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Correlation between regional oxidative stress markers of the cerebrum and spatial learning in tocotrienol-mediated protection against light-to-moderate doses of ethanol exposure

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Tocotrienol, a multipotent nutraceutical with antioxidative, anti-inflammatory and neuroprotective properties, could be used to maintain the cognitive functions even in the presence of neurotoxicants. Oral supplementation of two doses of tocotrienol was used during the three doses of ethanol exposure (comparable with low-to-moderate doses of alcohol consumption in human), and learning, retention, and utilisation of navigation performances were evaluated and correlated with the level of oxidative stress markers in cerebral regions. Rats received ethanol exposure for 4 weeks and tocotrienol supplementation for 4 weeks of ethanol exposure and continued for 2 more weeks. The significant decrement in weight gain during the experimentation was observed only in the groups receiving the highest amount of ethanol exposure (0.6 mg/kg body weight). Only the group exposed to ethanol at 0.4 mg/kg bw demonstrated alterations in acquisition time and post-48 h retention time of Morris water maze navigation task. Significant influences of ethanol exposure and tocotrienol supplementation were observed in the probe test using the Morris water maze. The correlation between oxidative stress parameters of cerebral regions and probe test did not provide any significant information; however, indicated that investigated domains of cognition most likely were associated with frontal cortex and temporal cortex functions.

Keywords: Cerebralregions, Ethanol, Morris water maze, Oxidative stress, Tocotrienol

Tocotrienols (T3), minuscule constituents of natural vitamin E, are tocochromanols with unsaturated geranylgeranyl-derived side chain[1]. T3s are well-accepted multipurpose nutraceuticals although regular consumption is not substantial[2,3]. Even though it has limited bioavailability, particularly through oral intake, its antioxidative[4] and neuroprotective effects are well documented[1,5,6]. Oral supplementation with graded doses of T3 demonstrated protection against behavioral and biochemical changes in diabetic neuropathy rat model[7]. Similarly, attenuations of ethanol-induced decay in spatial memory by tocotrienol supplementation were also reported in a dose-dependent fashion in rat pups[8].

The behavioral disturbances observed in case of chronic ethanol exposure were suggested to be the result of enhanced neuronal death because of the increased level of oxidative-nitrosative stress and inflammatory responses[9]. Suppression of these factors was suggested to be the possible mechanism of T3-induced neuroprotection against ethanol-mediated neuronal damage[10]. Long-term supplementation with T3, in the form of TRF, demonstrated improvement in neuronal functions and behavioral performances[11]. T3 supplementation was also found to protect against oxidative stress-mediated activation of protein kinase c signalling[10]. Apart from being an antioxidant, the role of T3 in signal transduction, cell membrane lipid metabolism was reviewed by Durani *et al*.[11]

Frequently, spatial memory was ascribed as functions of the hippocampus. It was suggested that worsening of spatial learning performances with aging was due to deterioration in hippocampal functions[12]. While identifying the role of dietary antioxidants in the reversal of cognitive performances, Joseph *et al*. demonstrated that only hippocampus of all tested brain regions had different vitamin E content when compared with suitable control group[13]. On the other hand, oxidative damages in numerous brain regions were identified as a possible link to impairments in cognitive and psychomotor functions[14]. They also suggested that exogenous antioxidants could be useful in slowing or reversal of the oxidative damage and eventually protect or restoration of brain functions[14]. However, dose-dependent nonuniformity of vitamin E uptake by brain regions was indicated[15], while involvement of

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several brain regions (including neocortex) apart from the hippocampus had been suggested for declination of cognitive functions[15]. Other researchers noticed that alterations in oxidative stress parameters and neuroinflammatory markers in response to ethanol exposure were higher in cerebral cortex compared to that of hippocampus; however, the dose-dependent reversals of those parameters by T3 supplementation were comparable for both brain regions[9]. Citing the linear relationship of cerebral acetylcholinesterase activities with behavioral impairments in acute ethanol exposure to zebra fish[16], T3-mediated protection against both biochemical and behavioral deteriorations were demonstrated[9]. T3 was also found to protect astrocytes[17] and neuronal cells[18] from glutamate-induced cell death.

The frontal cortex is the site of complex cognitive functions, while it is the most insulted area in an alcoholic brain with a significant neuronal loss in superior frontal cortex[1]. Accordingly, the current study was taken up to evaluate the pertinence of simultaneous T3 supplementation in providing protection against the deterioration in cognitive performances caused by low to moderate doses of ethanol exposure in adult rats and to correlate the oxidative stress markers in regions of the cerebral cortex with the performances in the spatial navigation task.

Materials and Methods

Materials

Oryza tocotrienol©-90 was kindly donated by the Oryza Oil & Fat Chemical Co. Ltd., Japan. Other chemicals were of analytical grade and procured from reputed companies.

Animal maintenance and treatment

Male albino Wistar rats weighing 120-140 g were obtained from NCLAS, National Institute of Nutrition, Hyderabad, maintained and treated in the Central Animal House of NRI Medical College & General Hospital and the procedures were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). All the experiments were carried out in four phases – Et-0, Et-I, Et-II and Et-III with 0, 0.2, 0.4 and 0.6 g ethanol exposure/kg body weight, respectively. In each phase, after one week of acclimatisation, rats were randomly divided (with the help of Random Allocation Software Version 1.0, May 2004) into three groups with 6 animals each – receiving sham treatment (T_0), 10 mg T3/day (T_{10}) and 20 mg T3/day (T_{20}). The treatment with ethanol continued for 4 weeks (total 28 days), and supplementation with T3 started along with ethanol exposure and continued for 2 more weeks after the cessation of ethanol exposure (total 42 days). Oral feeding of ethanol and distilled water was done in the morning session, while, oral feeding with T3 supplementation and sham feeding were done in the evening session daily. Doses and duration of T3 supplementation and ethanol exposure were established from our earlier studies. Thus, the study was planned as per the following paradigms – animals exposed to pro-oxidant only (T_0 animals of Et-I, Et-II , and Et-III groups), animals supplemented with antioxidant only (T_{10}, T_{20} animals of Et-0 group), animals exposed to pro-oxidant and also supplemented with antioxidant (T_{10} and T_{20} animals of Et-I, Et-II, and Et-III groups) and animals neither exposed to pro-oxidants nor supplemented with antioxidants (T_0 animals of Et-0 group),

Behavioral analysis

Morris water maze (MWM) is a significant tool in neuroscience research, which provides a means of neurocognitive evaluation using simple behavioral task modules. The test was performed following the protocol described elsewhere[19]. Rats were trained to swim to a visible platform in a circular pool (180 cm diameter and 60 cm in height) located in an experimental room. The pool was divided into four equal quadrants and filled with water to a height so that the rats were not able to touch the bottom of the pool. During the acquisition phase, a square platform (9 cm) was placed in one of the quadrants of the pool approximately 2 cm below the water. The water was made opaque by adding a nontoxic dye (titanium dioxide), and four equidistant locations were identified around the edge of the pool (A, B, C, and D) and were used as starting points. Animals received a training session consisting of four trials with a gap of 5 min between trials on day 40. Four different starting positions were used for the successive training sessions in random orders. Rats were released into the maze facing towards the wall of the pool and latencies to find the platform was recorded for a maximum period of 90 sec. If the rat did not find the platform within 90 sec, it was guided to the platform and allowed to remain there for 20 sec. The time required for the rat to find the platform on the first occasion was considered as the acquisition time.

The time is taken to find out the platform (retention time) was again assessed on day 41 (24 h after the acquisition time) and day 42 (48 h after the acquisition time). On the day 43, the platform was removed from the pool and rats were placed into the pool and allowed to explore the pool for 5 min. The time spent in the target quadrant where the platform placed was noted, the changes in time spent by the rats at the target quadrant were used as an indicator of spatial memory.

Isolation of regions of the cerebral cortex

Overnight fasted rats was sacrificed by cervical dislocation. The whole brain was removed, washed with ice-cold saline. Frontal cortex (FC), temporal cortex (TC) and rest of the cerebral cortex (RC) were immediately separated as described elsewhere[20] and preserved in the ice-chamber for biochemical processing.

Biochemical parameter

The homogenized brain tissues were used for the determination of reduced glutathione content, level of lipid peroxidation, activities of catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase as mentioned elsewhere[4]. Glutathione-dependent superoxide and peroxide handling capacity was evaluated by the ratio of glutathione peroxidase and superoxide dismutase activities, while, glutathione-independent superoxide and peroxide handling capacities were calculated from the ratio of catalase and superoxide dismutase activities.

Statistical analyses

The data are expressed as the mean of six observations ± standard error of the mean, if not mentioned otherwise. Box and whisker plots have been used to present the data graphically showing the median value (bold horizontal line), interquartile range (boxes on either side of the line) along with range (dotted lines) or outliers (small circles), if any. Influences of the ethanol exposure and T3 supplementation was carried out by two-way ANOVA. The differences between the groups were analyzed by Tukey's post-hoc test accepting the probability of 5% or less as significant using PAST statistical software (ver. 3.12; Copyright: Ø. Hammer 1999-2016)[21].

Results

During the 4 weeks of ethanol exposure in Et-0 phase, the T_0 and T_{10} groups of animals showed comparable ranges in body weight gain (26-44%), while the T_{20} groups of animals showed a little higher gain (30-56%). However, during the next 14 days, percentage body weight gain of those animals averaged 17.89 ± 0.84, 16.16 ± 1.42 and 11.99 ± 1.29 in T_0, T_{10}, and T_{20}groups, respectively. Interestingly, a substantially higher increment in body weight was observed during the only T3 supplementation period (post ethanol-exposure)in T_{20} groups (17.95 ± 1.79) compared to that of T_0 group (12.59 ± 1.41) and T_{10} group (11.63 ± 2.49) in the Et-I phase of the experimentation. The changes in body weight during ethanol exposure of this ranged 34-41%, 24-66% and 47-54% for T_0, T_{10} and T_{20} groups, respectively. Accordingly, there was a notable difference in the overall gain in body weight of the T_{20} group of animals of Et-I phase (Fig. 1). Both changes during ethanol exposure and subsequent only T3 supplementation, all animal groups of Et-II phase experimentation demonstrated comparable changes in body weight (Fig. 1). On the contrary, even though there was no difference between the groups, body weight gain during the first 4 weeks were so less (T_0: 22.69 ± 3.74; T_{10}: 24.69 ± 1.22; T_{20}: 21.17 ± 1.16) that overall gains in body weight were significantly less in Et-III phase compared to other three phases of experimentation (Fig. 1).

On the day of learning, the acquisition time was found to be highest in T_0 animals of Et-III phases, most of the animals could not find the platform on their own. The performances were relatively better in T_{10} and T_{20} animals, where 50% animals in the T_{10} group and one animal in the T_{20} group could not find

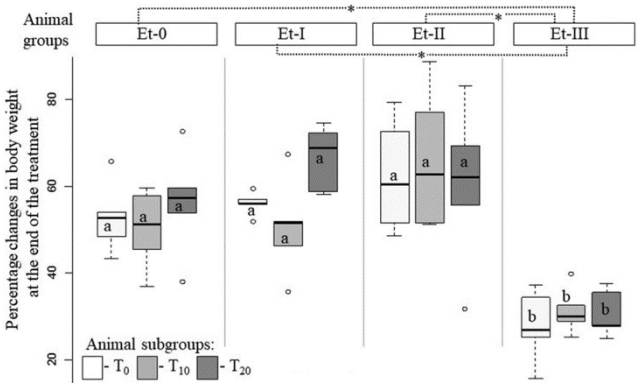

Fig. 1—Box and whisker plot of changes in body weight of rats during the treatment period in comparison to the initial body weight. The animals were grouped as per doses of ethanol exposure and subgrouped as per doses of tocotrienol supplementations. *indicates significant (P <0.05) difference between the ethanol exposure groups, while dissimilarity in letters assigned to each box indicates statistical significance (P <0.05) between the tocotrienol supplementation groups.

the platform on their own. Similarly, one animal in the T_{20} group of Et-0 phase and two animals from the T_{10} group and one animal from the T_0 group of Et-II phases could not find the platform on their own. Even though other animals of different groups of these phases (Et-0, Et-I and Et-III) could locate the platform themselves within stipulated 90 sec, however, their escape latency was quite high (Fig. 2). On the contrary, all the animals of Et-II phase demonstrated low escape latency, and statistically, this phase of experimentation was found to be significantly different from the other three phases (Fig. 2). Accordingly, the influence of ethanol exposure was found to be statistically significant for escape latency or acquisition time of MWM experimentation. However, the influence of T3 was not found statistically significant for the same. Notably, acquisition times for T3-supplemented animals (T_{10} and T_{20} groups) were statistically low in comparison to the acquisition time of T_0 in animals of phase Et-II experimentation.

The time to locate the same platform hidden in the same place had been evaluated after 24 h of the acquisition session and presented as retention (after 24 h) time in (Fig. 3). Interestingly, with the increase in ethanol doses, there was nearly a gradual decline in the median values of retention time. Eventually, the statistically significant influence of ethanol exposure was noted for the parameter, without any influence of T3 supplementation. During the Et-II phase of the study, one animal from the T_0 group failed to find the platform, even though the same animal could locate the platform within a reasonable time during the acquisition session and trials.

Likewise, the significant influence of ethanol exposure only was observed in the post 48 h retention time also. Required time for all the animals to find out the hidden platform (Fig. 4) was remarkably lower than their respective post 24 h retention time (Fig. 3). Statistically, post 24 h retention time of Et-III phase of the study was significantly lower from that of the Et-I phase of the study (Fig. 3), while, Et-II phase was significantly lower from Et-0 phase for 48 h retention time (Fig. 4).

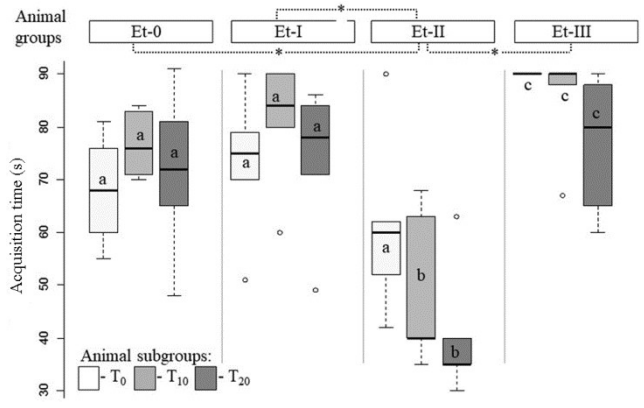

Fig. 2—Box and whisker plot of Acquisition time of the rats on first day of Morris Water Maze test. The animals were grouped as per doses of ethanol exposure and subgrouped as per doses of tocotrienol supplementations. *indicates significant (P <0.05) difference between the ethanol exposure groups, while dissimilarity in letters assigned to each box indicates statistical significance (P <0.05) between the tocotrienol supplementation groups.

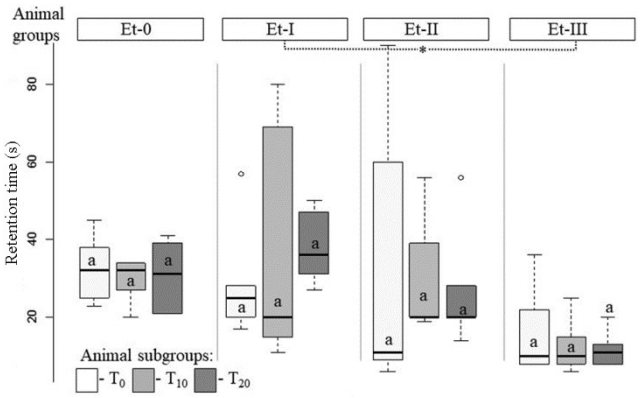

Fig. 3—Box and whisker plot of Retention time (after 24 h) in Moris Water Maze test. The animals were grouped as per doses of ethanol exposure and subgrouped as per doses of tocotrienol supplementations. * indicates significant (P <0.05) difference between the ethanol exposure groups, while dissimilarity in letters assigned to each box indicates statistical significance (P <0.05) between the tocotrienol supplementation groups.

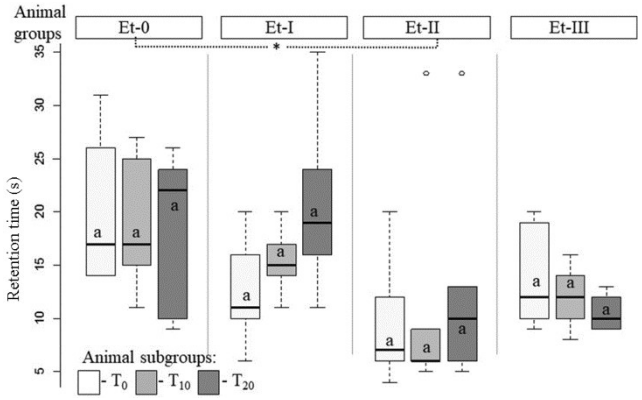

Fig. 4—Box and whisker plot of Retention time (after 48 h) in Moris Water Maze test. The animals were grouped as per doses of ethanol exposure and subgrouped as per doses of tocotrienol supplementations.* indicates significant (P <0.05) difference between the ethanol exposure groups, while dissimilarity in letters assigned to each box indicates statistical significance (P <0.05) between the tocotrienol supplementation groups.

In the absence of any hidden platform, when the animals had to search for the platform, the time spent in the target quadrant by three groups (T_0, T_{10}, and T_{20}) of animals in 4 phases (Et-0, Et-I, Et-II and Et-III) of the study were presented in (Fig. 5). Animals of Et-0 phase spent maximum time in the target quadrant, and statistical analyses showed that the time spent by the animals of this phase was significantly higher compared to other three phases of experimentation. However, only statistically insignificant differences were observed between the T3 groups of animals within Et-0 phase. On the other hand, similar intergroup differences were not observed in Et-II phase of the study, while this phase demonstrated the lowest time spent among the phases. No statistically significant difference was documented between Et-I and Et-III phases of the study. Nonetheless, both these phases demonstrated (Fig. 5) statistically significant difference between the sham supplementation group (T_0) and T3 supplementation groups (T_{10} and T_{20}) without having any intra-T3 supplementation difference (T_{10} *vs* T_{20}). In this context, the difference between the T_0 animals of Et-I and Et-II was noteworthy, especially when T_0 animals of Et-0, Et-I, and Et-II, Et-II were comparable about the time spent in the target quadrant (Fig. 5). The significant influence of ethanol exposure as well as the interaction between ethanol exposure and T3 supplementation was observed in the variance analysis.

Levels of oxidative stress markers like reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-dependent superoxide and peroxide handling capacity (GD-SPHC) and glutathione-independent superoxide and peroxide handling capacity (GI-SPHC) of FC, TC and RC of rats exposed to highest level of ethanol exposure (Et-III) are represented in (Table 1). As the Et-III groups of animals demonstrated the most significant alterations in the probe test of the MWM navigation task, data from Et-III group only along with their correlation with probe test performance were documented here. The significant influence of differential responses of the regions of the cerebral cortex was seen in all the tested parameters. Interestingly, none of the studied parameters of T_0 and T_{20} animals showed any statistically significant regional difference. On the other hand, none of the studied parameters of RC significantly differed between the T3 supplementation groups, even though, statistically significant differences between T3 supplementation groups were not much in other regions (FC and TC) as well. Influences of T3 supplementation were found to be statistically insignificant for activities of GPx and GR. Although no specific pattern was observed in the correlation coefficients of specific brain regions, frontal cortex and temporal cortex of T_{20} group were showing positive correlations with all the biomarkers and of all correlations with lipid peroxidation were minimum.

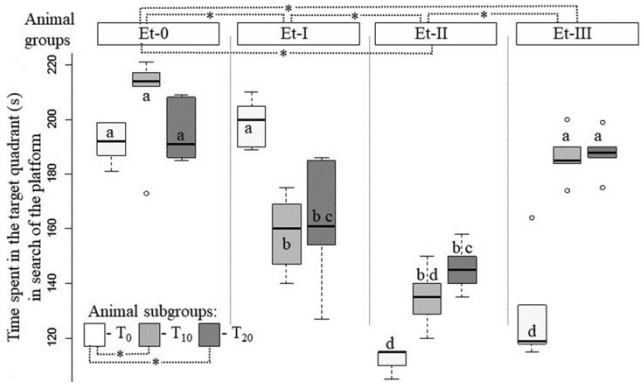

Fig. 5—Box and whisker plot of Total time spent in the target quadrant in search of the platform in Moris Water Maze test. The animals were grouped as per doses of ethanol exposure and subgrouped as per doses of tocotrienol supplementations. *indicates significant (P <0.05) difference between the ethanol exposure groups, while dissimilarity in letters assigned to each box indicates statistical significance (P <0.05) between the tocotrienol supplementation groups.

Discussion

Cognitive performances include both learning and memory[22]. Processing of information for acquisition, retention, and application involves a lot of neuronal networks. Functional impairments in neurons essentially lead to cognitive diminution and likely to be mediated through derangement of synaptic platsticity[23]. Consumption of ethanol leads to dose-dependent neurological malfunctioning because of its generalised depressant effects on the central nervous system[24]. As such, there are many domains of cognitive functions. Domain-specific impacts of acute low-to-moderate doses of ethanol in rhesus macaques were reported[25]. Though author could not ascertain the similarity of cognitive loss across the species, some degree of resemblance was suggested. Considering various aspects of cognitive decline in response to ethanol exposure, domain-specific differential response of ethanol had also been suggested[24]. Likewise, the cognitive decline in acute ethanol exposure as well as post-intoxication was also

Table 1—Correlation coefficients between regional levels of oxidative stress parameters in cerebrum andperformance scores on probe test of Morris water maze navigation task.

Reduced glutathione level (μmole/g cerebral tissue)							
T3 supplementation*		T_0(0 mg/day)[a]		T_{10}(10 mg/day)		T_{20}(20 mg/day)[a]	
Cerebral regions*	FC [b c]	1.11 ± 0.14	*0.34*	1.45 ± 0.08[e]	*−0.82*	1.36 ± 0.11	*0.60*
	TC [c]	0.71 ± 1.07[d]	*−0.07*	1.07 ±0.18	*−0.10*	1.33 ± 0.19[d]	*0.50*
	RC [b]	0.68 ± 0.08	*−0.25*	0.72 ± 0.05[e]	*−0.46*	0.97 ± 0.09	*0.40*
Lipid peroxidation level (nmole TBARS/g cerebral tissue)							
T3 supplementation*[I]		T_0(0 mg/day)[a b]		T_{10}(10 mg/day)[a]		T_{20}(20 mg/day)[b]	
Cerebral regions*[I]	FC [c]	22.14 ± 4.10	*0.16*	18.80 ± 4.95	*−0.47*	15.82 ± 2.19	*0.36*
	TC [d]	21.72 ± 3.80 [e]	*0.43*	14.93 ± 3.47	*−0.22*	12.23 ± 1.55 [e]	*0.33*
	RC [c d]	13.70 ± 1.34	*0.25*	8.60 ± 0.91	*−0.85*	9.46 ± 1.37	*0.35*
Catalase activity (μmol H_2O_2 decomposed/hr/g cerebral tissue)							
T3 supplementation*		T_0(0 mg/day)[a]		T_{10}(10 mg/day)[a]		T_{20}(20 mg/day)	
Cerebral regions*	FC [b]	23.88 ± 6.13	*0.13*	32.74 ± 3.14	*−0.37*	27.72 ± 2.28	***0.90***
	TC	17.64 ± 1.80	*0.00*	27.69 ± 6.12	*0.25*	24.06 ± 3.07	*0.64*
	RC [b]	16.89 ± 1.67	*−0.65*	21.02 ± 3.17	*0.11*	21.91 ± 1.98	*0.34*
Superoxide dismutase activity (SOD Units /g cerebral tissue)							
T3 supplementation*		T_0(0 mg/day)[a b]		T_{10}(10 mg/day)[a]		T_{20}(20 mg/day)[b]	
Cerebral regions*	FC [c]	6.65 ± 1.72[e]	*0.28*	10.69 ± 0.90[e f]	*−0.05*	8.28 ± 0.84	*0.48*
	TC [d]	4.03 ±0.69	*0.19*	7.93 ± 1.32	*0.29*	6.79 ± 0.89	*0.47*
	RC [c d]	3.14 ± 0.33	*0.51*	4.67 ± 0.27[f]	*−0.29*	4.89 ± 0.34	*−0.01*
Glutathione peroxidase activity (nmole NADPH oxidized/min/g cerebral tissue)							
T3 supplementation		T_0(0 mg/day)		T_{10}(10 mg/day)		T_{20}(20 mg/day)	
Cerebral regions*	FC [a]	34.23 ± 9.89	*0.12*	42.51 ± 3.79	*0.30*	35.41 ± 4.03	*0.75*
	TC [b]	19.96 ±2.81	*−0.11*	28.04 ± 3.14	*0.50*	28.72 ± 3.11	*0.68*
	RC [a b]	23.39 ± 3.04	*−0.77*	25.94 ± 2.75	*−0.03*	24.31 ± 2.11	*−0.36*
Glutathione reductase activity (nmole NADPH oxidized/min/g cerebral tissue)							
T3 supplementation		T_0(0 mg/day)		T_{10}(10 mg/day)		T_{20}(20 mg/day)	
Cerebral regions*	FC [a]	22.06 ± 6.22	*0.16*	31.32 ± 3.11	*−0.17*	25.58 ± 2.76	*0.54*
	TC [b]	19.22 ± 2.66	*−0.19*	26.37 ± 4.48	*0.22*	22.06 ± 4.20	*0.29*
	RC [a b]	20.92 ± 2.18	*−0.73*	23.03 ± 2.07	*−0.63*	22.02 ± 1.34	*−0.16*
Glutathione-independent superoxide and peroxide handling capacity (Ratio values)							
T3 supplementation*		T_0(0 mg/day)[a b]		T_{10}(10 mg/day)[a]		T_{20}(20 mg/day)[b]	
Cerebral regions*	FC [c]	4.12 ± 0.55	*−0.70*	3.17 ± 0.13	*−0.93*	3.38 ± 0.16	*0.50*
	TC [d]	4.64 ± 0.54	*−0.55*	3.51 ± 0.24	*0.01*	3.38 ± 0.20	*0.67*
	RC [c d]	5.71 ± 0.96	*−0.87*	4.42 ± 0.45	*0.29*	4.49 ± 0.32	*0.40*
Glutathione-dependent superoxide and peroxide handling capacity (Ratio values)							
T3 supplementation*		T_0(0 mg/day)[a b]		T_{10}(10 mg/day)[a]		T_{20}(20 mg/day)[b]	
Cerebral regions*	FC [c]	5.31 ± 0.56	*−0.72*	3.95 ± 0.16	*0.54*	4.18 ± 0.21	*0.80*
	TC [d]	5.46 ± 0.63	*−0.67*	3.79 ± 0.23	*0.15*	4.29 ± 0.12	*0.38*
	RC [c d]	8.29 ± 2.06	*−0.79*	5.53 ± 0.40	*0.13*	4.97 ± 0.27	*−0.47*

Data are mean ± SEM of six observations. Data presented in italics are the correlation coefficients (bold faces are statistically significant). *indicates significant influence (P <0.05) of regional difference and dose of T3 supplementation. [I]indicates significant influence (P <0.05) of the interactions between brain regions and supplementation groups. Similar superscript letters indicate significant differences (P <0.05) in parameter values between either supplementation groups or brain regions, as shown in the table. FC = Frontal cortex, TC = Temporal cortex, RC = Rest of the cerebral cortex.

noted[26]. Recently, cognitive decline was demonstrated in acute and chronic ethanol intoxications; while, those changes were associated with altered neuronal network connectivity in different brain regions[27]. Several mechanisms were proposed to explain the etiopathogenesis of alcohol-induced cognitive deficits[1]; such as altered synaptic plasticity[28]. Considering the antioxidative, anti-inflammatory and neuroprotective function of T3[29] along with reports of enhanced expression of synaptogenic proteins by T3 rich fractionsupplementation[23], the present study was planned to evaluate the spatial cognitive function in rats exposed to low-to-moderate doses of ethanol and supplemented with T3 (concurrent and post-intoxication).

It was demonstrated that T3 could counter the oxidative stress and decline in cognitive performances[29], possibly based on its physico-chemical properties. Similarly, supplementation with T3 improved the superoxide and peroxide handling capacities (SPHC) of serum and different brain regions as well as mended the neurobehavioral performances towards betterment[5]. Supplementation of T3 was also beneficial against neuropathies associated with diabetes, alcoholism or against cognitive deterioration induced by chronic alcohol exposure and streptozoticin and glutamate-induced neurodegeneration[30]. On the other hand, a cross-sectional, multi-center study (Add Neuro Med Consortium) found increased odds of clinical cognitive deterioration with lower levels of plasma T3s along with tocopherols and suggested that the reduced levels of vitamin E were because of their consumption to antagonise the oxidative or nitrosative chemicals[31]. A systematic review[32] also supported this; however, mostly in favor of T3. The inability of tocopherol to prevent the neurobehavioral deterioration had been demonstrated recently, even though it mostly stalled the oxidative stress during the process[33]. Hence, countering the oxidative stress and providing neuroprotection by supplementation with T3 could be possible; though, they may or may not be related phenomena.

The decrease in escape latencies for almost all rats indicated the animals had learned the navigation tasks during the acquisition phase of MWM training. When fed with 200 mg of T3 rich fraction (TRF)/kg of body weight for 3 months, aged Wistar rats demonstrated improved spatial learning and memory along with reduced anxiety[34]. However, during the acquisition phase, the escape latency was not improved in comparison to the non-supplemented aged rats. In the current study also, no improvement in acquisition performance during the navigation task was observed in most of the phases of the study. Interestingly, Et-II phase study demonstrated a significant reduction in acquisition time and, interestingly, a significant impact of T3 supplementation was also observed only in this phase. Even though there was no significant difference between the T3 supplemented groups and sham-supplemented group, the distribution of data in T_{20} groups showed a tendency towards the lower values for acquisition time during the Et-III phase of the study. One study also documented significantly reduced escape latency only after 3 days of trials when male rats, born from TRF supplemented dam and were supplemented with TRF for 16 weeks[22]. Neither beneficial effects of T3 nor the detrimental effect of ethanol exposure on acquisition time was observed during the Et-0 and Et-I phases of the study. In case of elevated plus maze study, nevertheless, ethanol-induced worsening, as well as T3-mediated protection in acquisition time, were observed in other study[3]. In addition to this, insignificant improvements in acquisition times were also documented in Et-II and Et-III groups in the absence of T3 supplementation[3]. The current study also demonstrated statistically insignificant improvement in the acquisition time of T_0 group in Et-II phase of experimentation; however, significantly higher acquisition time was recorded in Et-III study. Therefore, alteration in the acquisition time of MWM study was found to be dose-specific for ethanol exposure, but not for T3 supplementation.

Insignificant improvement of acquisition time, even in the T_0 group in Et-II phase suggested some improvement in the cognitive performance by the ethanol exposure. While a cognitive decline in heavy ethanol exposure was well-accepted, beneficial effects of moderate doses of ethanol exposure was also suggested in some studies. Observations from the Framingham Heart Study indicated that moderate consumption of alcohol likely to improve the cognitive test performance in gender-specific manner[35]. Another study suggested that modulation of cognitive manifestation of alcohol consumption by genetic predisposition and environmental factors[36]. On the other hand, better performances in several cognitive domains in seniors were associated with moderate and regular alcohol intake[37]. Our observations of post-24 h retention time and post-48 h retention time also suggested the same.

To evaluate the consolidation of memory, a probe test was carried out in MWM navigation task[38]. During the probe test, when the hidden platform was not available in the target quadrant, animal groups of Et-II phase spent minimum time compared to the other three phases of the study. Interestingly, these animals took the lowest time in the acquisition phase. Therefore, the dose of ethanol exposure to Et-II animals might have demonstrated the anxiolytic effect of ethanol and allowed the animals to come out of thigmotaxis and made them more explorative. Though the significant rise in time spent within the target quadrant by T_{10} and T_{20} animals of Et-II and Et-III phases of study might be linked to positive cognitive performance because of T3 supplementation, the cause of a significant reduction in same for the Et-I phase of the study was not apparent. Similarly, the correlation coefficients between regional oxidative stress markers and probe test performance did not provide any specific clue for a particular oxidative stress marker; except that the frontal cortex and temporal cortex in the T_{20} group of animals are more likely to be associated with the probe test performances. This observation again suggested that the improvement or protection of cognitive function by T3 supplementation was independent of antioxidative property of T3.

Considering the oxidative stress parameters in the regions of the cerebral cortex of Et-III group of animals, T3 supplementation improved the antioxidant (GSH) level and reduced the effects of oxidative stress (LPO) in all the three studied regions and both the doses of T3 supplementation. Nevertheless, only TC of T_{20} group demonstrated the changes to be statistically significant when compared with T_0 animals. Similarly, increased activities of catalase, SOD, GPx, and GR were also observed in all the cerebral regions at both doses of T3 supplementation, with statistical significance only in case of SOD activity (T_0 *vs* T_{10}).Out of the 3 types of SOD, cytosolic Cu-Zn SOD (SOD1) and mitochondrial Mn-SOD (SOD2) are present in mammals[39] and involved in conversion of superoxide into H_2O_2.Catalase neutralizesH_2O_2 into water and O_2. GPx also removes H_2O_2, nevertheless, with the help of GSH. While GPx takes care about H_2O_2 of cytosol and mitochondria, catalase takes care of peroxisomal H_2O_2[40]. Overall, combined efforts of SOD, GPx and catalase provide the capacity to handle the superoxide and peroxides and protect cells from oxidative stress. Accordingly, glutathione-dependent and glutathione-independent SPHCs were also compared in the present study with the two doses of T3 supplementation. In addition to these changes, both glutathione-dependent and glutathione independent SPHCs were decreased in all the three tested cerebral regions upon supplementation with T3. Notably, there was no statistically significant difference between T_{10} and T_{20} animals in either of the oxidative stress parameters and their handling capacities in any of the cerebral regions. This, corroborate our earlier unpublished observation of T3 supplementation suggesting no additional benefit of T3 over the used dose of 10mg /day T3 (≈ 50 mg Oryza –Tocotrienol©-90/Kg body weight of rat) in terms of preventing oxidative stress. However, all positive correlation coefficients in FC and TC of T_{20} supplemented group (Table 1) suggested the importance of higher doses of T3 supplementation for neuroprotection particularly against the higher doses of pro-oxidant insults (Et-III of this study).

Observations from the present study should be interpreted keeping in mind that the only specific navigation tasks of MWM behavioral protocol were utilized. Besides, the study was carried out in four different phases; hence, the comparison between the phases of the study might not reflect the actual situation. Identification of specific and effective cognitive domain is must to carry out the mechanistic evaluation of T3 supplementation on neurobehavioral performances, in the future. Based on the observations from the current study, expression study of antioxidant markers in frontal and temporal cortices should provide fruitful insight towards the applicability of T3 supplementation regime for cognitive disorders.

Conclusion

The present study indicated that concurrent and post-intoxication oral supplementation with T3 could provide some domain-specific protection against cognitive deterioration caused by low-to-moderate doses of ethanol exposure. The study also indicated the possibilities of the beneficial effects of low-to-moderate doses of ethanol within the specified duration of exposure, particularly in some domains of cognitive performances; however, anxiolytic effect of ethanol exposure could also be responsible for the same. Oral supplementation with T3 with current doses and duration provided benefits against oxidative stress in different cerebral regions; nevertheless,

changes in oxidative stress biomarkers of most of the cerebral regions were not found to be associated with cognitive performances.

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