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Effect of cadmium on lactate dehyrogenase isoenzyme, succinate dehydrogenase and NA$^+$-K$^+$-ATPase in liver tissue of rat

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Abstract: *Cadmium is known to be an environmental and industrial pollutant. Exposure to cadmium is known to affect various tissues. The purpose of this study is to determine the toxic effects of cadmium on lactate dehydrogenase (LDH) isoenzymes. Administration of cadmium chloride (3 mg kg^{-1}) resulted in the reduction of LDH_4 fraction of liver tissue indicating the inhibitory effect of cadmium. The LDH_1, LDH_3 and LDH_5 fractions showed decreasing trend initially but there was a significant increase later. In contrast the LDH_2 fraction showed increasing trend after 24 hr from the last dosage it was elevated after 30 days. The total activity of LDH in liver showed gradual reduction in both groups. The activity of succinate dehydrogenase (SDH) the mitochondrial enzyme has been observed to be inhibited by cadmium with the possibility of interfering with energy transport mechanism. Na$^+$- K$^+$ - ATPase activity of liver also showed similar decrease after cadmium administration which may lead to general deficit in cell membrane transport. As cadmium shows drastic decrease in all the enzymes studied, the LDH_2 fraction which sharply rises from the control may be used as a marker enzyme to asses the liver injury.*

Key words: *Cadmium, Lactate dehydrogenase, Rat, Liver, Succinate dehydrogenase, Na$^+$- K$^+$- ATPase*
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Introduction

Cadmium is a non-essential trace element, which is toxic to many plants and animals. Cadmium is an industrially used substance with negative long time effects on human health (Godt *et al*., 2006). Acute toxicity may result from the ingestion of cadmium through contaminated foods and beverages (Yoon *et al*., 2008; Pratap and Bonga, 2007). Mammalian organisms are exposed to cadmium by its technological utilization and the epidemiological studies shows that cadmium is one of the most toxic of the heavy metals to humans (Shadi *et al.,* 2001). Exposure to sublethal concentration of cadmium to the fishes leads to haemolysis (Karuppasamy *et al.,* 2005). Cadmium produces toxic lesions in various tissues (Friberg *et al.,*1986) the largest amount of this metal is deposited in the liver and kidney tissues (Marafante, 1976). It causes morphological and functional changes in the liver (Kopp *et al*., 1983; Bomhard *et al*., 1984). Cadmium ingestion causes testicular abnormalities in early sperm development in mice (Haffor and Abou-Tarboush, 2004). Cadmium inhibits the activity of enzymes by binding to their sulfhydryl groups (SH), consequently causing the peroxidative destruction of cell membranes (Chung and Maines, 1987). Cadmium is also known to alter several physiological activities such as alteration in carbohydrate metabolism in rat, mice and rabbit (Ghafghazi and Mennear, 1973).

Lactate dehydrogenase (LDH) a marker cytoplasmic enzyme is known to be affected by cadmium (Tanaka, 1982; Stewart *et al*., 1984; Itoh,1985; Sugihira *et al*.,1987; Xu *et al*., 1989). As the enzyme appears to be the reliable marker for metabolic abnormalities, it is not clear whether all the five fractions of LDH isoenzyme are affected by cadmium or not. Earlier studies revealed that cadmium affects mitochondrial enzymes especially those of citric acid cycle and electron transport chain like succinic dehydrogenase(SDH) (Prasada Rao, 1983; Toury *et al*., 1985; Weilgus Serefinska *et al*., 1989) and enzymes involved in active transport of ions and energy production like Na$^+$-K$^+$-ATPase of the liver tissues. The most sensitive cellular targets of cadmium seem to be ion transport and signal transduction (Rasheed *et al.,* 1984). The present study was designed to evaluate the toxic effects of cadmium on LDH isoenzymes. To this end total activity of lactate dehydrogenase enzyme (1.1.1.27), succinate dehydrogenase (1.3.99.1) and Na$^+$-K$^+$-ATPase and the amount of deposited cadmium were determined in the liver tissues of rat following cadmium treatment.

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Materials and Methods

Animal treatment: Adult male Wistar rats aged 10-13 weeks, weighing about 220-250 g were obtained from Kings Institute of Preventive Medicine, Chennai. They were housed in stainless steel wire bottom cages at the temperature of 25-28°C and photoperiod of 12 hr day / 12 hr night. They were fed rat pellet diet and water *ad libitum.* Rats were allocated randomly into two groups of 16 each and received nephrotoxic dose (Katsuta *et al.,* 1993) of 3 mg kg^{-1} of cadmium chloride by means of subcutaneous injections on alternative days over a period of 15 days as follows : (I) Control group, saline solution; (II) Experimental Group I, cadmium chloride solution in physiological saline at a dose of 3 mg kg^{-1} body weight on alternative days. Twenty four hours after the last dosage a group of eight animals were anesthetized and sacrificed by cervical dislocation

and these were treated as experimental Group I animals. Exactly thirty days after the last dose, a group of eight animals from the Group II were sacrificed and they were treated as experiment Group II animals. The excised liver tissues were isolated and cleaned from adhering matters and washed with ice cold deionized water and stored at -80°C for the biochemical studies.

Biochemical studies: The biochemical assays of lactate dehydrogenase, succinic dehydrogenase, and NA^+-K^+- ATPase were carried out with these tissues. LDH was assayed electrophoretically using PAGE (Poly Acrylamide Gel Electrophoresis) following the method of Dietz (1972). Isoenzyme bands were estimated densitometrically and their relative contributions were calculated and expressed as a percentage contributions of total activity. Specific total activity of LDH (EC 1.1.1.27) was measured by the method of King (1965) and the units were expressed as Units g^{-1} wet tissue. Succinic dehydrogenase activity was determined by the method of Nichlas *et al.* (1960) and the activity was expressed as MI Units g^{-1} tissue min^{-1}. Activity of Na^+-K^+-ATPase was determined employing the method of LeBel *et al*. (1978) as modified by Yamaguchi *et al*. (1979) and the activity was defined as mg pi liberated μg^{-1} protein min^{-1}. The cadmium content of liver tissue was measured by using atomic absorption spectrophotometer (THE VARION TECHTRON AA-60) and expressed as $\mu g\ g^{-1}$ wet tissue. Data were analyzed by students "t" test (Zar, 1984) and a value of $p<0.05$ was considered significant in all cases.

Results and Discussion

Cadmium, the metallic pollutant, has been found to bring about changes in all the five fractions of lactate dehydrogenase isoenzymes. LDH is known to play an important role in several metabolic pathways and by delicately balancing the equilibrium in the anabolism and catabolism of carbohydrates. The results show that LDH_4 is the main isoenzyme fraction in the fat storing cells of liver tissue (Kassner *et al*., 1988). A decline in LDH activity of mouse pre embryos after cadmium administration cause LDH degradation and thus interfere in general energy metabolism (Yu and Chan, 1986). Such reduction may lead to prevention of regeneration of NAD^+ resulting in the inhibition of glycolysis. In the present study also it is evident that cadmium brings about such an effect on LDH_4 fraction as its percent activity significantly decreased from 61.05±1.420 to 32.17±1.013 in experimental Group I animals and it further declines to 25.00%± 0.80 in experimental Group II animals. It is also significant ($p<0.05$) to note that only LDH_4 activity showed a continuous reduced percent activity, which is reflected in percent total LDH activity of liver tissue (Table 2). There is a gradual decrease in the total LDH activity in experiment group I and experiment Group II animals suggesting that cadmium plays a crucial role in preventing the activity of LDH. Such reduction in the activity of LDH may be due to the interaction of cadmium with free SH groups of LDH as it is known that the SH groups have strong affinity towards metallic cadmium (Vallee and Ulmer, 1972). The interaction of cadmium with SH groups indicates that Cd^{2+} ions interact with SH groups that are important for energy linked reactions and calcium transport resulting in hypertension and related diseases (Jay and Michel,1986). The percent activity of LDH_1 fraction decrease in experiment Group I animals from the control value indicating the possibility of cadmium interference. However, there is an increase in its activity in experimental Group II animals suggesting the induction of this fraction by the prolonged treatment with cadmium. It is possible that LDH_1 which showed a tremendous increase in experiment Group II animals may be related to antioxidant defense mechanism in the case of liver tissue, as has been suggested by Buckley and Basset (1987).

It is highly significant to note that there is an increase in secondary liver neoplasia suggesting that it may be marker for liver diseases (Castaldo *et al*., 1991). The present study also supports such a possibility that the fraction LDH_2 might be used as a marker enzyme for the liver injury as it is evidenced from elevated percent level of 32.67±1.177 compared to the control liver tissue. LDH_3 and LDH_5 fractions respond similarly to cadmium. They show significant

Table - 1: The percentage LDH isoenzyme activity in liver tissue of the control and cadmium chloride treated male Wistar rats

Lactate dehydrogenase (LDH) isoenzymes	Control	Experiment Group I	Experiment Group II
LDH_1	07.88 ± 0.475	02.39 ± 0.287	21.31 ± 0.632*
LDH_2	07.31 ± 0.355	11.74 ± 0.364*	32.67 ± 1.177**
LDH_3	19.26 ± 0.470	25.44 ± 0.992	02.84 ± 0.052
LDH_4	61.05 ± 1.420	32.17 ± 1.013*	25.00 ± 0.801
LDH_5	04.50 ± 0.159	28.26 ±1.510***	18.18 ± 0.533

Values are given in mean ± SE and significant at * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Table - 2: The Liver LDH, SDH, Na^+-K^+-ATPase activity percentage of the control and cadmium chloride – treated male Wistar rats

Parameters	Control	Experiment Group I	Percentage change	Experiment Group II	Percentage change
LDH	0.491 ± 0.0118	0.453 ± 0.0086	-7.73*	0.386 ± 0.0163	-21.38**
SDH	2.588 ± 0.0298	0.751 ± 0.023	- 70.98***	1.139 ± 0.0523	-55.98***
Na^+-K^+-ATPase	0.0309 ± 0.0016	0.0234 ± 0.0014	-24.27**	0.0255 ± 0.0017	-17.47*

Values are given in mean ± SE, Values are significant at * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

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increase in their activity in experiment Group I animals but drastic decrease was observed in experiment Group II animals. The decrease in the activity of LDH_3 fraction is so steep that it amounts to only 02.84 ± 0.052 which is well below the normal level suggesting complete inactivity of this enzyme because of cadmium interaction. The decrease in LDH_5 fraction is not as steep as that of LDH_3 but still significant enough to merit our attention in relation to carbohydrate metabolism.

The data given in Table 2 reveals total percent activity of LDH, SDH and Na^+-K^+-ATPase. Cadmium has tremendous influence on SDH enzyme associated with carbohydrate metabolism. The reduction in SDH activity is so severe that it is 70.98% in experiment Group I animals and 55.98% in experiment Group II animals. A similar decrease in the SDH of the liver tissue has been reported by Prasada Rao (1983) after the intraperitoneal injections of cadmium with a dosage of 3 mg kg^{-1}body weight. . Bracken and Sharma (1985) also noticed a decrease of 15% in SDH activity and 47% cytotoxicity in liver. Wielgus-Serafinska *et al*. (1989) reported similar inhibition of SDH activity by cadmium. A progressive slackening of SDH activity has been observed, Toury *et al*. (1985) suggested that this is due to membrane fixation of Cd^{2+} ions on the flavoprotein or SH groups associated with SDH. The reduction on SDH activity is noticed in liver tissues may be attributed to binding of cadmium to SH groups, ultimately leading to the interference with energy transport mechanism.

Evidence to such a suggestion is obtained by the inhibition by cadmium on the activity of Na^+-K^+-ATPase which is an integral part of Na^+-K^+-pump hydrolyzing ATP molecules which provide energy needed for active transport of cations. The activity of Na^+-K^+-ATPase is reduced by 24.27% in experiment Group I animals indicating the inhibitory effect of cadmium on this enzyme. Such inhibition may lead to reduction in the active transport of Na^+-K^+-ATPase activity to a maximum extent between 30 min to 2 hr after cadmium administration (Ahammadsahib *et al*.,1989). Kramer *et al*. (1986) also studied *in vitro* inhibition of Na^+-K^+-ATPase by trace metals and suggested that accumulation of cadmium in the tissue may cause serious health hazards by producing a general deficit in cell membrane transport.

To conclude, cadmium in the liver inhibits the activity of the enzymes LDH, SDH and Na^+-K^+-ATPase. The induced activity of enzyme which is also exhibited in LDH isoenzyme fraction and the enhanced activity of LDH_2 fractions both in experiment Group I and experiment Group II animals suggests that this fraction may be used as a marker for the liver injury. Further studies are required to confirm the role of LDH isoenzymes as the marker enzyme for the heavy metal toxicity.

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