INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

EFFECT OF ENDOSULPHAN ON LACTIC DEHYDROGENASE ACTIVITIES IN THE VITAL TISSUES OF SWISS ALBINO MICE

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ABSTRACT

Healthy adult female albino mice Mus musculus of Swiss strain were exposed to sub lethal concentration of 6 mg/kg body weight of endosulphan for 21 days, 60 days and 90 days respectively. The fluctuations of the variation in Lactic dehydrogenase (LDH) in brain,liver and kidney were estimated. In acute doses LDH increase in all tissues, whereas on subchronic and chronic period there was decreased trends.

Key words: Endosulphan toxicity, Biochemical Changes, LDH , Mus musculus.

INTRODUCTION

In recent years man has become more aware of his environment. This probably relates to the realization that if we continue depositing our waste product at our current rate as well as the overexploitation of our environment, our world, as we know, will have a finite time. The action of chemicals upon environmental system forms the basis of Environmental toxicology.

Urbanization results in the production of new synthetic chemicals. It is inevitable. It has been recorded that there has been an increase of 255 % in the production of synthetic chemicals in the past fifteen years in to the bio world[1]. According to an estimate, seven hundred to three thousand new chemicals are annually added in the environment. Thus chemical pollution is a thrust area for scientific study and so every piece of research is highly needed for the survival of living world.

Living beings arouse on this planet by constant accumulation and dissipation of energy from various matter which are evolved in this biosphere from time immemorial. Exposure to different artificial chemicals either toxic or non toxic by man in this age of modernization is increasing. Introduction of any foreign compound or chemical cause deleterious effect on the living system in the food chain. This will evoke and produce more and more new ailments. But we can reduce the intensity by a judicious use of these chemicals.

The target organs of the xenobiotics are brain, liver and kidney. In the present investigations brain, live and kidney were selected. Brain is the controlling device, the central processing unit (CPU) as well as major lipophilic organ where accumulation of endosulphan may occur. All organochlorines are lipophilic. So maximum accumulation as well as metabolic residues are remaining in the brain. So brain was selected as an organ for this specific study.

Similarly liver is the major metabolic organ. Most of the metabolic enzymes are present

in the liver maximum. So any type metabolic changes can be easily be studied qualitatively and quantitatively by studying the liver tissues.

Kidney is one of the major detoxifying and excretory organ. So variations in the general metabolism can easily be assessed by the kidney tissues.

Endosulpan is a toxicant which is used to drive away the insects and to increase the productivity of the food requirements of the human beings. So this chemical enters into the living system mainly through the food/nutrients, diets. The endosulphan can/may change the chemical nature of these nutrients through food chain as well as to the experimental model. Therefore the evolution of these nutrients in the biological system is equally significant. So the variation in the total lipid total protein and total glycogen in the selected tissues, brain, liver and kidney were studied. Along with these the enzymes related with neurotransmission, oxidative metabolism, cell permeability and general cell metabolism were also studied at different levels of toxicity of the toxicant.

One or two days of exposure to a toxicant is not at all a problem/disturbance to the biological world because all living organism have highly adaptive[2,3,4], but beyond the permissible limit of exposure to any sort of toxicant can cause delterious impact[5].

MATERIALS AND METHODS

Experimental Animals

Healthy adult female albino mice Mus musculus of Swiss strain, two months of age weighing 25-26 gms were used. Mice were obtained from the stock inbred colony which was maintained by mating brothers and sisters. The animals were housed in polypropylene cages and maintained on a normal laboratory diet. Water was given ad libitum. They were maintained throughout the experiment on 12 hr light -dark cycle.

Experimental Design

The animals were divided into four groups. The LD_{50} values were calculated by Graphical method (Probit analysis) [6]. Thus the sub lethal concentration of endosulphan was calculated as 6mg/kg body weight. The first group of animals was maintained as control. The second group of animals was administered with endosulphan up to 21st day, the third group were exposed up to the 60th day and fourth group up to the 90th day. Endosulphan were administered subcutaneously to the mice of all experimental groups once daily in the morning hours.

The feed intake was calculated by developing feeding index after modifying to suit the present experiment[7]. The feeding index was calculated in all cages so as to ensure the feed intake to be approximately same in all cages. The weights of animals were also recorded daily in both the control and the experimental group.

After the treatment the animals were sacrificed by decapitation method along with control on 1st day 22nd day, 61st day and 91st day respectively. Then the tissues were assayed for various biochemical, histological and residual analysis.

The biochemical Studies of brain liver and kidney of control and treated animals groups were carried out by using standard methods. The optical density readings of the bio chemical estimations were taken on Spectrophotometer and photoelectric colorimeter.

OBSERVATIONS AND RESULTS

Results of lactic dehydrogenase estimations were depicted on tables 1,2&3 .

On 21st day there was an increase of lactic dehydrogenase activity in all tissues. The increase observed were 24.8%, 19.3% and 19.6 % in brain, liver and kidney respectively.

On 60th day, an increase of LDH was observed in brain and while it was decreased in the liver and kidney.

On 90th day the LDH amount was decreased in all the tissues.19% in brain, 31% in liver and 42 % in kidney.

DISCUSSION

Cellular respiration is the set of reactions occurring within mitochondria, that include the tricarboxylic acid cycle and electron transport. Lactic dehydrogenase considered as marker of citric acid cycle and anaerobic respiration.

Lactic dehydrogenase is a tetrameric enzyme with perfect dihedral symmetry. LDH display nearly absolute specificity, transferring a proton into the "incorrect" pro-s-position no more than once in 5 x 10^7 catalytic cycles. This suggests a difference in transition state energies of about 40 KJ/mol for two isomers[8].

Alterations in carbohydrate metabolism are produced due to the stress caused by endosulphan. Endosulphan and other organochlorine pesticides have the ability to disrupt the respiratory functions of the organisms[9]. This leads to the development of internal hypoxic conditions which leads to anaerobic metabolism, indicated by the changes in the LDH activities.

Animal exposure to sub lethal concentrations of a toxicant can produce stress and disturbances of the normal animal physiology. Decrease in the lactic acid, tissue glycogen and various other metabolic enzymes after exposure to endosulphan where reported[10]. Lactate, a measure of anaerobic metabolism, has been widely used and increase of anaerobic metabolism has been shown to be a rapid and clear response against depletion of energy[11]. As in the case of LDH the binding affinity of endosulphan may be towards SDH than the enzyme substrate, so blocked productive complex hence the decline in metabolic activities.

The Lactic dehydrogenase is pivotal cellular metabolic enzyme between glycolytic pathway and tricarboxylic acid cycle. During the acute and subchronic exposure, elevation in LDH suggested that there is a shift in the respiratory metabolism from aerobic form to hypoxic anaerobic form following exposure to endosulphan. So, alterations observed in LDH activities suggested impairment in carbohydrate metabolism in the mice[12].

The present study revealed that decreased LDH activities at the chronic exposure in the tissues of liver brain and kidney. Activities of the respiratory chain linked enzymes are inhibited at levels which corresponded to the concentration of endosulphan used *in vitro*. Both respiratory control ratio (RCR) and the ADP to Oxygen ratio fell sharply at endosulphan concentrations above 10 micrograms /ml[13].

In the present study, the histopathological observations also clearly indicate the characteristic features of the cell death. Markers of the release of intracellular components such as the LDH detect the damage of outer cell membrane and are accepted as markers of cell death[14,15,16].

These marker enzymes mainly involved in the membrane transport[17]. Endosulphan and its metabolites interact with membrane lipids of sub cellular organelles like respiratory chain enzyme LDH and thereby affect the ion transport system[18.19].

The results of present study indicated that endosulphan possess the dual properties of an uncoupler of oxidative phosphorylation and an inhibitor of electron transport chain. Endosulphan, being lipophilic, like other organochlorine pesticides[20,21]. It might be interacting primarily with the mitochondrial lipoprotein surface resulting in structural damage and changes in the ionic permeability[22,23].

The inhibition of mitochondrial respiration was found to occur in parallel to the inhibition of enzyme activities of the respiratory electron transport chain of mitochondria such as LDH thus the result of the present study indicates that the suppression of both the aerobic and anaerobic pathways of carbohydrate metabolism by the toxicant. And also it can be suggested that a limited supply of energy is ensured during pesticide toxic stress for the sustenance of vital activities of the animal through these pathways.

CONCLUSION

The results of present study indicated that endosulphan possess the duel properties of an uncoupler of oxidative phosphorylation and an inhibitor of electron transport chain. Thus, inhibition of mitochondrial respiration was found to occur in parallel to the inhibition of enzyme activities of the respiratory electron transport chain of mitochondria. Suppression of both aerobic and anaerobic pathways causes the variation in LDH activities.

Table 1: Lactic dehydrogenase Concentration in brain (aa/ min.)

Period	Control	Treated	Value of 't' statistics
0 day	0.287 ± 0.007	0.286 ± 0.015	0.654
After 21 days	0.281 ± 0.005	0.349± 0.0150	19.426**
After 60 days	0.281 ± 0.005	0.328 ± 0.0111	7.165**
After 90 days	0.283 ± 0.005	0.248 ± 0.0179	8.268**

**Significant at 1 percent level (P ≤ 0.01)
Values indicate Mean ± S.D

Table 2: Lactic dehydrogenase concentration in liver (aa/ min)

Period	Control	Treated	Value of 't' statistics
0 day	0.314 ± 0.0088	0.314 ± 0.0073	0.000
After 21 days	0.321 ± 0.006	0.383 ± 0.0053	20.771**
After 60 days	0.320 ± 0.0071	0.267 ± 0.0158	9.238**
After 90 days	0.317 ± 0.0071	0.219 ± 0.0078	27.828**

** Significant at 1 percent level (P ≤ 0.01)
Values indicate Mean ± S.D

Table 3: Lactic dehydrogenase concentration in in kidney (aa/ min.)

Period	Control	Treated	Value of 't' statistics
0 day	0.324 ± 0.0088	0.322 ± 0.0083	0.712
After 21 days	0.323 ± 0.005	0.379 ± 0.019	8.481**
After 60 days	0.322 ± 0.0083	0.285 ± 0.0053	10.495**
After 90 days	0.319 ± 0.0078	0.187 ± 0.005	42.746**

** Significant at 1 percent level (P ≤ 0.01)
Values indicate Mean ± S.D

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