

Assessment of Changes in the Dopaminergic Neurotransmitter System Due to Acute Withdrawal after Chronic Ethanol Consumption During the Course of Ginger Administration

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Abstract: The objective of the study was to examine the effect of chronic ethanol exposure and ethanol withdrawal on the release of dopamine (DA) metabolism in ethanol-maintained reinforcement in dependent rats and possible protective role of ginger extract on monoamine system in the aforementioned conditions. Ethanol was given by a liquid diet for 42 days along with ginger extract administration. The concentration of ethanol was maintained at 2% (w/v) and ginger extract was 200mg/kg bw throughout the period of exposure. Various brain regions were assayed for levels of DA, 3,4-Dihydrophenylacetic acid (DOPAC) and Homovanillic acid (HVA). Results revealed significant decrease in DA and 5-HIAA levels during the first 72 h of ethanol withdrawal which suggest that the levels of DA and its metabolites are altered by ethanol withdrawal. In Conclusion, daily alcohol consumption may have facilitated dopamine release, however, dopamine and its acid metabolites were reduced after abruptly stopping chronic ethanol administration. All these changes, as well as ethanol-withdrawal behavioural signs, were not observed in rats given ginger extract administration along with ethanol. By this experiment it is possible to predict a prevention or reversal of withdrawal-induced neurochemical deficiencies due to ginger administration after subjected to withdrawal from ethanol consumption.

Key words: Ethanol-Withdrawal • DA • DOPAC • HVA • Ginger • Brain Regions

INTRODUCTION

Dopamine represents 50% of the total catecholamines content in the CNS. The highest levels of dopamine are found in the neo-striatum and nucleus accumbens. The presence of higher amounts of DA in the brain, especially the striatum, was discovered by Carlsson, 1959 [1]. Subsequently, extensive studies were conducted on DA and its metabolism [2,3]. All these studies indicate that DA is a central neurotransmitter. Dopamine also acts as an important neurotransmitter in the peripheral nervous system affecting both gastrointestinal and cardiovascular activity [4].

The vast majority of dopaminergic cells arise from the midbrain, although some also arise from the diencephalons. The midbrain dopaminergic neurons of the substantianigra pars compacta and the ventral tegmental area provide substantial dopaminergic inputs to the cortex, caudate-putamen, nucleus accumbens,

amygdale and septum [5]. The major dopaminergic tract originates in the zona compact of the substantianigra and sends axons to the caudate nucleus and putamen of the striatum. Nearly 80% of the brain dopamine is found in the corpus striatum.

Dopamine synthesis was dependent on the amino acid precursor tyrosine, the first and most important rate limiting step in the biosynthesis of catecholamines is the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) in the presence of tyrosine hydroxylase. The subsequent decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase results in the formation of Dopamine. After the synthesis, DA is transported to storage vesicles or is metabolized in the cytoplasm [6]. DA nerve terminals possess high affinity DA uptake sites which play an important role in the inactivation and recycling of DA released into the synaptic cleft by actively pumping extracellular DA back into the nerve terminal [7].

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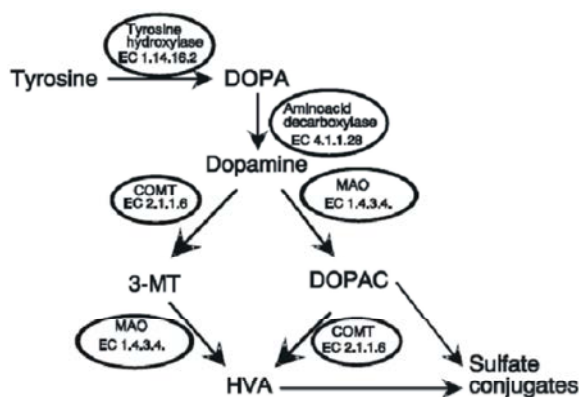
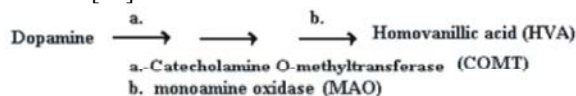


Fig. 2.3: Simplified presentation of the synthesis of dopamine and its major metabolic routes. COMT = catechol-O-methyltransferase, MAO = monoamine oxidase, 3-MT = 3-methoxytyramine, HVA = homovanillic acid, DOPAC = 3,4-dihydroxyphenylacetic acid

The main DA metabolizing enzymes are monoamine oxidase (MAO), catechol-O-methyltransferase (COMT) and aldehyde dehydrogenase (ADH) and the main metabolites of DA are 3, 4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA). DA released into the synaptic cleft can be inactivated both by reuptake into the dopaminergic nerve terminals, or is metabolized intraneuronally to corresponding aldehyde by MAO. Subsequently the formed aldehyde is oxidized to DOPAC by AH [7, 8]. About 90% of DA is metabolized to DOPAC in rat brain [9]. After the diffusion from the neurons, DOPAC can be further metabolized to HVA by COMT. 3-MT, the extraneuronal DA metabolite, is formed from released DA by COMT and is further metabolized to HVA by MAO and AH. Thus, the tissue and extracellular concentration of 3-MT is being used as an index of DA release [8-11]. The rate of catecholamine depletion after synthesis/inhibition is used as an index of catecholamine turnover [12].



The present study aims to determine changes in dopaminergic system both in conditions of chronic ethanol consumption and ethanol withdrawal. since dopamine agonists prevented ethanol withdrawal seizures and effectively proved to be treatment of ethanol withdrawal seizures in rats, this study has been designed to investigate the dopamine agonistic property of ginger (*Zingiber officinale*), a natural herb used for centuries

worldwide, in treating various health ailments. Since Alcohol withdrawal encapsulates the key clinical findings of anxiety, tremors, headache, disorientation, agitation, delirium, hallucinations (tactile, visual, auditory), insomnia, anorexia, nausea, vomiting, diaphoresis, hyper-reflexia, tachycardia, hypertension, arrhythmia or myocardial infarction, seizures, hyperventilation, differences in mental functioning, change in cognition and potentially fatal convulsions, all of which are controlled by prominent centers in regions of brain like cortex, cerebellum, hippocampus and pons medulla, these brain regions are selected for the current study.

MATERIAL AND METHODS

Collection of Plant Material: The rhizomes of *Z. officinale* used in this work are purchased from local vegetable market, Tirupati, Aandhra 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 was 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, Banglore were 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 12-24 h light-dark cycle is maintained. The animals were 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, CPCSEA No. 438 / 01/a / CPCSEA / IAEC / SVU / KSR-1 (dt: 11.09.2008).

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 with minor modification [13]. 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/kgbw 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 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+ AGE 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 predetermined treatment duration, the animals are sacrificed by cervical dislocation and the brain tissues viz. Cerebral Cortex (CC), Cerebellum (CB), Pons Medulla (PM) and Hippocampus (HC) was immediately isolated, frozen in liquid nitrogen and were stored at -80°C until analyses.

Experimental Investigation

Ethanol Withdrawal Scores: Rating scales are determined according to Gray *et al.* [14], with minor modification. For withdrawal testing, 72 hrs after termination of last dose of ethanol administration, rats are exposed to an audiogenic stimulus (100 dB) for 1 min. The intensity of the seizures was scored as follows: seizures were rated on a five-point scale ranging from 1 to 5. A score of 1 was assigned to rats showing only wild running. The rats showing tonic and tonic-clonic seizures in addition to wild running were given scores of 2 and 3, respectively. A score of 4 was assigned to the rats with longer lasting periodic (>90 s) tonic-clonic seizures. A score of 5 was given if mortality occurred. The intensity of the parameters is expressed as a median value.

Neurochemical Changes

Dopamine (DA) Estimation: Dopamine was estimated by the method of Kari *et al.* [15]. The amine content of each sample was calculated by the method of Ansell and Beeson, [16] and the content was expressed as µg/gm wet wt. of tissue.

3,4-Dihydroxyphenylacetic Acid (DOPAC) Levels: DOPAC was estimated according to Murphy *et al.* [17] and expressed in µmoles of Dopamine / gm wet tissue.

Homovanillic Acid (HVA) Levels: After the sample has been read for HIAA, 1ml of the same is transferred to another tube for estimation of HVA following the method of Anden *et al.* [18] and expressed as µg of HVA / gm wet weight of tissue.

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

Withdrawal Syndrome Scores: The median values of each behaviour is summed for an individual observation period as shown in Fig. A.

Neurochemical Changes: The following changes in the monoamine 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.

Dopamine: An increase ($p < 0.001$) in the levels of dopamine following chronic ethanol treatment is indicated from the results as below (Table 1):

HC > CB > CC > PM

Following the ethanol withdrawal, decreased DA levels are observed in all areas of the brain when compared with saline controls. CB recorded the highest decrease (45.47%), followed by HC (42.87%), PM (39.96%) and CC (36.56%).

CB < HC < PM < CC

Table 1: Dopamine Metabolism

Groups	DA				DOPAC				HVA			
	CC	CB	HC	PM	CC	CB	HC	PM	CC	CB	HC	PM
Control	3.334±0.76	2.905±0.69	3.839±0.73	2.147±0.52	1.684±0.06	1.499±0.24	1.931±0.40	1.227±0.19	3.74±0.06	3.827±0.28	4.548±0.42	3.241±0.95
Ethanol	4.432 ^a ±0.32	4.007 ^a ±0.14	5.643 ^a ±0.11	2.596 ^a ±0.26	2.067 ^a ±0.34	1.888 ^a ±1.06	2.638 ^a ±0.23	1.463 ^a ±0.10	4.97 ^a ±0.63	4.434 ^a ±0.04	5.671 ^a ±0.41	3.615 ^a ±0.06
Ethanol+AGE	3.101 ^b ±1.18	2.914 ^b ±0.85	3.886 ^b ±1.6	2.049 ^b ±0.13	1.618 ^b ±0.15	1.543 ^b ±0.29	2.024 ^b ±0.07	1.359 ^b ±0.12	3.91 ^b ±0.88	3.879 ^b ±1.17	4.676 ^b ±1.20	3.287 ^b ±1.59
EW	2.115 ^{ab} ±1.04	1.584 ^{ab} ±0.43	2.193 ^{ab} ±1.2	1.289 ^{ab} ±1.81	1.536 ^{ab} ±0.09	1.274 ^{ab} ±0.18	1.678 ^{ab} ±0.34	1.079 ^{ab} ±0.08	3.23 ^{ab} ±0.39	2.914 ^{ab} ±0.34	2.884 ^{ab} ±0.53	3.041 ^{ab} ±0.29
EW + AGE	3.116 ^{bc} ±0.42	2.923 ^{bc} ±0.127	3.724 ^{bc} ±1.24	2.038 ^{bc} ±0.87	1.579 ^{bc} ±0.18	1.567 ^{bc} ±0.41	1.951 ^{bc} ±0.08	1.253 ^{bc} ±0.06	3.76 ^{bc} ±1.62	3.913 ^{bc} ±1.93	4.517 ^{bc} ±1.81	3.271 ^{bc} ±1.56

Values are expressed in µg/gm wet weight of tissue. All the values are mean±SD of six individual observations. Values with ^a are significant at P<0.05 compare to SC; ^b are significant at P<0.05 compare to EtoH; ^c are significant at P<0.05 compare to EW; ^d are significant at P<0.001 compare to SC in Scheffe's test

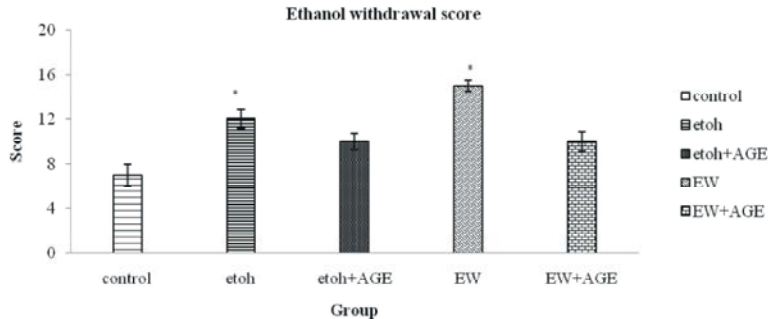


Fig. A: Ethanol withdrawal scores Values are expressed as score /experimental group. All the values are mean±SD of six individual observations. * indicates values are significant at $p<0.001$ compare to SC in Scheffe's test

Pretreatment with ginger extract at the dose of 200mg/kg bw, to EW animals (EW+AGE group) showed a significant ($p<0.05$ & $p<0.01$) increase in Dopamine levels in all brain regions of rats in comparison with both EW and EtOH groups.

3,4-Dihydroxyphenylacetic Acid (DOPAC): The concentrations of DOPAC were significantly increased ($p<0.001$) during chronic EtOH as follows (Table 1):

HC > CB > CC > PM

There is a significant ($p<0.001$) decline in DOPAC concentrations in the withdrawal rat brain with the highest decrease in hippocampus followed by cerebellum, pons and cerebral cortex regions that can be represented as follows

HC (-36.39%) > CB (-32.52%) > PM (-26.24%) > CC (-25.68%)

Comparisons of experimental groups treated with ginger extract are not found to be statistically significantly different from the saline controls with respect to DOPAC levels.

Homovanillic Acid (HVA): The concentrations of HVA were significantly increased ($p<0.001$) during chronic EtOH in the order (Table 1):

CC > HC > CB > PM

There is a trend of drop in HVA concentrations in the brain regions during the withdrawal syndrome. HC recorded the highest decrease (49.14%), followed by CC (35.01%), CB (34.28%) and PM(-6.17%).

HC < CC < CB < PM

On pretreatment with ginger extract during EW as well as treatment of ginger extract during chronic ethanol administration the HVA level are found not significantly different from the normal controls($p<0.001$).

DOPAC/DA: Ethanol intoxicated rats the ratios of DOPAC/DA in the brain regions decreased ($p<0.001$) in the following order (Fig. B):

HC (-66%) > CB (-48.07%) > CC (-37.25%) > PM (-31.57%)

However in case of ethanol withdrawal group, DOPAC/DA ratio recorded changes that are statistically significant at $p<0.001$ in this sequence

HC (+64.7%) > CB(+53.84%) > CC (+37.25%) > PM (+31.57%)

Groups treated with the ginger extract on comparing with the normal controls are not statistically significant from controls.

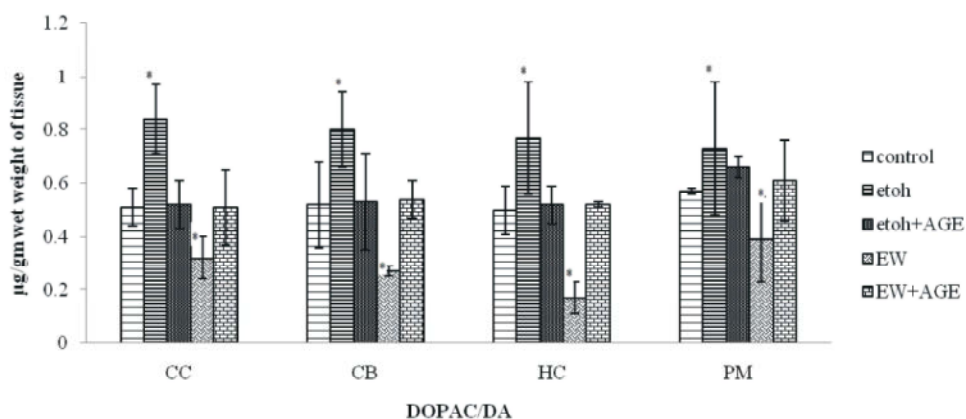


Fig. B: Changes in DOPAC/DA ratio in different brain regions due to pretreatment of ginger extract prior to ethanol withdrawal. Values are expressed in $\mu\text{g} / \text{gm}$ wet weight of tissue. All the values are mean \pm SD of six individual observations. * indicates values are significant at $P < 0.001$ compare to SC in Scheffe's test

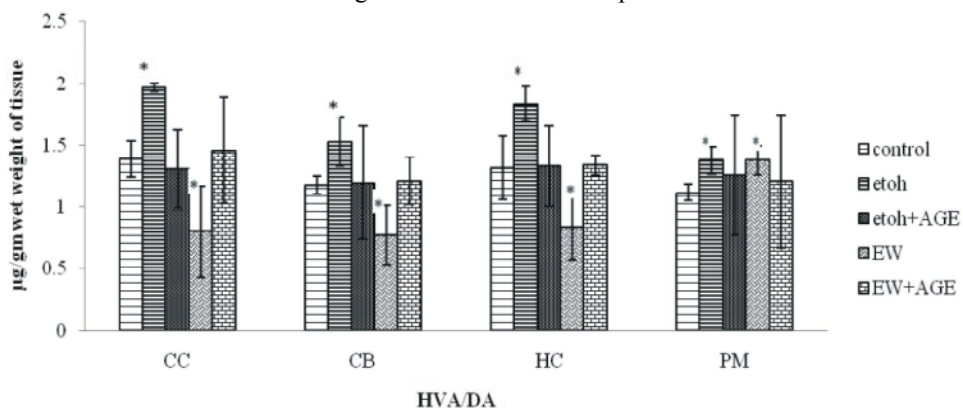


Fig. C: Changes in HVA/DA ratio in different brain regions due to pretreatment of ginger extract prior to ethanol withdrawal. Values are expressed in $\mu\text{g} / \text{gm}$ wet weight of tissue. All the values are mean \pm SD of six individual observations. * indicates values are significant at $p < 0.001$ compare to SC in Scheffe's test.

HVA/DA: HVA/DA ratio is increased ($p < 0.001$) by chronic ethanol treatment in the following order (Fig. C):

CC(+41.72%) > HC(+39.39%) > CB (+29.66%) > PM(+23.21%)

While during ethanol withdrawal there is a significant decrease ($p < 0.001$) in the ratio, the decrease is greater in CC and is represented as follows

CC(-42.37%) > HC(-36.37%) > CB(-34.74%) > PM(-23.21%)

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

DISCUSSION

Dopamine is a neuromodulator that is used by neurons in several brain regions involved in motivation and reinforcement, most importantly the nucleus accumbens (NAc). The mesolimbic dopamine pathway, which projects from the midbrain to the nucleus accumbens, a part of the limbic system is thought to be involved in pleasure as well as delusions, psychosis and drug abuse. Dopamine is involved in reinforcement, generation of pleasure, development of drug addiction and so forth [19]. Dopamine alters the sensitivity of its target neurons to other neurotransmitters, particularly glutamate. In addition, dopamine can affect the neurotransmitter release by the target neurons. Dopamine-containing neurons in the NAc are activated by motivational stimuli, which encourage a person to

perform or repeat a behaviour. Even low alcohol doses can increase dopamine release in part of the NAc. This dopamine release may contribute to the rewarding effects of alcohol and may thereby play a role in promoting alcohol consumption. The dopamine in the reward pathway may instigate craving for drugs or alcohol and it may also reinforce habitual drug use. Alcohol-related stimuli maintain the motivational significance even after repeated alcohol administration, which may contribute to the craving for alcohol observed in alcoholics.

The results indicated an increased turnover rate of DA following chronic EtOH treatment. These rats had blood ethanol levels higher than 200 mg/dl (data not shown). The concentrations of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) significantly increased in the EtOH-dependent rats while still intoxicated. However, the effects observed from previous studies are contentious. Budygin *et al.* [20] showed that ethanol exerts a profound effect on DA neurons, resulting in a suppression of DA neurotransmission in the striatum at high doses (around 5 g/kg). Others [21] demonstrated that DA and DOPAC levels significantly decreased in the striatum of rats chronically receiving alcohol. In the present study, the group of rats that showed overt signs and responses of EtOH withdrawal syndrome [22, 23], little or no traces of EtOH was found in the blood. Both DOPAC and HVA reverted to normal concentrations in the ethanol-dependent rats. The drop in HVA and DOPAC concentrations in the brain indicates that during the withdrawal syndrome, dopamine turnover was lower as compared to intoxicated rats, suggesting a decline in dopamine turnover rate following ethanol elimination from the blood. These findings were similar to the previously published study [24] which also showed similar reduction in DA turnover rate and release during withdrawal. However, the literature is not conclusive about changes occurring in HVA levels, with some investigators reporting an increase [25] or a decrease [26] in HVA levels, whereas in the present study, an increase in HVA levels during EW in all the selected brain regions is observed.

Dopaminergic lesioning of the prefrontal cortex leads to cognitive dysfunctions. Dopamine depletion of the mesoseptal dopaminergic projections leads to a decrease in working memory [27] and lesioning of the mesoaccumbens dopaminergic projections cause attention deficit and alterations in locomotor activity [28-30]. These findings give insight to dopamine's key

role in Schizophrenia, Parkinson's disease, Huntington's chorea, manic-depressive illness and tardive dyskinesia.

The substantial action of DA was first demonstrated on cortical neurons by Krnjevic and Phillis, 1963 [31]. The comparable depressant actions of DA were also observed subsequently in some other regions of forebrain such as hippocampus [32] and striatum [33]. In the cerebellar cortex the potency of DA is comparable to that of NE [34] but in the lower brainstem and the spinal cord, DA shows low potency than NE [32], whereas in other areas it shows depressant action. Alcohol causes an increase in dopamine in the area of the reward pathway [35]. This reward pathway is comprised primarily of the nucleus accumbens, the VTA (ventral tegmental area) and a part of the prefrontal cortex.

For DA, electrophysiological [36, 37], neurochemical [38-41] and behavioural [38, 42] evidence indicates that relevant doses of ethanol activate the mesolimbic DA reward pathway. Direct evidence of role for DA in ethanol reward is evident from findings that operantly self-administered ethanol stimulates DA release in the NAC [43], that rats will self-administer ethanol directly into the ventral tegmental cell body region of the meso-accumbens DA reward pathway [44] and that operant responding for ethanol is modified by pharmacological agents that interact with DA neurotransmission [45,46]. Finally, alcohol preference in genetic models of alcoholism has been linked to reduced DA content in the NAC [47,48] as well as heightened sensitivity to the DA release-enhancing and locomotor activating effects of ethanol [49-51].

Barak *et al.* [52], established that rats were given intermittent 24h access to alcohol solutions three times a week for 7 weeks will develop alcohol dependence. Then NAc extracellular dopamine levels and its response to VTA GDNF injections were measured. The authors showed that NAc dopamine levels in alcohol-dependent rats were significantly decreased after 1 h of withdrawal and remained low 24 h later. VTA GDNF injections (10 µg/side) in 24h-withdrawn rats restored NAc dopamine to control (normal) levels. Most relevant to the dopamine-depletion hypothesis is the finding that VTA GDNF injections normalized the decreased NAc dopamine levels during alcohol withdrawal and also decreased alcohol reward. Dopaminergic transmission has been suggested to be a main mechanism mediating reinforcement, withdrawal and craving associated with alcohol addiction [53]. Results indicate that during the ethanol-withdrawal syndrome the mesolimbic dopaminergic system is tonically reduced in activity, as indexed by electrophysiological and biochemical criteria.

Considering the role of the mesolimbic dopaminergic system in the reinforcing properties of ethanol, the depressed activity of this system during the ethanol-withdrawal syndrome may be relevant to the dysphoric state associated with ethanol withdrawal in humans.

In the present study HVA/DA ratio increased by chronic ethanol administration, while the DOPAC/DA ratio decreased significantly in the ethanol withdrawal group. Changes observed in DOPAC/DA and HVA/DA ratios correlated well with changes in DA concentration. However, at this point of time, it will be a haste to speculate on the role of ginger in improving brain monoamines during withdrawal. The results obtained for the cerebral cortex and hippocampus are most remarkable for the large variability observed in the DOPAC and HVA concentrations in relation to DA occurring in these brain regions due to withdrawal.

The present findings establish ginger as playing a prominent role in maintaining the dopaminergic system during ethanol withdrawal. A 2005 study found a dopamine-sparing effect when zingerone (a phytochemical found in ginger) was tested. A lack of dopamine is associated with several key characteristics of depression such as, "learned helplessness" and lethargy (lack of energy). Similar studies also pointed to a possible anti-anxiety effect of a specific ginger root extract. Zingerone, one of the principal components of ginger, treatment proved of possible value in treatment of Parkinson's disease, a disease of dopamine deficiency, according to studies of Kabuto *et al.* [54]. The benzene fraction (BF) of a petroleum ether extract of dried rhizomes of ginger, which contained anticonvulsant principle(s), was screened for anxiolytic and antiemetic activity [55].

CONCLUSION

In conclusion, this study has examined the effect of ginger extract on chronic ethanol consumption and withdrawal in dopamine activity in male wistar rat. Differences found between the ethanol naive and chronic ethanol and withdrawal groups demonstrate that the dopamine system is susceptible to modulation by chronic ethanol consumption and withdrawal that can be challenged by ginger extract administration. The exact mechanisms as to how these neurochemical changes due to ginger administration occur during ethanol withdrawal require clarification and awaits confirmation. This topic needs further elaborate studies at electrophysiological, neurochemical, receptor, molecular and behavioural levels.

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