoles of 7-hydroxy-6-methoxycoumarin /mg of protein /hr; @= μmoles of P-hydroxyphenylacetaldehyde /mg of protein /hr.

Values with <sup>a</sup> are significant at  $p<0.05$  compared to Control; <sup>b</sup>are significant at  $p<0.05$  compared to EtoH; <sup>c</sup> are significant at  $p<0.05$  compared to EW; \*are significant at  $p<0.001$  compared to Control in Scheffe's test

### Chronic Ethanol Treatment:

CC(-46.21%) > CB (-31.13%) >  
HC (-22.79%) > PM (-20.76%)

### Ethanol Withdrawal:

CC (+68.36%) > CB (+57.14%) >  
HC (+49.98%) > PM (+32.93%)

Treatment with ginger extract during chronic ethanol treatment and pretreatment during ethanol withdrawal prevented any significant changes in comparison with the controls.

### Monoamine Degrading Enzymes

**Catechol-O-Methyltransferase (COMT) Assay:** Increased enzyme activity is observed during withdrawal while during chronic ethanol administration the activity is found decreased (Table 3).

The changes observed can be sequenced as follows: chronic ethanol treatment :

CC(-38.14%) > CB (-25.88%) >  
HC (-23.97%) > PM(-20.81%)

### Ethanol Withdrawal as:

CC(+58.36%) >CB (+52.47%) >  
HC (+50.92%) > PM(+41.93%)

The chronic ethanol treatment and ethanol withdrawal groups on treatment with ginger extract didnot record any significant changes in the COMT activity in comparison with the saline control.

**Monoamine Oxidase (MAO) Assay:** During chronic ethanol treatment, a decrease is observed from the investigation in the monoamine oxidase activity. The decrease in activity of MAO in different brain regions can be summarized as below (Table 3):

### During Chronic Ethanol Treatment:

HC (-38.09%) > CC(-36.49%) >  
CB (-31.78%) > PM(-30.04%)

During Ethanol withdrawal as well as in the groups treated with the ginger extract minor changes are recorded that are statistically insignificant in comparison with the normal controls.

### DISCUSSION

Among the several neurotransmitter systems known to be associated with the pharmacological effects of alcohol, DA and 5-HT have received particular attention because of their apparent role in the motivational effects of EtOH [7, 8,39-41]. The involvement of biogenic amines in the mediation of central and peripheral effects of ethanol has been postulated by several authors [42-44]. Increased turnover rates of several amines are found to be associated with increased concentrations of their metabolites [45-46]. Neuroadaptation, the compensatory changes in neurochemical systems that are activated by alcohol underlie the symptoms of withdrawal after chronic administration. The present study investigated the effects of repeated and prolonged ethanol administration (42 days) at a dose of 2 g/kg, *po* and 72 h after ethanol withdrawal, on the levels of DA, 5-HT and their metabolites in the selected rat brain regions.

This investigation reports decreased Serotonin levels in brain regions of withdrawal rats which is consistent with the several lines of evidence that showed reduced 5-HT and 5-HIAA levels in rodent whole brain, as well as in limbic and striatal tissue preparations after ethanol withdrawal [6,47-49]. Lowered levels of 5-HIAA in connection with decreased levels of serotonin in the central nervous system is often associated with depression in Parkinson's disease and dysthymia [50]. Berggren *et al.*, [51] observed a negative correlation between prolonged and excessive alcohol consumption

and central serotonergic neurotransmission due to the toxic effect of alcohol on 5-HT neurons. Due to ginger pre-administration in the withdrawal group, the serotonin levels are increased and 5-HIAA levels decreased in comparison with the EW group. It is reported that anxiolytic drugs work by increasing 5-HT and decreasing 5-HIAA levels [52]. Taking into consideration the propelling role of 5-HT on DA release in the nucleus accumbens (NAc), an extraneuronal deficit of serotonin may be expected to exacerbate a DA - dependent reward deficit [53-56]. In milieu, it is remarkable to note that the progression of the extracellular deficits in the present study paralleled closely with the emergence of withdrawal symptoms such as hyper-irritability, behavioral inhibition and anxiogenic effects, which are similar to the effects observed in similar work by earlier researchers [57-59]. In addition, it is proved that several ginger components exhibit serotonin receptor-blocking activity [60-61]. Thus, the observed cycle of behavioral and neurochemical changes by ginger extract treatment may be a reflective of the role for ginger in reversing the depleted DA and 5-HT availability that accompany ethanol withdrawal. A decreased tendency in 5-HIAA/5-HT ratio was observed in the hippocampus at the chronic EtOH dose, which was consistent with the previous findings [62].

Catecholamine biosynthesis is regulated at the tyrosine hydroxylase step [63]. The results suggested that EW could reduce the tyrosine hydroxylase activity while still decreasing the dopamine levels. There is evidence which suggests that depletion of brain dopamine leads to parallel decreased tyrosine hydroxylase activity [64]. Dopamine- $\beta$ -hydroxylase (DBH) activity was found to be high during EW and less during chronic ethanol intoxication. It is well established that DBH inhibition increases tissue levels of DA [65] which supports the results observed in this investigation. Ginger administration produced significant changes in the activity of both these enzymes in ethanol treated and withdrawal groups with respect to their saline controls as well as in relation to each other.

Catecholamines are quickly inactivated, metabolized by the enzymes catechol-oxy methyltransferase (COMT) and monoamine oxidase (MAO). The activity of these enzymes is found to be reduced in ethanol intoxicated rats while co-administration of ginger along with chronic ethanol treatment in both the withdrawal groups does not cause statistically significant changes from saline control. Conversely, an increased activity of COMT is observed during EW. A functional polymorphism in this enzyme resulting in an increased activity has been

associated with alcohol dependence and polysubstance use [66, 67]. Visual and auditory disturbances among people with alcohol dependence in withdrawal symptoms suggest that COMT activity could partially affect the appearance of delirium tremens in these individuals [68]. COMT inhibitors are found to reinforce and prolong the effects of catecholamines.

As summarized above, EW works in every way substantiating the impairment of monoaminergic transmitter systems conducting to the after effects, craving and reversal, while ginger administration challenges the onset of these effects of ethanol. Generalizations are complex, although monoamine system shows a trend of maintaining normalcy due to ginger administration in all the brain regions under study. More importantly, these results suggest the role of ginger mitigating withdrawal-associated deficiencies in monoamine function in brain regions that have been implicated in the acute reinforcing effects of alcohol and other substances of abuse. Unfortunately, the mechanism that links ginger usage in the improvement of regional monoamine metabolism in the rat brain remains yet to be ascertained. The results obtained for the cerebral cortex and hippocampus are most remarkable for the large variability observed in the 5-HIAA concentrations in relation to 5-HT occurring in these brain regions due to withdrawal.

It is believed that 5-HT<sub>3</sub> receptor modulates the release of several neurotransmitters including acetylcholine, cholecystokinin, dopamine glutamate, norepinephrine and particularly  $\gamma$ -aminobutyric acid [69, 70] and ginger prevents this because of its well established anti 5-HT<sub>3</sub> receptor effects (serotonin receptor-blocking activity) [71]. Ginger has long been used in folk medicine as a treatment for anxiety and depression. Ginger is reportedly identified as containing 1-(3'-methoxy-4'-hydroxyphenyl)-5-hydroxyalkan-3-ones, known as [3, 6, 8, 10] and [12] gingerols (having a side-chain with 7-10, 12, 14, or 16 carbon atoms, respectively) and their corresponding dehydration products, which are known as shogaols. Qualitative analysis by thin-layer chromatography, qualitative and quantitative gas chromatography and high performance liquid chromatography analyses of ginger oils revealed presence of gingerols, shogaols,  $\alpha$ -zingiberene,  $\beta$ -bisabolene,  $\beta$ -sesquiphellandrene and *ar*-curcumene and sesquiterpene hydrocarbons that include (-)-zingiberene, (+)-*ar*-curcumene,  $\beta$ -bisabolene and (-)- $\beta$ -sesquiphellandrene. Monoterpene aldehydes and alcohols are also found to be present. Ginger is believed to act by "mimicking the serotonergic effects of

antidepressant medication". These components may be concerned in alleviation of depression and mitigating craving due to influence on monoamine changes in brain tissues in alcohol treated rat groups.

Studies from several investigators showed that zingerone and other derivatives from ginger inhibit the release of monoamine neurotransmitters, especially serotonin [23,72]. Considering the findings of the above mentioned studies and data from the present study, it can be suggested that ginger influences the levels of monoamine neurotransmitters reducing motivational effects and craving due to ethanol withdrawal as well as origin of alcohol withdrawal syndrome.

This study is the first of its kind involving ginger in ethanol withdrawal and the inexplicable influence of ginger on serotonin and monoamine enzyme systems, which makes it difficult to draw definitive conclusions about the manipulating effects of dietary ginger on the monoamines of rat brain. This interplay of monoaminergic pathways in EW under the influence of ginger needs further elucidation. The overall effect of ginger extract administration during ethanol withdrawal in relation to these monoaminergic neurotransmitter systems, has to be thoroughly investigated further, which can turn out to be beneficial, embarking an era of pristine therapies for drug withdrawal, particularly, ethanol withdrawal.

**Declaration of Interest:** None Declared.

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