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Taurine improves lambda cyhalothrin induced biochemical alterations in Wistar rat liver

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Abstract: Taurine is a major intracellular free β-amino acid, which can protect the body against toxicity. Lambda-cyhalothrin, a third-generation type II pyrethroid. is used predominantly in agriculture production and animal husbandry. The aim of the present study was to investigate lambda cyhalothrin-induced biochemical changes in rat liver and to search out the possible role of taurine for the attenuation of hepatotoxic biomarkers. Male rats were randomly divided into six groups and lambda cyhalothrin was orally administered at two dose levels (10.83mg/body wt., 15.17mg/body wt.) alone and in combination with taurine pretreatment (50mg/kg body wt) for 14 consecutive days. A significant change in blood glucose level with a marked decline in glycogen content were indicated the hepatic dysfunction in lambda cyhalothrin treated rats. This was also confirmed by the altered activities of serum hepatic biomarker enzymes and lipid profiles in LCT intoxicated rats. Pre-treatment of taurine mitigated the abnormalities. These findings pointed out the toxic effect of lambda cyhalothrin in rat liver and also revealed the protective action of taurine against this pyrethroid.

Key words: Taurine; Lambda cyhalothrin; Blood glucose; Serum hepatic biomarker enzymes.

Introduction

Taurine (2-aminoethane sulphonic acid) is a major free intracellular non- protein sulphur amino acid [1]found in milimolar concentrations in many animal tissues[1]. Taurine is present in the liver at high concentrations [2]. Several studies have reported that taurine has a defensive effect against chemically-induced hepatotoxicity[3-6]. Moreover, by trim down oxidative stress, enhancing mitochondrial activity and modulating cytoplasmic and mitochondrial calcium homeostasis taurine has been found to prevent toxin-mediated hepatic injuries[7]. Furthermore, taurine has been accounted to function as an antioxidant in biological systems, scavenge ROS, attenuate lipid peroxidation and as a consequence, stabilizes biological membranes[8,9].

Environmental pollution from pesticides is a vital topic that attracts broad spread public concern. In spite of harmful effects of pesticides on environment and other living organisms, currently the most efficient and perhaps the only useful way to fight against pests is chemical pesticides [10,11]. India is one of the principal users of agricultural pesticides to increase crop yield and in vector control program. The use of pesticides above the safe level may create serious threat to nontarget organisms in the environment.

A significant amount of pesticides and their metabolites become mixed in ponds; rivers and run-off from pastures. They are potentially toxic to animals[12]. Humans, as agricultural workers, or via food consumption are potentially exposed to these pesticides either directly or indirectly. In addition to careless contact at the time of spraying, entering sprayed farms, consuming foods infected by pesticides, drinking pesticide contaminating water, can cause diseases and abnormalities. Synthetic pesticides have always been under concern because of their effects on human-used agricultural products and their threat and risk to human beings' health[13]. Synthetic pyrethroids are the latest major class of broad-spectrum organic insecticides used in agricultural, veterinary and household applications. Due to their low mammalian toxicity, rapid breakdown in soil and remaining steady in sunlight, synthetic pyrethroids turn into a friendly group of insecticides but their volume of production point out that in future the misapplication and accidental contact may be very high.

Lambda-cyhalothrin (LCT), a synthetic type II pyrethroid is broadly used in agriculture, public health, house and in gardening for pest management[14]. Besides, lambda-cyhalothrin acts on farm animals to prevent and manage

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ectoparasites and in public health agendas. Residues of lambda-cyhalothrin have been accounted in vegetables and fruits, milk and blood of dairy cows and also in cattle meat [15,16]. The objectives of the present study are to highlight the effects of taurine on biochemical parameters of liver in male Wistar rats co-exposed to lambda cyhalothrin.

Materials and Methods

Chemicals and reagents

Lambda cyhalothrin 5% emulsifiable concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine was purchased Sigma Aldrich Inc. USA. All other chemicals used were of analytical grade and were purchased from Merck India Ltd, Himedia India Ltd., etc.

Animal mode

Wistar albino male rats (weighing 130-150 g) were maintained on the standard laboratory feed and water throughout the period of experimentation i.e. 14 consecutive days. Experimental protocol was approved by the Institutional Animal Ethical Committee, registered under CPCSEA. All animal treatment and surgical procedures were carried out according to the relevant laws and guidelines of the CPCSEA.

Treatment protocol

A commercial formulation of lambda cyhalothrin 5% emulsifiable concentrate (EC) was used for our experiments. The animals were divided into six groups of six rats each. Group I: Distilled water control (no treatment). Group II: Taurine control (50mg/kg body wt). Group III: Lambda cyhalothrin low dose (10.83mg/body wt. i.e. 1/7th of LD_{50} value). Group IV: Taurine (50mg/kg body wt) +lambda cyhalothrin low dose (10.83mg/body wt.). Group V: Lambda cyhalothrin high dose (15.17mg/body wt. i.e. 1/5 of LD_{50} value). Group VI: Taurine (50mg/kg body wt) + lambda cyhalothrin high dose (15.17mg/body wt.)

We followed the dose 75.85-mg/kg body weight as the oral LD_{50} of lambda cyhalothrin in male rats[17] and it was also verified in our laboratory. Dose levels for the present treatments were finalized according to our preliminary investigations. In our experiments taurine was applied at the dose level of 50 mg/kg body wt. This dose was effectively used to ameliorate the toxicity induced by various xenobiotics [18,19]. Taurine was pre-treated before 1 hr of LCT exposure to build antioxidant pool or advanced protection in animal body before LCT exposure. Lambda-cyhalothrin, taurine and distilled water were administered once daily by oral gavage for 14 consecutive days. Animal's weight was taken daily and the dose was adjusted accordingly.

Determination of blood glucose level

Blood glucose was estimated by Nelson method adapted from Somogyi's method (Nelson, 1944)[20]. The blood was deproteinized by adding 9.5 ml of 5% zinc sulphate solution in 1 ml of blood sample. Then 9.5 ml of 4.5% barium hydroxide solution was included and the mixture was allowed to stand for 15 min for complete precipitation and was filtered. For the preparation of sample, standard and blank, 0.5 ml of blood filtrate, 0.5 ml of 0.025mg/ml of glucose and 0.5 ml of distilled water were taken respectively in separate test tubes and 1ml of alkaline copper reagent were added in each test tubes and were boiled in a boiling water bath for 20min. Then those were allowed to cool in room temperature and 1 ml of arseno-molybdate reagent was mixed to each test tubes and volume was made upto 10 ml. Readings were taken at 540 nm in spectrophotometer.

Estimation of liver glycogen

Tissue homogenate (100 mg/ml in hot 80% ethanol) was centrifuged at 8000 × g for 20 min. The residue was collected and dried in a hot water bath. To the collected residue, 5ml of distilled water and 6ml of 52% perchloric acid were mixed. The extraction process was done at 0^0C for 20min. Then the collected material was centrifuged at 8000×g for 15 min. 0.2 ml of supernatant was transferred in a graduated test tube to make 1 ml volume by distilled water. Standards in graded concentrations were prepared by using working standard solution and all these volumes were made up to 1ml by distilled water. Then 4 ml of anthrone reagent was added to all test tubes and were heated in boiling water bath for 8 minutes. After cooling in a room temperature, the reading was noted at 630 nm. The amount of sample glycogen was measured from standard curve, prepared with standard glucose solution. Glycogen present in sample was expressed in µg of glucose/ mg of tissue [21].

Activity of serum Glutamate-oxaloacetate transaminase (SGOT) and Glutamate-pyruvate transaminase (SGPT)

To prepare sample for SGOT, 1 ml of buffer substrate (prepared by taking 2.66gm aspartic acid, 60mg α-ketoglutaric acid and, 20.5ml of 1(N) NaOH and volume was made upto 100ml by 0.1M phosphate buffer, pH7.4) and for the preparation of sample for SGPT, 1 ml of buffer substrate (containing 1.78 gm DL-alanine, 30mg α-ketoglutaric acid, 20 ml of 0.1M phosphate buffer and 1.25ml of 0.4(N) NaOH) were taken and allowed to wait for 5min at 37^0C. Then 0.2ml of sample serum was added and incubated for 60 min at 37^0C. To prepare standard, 0.2 ml of working standard (200µM/100 ml) was receive in a test tube and 0.8ml of buffer substrate was added. For blank, 1.0 ml of buffer substrate was taken. In each of sample, standard and blank test tubes, 1ml

of DNPH solution were mixed and allowed to wait for another 20 min. Then 10ml of 0.4(N) NaOH was mixed and allowed to stand for 10 minutes. Finally, the readings were taken in spectrophotometer (UV-245 Shimadzu, Japan) at 520 nm [22].

Estimation of serum alkaline phosphatase (ALP)

For sample preparation, 1.0ml of PNPP buffer (1mM PNPP in 0.02M Tris alkaline buffer, pH 7.5), 0.25ml of sample serum, 1.75ml of redistilled water were taken in a centrifuge tube and mixed. To prepare standard, four standard solutions were prepared by mixing 5, 10, 20, 40 µg/ml of PNP (10mg% PNP) and 3.85, 3.80, 3.70, 3.50ml redistilled water respectively and 0.01 ml of 0.1M NaOH in each standard solution. All samples and standard solutions were incubated at 37^{0}C for 30 min. Then 0.1ml of NaOH and 0.9ml of redistilled water were added to each sample and standard solution and was centrifuged at 2000 rpm for 10min. The reading of each supernatant was taken in spectrophotometer at 420 nm. Amount of PNP liberated was measured in a spectrophotometer (UV-245 Shimadzu, Japan) at 420 nm against blank [23].

Estimation of serum lactate dehydrogenase

Lactate dehydrogenase, a cytoplasmic enzyme, was estimated by measuring the change in absorbance at 340 nm in solution containing NADH and pyruvate [24].

Serum bilirubin and Lipid Profile Studies

Serum bilirubin was determined[25] using commercial diagnostic reagent kit. Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL) were estimated [26] by using commercial diagnostic reagent kit. Very low density lipoprotein (VLDL) was calculated using following formula (TG/5) given by [27]. LDL concentration (mg/dl) was estimated indirectly from the measured levels of TG, HDL, and TC using equation LDL = TC - (VLDL+HDL).

Statistical analysis

All the parameters were assayed in triplicate manner. The data was expressed as Mean±SEM, the differences between the means of each group were tested using a one-way ANOVA test (using a statistical package, Origin 6.1, Northampton, MA). P<0.05 was considered to indicate a statistically significant difference.

Results

Blood glucose and liver glycogen content

Biochemical analysis showed a significant increase ($p < 0.001$) in blood glucose (fig 1) with a marked reduction in serum glycogen (fig 2) in LCT-exposed group. However, pre-treatment of taurine can able to prevent lambda cyhalothrin induced toxicity significantly.

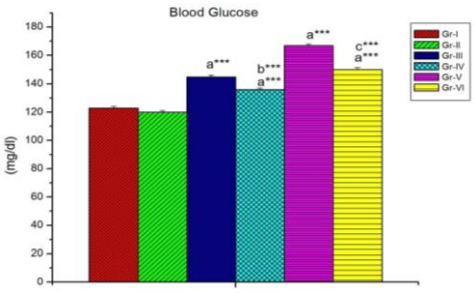

Fig-1 shows the effects of LCT and taurine on blood glucose. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*** indicates p<0.001).

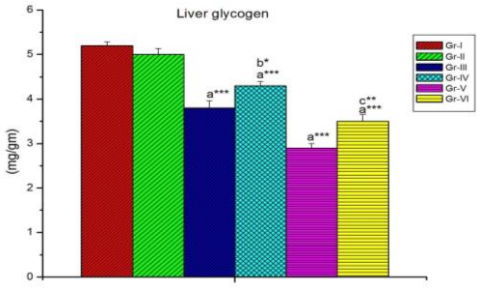

Fig-2 shows the effects of LCT and taurine on liver glycogen level. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001)

Estimation of serum enzymes

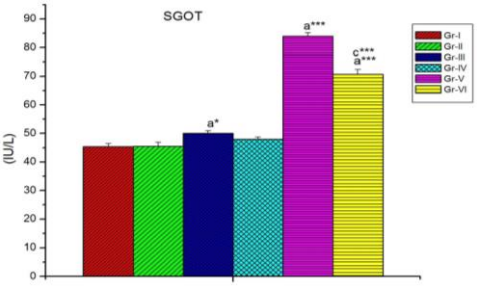

Fig-3 shows the effects of LCT and taurine on serum Glutamate-oxaloacetate transaminase (SGOT). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (* indicates p<0.05, *** indicates p<0.001).

As shown in fig-3,4 lambda cyhalothrin induced toxicity was confirmed by significant($p<0.001$) elevated activities of serum glutamate-oxalacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in fig 5,6. No significant changes were observed after taurine treatment alone. On the other hand, pre-treatment with taurine along with lambda cyhalothrin had significantly reduced the above stated serum enzymes.

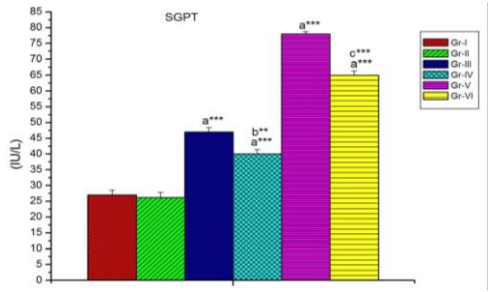

Fig-4 shows the effects of LCT and taurine on serum Glutamate-pyruvate transaminase (SGPT). Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (** indicates $p<0.01$, *** indicates $p<0.001$).

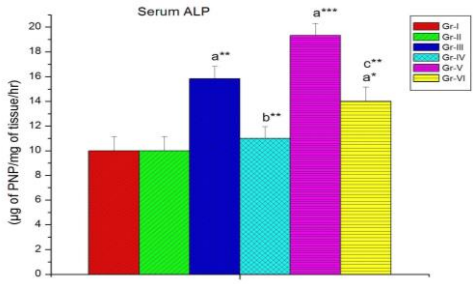

Fig-5 shows the effects of LCT and taurine on serum alkaline phosphatase activity. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (* indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$).

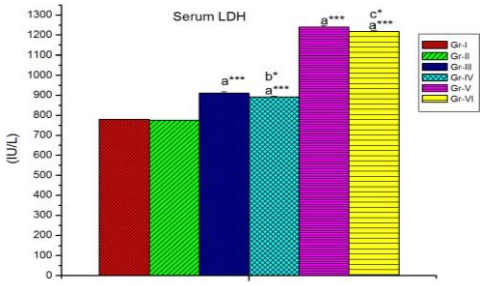

Fig-6 shows the effects of LCT and taurine on serum lactate dehydrogenase activity. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (* indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$)

Determination of lipid profile and serum bilirubin

Table 1 reflect the significant elevated level of serum total cholesterol (TC), serum triglyceride (TG), serum low density lipoprotein (LDL), serum very low density lipoprotein (VLDL) along with reduced high density lipoprotein cholesterol (HDL) level in lambda cyhalothrin intoxicated rat (table 1). The significant elevation ($p<0.001$) in total serum bilirubin level in pyrethroid treated groups compared to control were observed in this experiment (table 1).

Table 1: Effect of LCT and taurine on lipid profile and serum bilirubin in male rats.

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Total Cholesterol (mg/dl)	120±2.4	115±1.5	135±1.59a***	121.33±1.62b***	145±1.29a***	126 ±1.32c***
Triglyceride (TG) (mg/dl)	83.66±1.5	80±1.06a**	124.5±1.33a***	92±1.13b***	135±1.52 ***	126±1.18a***c***
High density lipoprotein cholesterol (HDL) (mg/dl)	48±1.5	52.83±1.19 a*	34±1.18 a***	40±0.93a**b***	25±1.09 a***	31±0.96 a***c**
Very low density lipoproteins (VLDL) (mg/dl)	16.73±0.29	16.0±0.21	24.9±0.27a***	18.4±0.22a**b***	27±0.30a***	25.2±0.24a***c***
Low density lipoproteins (LDL) (mg/dl)	55.26±2.2	46.6±1.8a*	76.1±2.5a***	62.93±1.47a*b**	93±1.5a***	69.8±1.98a**c***
Bilirubin level (mg/dl)	0.3±0.05	0.4±0.06	1±0.07a***	0.5±0.02a**b***	1.7±0.1a***	1.2±0.1a***c***

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups, superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates $p<0.05$, ** indicatesp<0.01, *** indicates $p<0.001$).

Discussion

Biochemical parameters are the sensitive indices and important tools for toxicological studies. In the present study, increased fasting blood glucose levels in lambda cyhalothrin-exposed rats were observed compared to the control group in the present study. The increased blood glucose level may be due to the interference of carbohydrate metabolism resulted from the alteration of the catecholamine levels [28] or phosphorylase activities or due to less peripheral glucose utilization.

Observed reduced hepatic glycogen level in the present study may be a consequence of abruptly increased catabolism to meet higher energy demands caused by LCT intoxication.

Raised levels of serum transaminase enzymes are indicators of cellular leakage and loss of functional integrity of hepatic cell membrane. Hepatocellular necrosis leads to high level of serum aspartate transminase, alanine transaminase. Elevated level of alanine transminase in the serum is a prominent index of liver damage. The increased levels of enzymes were normalized to a good extent after 14 days pre-treatment of taurine pointed out that it provided protection by stabilizing the structural integrity of the hepatocellular membrane against lambda cyhalothrin. Level of serum alkaline phosphatase is associated to the hepatocytes functioning. Bile canaliculi cells lining usually synthesize increased serum alkaline phosphatase in response to cholestasis and increased biliary pressure [29]. Lambda cyhalothrin administration increased serum ALP level and it was brought to near normal level by taurine treatment. Hepatic damage was also reflected in LCT treated group through the altered serum lactate dehydrogenase, a sensitive intracellular enzyme and an indicator of liver cell damage[30]. Pretreatment with taurine caused apparent normalization in the ALP and LDH level.

In the present study, increased total bilirubin level in LCT exposed rats may be due to increased haemoglobin percentage resulted from increased destruction of red blood cells. LCT may also block the biliary tract in treated rat which is another possible mechanism of LCT toxicity. In studies observing the role of taurine in improving hyperbilirubinemia, it may be assumed that taurine may improve excretion of bile, blood flow, and enhances the functions of hepatocytes [31,32].

In LCT exposed rats, the rise in total serum cholesterol level could be due to obstruction in liver bile ducts causing decline or interruption of its secretion to the duodenum consequently producing cholestasis[33]. The disturbance in lipoprotein formation is one of the factors leading to accumulation of cholesterol in pesticide treated mice[34] and it may be a reason for elevated cholesterol in LCT exposed rats. Administration with taurine may decrease the levels of total cholesterol. The hypocholesterolemic effect of taurine has been reported in mice [35], rats [36,37], and humans[38], but its mechanism is not well established. Taurine takes a part in conjugation reaction with bile acids in the liver. Taurine increases bile acid synthesis[39] by simultaneous upsurge in the mRNA expression and activity of cholesterol 7α-hydroxylase, a rate-limiting enzyme in hepatic bile acid synthesis[40]. The primary mechanisms accountable for the hypocholesterolemic action of taurine may be due to the increased conversion of cholesterol into bile acids through the activation of cholesterol 7α-hydroxylase.

Triglycerides are free fatty acids esters of glycerol. Liver causes biosynthesis and assimilation of lipoproteins like LDL and VLDL through which triglycerides are secreted into circulation [41]. Rise of these lipoproteins causes increase of serum triglyceride. In the present study, pre-treatment of taurine significantly alleviates lambda cyhalothrin induced rise in the liver triglyceride level. Decrease serum HDL is associated with elevated serum cholesterol level in LCT treated rat because HDL mainly plays an important role in cholesterol efflux from tissues [42]. Pretreatment of taurine tries to normalize the above said changes.

Conclusion

It may be concluded from the present study that the lambda cyhalothrin exposure produced biochemical alterations in treated rats liver. Collectively, our data also suggested that taurine pretreatment played an ameliorative role in lambda cyhalothrin mediated biochemical alterations in liver. The mechanism underlying protection of taurine may be related to its function as a direct antioxidant by scavenging ROS and also by the stimulatory effects on hepatic bile acid synthesis.

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