Original Paper

Effect of Cypermethrin on Selected Dehydrogenase Enzymes in Muscle and Heart Tissues of Albino Rats

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ABSTRACT

This study reveals significant variations in dehydrogenase enzymes on administration of oral, sublethal (41mg/kg) doses of cypermethrin as single, double and multiple doses with 48hr intervals. Glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) activities were increased, whereas succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities were significantly decreased in muscle and heart tissues of albino rats, in a dose and time dependent manner. G-6-PDH is a key enzyme of HMP pathway. This pathway serves to generate glycolytic intermediates for the production of energy to tolerate toxic stress. SDH is a vital enzyme of citric acid cycle, and catalyses the reversible oxidation of succinate to fumarate. LDH activity shows an increase during anaerobic conditions to meet the energy demands. MDH activity depends on fluctuations of oxidative metabolism, and also reflects the turnover of carbohydrates and energy output.

Key Words: Cypermethrin; Muscle; Heart; Albino rat; Glucose-6-phosphate dehydrogenase; G-6-PDH; lactate dehydrogenase; LDH; succinate dehydrogenase; SDH; malate dehydrogenase; MDH

Introduction

In his constant efforts to produce adequate food, man has been thwarted periodically by the ravages of pestilence and crop diseases. An increase in global food demand has resulted in a significant increase in the use of pesticides in agriculture. Today, synthetic pyrethroid pesticides account for over 30% of the global pesticides use.[1] Cypermethrin is one of the synthetic pyrethroids, initially synthesized in 1974, and marketed from 1977 onwards as a highly active insecticide, effective against a wide range of pests in agriculture, animal husbandry, and domestic situations.[2] Pyrethroids in general, have gained popularity over organochlorine and organophosphate pesticides due to their high efficacy against target species,[3] relatively low mammalian toxicity,[4] and rapid biodegradability.[5]

Poisoning with pyrethroids can cause facial paraesthesias, dizziness, headache, nausea, vomiting, and increased gastrointestinal secretions. Cypermethrin is in addition, a skin and eye irritant. Normally, symptoms should disappear after some days, but severely exposed patients may progress to muscular twitching, coma, and convulsions. In such cases, symptoms may persist for several days. Cypermethrin is classified by the World Health Organization (WHO) as 'moderately hazardous' (Class II).[6] It interferes with the sodium channels in nerve cells through which sodium enters in order to transmit nerve signals. These channels may then remain open for up to seconds, compared to the normal period of a few milliseconds, after a signal has been transmitted.

Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long-lasting trains of repetitive impulses in sense organs.[7] This results in

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prolonged depolarization of the nerve membranes causing convulsions, and eventually death.

The present study was undertaken to determine cypermethrin-induced alterations in glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) activities in muscle and heart tissues of albino rats.

Materials and Methods

1. ***Test Chemical***
   Technical grade cypermethrin (92% purity) was obtained from Tagros Chemicals India Limited, Chennai.
2. ***Animal Model***
   Healthy adult albino rats of same age group (70 ±5 days), and weight (175 ±10g) were obtained from the Indian Institute of Science (Bangalore), and maintained in laboratory conditions (28±2°C, with 12h light; 12h darkness).
3. ***Experimental Design***
   The animals were divided into four groups comprising ten rats in each group. The toxicity of cypermethrin was evaluated by static bioassay method of Finney,[8] and the LD_{50} of cypermethrin for albino rats was found to be 205 mg/kg. One-fifth of LD_{50} value (41mg/kg) was selected as the sublethal dose, and administered as single, double, and multiple doses, with one-day intervals in between. The first group of animals was used as vehicle controls, and adminstred corn oil. The second group of animals was administered a single dose of cypermethrin, while for the third group, a double dose was given, and for the fourth group, multiple doses were given orally. After a pre-determined time, the animals were sacrificed, and muscle and heart tissues were isolated and stored at -80°C for enzyme assays.
4. ***Enzyme Assays***
   Five percent homogenates of tissues were prepared in 0.25 ice-cold sucrose solution for estimation of glucose-6-phosphate dehydrogenase (G-6-PDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) enzyme activities, and these were centrifuged at 2500 rpm for 15 min in a refrigerated centrifuge at 4°C to remove cell debris. Only clear extracts were used as enzyme source. G-6-PDH activity was estimated by the method of Lohr and Waller[9] as modified by Mastanaiah et al,[10] while SDH and MDH activities were estimated by the method of Nachalas et al,[11] and LDH activity was estimated by the method of Srikanthan & Krishnamoorthy.[12] The reaction mixture was prepared containing 100 micromoles of sodium potassium phosphate buffer, 40 micromoles of INT, and 0.3 micromoles of NADP for G-6-PDH, 0.1 micromoles of NAD for LDH and MDH, and 40 micromoles of different substrates, which was initiated by adding 1 ml of required enzyme. The contents were incubated for 30 min at 37°C, and the reaction was stopped by adding 5 ml of glacial acetic acid and 5 ml of toluene, and kept overnight. The colour extract was read at 495nm, using Hitachi U-2800 model spectrophotometer.

Results and Discussion

The results of all enzyme activities of control, as well as experimental rats (subjected to cypermethrin toxicity) in muscle and heart tissues are depicted in **Tables 1 & 2**. The enzyme activities in experimental rats exposed to cypermethrin showed statistically significant ($p<0.05$) increase of G-6-PDH and LDH activities, whereas SDH, MDH activities were significantly ($p<0.05$) decreased. Alterations in oxidative enzyme activities revealed a dose and time dependent manner in cypermethrin-treated rats.

Increased glucose-6-phosphate dehydrogenase activity was observed in the tissues of rats treated with cypermethrin. G-6-PDH is the key enzyme of hexose monophosphate (HMP) pathway, and is used to generate NADPH and ribose-5-phosphate. If energy needs are high, this pathway serves to generate glycolytic intermediates for the production of energy.[13] The increased oxidation of glucose through switched-over HMP shunt by G-6-PDH is due to the prevalence of anaerobiosis.[14] The enhancement in the HMP shunt could be significant as a mechanism directed towards tissue repair, and cell regeneration and proliferation, whenever inflammatory responses take place.[15] This elevation in G6PDH activity has been noted in earlier studies also.[16]

The downward trend in SDH activity denotes fluctuations of oxidative metabolism, and also reflects the turnover of carbohydrates and energy output.[17] Low operation of glycolytic pathway, and reduction in pyruvate feeding into TCA cycle corroborate the reduced activity levels of SDH. There is significant depletion of SDH activity in tissues of rats treated with sub-lethal doses of cypermethrin. It appears evident that the damage caused by cypermethrin to the architectural dynamics of cells and their components could have caused elevation in G-6-PDH activity

In an earlier study it was shown that SDH activity was significantly decreased in gastrocnemius muscle of sodium fluoride-treated mice as compared to controls.[18] Several reports have also been published with regard to decreased SDH activity in muscles of mice.[19] Jacob Doss et al reported on the decreased SDH activity in the liver and brain of *Labeo rohita* exposed to cypermethrin.[20] Satyaparameshwar et al reported on the decreased SDH activity in selected tissues of fresh water mussel, *Lamellidens marginalis* exposed to copper sulphate.[21]

Decreased SDH activity of muscle and heart tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria, leading to depression of TCA cycle on exposure to cypermethrin.

Lactate dehydrogenase (LDH) is the key enzyme of anaerobic glycolysis, and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The activity of LDH increases during conditions favouring anaerobic respiration to meet the increased energy demands, and whenever aerobic oxidations are lowered.[22] Due to diminished TCA cycle enzymes activity, cypermethrin attenuates markedly the whole animal oxygen consumption,[23] and inhibits the activities of oxidative enzymes such as SDH and MDH.[24]

Increased LDH activity has been reported by Vasantha Sena in albino rats treated with sodium selenate.[25] Kamalaveni et al reported on the increased LDH activity in the liver of *Cyprinus carpio*.[26] Jacob Doss et al have reported increased LDH activity in the freshwater fish, *Labeo rohita* exposed to cypermethrin.[20]

The drop in MDH activity denotes fluctuations of oxidative metabolism, and also reflects the turnover of carbohydrates and energy output.[27] It is well known that any alteration in mitochondrial structure inhibits the activity of MDH.[28] Reduction in MDH activity may also be due to the inhibition exerted by oxaloacetate, because a decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria of CO_2 formation from acetate. This results in the accumulation of oxaloacetate, which in turn inhibits NAD-specific MDH.[29] The decrement of MDH suggests that there is a shift in the respiratory metabolism towards anaerobiosis. Murthy et al have reported that there is a shift in the respiratory metabolism towards the anaerobic state, which results in decreased oxidative metabolism and decreased MDH activity.[30] Similar decreased MDH activity in tissues of albino rats due to sodium selenate intoxication has been reported by Samson Raju.[31] In the present study, the activity levels of MDH showed inhib-

Table 1 Alterations in dehydrogenase enzyme activities in muscle tissues of control and cypermethrin-treated albino rats

Oxidative Enzymes (micromoles of formazan/mg protein/hr)	**Control**	**Single Dose**	**Double Dose**	**Multiple Dose**
G-6-PDH	0.624 ±0.04	0.733* ±0.04 (17.34)	0.866** ±0.04 (38.66)	1.082** ±0.02 (73.34)
SDH	0.463 ±0.03	0.531* ±0.037 (-17.4)	0.387** ±0.0305 (-39.87)	0.284** ±0.027 (-55.87)
LDH	3.793 ±0.072	4.259* ±0.046 (12.26)	4.673* ±0.030 (23.18)	4.824** ±0.050 (27.17)
MDH	0.686 ±0.02	0.573* ±0.040 (-16.37)	0.520* ±0.03 (-24.10)	0.419** ±0.023 (-38.89)

Values are mean ± SD (n=6); PC = Percent change; Values in parentheses indicate percent change over control;
NS = Non-significant; * and ** indicate significance of values at P<0.05 and P<0.01

Table 2 Alterations in dehydrogenase enzyme activities in heart tissues of control and cypermethrin-treated albino rats

Oxidative Enzymes (micromoles of formazan/mg protein/hr)	**Control**	**Single Dose**	**Double Dose**	**Multiple Dose**
G-6-PDH	0.388 ±0.04	0.420* ±0.02 (8.28)	0.493** ±0.02 (26.95)	0.589** ±0.04 (51.67)
SDH	0.264 ±0.02	0.237** ±0.034 (-10.34)	0.178** ±0.032 (-32.11)	0.144** ±0.022 (-45.41)
LDH	2.625 ±0.070	2.876* ±0.07 (9.56)	3.118* ±0.052 (18.79)	3.241** ±0.054 (23.47)
MDH	0.435 ±0.031	0.396* ±0.037 (-8.85)	0.363* ±0.031 (-16.39)	0.330** ±0.030 (-24.02)

Values are mean ± SD (n=6); PC = Percent change; Values in parentheses indicate percent change over control; NS = Non-significant; * and ** indicate significance of values at P<0.05 and P<0.01

ited pattern in selected tissues of rats exposed to cypermethrin stress. MDH is a NAD-dependent enzyme which converts malate to oxaloacetate, and reversible oxidation of fumarate to malate. Oxaloacetate also plays a significant role in CO_2 fixation and in gluconeogenesis.[22]

Conclusion

The present study clearly reveals significant alterations in oxidative enzymes due to cypermethrin exposure and oxidative stress in albino rats. It appears evident that long-term exposure to sublethal doses of pyrethroid pesticides can result in cell metabolism toxicosis.

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