

Original Article

THIAMINE DEPRIVATION DISTURBS CHOLINERGIC SYSTEM AND OXIDATIVE STRESS IN
LIVER OF MUS MUSCULUS

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ABSTRACT

Objective: Present study determines the change in the levels of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), reduced glutathione (GSH), lipid peroxidation (LPO), protein carbonyl content (PCC) and catalase (CAT) in liver of experimental groups. Change in total proteins of liver was also observed.

Methods: Experiment animals were divided in three groups among which group I was considered as control. Group II and group III were made thiamine deficient for 8 and 10 days respectively. Enzyme activities of the groups were determined.

Results: Activity of AChE ($p < 0.001$) and BChE ($p < 0.05$) significantly reduced in experimental mice exposed for 8 and 10 days as compared to control. Reduced levels of GSH were found in experimental groups as compared to group I. Elevation in TBARS level was determined significantly enhanced in group III as compared to control ($p < 0.01$) and group II ($p < 0.05$). In comparison to control, inter group analysis revealed significant ($p < 0.001$) increase of PCC in thiamine deprived groups. Significant decrease in the activity of CAT ($p < 0.001$) was recorded in treated groups, compared to control. Biochemical and molecular studies of total protein also showed the increase in protein level of treated mice compared to control.

Conclusion: Imbalance between free radical and endogenous antioxidants in the liver creates oxidative stress, disturbs the cholinergic system in liver, and affects the signaling of body which may lead to progression of hepatotoxicity.

Keywords: Cholinesterases, Liver, Thiamine deficiency, Total protein.

INTRODUCTION

Thiamine is a water soluble vitamin B1 that cannot be synthesized by mammals and thus thiamine can be obtained only from diet [1,2]. It plays a role in the synthesis of AChE, via the synthesis of acetyl CoA. As thiamine plays a role in the synthesis of AChE, it is likely that deficiency of thiamine will lead to alter the level of AChE. BChE has a synergistic relationship with AChE to maintain the cholinergic function of the body [3], thus the amendment of AChE levels in the body directly affects the level of BChE. AChE and BChE are cholinergic neurotransmitters and their role is to terminate action of the acetylcholine (ACh) and butyrylcholine in synapses terminals. It has been reported that AChE and BChE have some non cholinergic function also in the body.

These cholinesterases occurred in the polymorphic form in other organs of the body beside brain. They play role in various physiological functions such as cell division, cell-cell interaction and amine sensitive aryl acylamidase activity [4]. Thiamine is also reported to inhibit the LPO and free radical oxidations of oleic acid in rat liver microsomes while its deficiency endorses the LPO in the cell [5]. The major stable end product of LPO is thiobarbituric acid reactive substance (TBARS) [6]. Determination of TBARS levels in the cell act as an indicator of oxidative stress. LPO may be mutagenic and carcinogenic [7].

High levels of LPO products were found in liver of rats with thiamine deficiency [8]. The free radicals directly attack on the double bonds of polyunsaturated fatty acids in the cell membrane which induces LPO that had been implicated as important oxidative stress determining marker in various clinical disorders such as heart disease, diabetes, gout and cancer. The major defense system in the cell includes antioxidant enzymes, which convert active oxygen molecules into non-toxic compounds [9]. The imbalance between the oxidants and antioxidants, potentially leads damage in the cell called as oxidative stress [10].

Glutathione is the most abundant tripeptides nonenzymatic antioxidants present in the liver. It serves as substrate for glutathione peroxidase and GST. The functions of glutathione are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols [11]. As the oxidative stress increases, the carbonyl content of protein increases due to oxidation of protein. ROS give rise to a variety of modifications in amino acid residues of protein like cysteine, methionine, tryptophan, arginine, lysine, proline, and histidine [12,13].

The evaluation of protein carbonyl content in the cell acts as a physiological marker that shows oxidative stress [14,15]. CAT is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. The decomposition of hydrogen peroxide into water and oxygen is the key role of CAT in the cell [16].

It protects the tissue from highly reactive hydroxyl. The alteration in the activity of CAT enzyme directly affects the generation of free radicals causes oxidative stress [17]. Deficiency of thiamine may lead to oxidative stress which is the key feature of various diseases such as fatigue, muscular weakness, Wernicke's encephalopathy and neurodegeneration [2,18,19]. Liver acts as a province for detoxification, protein synthesis, hormone production and glycogen storage. The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of several metabolic functions [20]. Additionally, it is the key organ of metabolism and excretion, thus it is constantly and diversely exposed to xenobiotics because of its strategic placement in the body [17].

Bearing in mind above mentioned facts, the present investigation was destined to determine the changes in the echelon of cholinesterases, GSH, LPO, PCC, protein and CAT activity. Molecular study of protein was also determined in the liver of thiamine deficient mice.

MATERIALS AND METHODS

Chemicals

Pyriithiamine hydrobromide and acetylthiocholine iodide were purchased from Sigma (MO, USA). dithiothreitol, 5, 5'-dithiobis-2-nitrobenzoic acid, (DTNB), butyrylthiocholine iodide, EDTA, tris hydrochloride, trichloroacetic acid (TCA), proteinase K, 2-thiobarbutric acid were purchased from Sisco research laboratories (Mumbai, India). All other chemicals were of analytical grade.

Animal care and monitoring

Healthy Mus musculus (6-8 week old) were procured from C.C.S. Haryana Agricultural University, Hisar. They fed with pelleted diet (Hindustan Uniliver Limited) and water *ad libitum*. After 3-7 days of adaptation, mice were used for experimental purpose. Maintenance and treatment of animals were done in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

Experimental Design

The animals were divided into 3 groups, with minimum of 6 animals in each group.

Group I Normal pelleted diet + normal saline

Group II Thiamine deficient diet + pyriithiamine exposed for 08 days

Group III Thiamine deficient diet + pyriithiamine exposed for 10 days

Procedure

Induction of thiamine deficiency (TD)

Mice were made thiamine deficient by injecting the pyriithiamine hydrobromide (5 µg/10 g of body weight) intraperitoneally and fed with thiamine deficient pelleted diet. Control animals were fed with normal diet.

Biochemical Studies

Tissue preparation for determining the activity of AChE and BChE

After dosing, the experimental mice were sacrificed by cervical dislocation. Livers were removed and extraneous material was washed with normal saline (0.85%). The tissues were homogenized in the specific buffer (0.1M phosphate buffer). The samples were centrifuged at 3500 rpm for 10 min at 4°C. Supernatant were used for biochemical analysis.

Estimation of AChE

Thiocholine released from acetylcholine by the AChE, reacts with the DTNB, reducing it to the thiol which has an absorption maximum at 412 nm. The activity of AChE was assayed according to the method of Ellman *et al.*, (1961) [21].

Estimation of BChE

Ellman *et al.*, (1961) [21] method was used for determination of BChE activity. BChE releases thiocholine from butyrylcholine and reacts with DTNB reduced into thiol which shows maximum absorption at 412 nm.

Estimation of GSH level

GSH level served as an index for determining the extent of LPO. GSH was determined by Ellman's method [22].

Estimation of LPO

TBARS are harmful substances formed by LPO as degradation products of fats in foodstuff. Spectrophotometric method of Ohkawa *et al.*, (1979) [23] was used for LPO determination.

Estimation of PCC

Detection of protein carbonyl group with 2,4-dinitro phenylhydrazine (DNPH), which leads to formation of a stable dinitrophenyl (DNP) hydrazone product. The measurement of

protein carbonyls following their covalent reaction with DNPH was pioneered by Levine [24].

Estimation of CAT

CAT activity was measured by recording a decrease in absorbance of H₂O₂ at 240nm due to initial rate of disappearance of H₂O₂ after 15-20 seconds. The CAT activity was determined by Claiborne, (1985) method [25].

Extraction and Estimation of total proteins

The proteins were isolated from the liver tissue. The proteins were extracted and using acetone, the proteins were precipitated. The samples were centrifuged and pellet was resuspended in phosphate buffer (pH 7.4). For biochemical estimation of total proteins Lowry *et al.*, (1951) [26] method was used.

Molecular quantification of proteins

Expression of the protein samples were further quantified by SDS-PAGE [27]. The acrylamide (11%) gel was used for separation of proteins. The samples were mixed with sample loading buffer in ratio of 1:1 and heated for 5min at 95°C. Then samples were loaded in the wells and proteins were allowed to run at 100 V.

Data analysis and statistics

The data were represented as mean ± SD. The statistical analysis was done by using one way- analysis of variance (ANOVA) (SPSS editor 16). Inter group comparisons were made by post-hoc comparison analysis and significant level was measured at 95% and 99%.

RESULTS

AChE (Fig 1)

In the present study, AChE level significantly ($p < 0.001$) lowered in treated groups which were made thiamine deficient for 8 days and 10 days respectively as compared to control group. Group III exhibits maximum decrease in the activity of AChE, among all the groups.

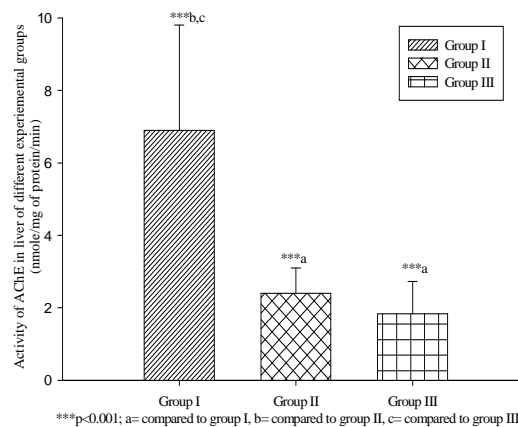


Fig.1: Effect of TD on AChE level in different experimental groups as mean ± SD. Results were expressed in nmole/mg of protein/min.

BChE (Fig 2)

When compared with group I, significantly reduced BChE levels were recorded in groups II and III ($p < 0.05$). Maximum decreased level of BChE was significantly ($p < 0.05$) observed in treated group exposed for 10 days as compared to control.

GSH (Fig 3)

GSH concentration was recorded to be reduced in group II and group III as compared to control group. The group which was exposed to thiamine deficiency for 10 days showed the minimum GSH concentration among all experimental groups.

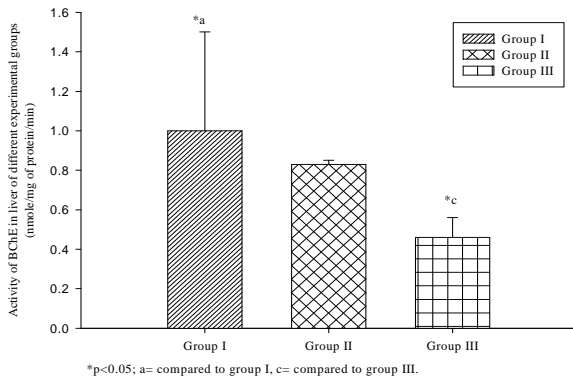


Fig. 2: Effect of TD on BChE level in different experimental groups as mean ± SD. Results were expressed in nmole/mg of protein/min.

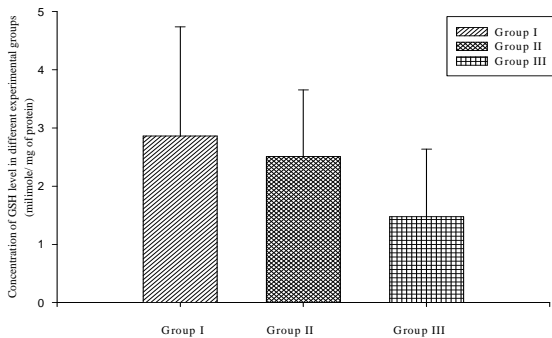


Fig. 3: Effect of TD on GSH level in different experimental groups as mean ± SD. Results were expressed in mmole/mg of protein.

LPO (Fig 4)

TBARS concentration elevated in groups II and III as compared to control group. In group III, a significant rise in TBARS level was recorded (p<0.01), compared to group I. The significant (p<0.05) enhancement of TBARS level was also calculated by group III in comparison to group II.

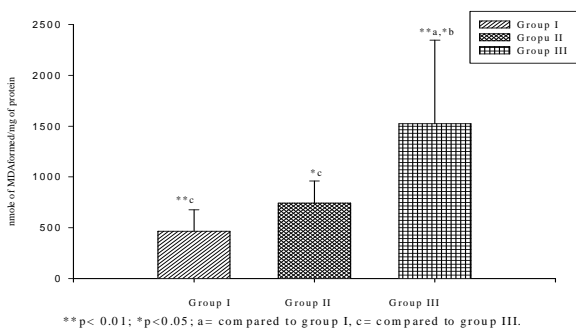


Fig.4: Effect of TD on LPO in different experimental groups as mean ± SD. Results were expressed in nmole of MDA formed/mg of protein

PCC (Fig 5)

PCC level significantly elevated in group II (p<0.001) and group III (p<0.001) as compared to control group. A significant reduced PCC level was obtained in group II as compared to group III (p<0.001).

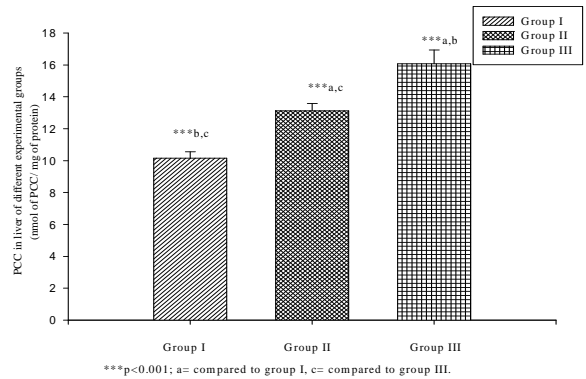


Fig. 5: Effect of TD on PCC level in different experimental groups as mean ± SD. Results were expressed in nmole of PCC/ mg of protein

CAT (Fig 6)

CAT concentration significantly decreased in groups II (p<0.001) and group III (p<0.001) as compared to control group. Among all the groups, maximum reduction of CAT activity was observed in thiamine deficient group exposed for 10 days.

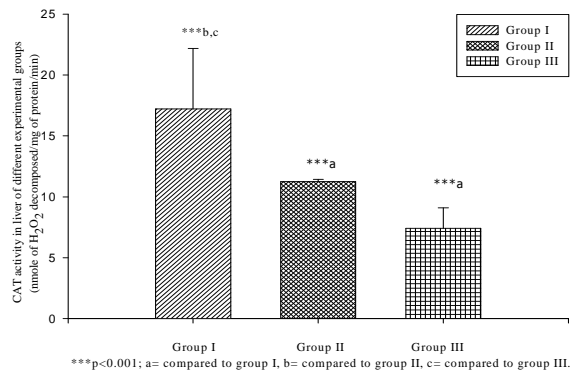


Fig. 6: Effect of TD on CAT activity in different experimental groups as mean ± SD. Results were expressed in nmole of H₂O₂ consumed/mg of protein/min.

Total protein content (Fig 7A)

As compared to control, enhancement in protein levels were observed in treated groups. Maximum increase was recorded in group III, among all the groups.

Molecular changes of protein (Fig 7B)

A visible change in the band patterns and their intensity was recorded for thiamine deficient groups (TD 8 and TD 10) as compared to control (7B). Differences in intensity of staining reflect relative abundance of individual polypeptides. The degree of protein level enhanced as exposure time of the dose increases. The maximum expression of protein level was observed in group exposed for 10 days with TD.

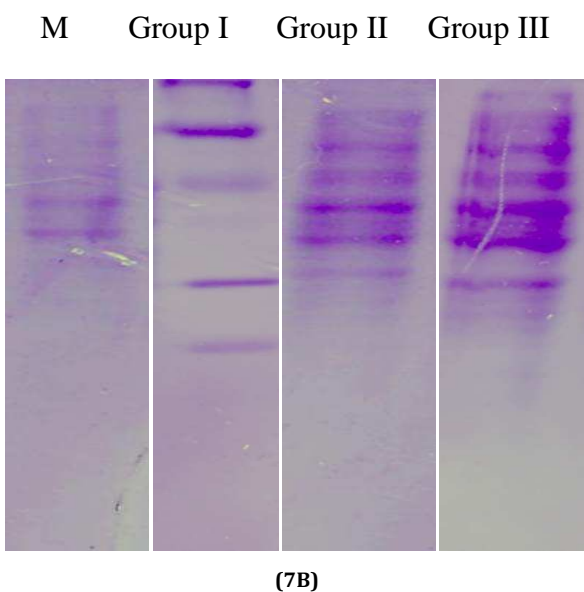
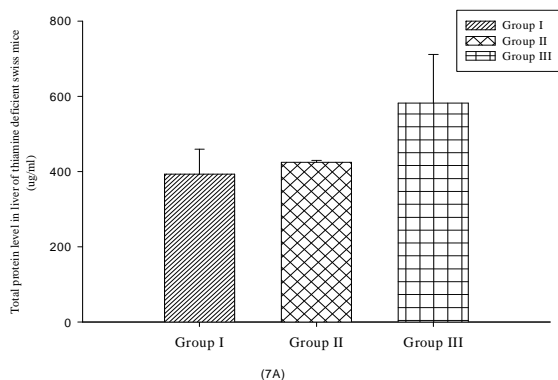


Fig. 7: Effect of TD on total proteins level in liver of *Mus musculus*. (A) Biochemical levels of proteins in liver of mice. Results were expressed in µg/ml of sample. (B) Molecular expression of total proteins in liver of mice.

DISCUSSION

Thiamine is an important antioxidant which is stored in the liver. Liver is a vital organ performing a variety of important roles such as detoxification, protein synthesis, hormone production and glycogen storage [9,17]. It is an organ which helps in the synthesis as well as breakdown of various molecules. It is also essential for the functioning of the heart, muscles, and nervous system therefore its deficiency may have far reaching effects on the body. Deficiency of thiamine can cause weakness, fatigue, psychosis and nerve damage. The present study exhibited decrease in the cholinesterase (AChE and BChE) in liver of treated groups. AChE plays a key role in cholinergic neurotransmission [28]. It is an important enzyme which regulates the effects of ACh at cholinergic synapses. AChE's main function is to terminate the effects of ACh after it is released. It is predominantly bound to the postsynaptic membrane. AChE has two sites which bind to the cationic and esteric domains of ACh, cleaving the molecule between these sites. ACh is degraded into choline and acetate by AChE. So when AChE is inhibited by TD, consequently ACh cleaving activity is interrupted. In other words, neurotransmission is disturbed which consequently leads to a number of neurological disorders [29]. BChE also has a same function as AChE. It is also inactivates the neurotransmitter ACh and hence acts as an efficient therapeutic target against neurological disorders [30]. Inhibition of BChE by deficiency of thiamine leads to disturbance in neurotransmission. However, the BChE is less efficient in ACh

hydrolysis at low concentration, but highly efficient at high ones [31]. The results of our study accounts the reduction in GSH level and increase in LPO may act as key markers of oxidative stress [29]. An increase in TBARS level in heart of TD rats is earlier considered as a presumptive marker of oxidative stress [32]. Maity *et al.*, (2008) [9] examined the increase in malondialdehyde (MDA) levels in liver suggested that enhanced LPO leads to tissue damage. Decreased GSH content in liver tissue was recorded. It could be the result of decreased synthesis or increased degradation of GSH by oxidative stress that prevails during diabetes. Sharma *et al.*, (2010) [33] calculate that decreased activity of GSH level [17] supports marked increase of LPO in liver of diabetic mice. The present investigation records increase in PCC level in the TD liver. This increase in protein carbonyl content may be due to generation of ROS. ROS are well known to convert amino groups of protein to carbonyl moieties [34,35]. Greater content of protein carbonyl groups in dystrophic pectoralis major muscle was examined by Murphy and Kehrer (1989) [36].

Data of the present study showed reduction in the CAT activity of thiamine deficient liver. The result was also supported with Paliwal *et al.*, (2011) [17]. Activity of CAT decrease could be due to higher level TBARS which may block the activity of membrane bound enzymes. Oxidative stress in the thiamine deficient brain mitochondria was elucidated by Sharma *et al.* 2013[10]. The broad spectrum of changes is reflected by the reduction in antioxidant enzyme activities such as SOD, CAT, GPx, as well as GSH levels. These changes are also accompanied by an enhancement in TBARS and PCC levels in the mitochondrial fraction of brain following TD [10]. TD leads to alteration in the level of proteins in liver. The thiamine dependent enzymes show reduction in their activity due to thiamine deprivation in mice. Decrease in enzyme activities in TD should go along with decrease in protein content whereas an increase was recorded in the protein levels of treated group compared to control (7A). Mice that were exposed to TD for 10 days exhibited maximum enhancement of total proteins in liver among the groups. It may be due to expression of stress proteins increases as the exposure time of deprivation of thiamine increases.

CONCLUSION

The present study examines the effect of thiamine deprivation on cholinergic system and total protein content of liver. In conclusion, the expression of stress proteins increases total proteins level in liver. TD disturbs the functions of cholinergic molecules also which directly affect the activity of brain in signaling. TD leads to cumulative damage of cholinesterases (AChE and BChE), oxidative stress in liver, which eventually leading to progression of neurotoxicity and hepatotoxicity.

CONFLICT OF INTEREST

There is no conflict of interest

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