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Original Article

Impact of Thiamine Supplementation in the Reversal of ethanol induced toxicity in rats

Vidhya A., Renjugopal V. and Indira M.*

Department of Biochemistry,
University of Kerala,
Kariavattom, Thiruvananthapuram – 695 581, India

Abstract

One of the molecular mechanisms of alcohol induced toxicities is mediated by oxidative stress. Hence our studies were focused on the effect of thiamine (antioxidant) in the reversal of alcohol induced toxicity and comparison of the reversal with abstinence. Administration of ethanol at a dose of 4 g/kg body wt/day for 90days to Sprague Dawley rats manifested chronic alcohol induced toxicity evidenced by decreased body weight, an increase in liver-body weight ratio, increase in activities of serum and liver aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT); decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the liver and brain. The levels of inflammatory markers, fibrosis markers and DNA fragmentation were also elevated in the serum, liver and brain. After ethanol administration for 90days, the reversal of the alcohol induced toxicity was studied by supplementing thiamine at a dose of 25 mg/100 g body wt/day. Duration of the reversal study was 30 days. The activities of AST, ALT, GGT, scavenging enzymes as well as markers of inflammation and fibrosis in serum, liver and brain were reversed to a certain extent by thiamine. Changes in neurotransmitter levels in brain were also reversed by thiamine supplementation. DNA damage was decreased and DNA content increased in thiamine supplemented group compared to abstinence group showing a faster regeneration. In short, histopathological and biochemical evaluations indicate that thiamine supplemented abstinent rats made a faster recovery of hepatic and neuronal damage than in the abstinence group.

INTRODUCTION

Ethanol manifests its harmful effects either directly or through derangements in metabolic, hormonal and nutritional mechanisms (1). Excessive generation of free radicals play an important role in alcohol induced cellular damage by changing their structures and functions or by contributing to the mechanisms that finally promote enhanced oxidative damage (2, 3).

***Corresponding author :**
Indira M., Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram – 695 581, India
E-mail : indiramadambath@gmail.com, Ph. 091-471-2308078

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Adolescents diagnosed with alcohol use disorder show neurodegeneration in the hippocampus, a region important for learning, memory, and mood regulation. Neurogenesis is impaired in alcoholics by two mechanisms: alcohol-inhibition of neural stem cell proliferation and alcohol effects on new cell survival (4). Liver is the major target of ethanol toxicity and the role of oxidative stress in the pathogenesis of alcohol related liver diseases has been repeatedly confirmed (5).

Thiamine, also known as vitamin B1, is a water-soluble vitamin now known to play a fundamental role in energy metabolism. Thiamine acts as an antioxidant preventing oxidative stress toxicity (6). The most common cause of thiamine deficiency in

affluent countries is alcoholism. Alcohol affects thiamine uptake and other aspects of thiamine utilization in alcoholics (7). In heavy drinkers malabsorption is frequent, due to severe chronic impairment of liver or pancreas (8).

One of the most important problem faced by those who have stopped alcohol consumption after chronic intake, is the recovery of damaged organs to normal level. Since thiamine is deficient in alcoholics and also since it acts as an antioxidant, it was designed to study the effect of thiamine in reversal of alcohol induced toxicity and compare it with normal recovery during abstinence.

Materials and Methods

Selection of animals

Male Sprague Dawley rats weighing between 150 and 200 g were used. Animals were housed in polypropylene cages. Cages were kept in a room that was maintained between 28 and 32°C and a 12 h dark and light cycle was maintained. The study protocol was approved by the Institutional Animal Ethics Committee. Animals were handled using the laboratory animal welfare guidelines (9). Rats were fed with rat feed (Lipton India Ltd). Water was given *ad libitum*. Thiamine was purchased from M/s SRL Ltd., Mumbai, India and ethanol from M/s Merck Ltd., Mumbai, India.

Ethanol diluted with distilled water (1:1) was given orally by gastric tube. Thiamine was freshly dissolved in distilled water and given orally by gastric tube.

Groups

A total of 44 animals were first grouped into two as follows :

Group 1 Control

Group 2 Ethanol 4g/ kg /day

After 90 days, ethanol administration was stopped and 6 animals in ethanol group and control group were sacrificed after overnight fasting and liver was collected for biochemical analysis.

The rest of the animals in the control group were divided into two groups as follows :

Group 1 A Control

Group 1 B Thiamine (25 mg/kg/day).

The rest in the ethanol group were divided into two groups as follows :

Group 2 A Abstinence

Group 2 B Thiamine (25 mg/kg/day)

Dose of thiamine was selected from previous reports (10).

At the end of the experimental period, animals were sacrificed after overnight fasting. The liver and brain was dissected out and cleaned with ice-cold saline, blotted dry and immediately transferred to ice-cold container for various evaluations. Blood was drawn during the sacrifice and cryopreserved for various enzymatic assays. The brain and liver were dissected in half for biochemical assays and the other half for histology. This is schematically presented in Fig. 1.

Biochemical methods

The activities of liver and serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined by the method of Reitman and Frankel as described by Wooten (11) Gamma glutamyl trans peptidase (GGT) in the serum was assayed by Szasz method (12). Activities of super oxide dismutase(SOD) and catalase (CAT) were determined in both brain and liver by the method of Kakkar et al (13) and Maehly and Chance (14) respectively. The activity of glutathione peroxidase (GPx) was determined by the method of Agergaard and Jense (15) The activity of glutathione reductase (GR) was determined by the procedure of David and Richard (16). Total protein from brain, liver and serum samples were estimated by the method of Lowry et al (17). Hepatic regeneration was determined by the estimation of the total DNA concentration. DNA

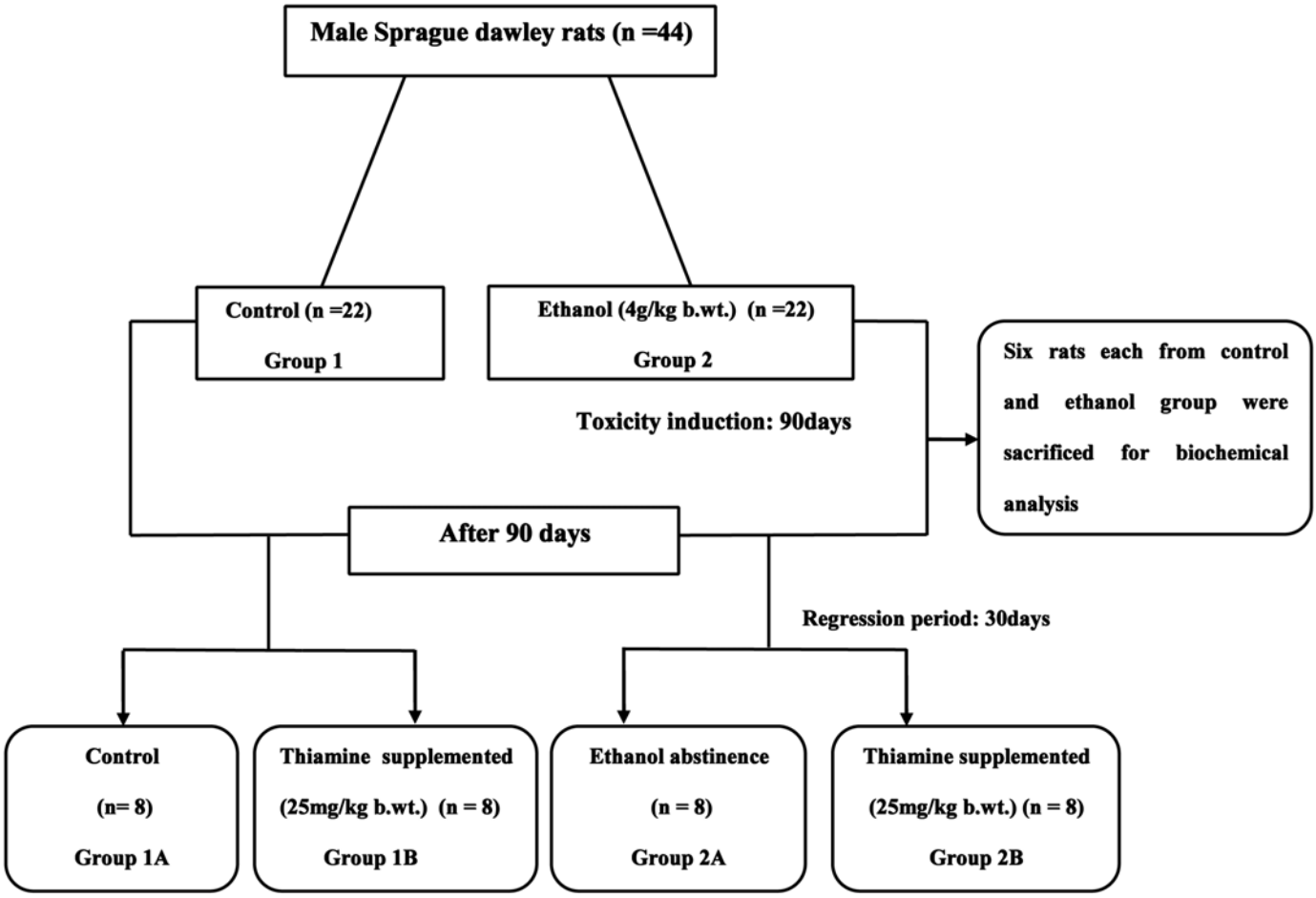

Fig. 1 : Experimental design.

concentration was determined fluorimetrically according to Burton et al (18). Quantitative estimation of DNA fragmentation was determined by colorimetric diphenylamine assay as described by Burton (18) Collagen and hydroxyprroline content were estimated by the method of Woesnner (19). Collagen linked fluorescence was estimated by the method of Monnier *et al* (20). Epinephrine, dopamine and serotonin were estimated according to the procedure of Schlumfjf (21). Activity of cyclooxygenase (COX) was carried out by the method of Kondo *et al* (22). Activity of lipooxygenase (LOX) was carried out by the method of Axelord *et al* (23). Activity of nitric oxide synthase (NOS) was carried out by the method of Miranda *et al* (24).

Monitoring the progression and reversal of hepatotoxicity

Change in the activity of hepatic toxicity marker, GGT, was assessed continuously. Blood was taken from the tail of rats at the end of every second week and the activity of GGT was determined for 12 weeks. During reversal period blood was taken from the tail of animals at the end of every week and activity of GGT was determined.

Histopathological studies

For histopathological studies, liver was fixed in Bouin's fixative and sections were taken in the microtome. Sections were stained using haematoxylin and eosin. Pathological changes were examined using a sensitive light microscope. Brain was dissected in half so that half was used for biochemical studies and half for histology. It was postfixed for 24 h in 4% paraformaldehyde then rinsed and stored in PBS until sectioning. Brains were sectioned coronally, at a thickness of 40 µm

from Bregma 1.2 through –7.0, on a vibrating microtome.

Statistical analysis

The results were analysed using a statistical programme SPSS/PC+, version 10 (SPSS Inc. Chicago, IL, USA). A one way ANOVA was employed for comparison among the six groups. Duncan's post hoc multiple comparison test of significant difference among groups were determined. $P \leq 0.05$ was considered significant. Mean values with the same superscript do not differ significantly. Significance accepted at $P<0.05$.

Results

Activities of Toxicity marker enzymes (Table-I)

Activities of AST, ALT and GGT in serum and liver were significantly increased in rats given ethanol. Abstinence from ethanol decreased the activities of these enzymes significantly. Thiamine supplementation further reduced their activities when compared to abstinence group. Groups 1, 1A and 1B did not show any difference in the activities of toxicity marker enzymes.

Concentration of Total DNA and percentage of DNA fragmentation in liver and brain (Table-II)

Ethanol administration resulted in significant reduction in total DNA content in both liver and brain tissues. Abstinence from ethanol increased the DNA content in liver, but not in brain. Thiamine supplementation increased the DNA content in both tissues as compared to ethanol group. Groups 1,1A and 1B did not show any significant difference in DNA level.

DNA fragmentation in ethanol fed group was much higher when compared to control group. Abstinence from ethanol decreased fragmentation to a small extent in liver, but not in brain. The percentage of fragmentation was decreased significantly by thiamine

TABLE I: Activities of Toxicity marker enzymes.

Groups	*ALT (µmoles of pyruvate (liberated/min/mg protein)*		*AST (µmoles of OAA (liberated/min/mg protein)*		*GGT (* units/mg protein)*
	Liver	*Serum*	*Liver*	*Serum*	*Serum*
1	15.76 ± 1.44^a	51.21 ± 4.90^a	17.3 ± 1.47^a	185.37 ± 17.77^a	5.29 ± 0.48^a
2	100.55 ± 9.17^b	165.10 ± 14.97^b	75.35 ± 6.55^b	536.02 ± 37.64^b	16.61 ± 1.51^b
1A	16.24 ± 1.68^a	56.09 ± 5.4^a	15.58 ± 1.51^a	201.88 ± 18.22^a	5.02 ± 0.44^a
1B	14.47 ± 1.31^a	49.73 ± 4.53^a	21.17 ± 1.93^a	189.64 ± 17.79^a	4.17 ± 0.33^a
2A	62.48 ± 5.61^c	117.67 ± 10.74^c	51.8 ± 4.49^c	408.56 ± 37.22^c	11.87 ± 1.12^c
2B	43.85 ± 3.82^d	94.64 ± 8.6^d	49.26 ± 4.45^d	292.96 ± 29.22^d	8.38 ± 0.80^d

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at $P<0.05$.
*Units – The molar absorption coefficient of p-nitroaniline at 450 nm is $9900/mol^{-1}/cm^{-1}$.
OAA – Oxaloacetic acid.

TABLE II: Concentration of Total DNA and % of DNA fragmentation in liver and brain.

Groups	*Liver DNA (mg/g tissue)*	*Brain DNA (mg/g tissue)*	*Liver DNA fragmentation (%)*	*Brain DNA fragmentation (%)*
1	2.05 ± 0.19^a	1.43 ± 0.11^a	15.2 ± 1.06^a	4.5 ± 3.5^a
2	0.82 ± 0.07^b	0.96 ± 0.08^b	58.8 ± 4.83^b	14.2 ± 1.26^b
1A	2.10 ± 0.19^a	1.48 ± 0.12^a	15.94 ± 1.08^a	4.52 ± 3.5^a
1B	2.16 ± 0.20^a	1.52 ± 0.12^a	14.8 ± 1.04^a	4.38 ± 3.2^a
2A	1.03 ± 0.09^c	1.07 ± 0.09^b	42.2 ± 3.6^c	12.94 ± 1.02^b
2B	1.14 ± 1.00^d	1.36 ± 0.10^a	38.12 ± 2.7^d	7.14 ± 6.04^a

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at $P<0.05$.

supplementation in both tissues as compared to ethanol group. Groups 1, 1A and 1B did not show any significant difference in DNA fragmentation.

Activities of Superoxide dismutase and Catalase (Table-III)

Activities of Superoxide dismutase and catalase were reduced significantly in rats given ethanol compared to normal in both brain and liver tissues. Abstinence from ethanol increased the activities of these enzymes when compared to the ethanol group. Thiamine supplementation significantly increased the activities of these enzymes when compared to abstinence group. Supplementation of thiamine to control did not alter the activities of the enzymes in liver, but increased significantly in brain. Control groups 1 and 1A did not show any significant difference.

Activities of Glutathione peroxidase and Glutathione reductase in liver and brain (Table-III)

Activities of glutathione peroxidase and glutathione reductase showed a significant decrease in ethanol fed rats when compared to normal. Abstinence and thiamine supplementation reversed the activities to a significant level when compared to the ethanol group. More significant increase was found with thiamine supplementation. Thiamine supplementation to normal rats increased the enzyme activities in brain and liver significantly. Control rats 1 and 1A did not show any significant difference in the activities of these enzymes.

TABLE IV : Concentrations of Hydroxyl proline, Total collagen and Collagen linked fluorescence in liver.

Groups	*Hydroxyl proline (mg/100 g protein)*	*Total collagen (mg/100 g protein)*	*Collagen linked fluorescence (AU/µmol of hydroxyproline)*
1	9.54±0.52[a]	69.21±5.99[a]	29.77±2.80[a]
2	19.19±1.74[b]	134.23±12.15[b]	61.62±5.61[b]
1A	9.72±0.62[a]	70.08±6.01[a]	30.98±2.91[a]
1B	9.95±0.93[a]	69.82±6.16[a]	28.18±2.62[a]
2A	15.68±1.49[c]	115.84±11.31[c]	49.35±4.48[c]
2B	13.89±1.40[d]	102.12±10.01[d]	42.21±3.91[d]

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at P<0.05.

Concentrations of Hydroxyproline, Total collagen in liver and Liver Collagen linked fluorescence (Table-IV)

Total liver collagen content, hydroxyl proline and collagen linked fluorescence were increased significantly in rats fed with ethanol as compared to control rats. Abstinence and thiamine supplementation reduced both collagen and hydroxyl proline significantly when compared to ethanol. More significant reduction occurred in thiamine supplemented group as compared to abstinence group. Groups 1, 1A and 1B did not show any significant difference in collagen and hydroxyl proline level.

Activities of COX and LOX in monocytes, NOS in serum (Table-V)

The activities of the enzymes-LOX, COX and NOS were significantly increased in ethanol administered

TABLE III : Activities of Superoxide dismutase, Catalase Glutathione peroxidase and Glutathione reductase in liver and brain.

Groups	*Superoxide dismutase (units/mg protein)*		*Catalase (*units/mg protein)*		*Glutathione peroxidase (µmole NADPH oxidized/min)*		*Glutathione reductase (µmole NADPH oxidised/min)*	
	Liver	*Brain*	*Liver*	*Brain*	*Liver*	*Brain*	*Liver*	*Brain*
1	82.67±8.34[a]	10.55±0.94[a]	64.21±6.15 [a]	8.22±0.75[a]	12.11±1.17[a]	4.27±0.37[a]	20.75±1.87[a]	52.15±4.67[a]
2	27.24±2.62[b]	3.81±0.36[b]	45.87±4.39[b]	2.93±0.26[b]	5.93±0.56[b]	0.88±0.07[b]	3.32±0.32[b]	32.98±2.99[b]
1A	86.01±8.12[a]	12.04±0.87[a]	67.84±6.40[a]	8.84±0.77[a]	13.02±1.43[a]	4.37±0.34[a]	24.04±2.21[a]	52.29±4.68[a]
1B	87.44±8.38[a]	13.26±1.18[c]	60.10±5.76[a]	10.51±0.96[c]	14.32±1.45[c]	5.40±0.49[c]	26.16±2.24[c]	56.48±4.86[c]
2A	35.54±3.4[d]	6.08±0.47[d]	52.01±4.98[d]	4.12±0.37[d]	7.78±0.66[d]	1.88±0.56[d]	8.16±0.76[d]	40.06±3.35[d]
2B	46.69±4.3[d]	8.63±0.79[e]	56.46±5.15[d]	7.25±0.65[e]	8.46±0.80[e]	3.02±1.84[e]	12.34±1.14[e]	46.87±3.93[e]

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at P<0.05.
Units - Enzyme concentration required to inhibit the chromogen production by 50% in one min.
* Units - Velocity constant/s.

TABLE V : Activities of Cyclooxygenase and Lipoxygenase in monocytes, Nitric oxide synthase in serum.

Groups	*Cyclooxygenase (µmoles of MDA liberated/mg protein)*	*Lipoxygenase (*Units/mg protein)*	*Nitric oxide synthase (#Units/mg protein)*
1	0.41±0.04[a]	1.33±0.13[a]	1.17±0.11[a]
2	2.89±0.26[b]	4.84±0.45[b]	5.97±0.56[b]
1A	0.40±0.04[a]	1.10±0.08[a]	1.14±0.10[a]
1B	0.42±0.04[a]	1.10±0.08[a]	1.11±0.10[a]
2A	2.29±0.2[c]	3.91±0.35[c]	4.31±0.39[c]
2B	1.15±0.10[d]	2.48±0.22[d]	4.12±0.37[c]

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at P<0.05.
*Units - OD shift/min/mg protein.
#Units - Change in OD/min/mg protein.

rats when compared to control rats. Abstinence group showed significant reduction in the activities of these enzymes compared to ethanol group. Thiamine supplementation significantly reduced the activities of COX and LOX when compared to abstinence. But it did not reduce NOS activity as compared to abstinence group. Groups 1, 1A and 1B did not show any significant difference in the activities of COX, LOX and NOS.

Concentration of Neurotransmitters and activity of Transketolase (Table-VI)

Epinephrine level showed significant reduction in ethanol administered rats when compared to control. Epinephrine was increased to a significant level by abstinence from ethanol and thiamine supplementation as compared to ethanol group. Dopamine and serotonin levels showed a significant increase in ethanol administered group as compared to control group. Abstinence from alcohol did not change the dopamine and serotonin level as compared to ethanol group. Thiamine supplementation reversed the changes in epinephrine, dopamine and serotonin to normal levels. There was no alteration in the levels of epinephrine, serotonin and dopamine in groups 1, 1A and 1B. Activity of transketolase was decreased significantly in ethanol administered group as compared to control. Abstinence from ethanol did not alter the activity of transketolase. Thiamine supplementation increased its activity significantly. Thiamine supplementation to control group significantly increased the activity of this enzyme. Groups 1 and 1A did not show significant difference in the activity of transketolase.

Monitoring the activity of GGT

GGT activity showed a steady increase during ethanol administration (Fig. 2). Abstinence from ethanol resulted in a decrease in GGT activity. Thiamine supplementation during reversal period resulted in a faster reduction in GGT activity as compared to abstinence group. Thiamine supplementation in control group did not affect GGT activity significantly.

Histopathology of liver and brain

After ethanol administration liver sections showed extensive hepatocellular damage as evidenced by necrosis, ballooning of hepatocytes, steatosis, vacuolization and dilation of sinusoids. The histological features of the liver in the control group showed a normal liver architecture and cell structure. The histological features of the liver in the control group (1A) showed a normal liver architecture and cell structure. Thiamine supplementation to control

TABLE VI : Concentration of Neurotransmitters and activity of transketolase.

Groups	*Epinephrine (µM/g tissue)*	*Dopamine (µM/g tissue)*	*Serotonin (µM/g tissue)*	*Transketolase (*units/ml hemolysate)*
1	3.89±0.35[a]	4.69±0.43[a]	1.64±0.15[a]	625.22±59.88[a]
2	1.86±0.17[b]	10.21±0.93[b]	2.27±0.2[b]	466.43±42.11[b]
1A	3.98±0.36[a]	4.72±0.44[a]	1.72±0.15[a]	631.64±60.44[a]
1B	4.02±0.37[a]	4.94±0.45[a]	1.76±0.17[a]	702.56±68.34[c]
2A	2.18±0.20[c]	9.24±0.84[b]	2.13±0.19[b]	486.76±44.86[b]
2B	3.60±0.29[a]	4.78±0.59[a]	1.79±0.17[a]	544.64±51.42[d]

Values are given as mean±SD of six rats in each group. Mean values with the same superscript do not differ significantly. Significance accepted at P<0.05.
*Units - µg sedoheptulose-7-phosphate formed/ml hemolysate.

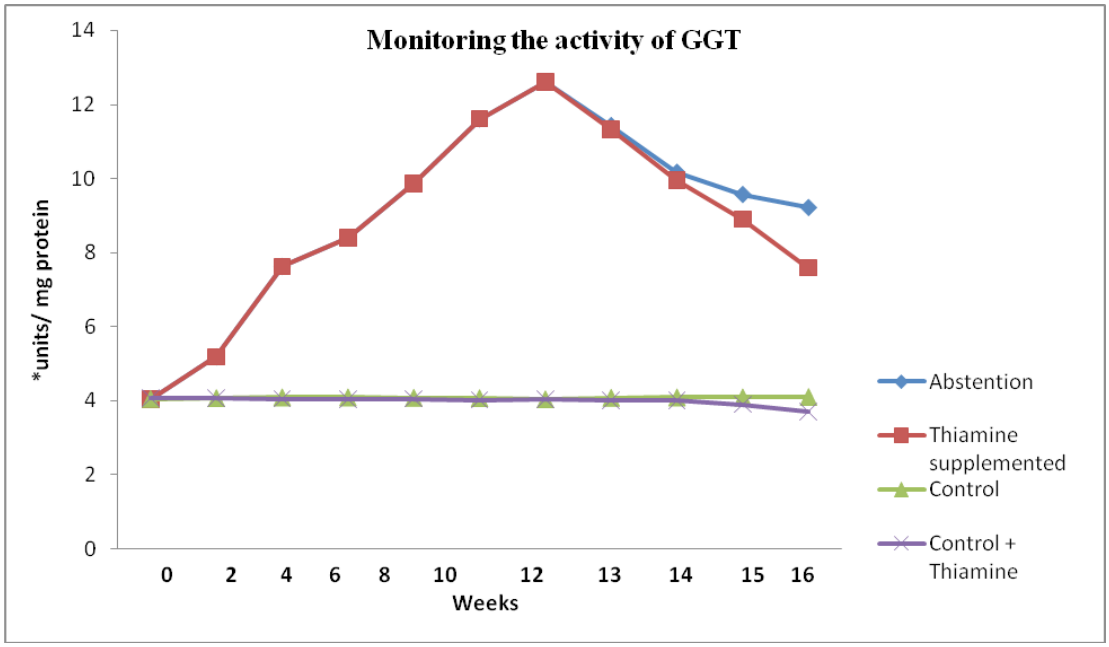

Fig. 2 : Monitoring the activity of GGT. Abstention period starts after 12th week. *Units – The molar absorption coefficient of p-nitroaniline at 450 nm is 9900/mol^{-1}/cm^{-1}.

rats did not alter normal liver structure. Abstinence from ethanol resulted in reduction in hepatic damage as evidenced by mild steatosis. There was mild reduction in histopathological changes by thiamine treatment.

After ethanol administration brain sections showed that the number of degenerated neurons increased in comparison with control. The histological features of the brain in the control group showed a normal architecture and cell structure. Brain in group 1A and 1B also showed normal structure. Abstinence from ethanol resulted in reduction in neuronal damage as evidenced by mild sclerosis. The histopathological changes were ameliorated by thiamine treatment. Damage is of lesser degree in thiamine treated group.

Discussion

Alcoholism is a problem affecting a large segment of the society. Treatment to alcoholism should have different approaches ranging from abstinence, medication, psychological and spiritual. Formulation of an effective and, economic way to recover from the various toxicities induced by alcohol is of particular importance for a healthy society. One of the molecular mechanisms of alcohol induced toxicities is mediated by oxidative stress. Hence our studies were focused on the effect of thiamine in the reversal of alcohol induced toxicity and comparison of the reversal with abstinence. We observed that administration of thiamine caused a faster recovery of hepatic and neuronal damage than the abstinence group.

Ethanol administration caused significant increase in the activities of toxicity markers ALT, AST and GGT indicating ethanol induced toxicity. But post-treatment with thiamine reduced ethanol induced toxicity as indicated by drop in activities of marker enzymes. This is in line with earlier reports (25). Activity of serum GGT, the most significant marker of hepatotoxicity, which showed rapid increase in its activity during ethanol administration, was reversed more significantly by the administration of thiamine when compared to abstinence group indicating that thiamine supplementation leads to a faster recovery of hepatic damage than abstinence.

There was a significant reduction in the activities of scavenging enzymes upon ethanol administration. The activities of SOD, CAT, GPx and GR increased significantly by thiamine supplementation as compared to abstinence group. These observations

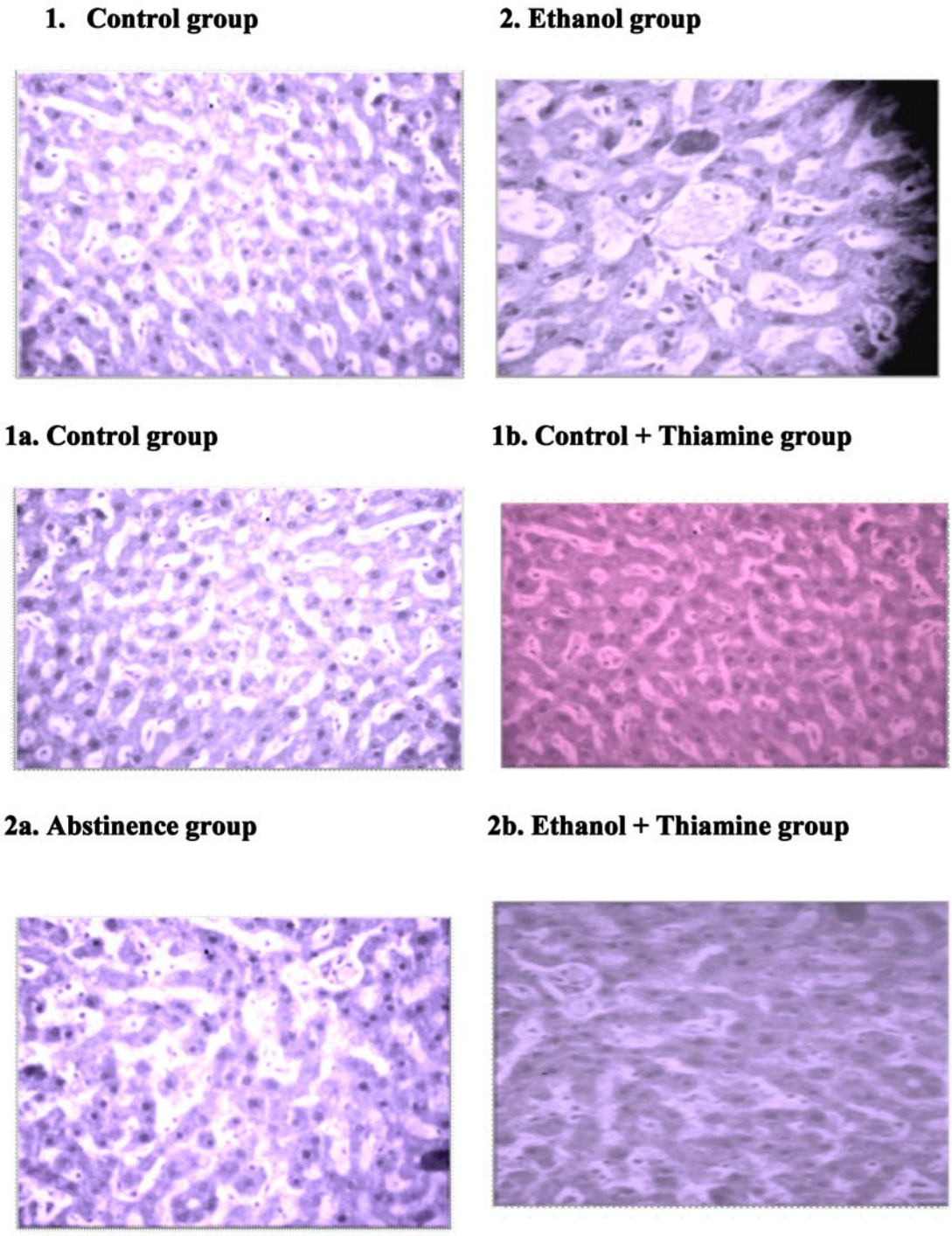

Fig. 3 : Histopathology of Liver.

1 : Microphotograph of liver of the Control group (1) (40X). This slide shows the structure of normal liver. Normal hepatocytes are seen.

2 : Microphotograph of liver of the Ethanol treated group (40X). Damage is noted in the slide as ballooning of hepatocytes, steatosis, vacuolization and dilation of sinusoids.

1a : Microphotograph of liver of the Control group (40X).
This slide shows the structure of normal liver. Normal hepatocytes are seen.

1b : Microphotograph of liver of the Control + Thiamine treated group (40X). This slide shows hepatocytes with normal structure

2a : Microphotograph of liver of the Ethanol abstinence group (40X). Marked damage to hepatocytes is noted.

2b : Microphotograph of liver of the Ethanol + Thiamine treated group (40X).
Mild ballooning of hepatocytes and mild portal triaditis noted.

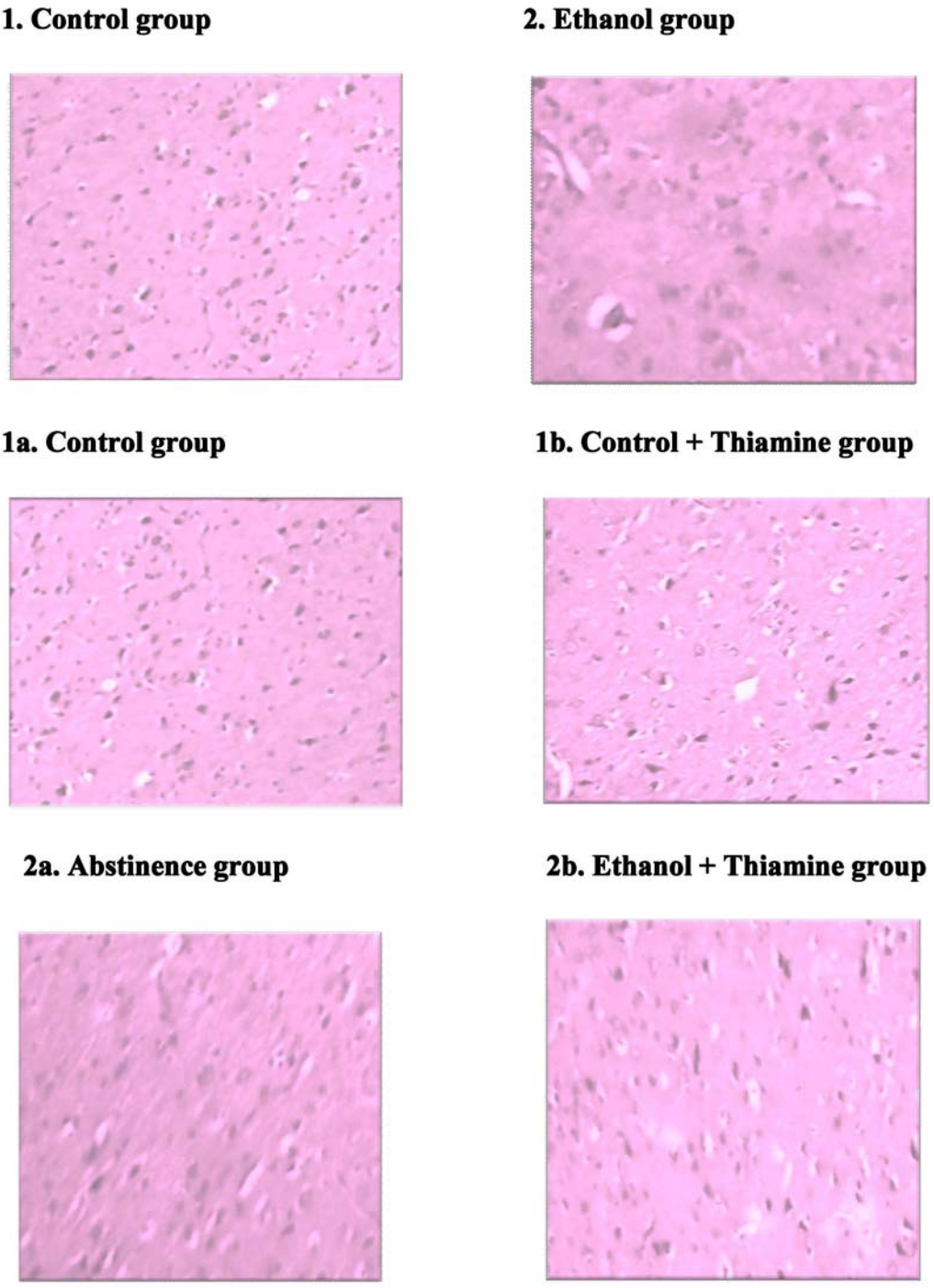

Fig. 4 : Histopathology of Brain.

1 : Microphotograph of brain of the Control group (10X × 10).This slide shows the structure of a normal brain cell

2 : Microphotograph of brain of the Ethanol group (10X × 10).Extensive sclerosis in the choroid plexus was observed. Degeneration of neurons was observed.

1a : Microphotograph of brain of the control group (10X × 10).Normal neurons.

1b : Microphotograph of brain of the Control + Thiamine group (10X × 10). The cells were similar to that of control.

2a : Microphotograph of brain of the Abstinence group (10X × 10). Moderate sclerosis in the choroid plexus was observed.

2b : Microphotograph of brain of the Ethanol + Thiamine group (10X × 10). The cells were almost similar to that of control. Regeneration of brain cells was observed.

are in agreement with findings of Jung and Kim (26) which showed that thiamine may act as a potent antioxidant as it scavenges free radicals such as 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation relatively slowly. The fact that simultaneous supplementation of ascorbic acid and thiamine ameliorated the liver damage in arsenic treated mice, indicating that prevention was attributed to apoptotic inhibition by increased levels of GP_X, SOD and GSH. It has been shown that thiamine increased the antioxidant enzyme-SOD and CAT levels in arsenic intoxicated animals (27). Thiamine induced elevation of GR activity in myocardial hypertrophy (28).

The present study demonstrated that ethanol exposure results in significant DNA damage in rat liver. Thiamine supplementation reduced the level of DNA damage induced by ethanol as compared to abstinence. This is in line with the reports that thiamine when supplied along with ascorbic acid reversed the DNA damage induced by lead in rat liver (29) and mice testes (30). This may be due to reduction in the production of reactive oxygen species by thiamine.

Ethanol administration led to fibrosis as indicated by increased levels of collagen and hydroxyproline. This is in agreement with earlier reports proved that thiamine supplementation prevented diabetes-induced cardiac fibrosis in experimental diabetes, probably by suppression of hexosamine biosynthesis pathway (31).

In the present study there was significant increase in the activities of COX and LOX upon ethanol administration. Thiamine supplementation decreased the activities of these enzymes as compared to abstinence indicating reduction in alcohol induced inflammation. This is in line with the studies of (32) that decrease in the activity of thiamine dependent-ketoglutarate dehydrogenase is associated with release of proinflammatory cytokines.

Alcoholism results from interplay between genetic and environmental factors, and is linked to brain defects and associated cognitive, emotional, and behavioral impairments. Studies indicate that the frontal lobes, limbic system, and cerebellum are particularly vulnerable to damage and dysfunction (33) Alcohol-induced changes in NOS may alter immunocompetence, response to injury in the central nervous system, and may be involved in ethanol-mediated neurodegeneration and neurotoxicity (34). Alcoholics were reported to have significantly altered hypothalamic-pituitary-adrenal axis functioning up to 3 weeks following the cessation of drinking, with a more subtle impairment present for greater than 6 months following abstinence it was reported (35, 36) that abstinence-associated tissue volume gains were found to be significant in focal parts of the fronto-ponto-cerebellar circuit that is adversely affected by heavy drinking. This is in agreement with reports that indicate rapid recovery of ventricular volume and metabolite levels with removal of the causative agent (37). Thiamine supplementation reversed the changes in neurotransmitter levels in brain as compared to abstinence group. Ameliorated sclerosis in the thiamine treated group supports this finding. Eventhough thiamine is a well known antioxidant its neuroprotective mechanism has to be further elucidated. An earlier study suggested that acute thiamine deficiency, both increases brain 5-HT synthesis and impairs 5-HIAA efflux from the brain. There is a close correlation between neurological manifestations and changes in brain 5-HT metabolism in acute thiamine deficiency (38). In accordance with earlier reports (39), the activity of transketolase was significantly decreased in ethanol administered rats. This may be due to depletion of thiamine which is a cofactor for transketolase activity. It has been shown that metabolic disturbance of thiamine causes the depression of the enzymatic activity in experimental liver injury (40). Supplementation of thiamine restored the activity of this enzyme to a certain extent.

Hepatotoxicity induced by ethanol is further confirmed by abnormal histological findings. Toxicity manifestations by ethanol in the liver tissue are revealed by morphological changes such as inflammation around portal triad (Triaditis) with severe fatty degeneration. Thiamine supplementation reduced the morphological changes to a certain extent. Damage was milder as compared to abstinence group. Toxic manifestations are shown by morphological

changes such as sclerosis in brain. Thiamine supplementation resulted in significant reduction in sclerosis and increased regeneration as compared to abstinence group.

From this study it can be concluded that thiamine supplementation have a curative effect on ethanol induced hepato and neuro toxicity. Abstinence from ethanol reversed many of these effects produced by ethanol administration. But thiamine supplementation made the recovery faster compared to abstinence alone. Thus thiamine supplementation can be done for a speedy recovery from ethanol induced toxicity in abstaining subjects.

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