Effect of cypermethrin on total carbohydrates, glycogen, pyruvate and lactic acid contents in liver and kidney tissues of albino rats

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SUMMARY

Cypermethrin is a synthetic pyrethroid insecticide that has insecticidal activity, low avain and mammalian toxicity that affects the nervous system of vertebrates and invertebrates by affecting voltage depending sodium channels and inhibiting ATPase enzymes. This study has revealed significant variance in total carbohydrates, glycogen, pyruvate and lactate content in liver and kidney tissues of albino rats after administration of cypermethrin. With the oral sublethal dose (41mg/kg) of cypermethrin as single dose, double dose, multiple doses with 48 intervals, the total carbohydrate, glycogen, pyruvate contents decreased where as lactic acid content increased in liver and kidney tissues of albino rats. In the present study, significant decrement was observed in total carbohydrates, glycogen and pyruvate contents due to higher energy demands under cypermethrin toxicity.

Key words : Cypermethrin, Total carbohydrates, Glycogen, Pyruvate, Lactic acid, Albino rat

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Pesticides occupy rather a unique position among many chemicals by increasing food and fibre production and by reducing the occurrence of vector borne diseases. An increase in global food demands has resulted in a significant increase in the use of pesticides in agriculture. If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre, and amelioration of vector-borne diseases but their debits have resulted in serious health implications to man and his environment. There is now overwhelming evidence that some of these chemicals do pose potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993). No segment of the population is completely protected and the burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world-wide deaths and chronic illness due to pesticide poisoning number about 1 million per year (Environews Forum, 1999). In humans, exposure to pesticides has been associated with cancer (Dich *et al.,* 1997).

Pyrethroids and pyrethrins are used in a wide array of indoor and outdoor applications, including medicinal, veterinary and agricultural usages (ATSDR, 2003). Pyrethroids usage has been estimated at 23% of the worldwide insecticide market.

Cypermethrin was initially synthesized in 1974 (Elliott *et al.,* 1974) and first marketed in 1977 as a highly active synthetic pyrethroid insecticide, effective against a wide range of pests in agriculture, public health and animal husbandry. These compounds have gained popularity over organochlorine and organophosphate pesticides due to their high efficacy aginst target species (Eillott *et al.*, 1978) and their relatively low mammalian toxicity (Parker *et al.*, 1984) and rapid biodegradability (Leahey, 1985).

Cypermethrin acts as a stomach and contact insecticide. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry and its structure is based on pyrethrum, a natural insecticide which is extracted from chrysanthemum flowers, but it has a higher biological activity and is more stable than its natural model.

Cypermethrin is classified by the World Health Organization (WHO) as 'moderately hazardous' (Class II) (WHO 1994 - 95). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can 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.

The pyrethroids are highly toxic to aquatic organisms and fish as well as to bees – with the same mode of action in each organism. The

LC_{50} values for small fish and other aquatic organisms typically lie below 1 mg/kg. For use with conventional hydraulic sprayers, buffer zones of 16-24 m are needed to reduce mortality of butterflies in the surroundings (Davis, 1993).

Therefore, the present study was designed to determine the cypermethrin induced alterations in total carbohydrates, glycogen, pyruvate and lactic acid contents in liver and kidney tissues of albino rats.

MATERIALS AND METHODS

Test chemical:

Technical grade cypermethrin (92% purity) was obtained from the Tagros Chemicals India limited, Chennai.

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, India) and maintained in laboratory conditions (28±2 °C and with 12h light; 12h darkness).

Experimental design:

The animals were divided into four groups consisting of ten rats in each group. Toxicity of cypermethrin was evaluated by static bioassay method (Finney, 1971) and the LD_{50} of cypermethrin to albino rats was found to be 205mg/kg bw. 1/5 of LD_{50} value (41mg/kg bw) was selected as sublethal doses and administered as single, double and multiple dose with one day interval in between. The first group of animals was treated as vehicle controls and adminstred with corn oil. To the second group of animals single dose of cypermethrin was given. To the third group, a double dose was given while to the fourth group, multiple doses were given orally. After stipulated time, the animals were sacrificed and the tissues like liver and kidney were isolated and stored at -80^0C for biochemical analysis.

Estimation of biochemical parameters:

Total carbohydrates, glycogen were estimated by the method of Carrol *et al.* (1956), pyruvates was estimated by the method of Friedman and Haugen (1943) and Lactic acid was estimated by the method of Barker and Summerson (1941).

RESULTS AND DISCUSSION

The results of total carbohydrates, glycogen, pyruvate and lactic acid contents of the control and experimental rats under cypermethin are mentioned in Table 1. The experimental rats exposed to cypermethrin showed statistically significant ($p<0.05$) decrease of total carbohydrates, glycogen, puruvate contents, where as lactic acid content was significantly ($p<0.05$) increased. Alterations in carbohydrate metabolic profiles were in the form of a dose and time dependent manner in treated rats.

Carbohydrates are one of the main dietary sources of energy for the body. Carbohydrates are an essential part of a healthy diet. The major function of carbohydrates in metabolism is to make available to be oxidized energy for other metabolic process (Martin *et al.*, 1983). They are stored as such in the body of an animal as glycogen. Glycogen is considered as one of the major sources of energy and maintenance of its reserves is an important feature of cellular metabolism (Turner and Manchester, 1972). The TCA cycle is the main pathway for the oxidation of carbohydrates, lipids and proteins (Hansford, 1980).

The levels of total carbohydrates were found to decrease continuously throughout the time course study. The reduction in total carbohydrate content in experimental animals may be due to higher energy demand under cypermethrin toxicity. The organisms need high energy to tolerate the stress conditions. Similar sort of changes in carbohydrate levels have been reported in several stress conditions (Nadhamuni Chetty, 1992).

The disturbance in the carbohydrate metabolism caused by the action of toxic compounds and compensatory shift from aerobic to anaerobic segment seems to be inevitable in tissue cells for survivability (Vasioles *et al.*, 1976). The decrease in carbohydrate levels signifies its utilization possibly to meet higher energy demands. Several workers have reported the reduced carbohydrates metabolism, (Hameed *et al.*, 2006); Jeeva Selvasundari and Vengadesh Perumal, 2006, Chandra Mouli, 2008).

A significant decrease in the total carbohydrates levels in the rat to sublethal concentration of cypermethrin, observed in the present study envisages the possibility of their rapid utilization for more energy requirements during induced cypermethrin stress.

Glycogen is a major source of energy in animal tissues. The maintenance of glycogen reserve is an essential aspect of the normal metabolism (Turner and Manchester, 1972). Increased glycolytic activity and decreased glycogen content in liver was observed (Ostroukhova, 1965). The high energy demand in pesticide treated animals was possibly to meet the enhanced protein synthesis (Cappons and Nicholls, 1975). The fall in

Table 1 : Biochemical changes in liver and kidney tissues of control and cypermethrin treated albino rats

Parameters selected	Liver				Kidney			
	Control	Single dose	Double dose	Multiple dose	Control	Single dose	Double dose	Multiple dose
Total carbohydrates	98.415	75.866*	57.419**	43.803**	26.078	21.379*	17.753**	14.925**
(mg/gm wet wt. of	±0.132	±0.109	±0.134	±0.493	±0.322	±0.182	±0.146	±0.211
tissue)		(-22.91)	(-41.65)	(-55.49)		(-18.01)	(-31.92)	(-42.76)
		P<0.05	P<0.01	P<0.01		P<0.05	P<0.01	P<0.01
Glycogen	18.190	11.872**	8.198**	6.592**	2.476	1.884*	1.346**	1.237**
(mg/gm wet wt. of	±0.376	±0.046	±0.103	±0.034	±0.143	±0.025	±0.043	.±020
tissue)		(-34.73)	(-54.93)	(-63.75)		(-23.89)	(-45.62)	(-51.46)
		P<0.01	P<0.01	P<0.01		P<0.05	P<0.01	P<0.01
Pyruvate	42.699	34.158*	28.421**	23.524**	26.492	21.639*	17.191**	14.552**
(μ moles/gm wet wt. of	±0.500	±0.169	±0.142	±0.107	±0.120	±0.113	±0.163	±0.427
tissue)		(-2.00)	(-33.43)	(-44.90)		(-18.32)	(-35.10)	(-45.07)
		P<0.05	P<0.01	P<0.01		P<0.05	P<0.01	P<0.01
Lactic acid	39.412	43.279*	49.970**	54.114**	31.117	34.767*	39.262**	42.531**
(mg/gm wet wt. of	±0.124	±0.106	±0.097	±0.093	±0.107	±0.085	±0.107	±0.085
tissue)		(9.81)	(26.78)	(37.30)		(11.72)	(26.17)	(36.68)
		P<0.05	P<0.01	P<0.01		P<0.05	P<0.01	P<0.01

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

glycogen content in all tissues indicate its rapid utilization by the respective tissues as a consequence of pesticide toxic stress. Reduction in glycogen content might be due to the elevated glycolytic activity in animals during cypermethrin stress. Decrease in glycogen level was reported in fish due to pesticide effect (Vijayavel *et al.*, 2006, Crestani *et al.*, 2005) and albino mice exposed to sodium fluoride (Jayasankar, 2007).

The significant decrease in the glycogen levels in the rat under different doses of cypermethrin exposure observed in the present study suggests a possibility of their rapid utilization of more energy requirements during cypermethrin stress.

Pyruvate is the terminate metabolite of glycolysis under aerobic conditions. The level of pyruvate indicates the efficiency of oxidative metabolism. The decrease in pyruvate level suggests the possibility of shift towards anaerobic dependence due to a remarkable drop in the aerobic segment. The decrease in pyruvate could be due to its conversion to lactate or due to its mobilization to form amino acids, lipids, triglycerides (Satya Prasad, 1983), in addition to its role as a detoxification factor (Tripathi and Singh, 2002).

The conversion of pyruvate to lactate leads to reduction in pyruvate levels. Cyclic AMP activates the phosphorylase system during stress conditions (Kalicharan and Gibson, 1972) and inhibit the pyruvate levels (Santhi, 1991), thus increasing the lactate (Surya Prakash, 1988). Further, several reports also indicated a similar decrease in pyruvate upon different stress conditions (Scifter, 2001 and Jayasankar, 2007).

In the present study, reduced level of pyruvate in the rat under cypermethrin stress indicates the conversion of pyruvate to lactate which in turn leads to decrease in oxidative metabolism as evidenced from decreased specific activities of MDH etc. enzymes of SDH and oxidative enzymes (Ravi Sekhar, 2008).

Lactate is the end product of anaerobic glycolysis. The rate of lactate production is considered as an index of physiological stress in biological system (Kozlouski *et al.*, 1985). The increased lactate levels were observed in different tissues in cypermethrin treated albino rats. The increased levels of lactate suggest the prevalence of anaerobiosis in the tissues and also indicate susceptibility to aerobic conditions. Another possible reason for hypoxic condition was known to be increased lactate levels (Johnson, 1981).

Under anaerobic conditions, lactate is formed from pyruvate. This reaction is important in the muscle when energy demands exceed oxygen supply. Glycolysis occurs in the cytosol (fluid portion) of a cell and has a dual role. It degrades monosaccharide to generate energy and it provides glycerol for triglyceride synthesis. The Krebs cycle and the electron transport chain occur in the mitochondria. Most of the energy derived from carbohydrate, protein and fat is produced via the Krebs cycle and the electron transport system. The increase in lactic acid in fish, exposed to pesticides was also supported by several authors (Sathyaparameshwar *et al.*, 2006 and Crestani *et al*., 2005).

Thus in the present investigation the carbohydrate profiles were altered due to intoxification of cypermethrin and oxidative stress in albino rats. Finally it can be stated that long term exposure to sublethal doses of pyrethroid pesticides can result in cell metabolism toxicosis.

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