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RESEARCH ARTICLE

IMPACT OF HEAVY METAL ZINC ON ENZYME STUDIES IN SELECTED TISSUES OF *ODONTOPUS VARICORNIS* (Dist.) (HEMIPTERA : PYRRHOCORIDAE)

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ABSTRACT

An attempt was made to find out the effect of heavy metal zinc (25ppm median lethal concentration) on fat body, testis, seminal vesicle and MAGR's of *Odontopus varicornis*. Zinc is the fourth most widely used metal in the world and also one of the ubiquitous elements in the world. Heavy metal zinc causes the destruction of the beneficial species directly or indirectly through breaking the biological food chain. Enzymes are the attractive indicators because they are more easily quantified than the other indicators. The metabolic pathway mainly depending on enzyme- activities may be affected due to the destruction under the stress, reflecting the changes of enzyme activities. Investigators were made on the TCA enzymes, SDH, GDH and MDH. The heavy metal, zinc brought drastic changes in the antioxidant enzymes to reduce the detoxification mechanism which intern causes the mortality of the insects. In conclusion, the heavy metal zinc decreased the enzymatic antioxidant levels drastically from the selected tissues of *Odontopus varicornis.*

INTRODUCTION

Nowadays modern research is oriented towards attaining health and economic benefits of mankind. Many experiments are actively going on in various fields like chemical research , medical research, engineering and biological research. The biological organisms are exposed to a great variety of natural and manmade toxic substances in the environment. Apart from the occupational and accidental aspects of hazardous chemical exposure to human, contaminants also obviously influence water, soil, air and the survival of a variety of plants and animals (Moore and Ramamoorthy, 1984). Such exposures cause adverse health effects ranging from sub lethal changes to death (Lu, 1985). Of the common toxic substances in practice, heavy metals constitute the most widely distributed group of highly toxic and persistent substances. Trace levels of some metals, however, are very essential for the metabolic activity and good health (Eisler, 1981). But when their bioconcentrations exceed the safe level, they act as metabolic inhibitors besides disrupting other physiological, biochemical and behavioural aspects (Vallee and Wacker, 1970). It is known that relatively low concentration of certain heavy metals appears to be toxic to all animals (Calamari, 1977 and Ellgaard *et al*., 1978). The increasing industrialization leads to continuous additional pollutants to the natural environment (Edwards, 1973). One of the common sources of heavy metal pollution is the discharge of effluents from the industries into the environment (Nriagu, 1980). Apart from the release of heavy metals by natural agencies, they also find their way through some other artificial sources such as mining, smelting and metal refining, which discharge a variety of metals into the aquatic and terrestrial environment (Carmody *et al*., 1973). The burning of fossil fuels containing heavy metal and the addition of tetraethyl lead to gasoline has now made environmental pollution, the major source of heavy metal poisoning (Klaassen, 1980).

Zinc is the fourth most widely used metal in the world and also one of the ubiquitous elements in the world among others. It means the concentration of zinc in the earth's crust is estimated as 70ppm (Abbasi, 1989). The occurrence of zinc has been reported in various rocks and soils with its concentration ranging from 100 and 300 ppm (Adriano, 1980). In natural freshwaters such as unpolluted springs, streams, rivers and lakes, zinc concentrations rarely exceed 0.5 ppm (Abbasi, 1989; Abbasi *et al*., 1998). have reported that 12.5 to 151 ppm of zinc in Jamaica Bay sediments in New York. Swedish forest lakes contained zinc levels ranging from 25 to 280 ppm (Johansson, 1988). Pure zinc is used mainly for protecting steel from rust (galvanization). Other very important fields of application are Zinc –base alloys, brass and bronze, zinc semi-manufactures and chemicals. Zinc oxide is used mainly for rubber processing products, pigment, and as intermediate for zinc chemicals. Zinc chloride is used as wood preserver, in soldering fluxes, for galvanization, and as dry battery filler, zinc phosphate is used as corrosion-inhibitive pigment and material for dental cements 1995. The sources of zinc pollution are natural as well as anthropogenic. The annual zinc input to the environment from weathering, erosion and other natural phenomenon is estimated to be the order of 8,00,000 tonnes (Nriagu, 1980). Anthropogenic sources contribute an estimated 414,000 tonnes of zinc per annum. On a global basis, the most important anthropogenic sources include air emissions from primary zinc production (99000 ton/year), wood combustion (75,000 ton/year), waste incineration (37,.000 ton/year), iron and steel production (35,000 ton/year), other atmospheric emissions (68,000 ton/year) and municipal waste water (1,00,000

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ton/year). (Abbasi *et al*., 1998). have observed an average yearly concentration of zinc 19.80 ppb higher than that of cadmium, lead, copper, nickel and cobalt.

In general, zinc compounds are used for various purpose: pesticides, fungicides, insecticides, wood preservatives, drugs, animal feed additives, astringents, catalysts, dietary supplements chemical reagents, metal plating agents, tanning agents, intermediates for zinc chemicals oil, additives flame retardants, pharmaceuticals, ointments, accelerators and vulcanizing agents. The world total mine production of zinc was in 1997 about 7.4 million tonnes, is 1987 the primary production of zinc was about 6.6 million tonnes, and in 1982 the production was about 5.7 million tonnes. Thus the production has increased significantly during the last fifteen years. Figures from the world smelter production of zinc indicate that the smelter production of zinc was stable (7.3 million tonnes) during the period 1989 to 1994 (Chapman *et al*., 1999; United Nations, 1995; Knight-ridder financial/ commodity research bureau, 1996; Eurostat, 1997). Important releases of zinc to the environment are for instance, discharges of smelter slags and wastes, mine tailing coal and bottom fly ash, municipal and industrial effluents, and the use of commercial products such as fertilizers and wood preservative that contain zinc. Important emission to air comes from zinc production and other metals production where zinc is present fuel combustion and incineration, weathering of rocks and soil particles area other important natural sources of zinc (Eurostate, 1998). Estimates for 32 countries in Europe of past and likely future air emission of arsenic cadmium, lead, and zinc have been made by European Environment Agency (EEA). The emissions of zinc to air have decreased for about 160000 tonnes in 1975 to about 60000 tonnes in 1991 (EEA, 1998). However according to Eurostat the total air emissions of zinc in 38 European countries was in 1990 only about 260000 tonnes. In EU -15 the air emission of zinc from EU – 15 has been estimated to 11115 tonnes in 1990 , of which 3400 came from Spain, 1800 from France , and 1600 from Germany (Eurostat, 1998). The air emission of zinc is expected to continue to decrease (EEA, 1998). Manure and atmospheric deposition are the main sources of zinc inputs to agriculture soil. For Swedish conditions it has been estimated that the removal of zinc from the soil with the harvest amounts to approximately 200 g/ha/year is returned to the soil by the atmospheric deposition in southern Sweden, and only 15g /ha/year in cases of commercial fertilizers is used . This means that the soils are slowly being depleted of zinc, with a risk of deficiency. This depletion can be counteracted by the use of farmyard manure, comes from zinc added to animal feed to prevent deficiency symptoms in the livestock. Zinc is found in the atmosphere at the highest concentrations in the smallest particle. Particles with small diameters and low densities may travel long distances from emissions sources. The possibility of long range transport indicates that life time in air is at least on the order of days. Zinc is removed from the atmosphere by dry and wet deposition (Landner and Lindestorm, 1998).

The major share of the total world production of zinc is used for the industrial applications such as zinc coating to protect iron and steel by hot-dip galvanizing, electrogalvanizing, spraying, painting and sherardizing. It is also used in the zinc alloys, brass, rolled sheets and strips, dry batteries, roofing outer fittings on buildings and printing processes. The concentration of zinc in the effluents of phosphate fertilizer industry was about 1.4 - 1.83 ppm (Muthukumar and Subramanyam, 1987). Ghee manufacturing 0.369 ppm (Ajmal and Khan, 1984). Metal processing unit 0.2-1463 ppm, zinc plating units 55-120 ppm, silver plating units 0-220 ppm, Rayon industry 250-1000 ppm and distillery 0.027-0.225 ppm (Sheham and Grrehfield, 1980). And Landfillieachates 0.6-370 ppm (Abbasi *et al*., 1998). Zinc is the 24th most abundant element in the earth's crust. Its average concentration is estimated to 70mg /kg. Volcanic rock contains around 70mg/kg and sedimentary rocks 15-100mg/kg. High zinc concentrations are found in shale's and clay sediments 80-120mg/kg. Zinc also occurs in phosphate rocks. The average zinc concentration in 91% of the phosphate reserves has been estimated to 239 mg/kg (Landner and Lindestrom, 1998; Kiekens, 1995). The total zinc concentration in soils is 10-300mg/kg with an average of around 50mg/kg dry weight kg. The concentration of zinc in solution is .however, compared to the total concentration in general very low. The zinc concentrations in most soils in Western Europe are consistent with the levels considered as background values, with exception for mined areas (Landner and Lindestrom, 1998; Kiekens, 1995).

Heavy metal pollution may cause the destruction of the beneficial species indirectly through breaking the biological food chains. The polluted water and air affects the life of aquatic, terrestrial animals and their habits (Anderson and Peterson, 1969). Growth (Korschgen and Murphy, 1969). Reproductive potentials (Jarvinen *et al*., 1976). And resistance to diseases through a variety of mechanisms in which the key physiological functions are either modified or suppressed. The majority of the insects are economically important and hence gaining knowledge of their reproduction is very essential. The various aspects of reproductive activities such as sexual excitement, mating, oviposition, transfer of seminal fluid during mating and the functions of male accessory reproductive glands have been studied in different orders of insects (Jarvinen *et al*., 1976; Ravisankar and Venkatesan, 1988; Padmanabhan, 1992; and Selvisabhanayakam, 1995). Studies on the fat bodies, testes, seminal vesicles and male accessory reproductive glands are very essential to understand the problems related to the reproductive physiology of insects.

Reproduction in insects is an essential physiological process from the view point of propagation of the insect species and as mentioned earlier, naturally results in the high biotic potential of insects, making them a successful group of animals. Reproduction always does not imply the bi-sexual methods, but other reproductive processes like parthenogenesis, polyembryony, etc. may also be involved (Nayar, 1963). In some insects parthenogenesis is a normal means of reproduction as reported in various species of insects except Odonata, Dermaptera, Neuropteran and Siphonaptera. The parthenogenesis occurring in insects may be arrhenotoky, in which only males are produced; Thelytoky, in which only females are produced (or) amphitoky producing the individuals of either sex. Besides such diversity occurring in the mode of reproduction, almost all insects possess a well-defined set of internal organs of reproduction, primarily consisting of paired gonads, genital ducts leading to a gonopore, and some accessory structures. The accessory structures include various glands and particularly in the female, the spermatheca for storing the sperm received from the male during copulation. Copulation among apposite

sexes is carried out by external genital organs which are described elsewhere (Chapman, 1972).

The male and female reproductive systems generally consist of paired gonads connected to a median duct leading to the gonopore. Accessory glands are often present which in the male are usually concerned with spermatophore formation and sperm maintenance and in the female provide glue for sticking the eggs to the substratum (or) provide the substance for a complex egg-case. The female has, in addition, a spermatheca for storing sperm after copulation. Each gonad typically consists of a series of tubes, each with a germinal area at the tip containing the primordial sex cells. From these spermatogonia (or) oogonia are produced, which can be seen in successive stages of development as they pass down the tube. Each secondary spermatogonium gives rise to four spermatozoa, but only a single oocyte is formed from each secondary oogonium. General review of insect reproduction are given by (Engalmann, 1970). And de (Wilde and de Loof, 1973a). While the structure of reproductive organs is described by Snodgrass (1935). The fine structure of insect sperm is reviewed by (Baccetti, 1972) and (Phillips, 1970). For reviews of aspects of oogenesis see (Hagedorn and Kunkel, 1979). (Engelmann,1970; Telfer, 1975; Telfer and Smith, 1970). Control of reproduction is reviewed by (de Wilde and de Loof, 1973b). And nutritional aspects are considered by (Johansson, 1968; Bell and Bohm, 1975). Discuss oocyte resorption.

Insects are not always sexually mature when they have completed the final moult to the adult stage and in species with an adult diapause there may be a considerable delay before mature sex cells are produced. Hence, it is necessary to distinguish between adult and becoming sexually mature (Chapman, 1972). However, sexual reproduction is by far the common and sexual differentiation on the basis of the external appearance, though possible in many insects is not always the rule. Enzymes are attractive as indicators because they are more easily quantified than the other indicators. Metabolic path way mainly depending on enzyme- activities may be affected due to the destruction under stress, reflecting the changes for enzyme activities. Glycolysis is known to yield pyruvate and glycophosphate. Pyruvate apart from being formed by glucose metabolism is also formed from the carbon skeletons of a number of amino acids such as serine, alanine and cysteine. α-ketoglutarate is found as a preferred energy substrate for the spermatozoa and as an accelerator for sperm respiration in insects. In addition, energy metabolism of reproduction of male insects has not been much studied. Investigations were made on the TCA enzymes, SDH, GDH, LDH and MDH in the fat body and the accessory reproductive gland of *Odontopus varicornis* before and after mating by (Selvisabhanayakam, 1995). A study of oxidative enzymes will reveal whether pyruvate is being utilized to supply energy or from other pathways.

The heavy metal, zinc acts as enzyme inhibitors, leading to hyperexcitability of the nervous system. It may also cause various side effects, e.g. change in DNA structure (Griffin and Hill, 1978), cause sperm malformations (Mathew *et al*., 1992). Generate reactive oxygen species (Bagchi *et al*., 1995), act as inducers of heat shock protein (Bagchi *et al*., 1996). Heavy metal, zinc and other xenobiotics may increase the level of free radicals (Freeman and Crapo, 1982) and influence (mobilise) an antioxidant defence system in tissues and cells. Antioxidants can so other neurotoxic effects of insecticides (Bagchi *et al*., 1993, 1996; Minakata *et al*., 1996). However, cytochrome P450 mediated detoxification of xenobiotics results in enhanced free radical content of cells. Heavy metal, zinc may be broken down in different ways, which can lead to different products of a higher or lower toxicity than the mother compound (Brattsten *et al*., 1986; Chambers *et al*., 1994). On activity of superoxide dismutase (SOD) and catalase (CAT), i.e. two important enzymes, which protect cells against free radicals.

Lactate dehydrogenase (LDH) is an important glycolytic enzyme being present in virtually all tissues (Kaplan and Pesce, 1996). It is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantine *et al*., 2001). Although, it is usually used as an index of anaerobic metabolism (Chamberlin and King, 1998). To show correlation between some enzyme activity and non-enzymatic compounds, the amount of glucose and protein was measured. Lactate dehydrogenase catalyzes the last step in glycol sis, as it reduces private to lactate. Succinct dehydrogenase is several studies have shown that metal trace elements are, at the cellular level, often involved in oxidative stress, which results from the production of reactive oxygen species (ROS). ROS includes the superoxide radical ($\bullet O^{2-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids (Damien *et al*., 2004). The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging of ROS. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and guaiacum peroxidase (GPx). Superoxide radicals that are generated are converted to H2O2 by the action of SOD, and the accumulation of H_2O_2 is prevented in the cell by CAT and GPx. It has been demonstrated that the activities of SOD, CAT, and GPx are induced both in plant species (Frazier *et al*., 2002a; Bhattacharjee, 1997,1998; Lee and Shin, 2003; Skorzynska-Polit *et al*., 2003, 2004; Pereira *et al*., 2002; Gallego *et al*., 1999) and in animal species (Sarkar *et al*., 1998; Rashed, 2001) by some external factors, but less is known about the activity of antioxidant enzymes in insects the active regulatory enzyme of the Tricarboxylic acid cycle.

Superoxide dismutase (SOD) reacts with superoxide radicals and converts them to H_2O_2, which is catalysed by catalase or GSH peroxidase (Kakkar *et al*., 1984). Superoxide dimutases are metalloproteins and play a vital role in protecting cells against oxidative damage. Glutathione peroxidase (GPx) an enzyme with selenium in the form of seleno cyseteine at its catalytic site catalyses the reduction of hydrogen peroxides and hydroperoxides to non toxic products. The reducing equivalent of glutathione is used as a substrate to form oxidized glutathione (Bruce *et al*., 1982). Phosphatases have been included in the list of detoxifying enzymes of insecticides; mostly of organophosphorus (Oppenoorth, 1985), however, fenvalerate and cypermethrin resistant larvae of *Helicoverpa armigera* (Hubner) showed higher activities of esterases, phosphatases and methyl paraoxon hydrolase compared with susceptible larvae (Srinivas *et al*., 2003). (Abdel-Hafez *et al*., 1985) have also reported the changes in the level of phosphatases in moths of *Pectinophora gossypiella* (Saund.) during the course of heavy metal poisoning. There are instances where phosphatases were not only detected in red flour beetle, *Tribolium castaneum*, but also changes in level of these enzymes upon exposure to cypermethrin and bifenthrin

insecticides were reported (Saleem and Shakoori, 1985, 1986, 1987, 1996; Tufail *et al*., 1994; Sohail Ahmed *et al*., 2004).

MATERIALS AND METHODS

Lactate dehydrogenase (L-lactate: NAD + oxido reductase). The enzyme LDH was assayed by the method of (King, 1965). The method is based on the ability of LDH to convert lactate to pyruvate, with the help of the co-enzyme nicotinamide adenine dinucleotide (NAD+). The pyruvate formed is made to react with, 2, 4-dinitrophenyl hydrazine in hydrochloric acid. The hydrazine formed turns into orange coloured complex in alkaline media and is measured at 420 nm.

The enzyme SDH was assayed by (Bernath and Singer, 1962) method. The measurement of succinate dehydrogenase activity was much less complicated with isolated floroprotein than when the enzyme was still attached to all, or part of electron transport chain. The activity of the enzyme is determined till the forward reaction-the oxidation of succinate. The isolated floroprotein may be assayed in the direction of succinate oxidation with phenazine methosulphate. In this assay, it is recommended that the activity is determined as varying dye concentrations and extrapolated to match with respect to the electron carrier. At fixed dye concentration, an uncertain fraction of the activity is measured, since the affinity of the dye for the enzyme may vary, depending on the treatment during isolation.

L-Glutamate, NAD+ oxidereductase deamination. The enzyme activity was assayed by the method of (Stretcker, 1965).

The fat body, testis, seminal vesicle and male accessory reproductive glands and other related organs were homogenised in 10 volumes of cold acetone and centrifuged. After washing, the precipitate with cold acetone, the residue was dried in a stream of dry nitrogen gas at room temperature. The powder from gland was extracted with 20 volume of 0.1 M phosphate buffer (pH 7.4). The reaction mixture in a final volume of 3.0 ml contained (final concentrations) 90 mM phosphate buffer (pH 6.4), 33 mM potassium glutamate, 0.33 mM NAD+ and 0.1 ml of enzyme extract. The reaction was started by adding enzyme extract to the reaction mixture equilibrated at 30°C. The change in extinction per minute at 340 nm was recorded in Bausch and Lamb Spectronic-20 UV visible spectrophotometer. The values were expressed as µ moles NAD+ reduced /min /mg / protein.

Superoxide dismutase (SOD) was assayed by method of (Kakkar *et al*., 1984).

NDH at equilibrium position of the reaction favours reduction of oxaloacetate to L-malate. The malate dehydrogenase activity was determined based on the measurement of the rate of oxidation of NADH (decrease in optical density at 340 nm) in the presence of the enzyme and excess oxaloacetate. The early rate of NADH oxidation was proportional to the concentration of the enzymes. The measurement was carried out in Spectronic 20 at 320 nm using cortex or silica cells of 1.0 cm light path.
The enzyme malate dehydrogenase was assayed by (Severo Ochoa,1955) method.

The assay was based on the inhibition of the formation of NADH - phenazine - methosulphate nitro blue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90 seconds, the reaction was stopped by adding of glacial acetic acid. The colour developed at the end of the reaction was extracted into n-butanol layer and measured at 520 nm.

Catalase (CAT) was assayed colorimetrically by the method of (Sinha,1972).

Dichromate in acetic acid was converted to per chromic acid and then to chromic acetate when to be hot in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm.
Glutathione peroxidase (GPx) was estimated by the method of (Rotruck *et al*., 1973).

A known amount of hemolysate preparation was allowed to react with H_2O_2 in the presence of GSH for a specific time period. Then the remaining GSH content was allowed to react with DTNB and the yellow colour developed was measured.

$$2GSH+H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

Statistical analysis

All the data were analyzed with student -*t*-test. The data were expressed as the mean ± SD for two different sets of experiments and results were considered as significantly at $P < 0.05$.

RESULT AND DISCUSSION

The MDH and GDH activities in the fat body of heavy metal, zinc treated insects were found to be increased significantly than that of control insects. (Table. 1).

In contrast, the LDH and SDH activities in the fat body of treated insects have decreased significantly than that of control insects. (Fig. 1).

Fig. 1 Enzymes activity on fat body in control and treated adult male insect, *Odontopus varicornis*

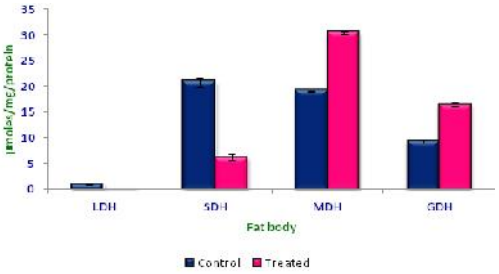


Fig. 1

The LDH activity in the fat body of control and treated insect of about 0.90 ± 0.056 to 0.12 ± 0.01 µmoles/mg/protein; The SDH activity in the fat body of control and treated insect of about 18.82 ±0.82 to 4.25 ± 0.60 µmoles/mg/protein; The MDH and GDH activity in the fat body of control and treated insect of about 17.04 ± 0.11 to 28.38 ± 0.34; 7.29 ± 0.23 to 14.42 ± 0.34 µmoles/mg/protein, respectively. The mean LDH, SDH, MDH and GDH content of the fat body of control and treated insects are compared for significance of difference and that the t-values 34.82, 35.28, -76.58 and -42.50 are significant at 0.05% levels. Therefore, it may be concluded that the LDH, SDH, MDH and GDH in fat body of control and treated insects were differ significantly. Similarly, the LDH and SDH activities in the fat body of treated insects have decreased significantly. But in the MDH and GDH activities in the fat

Table 1 Enzymes activity on fat body in control and treated adult male insect, *O. varicornis*

Fat body	Control (µmoles/mg/protein)	Treated (µmoles/mg/protein)	Percentage over control	't' value
LDH	0.96 ± 0.05	0.18 ± 0.01	-86.90	34.82*
SDH	20.82 ± 0.82	6.25 ± 0.60	-77.44	35.28*
MDH	19.04 ± 0.11	30.38 ± 0.34	68.52	-76.56*
GDH	9.29 ± 0.23	16.44 ± 0.34	99.78	-42.50*

Data represent values are mean ± S.D (n=6).*Significant at 0.05% level.LDH : Lactate dehydrogenase SDH : Succinate dehydrogenaseMDH : Malate dehydrogenase GDH : Glutamate dehydrogenase

body of treated insects were increased significantly than that of control insect, *Odontopus varicornis.*

The LDH and SDH in the testis of treated insects were found to be comparatively less than that of the control insects. But the MDH and GDH activities in the treated insects were found to be comparatively more than that of control insects. (Table. 2).

seminal vesicle of control and treated insects of about, 1.61 ± 0.22 to 0.78 ± 0.11; 9.33 ± 0.30 to 6.66 ± 0.38 and 8.52 ± 0.36 to 12.40 ± 0.58 and 14.14 ± 0.27 to 18.04 ± 0.69 µmoles/mg/protein, respectively. From the Table, it is clear that the t-values 8.35, 13.57, -14.04 and -12.93 were significant at 0.05% level. Therefore, it may be concluded that the LDH, SDH, MDH and GDH activities in the seminal vesicle of

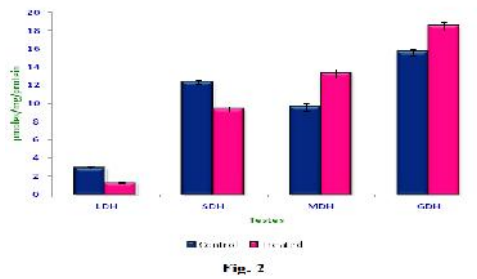
Table 2 Enzymes activity on testes in control and treated adult male insect, *O. varicornis*

Testes	Control (µmoles/mg/protein)	Treated (µmoles/mg/protein)	Percentage over control	't' value
LDH	2.93 ± 0.09	1.29 ± 0.11	-58.47	27.97*
SDH	12.31 ± 0.23	9.38 ± 0.27	-24.71	20.16*
MDH	9.60 ± 0.39	13.29 ± 0.49	43.93	-15.38*
GDH	15.61 ± 0.32	18.44 ± 0.46	20.35	-14.35*

Data represent values are mean ± S.D (n=6).*Significant at 0.05% level.LDH : Lactate dehydrogenase SDH : uccinate dehydrogenase MDH : Malate dehydrogenase GDH : Glutamate dehydrogenase

The LDH activities in the testes of control and treated insects were 2.91 ± 0.09 to 1.27 ± 0.11 µmoles/mg/protein. (Fig. 2).

Fig. 2 Enzymes activity on testes in control and treated adult male insect, *Odontopus varicornis*



The SDH activities in the control and treated insects were 12.31 ±0.23 to 9.39 ± 0.27µmoles/mg/protein. The MDH activities in the control and treated insects were 8.59 ± 0.39 to 12.28 ± 0.49 µmoles/mg/protein. The GDH activities in the control and treated insects of about 15.41 ± 0.32 to 18.24 ± 0.46 µmoles/mg/protein, respectively.

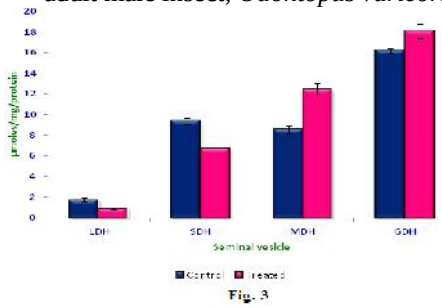
The mean LDH, SDH, MDH, and GDH activities in the testis of control and treated insects were compared for significance of difference. It is clear that the t-values, 27.97, 20.16, -14.38 and -12.35 were significant at 0.05% level. Therefore, it may be concluded that the LDH, SDH, MDH and GDH activities differ significantly in the testis of control and treated insect, *Odontopus varicornis*.

The MDH and GDH activities in the seminal vesicle of treated insects were increased significantly than the control insects. The LDH and SDH activities in the seminal vesicle of treated insects were found to be decreased than that of the control insects. The LDH, SDH, MDH, and GDH activities in the control and treated insects were differ significantly.

The MDH and GDH activities in the MARGs treated insects have increased significantly than that of control insects. (Table. 3).

The LDH and SDH activities in the MARG of treated insects have decreased significantly than the control insects. It is clear from the table that the t-values of MARGs are significant at 0.05% level. Therefore, it may be concluded that the LDH, SDH, MDH and GDH activities in the MARGs of control and treated insects differ significantly. (Fig. 3).

Fig. 3 Enzymes activity on testes in control and treated adult male insect, *Odontopus varicornis*



The widespread use of heavy metal has amounted the biochemical/ physiological changes, which may be of adaptive significance to the life of an animal. Among these changes, alteration in xenobiotics metabolizing enzymes has dominated the toxicological literature. Enzyme is a biocatalyst which accelerates biological reactions. However, the concept of biocatalyst is a very wide source of enzymes used common in plant and animal cells. Animal enzymes that are used currently are lipases, tripsin, rennin, etc. Energy is derived from the three major sources namely carbohydrates, proteins and fats, when they are oxidized (Gilmour, 1965; and Zubay, 1983). It is released from organic molecules, principally by oxidation.

Table 3: Enzymes activity on seminal vesicle in control and treated adult male insect, O. varicornis

Seminal vesicle	Control (µmoles/mg/protein)	Treated (µmoles/mg/protein)	Percentage over control	't' value
LDH	1.71 ± 0.22	0.88 ± 0.11	-52.61	8.36*
SDH	9.36 ± 0.30	6.68 ± 0.38	-29.68	14.57*
MDH	8.52 ± 0.36	12.40 ± 0.58	45.58	-14.04*
GDH	16.14 ± 0.27	18.04 ± 0.69	29.59	-12.93*

Data represent values are mean ± S.D (n=6).*Significant at 0.05% level. LDH : Lactate dehydrogenase SDH : Succinate dehydrogenase MDH : Malate dehydrogenase GDH : Glutamate dehydrogenase

Table 4 Enzymes activity on MARGs in control and treated adult male insect, *O. varicornis*

$MARG_1$	Control (µmoles/mg/protein)	Treated (µmoles/mg/protein)	Percentage over control	't' value
LDH	1.91 ± 0.05	1.28 ± 0.12	-32.87	11.96*
SDH	11.07 ± 0.31	7.64 ± 0.38	-30.97	17.14*
MDH	0.90 ± 0.02	1.49 ± 0.04	64.76	-35.35*
GDH	0.84 ± 0.02	1.52 ± 0.04	80.04	-36.94*

Data represent values are mean ± S.D (n=6).*Significant at 0.05% level. LDH : Lactate dehydrogenase SDH : Succinate dehydrogenaseMDH : Malate dehydrogenaseGDH : Glutamate dehydrogenase

Biologically, such energy yielding oxidations are accomplished by the removal of hydrogen and electrons from the substrates and their transfer to other acceptors within the cell (Gilmour, 1965). The activity of, MDH and GDH in the fat body of treated insects were increased than the control insects. In contrast, the activity of LDH and SDH in the test tissues of treated insects were decreased than the control insects.(Table.4).

This observation is in conformity with (Jayanthi, 2001). For *Macrobrachium malcolmsonii*, (Sumathi, 2002). For *Gryllotalpa africana* when exposed to endosulfan; (Rajathi, 2004). For *Sphaerodema rusticum* exposed to heavy metal mercury; (Rameshkumar, 2004). For *Laccotrephus ruber* exposed to zinc. This is supported by the observations, indicating the formation of new protein in the gland during stress. Thus, *Odontopus. varicornis* not only depends on glycolytic pathway to release energy but also derives its energy for the transfer of sperms to the female by other path ways.(Fig.4).

Fig. 4 Enzymes activity on MARGs in control and treated adult male insect, *Odontopus varicornis*

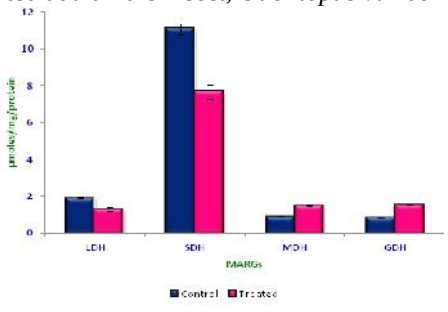


Fig. 4

Based on these findings, it is suggested that in this insects, energy may probably be supplemented through the oxidation of α-ketoglutarate as it has been shown for other insects such as *Apis mellifera* (Blum, 1962). *Plebiogryllus guttiventries* and *Chrysocoris purpureaus* (Ranganathan, 1970; 1980). *Serinetha augar* (Basker, 1988). *Aspongopus janus* (Padmanabhan, 1992). *Odontopus varicornis* (Selvisabhanayakam, 1995).

Lactate dehydrogenase (LDH) is an important glycolytic enzyme being present virtually in all tissues (Kaplan and Pesce, 1996). It is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantino *et al*., 2001). And it is used as an index of anaerobic metabolism (Chamberlin and King, 1998). Activity level of lactate dehydrogenase in *Culex* after treatment with DDT, malathion and cyfluthrin decreased 58.88%, 33.33% and 66.66%, respectively (Arshad *et al*., 2002; Nathan *et al*., 2005). Have showed that feeding of *Spodoptera litura* on *Ricinus communis* L. treated with azadirachtin and nucleopolyhedrovirus decreases the amount of this enzyme in midgut that demonstrates low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *Melia azedarach* on rice leaf folder (Nathan, 2006). Hence, using chemicals may decrease activity level of LDH. The activity of LDH in the fat body, testis, seminal vesicle and MARGs of treated insects were lower than the control insects. From these findings, it may be suggested that the decreased LDH activity is probably for the conversion of lactate to pyruvate. It is inferred from the present study, that the LDH activity in certain reproductive tissues, perhaps due to treatment with the heavy metal zinc, suggested that these changes might be due to the occurrence of more amount of pyruvate and less amount of lactate in their tissues. It is known that during intoxication, there could be oxygen dept and accumulation of lactate in the muscle but not in other organs; the pyruvate unutilized may be perhaps converted into lactate during energy demand. Further, the enhanced LDH in certain tissues with concomitant reduction of pyruvate indicates the conversion of pyruvate to lactate. In the present study, it has been observed that the SDH activity levels showed inhibition in all the reproductive tissues during treatment with the heavy metal, zinc than the control insects, suggesting that the decreased amount of glycogen and increased level of glucose signified their utilization for the energy requirement during the period of stress. Inhibition of oxidation of succinate or succinate dehydrogenase by insecticides and heavy metals are well known. DDT and other non-toxic DDT analogues inhibit the succinate oxidation in housefly (Anderson *et al*., 1954). Melathion inhibits succinate and pyruvate oxidation (O'Brien, 1956, 1969; Uthaman, 1980). Has also reported a decreased succinate dehydrogenase activity levels in the scorpion *Heterometrus falvipus* after cyanide treatment.

In the present investigation, the decreased level of SDH has been observed in all the tissues of intoxicated insects. It is known that each cell relies on numerous enzyme catalysed to maintain its metabolism. As SDH is a key enzyme in the TCA cycle, it is logical to assume that the inhibition of SDH activity, the metabolic pathway might switch over from aerobic to anaerobic to meet the increased energy demand during toxicity.

The SDH activity showed a decrease in the fat body which indicates disturb in enzyme synthesis. The heavy metal zinc perhaps disturbs the mitochondrial membrane of the fat body and all the reproductive tissues. This rupture leads to a decrease in the activity of membrane bound SDH. It is also suggested from the present study that along with decreased activity of SDH, the oxygen carrying capacity may be responsible for the decrease in the aerobic respiration. These results are in concomitant with the works of (Sumathi, 2002). Who has reported for inhibition of the activity of the dehydrogenase which may be due to the activity of changes in the mitochondrial membrane function in *Gryllotalpa africana* treated with the endosulfan.

Succinate dehydrogenase belongs to complex-II of respiratory chain and is present in inner mitochondrial membrane. In the present study, the activity of succinate dehydrogenase also decreased with 2,4-D treatment. Similar decline in the succinate dehydrogenase activity with dimilin, batex and K-othrine was observed in *Diplonychus indicus* (Raja and Venkatesan, 2001). In 2, 4-D herbicide in *Lipophis erysimi* (Sohal *et al*., 2008; Rup *et al*., 2006). The inhibition of SDH activity in *Odontopus varicornis* may be followed due to the reduction in O_2 consumption level, suggesting that the heavy metal affects the TCA cycle, which leads to disturbances in the respiratory metabolism of these insects.

In insects, two kinds of MDH cytosolic and mitochondrial are well known (Sacktor, 1975). Cytosolic MDH is oxidized into oxaloacetate by the action of cytosolic MDH, to yield malate. The malate enters into the mitochondria via a carrier and is oxidized there into oxaloacetate by the action of mitochondrial MDH. It is evident that the gland is assured to supply energy by the TCA cycle during energy demand when insects are intoxicated with the heavy metal, zinc. This suggested that more of the substrate either malate to oxaloacetate is combusted to sustain the TCA cycle.

In the present study, it has been observed that the pattern of activity of the respiratory enzyme, MDH is similar to the other respiratory enzyme SDH, which is found increased in all the reproductive treated tissues than the control tissues. These changes might be due to the supply of energy by the TCA cycle for the treated insect which requires energy during heavy metal intoxication. Similar results have been observed by (Sumathi, 2002). For *Gryllotalpa africana* when exposed to endosulfan; (Rajathi, 2004). For *Sphaerodema rustium* exposed to heavy metal mercury; (Rameshkumar, 2004). For *Laccotrephus ruber* when exposed to zinc. Further, the results were significantly decreased in the activity levels of SDH and MDH, in the fat body and other reproductive tissues and a reduction in the rate of oxidative metabolism at the mitochondrial level, as the heavy metals are known to block the respiratory center of the tissues leading to a condition similar to asphyxia (Rao *et al*., 1980; Vijay Joseph and Jayantha Rao, 1991). Have stated that SDH, MDH and LDH inhibited consequently on exposure to sublethal concentration of aldrin enhanced activities of LDH. Glutamate is the only amino acid for which specific and highly active dehydrogenase exists. This occurs principally through the amino transferases completed with the action of GDH (Smith *et al*., 1985; Van Der Berg, 1964). Has reported that the formation of α-ketoglutarate from glutamate in the flight muscles of house fly. Deamination of amino acids by GDH is the major route of protein metabolism. The MARGs in insects are known to elaborate proteinaceous seminal plasma or spermatophore (Davey, 1965; Odhiambo, 1969; Leopold, 1970; Selvisabhanayakam, 1995; Shivaji, 1998; and Latha, 1998). Though it was not possible to estimate the TCA cycle metabolites, the view of their highly labile and dynamic nature, an increase of GDH activity during heavy metal stress in the fat body and all the reproductive tissues, suggested that the glutamate may be utilized for the conversion of α-ketoglutarate to augment the energy resource for *Odontopus varicornis* when exposed to zinc.

In general, these are main pathway for the conversion of a-ammo acids to the corresponding L-keto acids through the formation of other acids by the enzymes L-amino acid dehydrogenase. Glutamate can be converted into a-ketoglutarate by the transmission with other keto acids. It is also known that deamination of glutarate brought about the action of GDH (NAD^+ dependent) yields ammonia and α-ketoglutarate, (Osanai *et al*., 1986) have demonstrated the similar metabolic pathway for energy supply to spermatozoa into spermatophore for *Bombyx mori.* In the present study, it has been shown that the dynamic nature of an increase in GDH activity in all the tissues of treated insects suggesting that the glutamate may be utilized for the conversion of α-ketoglutarate to meet out an extra energy demand by *Odontopus varicornis* during heavy metal intoxication. Similar results have been reported for *Melanoplus sanguinipes* (Chesseman *et al*., 1990). *Odontopus varicornis* (Selvisabhanayakam, 1995). *Macrobrachium malcolmsonii* (Jayanthi, 2001). *Gryllotalpa africana* (Sumathi, 2002). *Sphaerodema rusticum* (Rajathi, 2004). *Laccotrephus ruber* (Ramesh Kumar, 2004). Glutamate dehydrogenase is an enzyme that, in addition to its role in the energy metabolism in mitochondria, is involved in neuromuscular transmission for *Drosophila melanogaster*. On the basis of the observations made in the present study, it is evident that the inhibition of SDH and LDH activities and stimulation of MDH and GDH activities in all the target tissues of the reproductive system of *Odontopus varicornis* when intoxicated with heavy metal zinc. The metabolic pathway of this insect has shifted towards anaerobic side rather than aerobic side to meet the increase in energy demands during heavy metal treatment.

The main toxic effects in that of producing oxidative stress, linked with lipid peroxidation of cell membranes (Sarkar *et al*., 1998). Oxidative stress induces cellular damage caused by reactive oxygen species, which are scavenged by enzymes of the antioxidant system, such as SOD, CAT, and GPx (Donahue *et al*., 1997; Lagriffoul *et al*., 1998; Laurence *et al*., 2000; Khaper *et al*., 2003). SOD, CAT, and GPx activities are changed in response to reactive oxygen species (Sarkar *et al*., 1998; Fornazier *et al*., 2002a; Lee and Shin, 2003; Lijun *et al*., 2005). In the present study, it has been observed that the

activities of SOD, CAT and Glutathione in the treated tissues were found to be decreased than the control insects. The role of the fat body in insects is similar to the function of liver in vertebrates heavy metal can affect cell membrane fluidity and lipid content (Domenech *et al*., 1977; Antunes - Madeira and Madeira 1979; Blasiak, 1996; Debnath and MandaI 2000; Labana *et al*., 2001). Those data, as well as present study results, suggest that can modify fatty acid metabolism. Similarly, a decrease in fatty acid content of liver in fish after exposure to pesticides was observed (Knauer *et al*., 1996). Total SOD activity is almost always lower in treated insect, *Odontopus varicornis.* The toxicity of the pesticide may be caused by the unbalance between the free radicals and antioxidants. The survival rate of insects was very low (Zbigneiw *et al*., 2005). In the previous study (Adamski *et al*., 1996). It has been proved that mitochondrial SOD is more important during the response to the stress. Those observations confirm the important role of SOD during xenobiotic stress in insects.

The superoxide dismutase acts on superoxide radical (O_2^-) and provides the first line of defence against toxicity from free radicals generated during metabolism and helps in development of resistance (Paes *et al*., 2001; Wang *et al*., 2001; Nivsarkar *et al*., 1991). Have reported that alpha – terthienyl, an insecticide and phototoxic compound inhibits SOD activity completely in all the four instars of mosquito larvae in the presence of ultraviolet light. (Jiang *et al*., 1999). Have detected the activity of SOD in fourth instar larvae of cutworm, *Spodoptera litura* (Fab.) and compared the effects of the two photosensitive compounds, P-terthienyl and 1-phenyl-4- (3,4-methylene dioxy) phenyldiaethylene on superoxide dismutase activity under the specified conditions. Three types of superoxide dismutase were identified; Cu, Zn-SOD in mitochondrial fraction and Fe-SOD in post mitochondrial fraction in a dipteran, *Chironomus riparius* Mg. (Choi *et al*., 1999).

It has been measured in the present study, an increase in CAT activity in the control insects than the treated insects this might have been an effect of the high resistance of CAT, as compared to other cellular proteins. One may suggest that other metabolic pathways, which involve Glutathione, or Glutathione S-transferase, are active in detoxification of H_2O_2. CAT activity was significantly lower in treated groups, as compared to the value measured for the control. Such a low activity may be one of the factors that cause the constant decrease in survival of *Odontopus varicornis.* Similar results have been observed by (Naquie and Hasan, 1992). The SOD, CAT and Glutathione activities were found to be decreased in the treated insect than the control insect. The present study is consistent with that of (Adamski *et al*., 1996). Who have reported for *Spodoptera exigua* exposed to fenitrothion; (Carniewska *et al*., 1996). For *Rana esculenta* exposed to fenitrothion, (Zbigniew Adamski *et al*., 1996). For *Spodoptera exigua* and *Tenebrio molitar* exposed to fenitrothion; (Sumathi, 2002). For *Gryllotalpa africana* exposed to endosulfan; (Rajathi, 2004). For *Sphaerodema rusticum* when exposed to heavy metal mercury and (Ramesh Kumar, 2004). For *Laccotrephus ruber* exposed to zinc.

GPx detoxification system is one of the defense systems against exogenous as well as endogenous fractions. As such, lower level of GPx contents may favour an over production of free radicals and lipid peroxidases which may inturn induce the damage to cell membrane and cellular molecules (DNA and RNA) leading to a neoplastic changes.

The enzyme glutathione peroxidase (GPx) belongs to the family of selenoproteins and plays an important role in defence mechanisms of animals against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as a reducing substrate. (Lee, 1991). Has noticed an inhibition in glutathione peroxide activity under the influence of two plant phytotoxins (xanthotoxins and harmine) in cabbage looper, *Trichoplusia ni* (Hubner) and black swallow tail, *Papilio polyxenes* (Fab.). Similarly, (Leszczynski *et al*., 1993). Reported a decrease in the glutathione peroxide activity in grain aphid, *S. avenae* when fed on wheat cultivar containing high concentration of cereal allelochemicals (phenolic compounds and hydroxamic acid). (Wang *et al*., 2001). Have observed that glutathione peroxidase which is ubiquitously active in mammalian cells under oxidative stress was not active in any of the *S. frugiperda* cell lines.

However, induction of glutathione peroxidase reported in *Apis mellifera* Spibola with exposure to flumethrin was correlated to elevated oxidative stresses by (Neilson *et al*., 2000a). In the same year (Neilson *et al*., 2000b). Have related the activated glutathione peroxidase activity in *Coccinella septempunctata* L. To elevated oxidative stress caused due to low quality food. A significant increase in the glutathione peroxide activity in the present findings could be associated with its involvement in reducing the oxidative stress caused with kinetin application in the mustard aphid. The GPx is a cysteine rich protein and participates in the maintenance of cytoplasmic membrane and their status. The level of glutathione content of fat body and all reproductive tissues like testis, seminal vesicle and MARGs have been found that the decreased activity of this enzyme was attributed due to intoxication. In the present study, the cell damages were produced by heavy metal, zinc is evident by the decrease in the activity of antioxidant enzymes. It has been inferred from the present study that the median lethal dose of the heavy metal, zinc brought about some changes in the antioxidant enzymes to reduce the detoxification mechanism which intern causes mortality of this insect.

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