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Research Article

EFFECTS OF ENDOSULFAN AND FENVALERATE ON CARBOHYDRATE METABOLISM OF THE FRESHWATER FISH, *LABEO ROHITA* (HAMILTON)

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ABSTRACT

Freshwater fish *Labeo rohita* was exposed to two pesticides i.e., endosulfan an organchlorine and fenvalerate a synthetic pyrethroid. The LC_{50} values determined for endosulfan and fenvalerate at 24 hrs were 0.6876, 0.4749 μgL^{-1} respectively. The $1/10^{th}$ of 24 hrs, LC_{50} value of both the pesticides was selected as sublethal concentrations. The fish were exposed to lethal and sublethal concentrations for 24 hrs and 15 days and the changes in the carbohydrate metabolism such as total glycogen and the activities of enzymes Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH) and Malate Dehydrogenase (MDH) of vital organs such as brain, gill, kidney, liver and muscle were studied.

Keywords: Endosulfan, Fenvalerate, LDH, SDH, MDH.

INTRODUCTION

Long-term application of toxic chemicals including pesticides in different ecosystems, which due to their high efficacy and easy use have eliminated some biological methods of pest control, was caused by environmental pollution. Pesticides have been applied to fight against pests of plants, animals and humans. However, the introduction of pesticides to the natural environment has also some negative effects, including unintentional intoxication of useful insects, fish, birds, mammals, and other inhabitants of aquatic and terrestrial biocenoses [1]. Therefore in the present study, an attempt has been made to explore the effects of Endosulfan and Fenvalerate on carbohydrate metabolism of the freshwater fish *Labeo rohita* which is an edible fish of the local area.

Endosulfan ($C_9H_9Cl_6O_3S$) is a chlorinated hydrocarbon insecticide used to control pests in more than 60 countries around the world in agriculture [2]. India is one of the major producers of endosulfan. Since 1996-97 it produces an average of 8206 metric tons per annum totaling 41,033 metric tons produced during 1995-2000 [3]. In India alone, the agricultural consumption of endosulfan was estimated to be 5,200 metric tons in 1994-1995 [4].

Synthetic pyrethriods are another type of pesticides; these are also toxic to aquatic organisms. Pyrethroid insecticides have been used in agriculture for more than 30 years to control insect pests in a range of crops. Fenvalerate is one of the pyrethroid insecticide and most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. They account for approximately one fourth of the worldwide insecticide market [5].

MATERIAL AND METHODS

The freshwater fish *Labeo rohita* (Hamilton) is an edible and commercially valuable fish. Live fish of size 6-7 ±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2 ^{0}C in the laboratory for one week. The stock solutions for Endosulfan 35% Emulsifiable Concentrate (EC) and Fenvalerate 20% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg/100ml which were further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC_{50} values were calculated by Finney's Probit analysis (1971) [6].

Estimation of Glycogen

The amount of glycogen was estimated by the method of Kemp *et al.*(1954) [7]. The glucose obtained was converted into glycogen by the multiplication factor, 0.98 (Hawks, 1951) [8] and is expressed in mg of glycogen/g wet weight of the tissue.

Lactate dehydrogenase (LDH) activity was estimated by the method of Srikanthan and Krishna Murthy (1955)[9]. Succinate dehydrogenase (SDH) and Malate dehydrogenase (MDH) activity was estimated by the method of Nachlas *et al.*(1960)[10].

Glycogen

The levels of total glycogen and activities of LDH, SDH and MDH decreased in all the tissues compared to control.

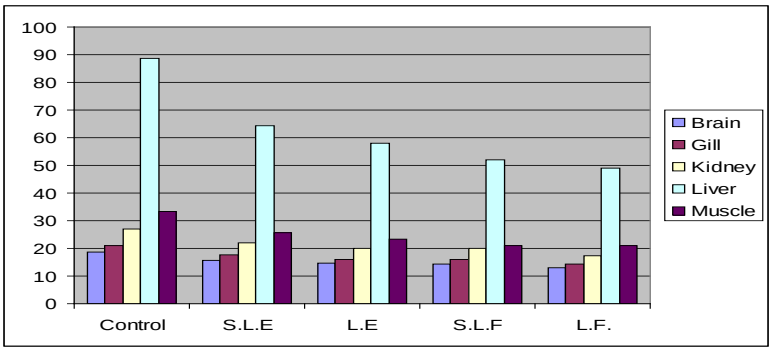

Fig. 1: Changes in the giycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

S.L.E – Sublethal Endosulfan, L.E – Lethal Endosulfan, S.L.F - Sublethal Fenvalerate, L.F – Lethal Fenvalerate

Table 1: Changes in the giycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

Organs	Control	Endosulfan – 24 hrs				Fenvalerate- 24 hrs			
		Sub –Lethal	%Change	Lethal	%Change	Sub-Lethal	%Change	Lethal	%Change
Brain	18.60± 0.02	15.64± 0.03	-15.91	14.66± 0.02	-21.78	14.42± 0.03	-22.47	13.01±0.001	-30.05
Gill	21.12 ±0.02	17.58± 0.02	-16.76	16.16± 0.01	-31.86	16.08± 0.02	-23.86	14.28±0.002	-32.38
Kidney	27.16± 0.01	22.08± 0.01	-18.70	20.10± 0.003	-25.99	20.02±0.002	-26.28	17.40±0.006	-35.93
Liver	88.60± 0.002	64.26± 0.004	-27.47	58.16± 0.003	-34.35	52.08±0.006	-41.21	49.10±0.005	-44.58
Muscle	33.24 ±0.01	25.68± 0.002	-22.74	23.19± 0.01	30.23	21.07±0.03	-36.61	21.08±0.004	-36.58

Values are the means of five observations: (±) indicates the standard deviation values are significant at P > 0.05

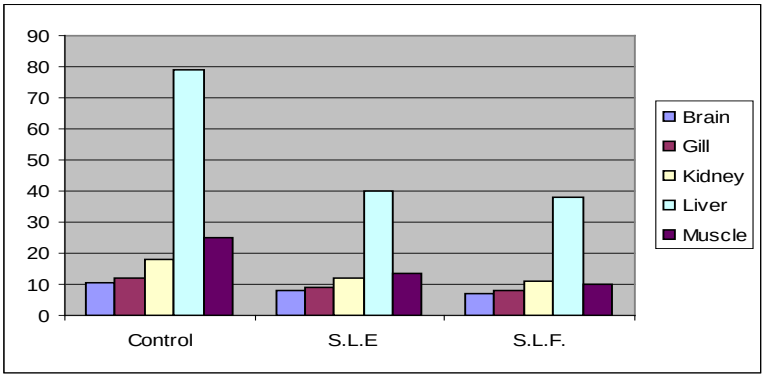

Fig. 2: Changes in the giycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days.

S.L.E – Sublethal Endosulfan; S.L.F - Sublethal Fenvalerate

Table 2: Changes in the giycogen (mg/g wet weight of tissue) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days

Organs	Control	Endosulfan		Fenvalerate	
		Sub-Lethal	% Change	Sub- Lethal	% Change
Brain	10.42± 0.01	8.16± 0.02	-21.68	7.06± 0.03	-32.24
Gill	12.07± 0.03	9.06± 0.01	-24.93	8.05± 0.024	-33.30
Kidney	18.07± 0.023	12.18± 0.01	-32.59	10.86± 0.02	-39.90
Liver	79.12± 0.01	40.05± 0.01	-56.84	38.11± 0.02	-58.93
Muscle	25.05± 0.01	13.26± 0.03	-33.86	10.08± 0.02	-49.72

Values are the means of five observations: (±) indicates the standard deviation values are significant at P> 0.05

The results indicated that the liver is vital organ of carbohydrate metabolism and were drastically affected by both endosulfan and fenvalerate. In almost all the tissues of fish brain, gill, kidney, liver and muscle tested at sublethal and lethal concentrations of both endosulfan and fenvalerate, a decrease in glycogen values was noticed during the exposure periods.

Carbohydrate metabolism is mainly concerns to fulfill energy demand of animals by its aerobic and anaerobic segment [11]. Among various tissues, higher glycogen content was observed in liver. This is due to the involvement of liver in glycogen synthesis and utilization. Glycogen is the major storage form of energy in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself[12]. Though brain tissue is metabolically active, lower glycogen content was observed since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities [11].

The results indicated that the liver is the vital organ of carbohydrate metabolism was drastically affected by both endosulfan and fenvalerate. In almost all the tissues brain, gill, kidney, liver and muscle tested at lethal and sublethal concentrations of both endosulfan and fenvalerate, showed a decrease in glycogen values were noticed during the exposure periods.

Significant depletion in glucose and glycogen levels in various tissues of freshwater teleost *Tilapia mossambica* under sublethal concentration of sodium arsenite and stated that these changes were tissue specific and time dependent[13]. The total glycogen levels of brain, liver, muscle, gill and kidney of *Labeo rohita* were decreased on exposure to sublethal concentration of cypermethrin[14]. Decrease in levels of liver glycogen in fish, *Heteropneustes fossils* under exposure to endosulfan and stated depletion in glycogen contents is greatly affiliated to cellular damage in hepatic cells[15]. A fall in glycogen levels in the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* on exposure to sublethal concentrations of chlorpyrifos[16]. Fall in glycogen levels in the freshwater fish *Labeo rohita* under the fenvalerate exposure[17]. The depletion in glycogen levels under exposure to kelthane an organochlorine insecticide in the freshwater fish *Channa punctatus*[18]. A significant decrease in glycogen content was observed in the tissues of fish *Channa punctatus* exposed to alachlor technical and lasso 50% EC formulation [19].

Carbohydrates are the primary and immediate sources of energy in stress condition, carbohydrate reserves depleted to meet the energy demand. Depletion of glycogen may be due to direct utilization for energy generation, a demand caused by pesticidal stress[20].

Cells contain enzymes that are necessary for their function. When the integrity of a cell is disrupted, enzymes escape into plasma/serum, where their activity be measured as a useful index of cell integrity [21].

Lactate Dehydrogenase

LDH is an important glycolytic enzyme which is present in the cells of almost all body tissues and changes in the enzyme activity may provide direct and indirect evidence of the cellular damage and can indicate the toxic mechanism. LDH is a terminal enzyme of anaerobic glycolysis, therefore, being of crucial importance to the muscular physiology, particularly in conditions of chemical stress, when high levels of energy may be required in a short period of time [22,23,24].

The significant changes in enzymes activity of LDH indicate damageto any or all organs producing this enzyme such as liver or kidney injuries [25,26].

Table 3: Changes in the LDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

Organs	Control	Endosulfan – 24 hrs				Fenvalerate- 24 hrs			
		Sub –Lethal	%Change	Lethal	%Change	Sub-Lethal	%Change	Lethal	%Change
Brain	0.18± 0.01	0.16± 0.03	-11.11	0.15± 0.02	-16.66	0.15± 0.01	-16.66	0.14± 0.02	-22.22
Gill	0.36± 0.04	0.30± 0.02	-16.66	0.27± 0.01	-25.0	0.28± 0.03	-22.22	0.23± 0.03	-36.11
Kidney	0.23± 0.01	0.20± 0.04	-13.04	0.19± 0.03	-17.39	0.18± 0.05	-21.73	0.15± 0.01	-34.78
Liver	0.47± 0.03	0.40± 0.01	-27.65	0.32± 0.04	-31.91	0.33± 0.01	-29.78	0.28± 0.04	-40.42
Muscle	0.42± 0.01	0.34± 0.02	-19.04	0.31± 0.02	-26.19	0.32± 0.02	-23.80	0.26± 0.01	-38.09

Values are the means of five observations: (±) indicates the standard deviation values are significant at P > 0.05

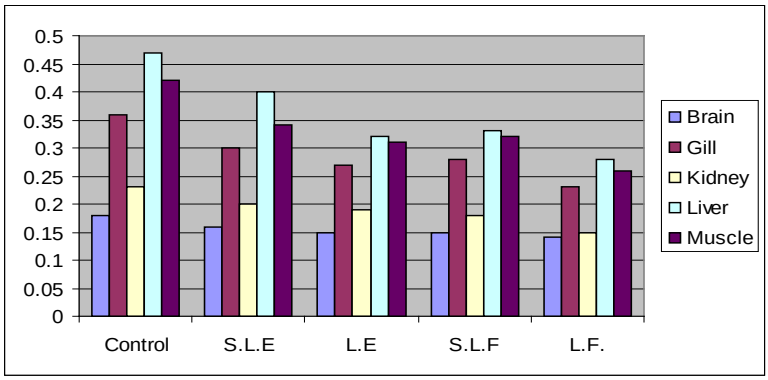

Fig. 3: Changes in the LDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs.

S.L.E – Sublethal Endosulfan, L.E – Lethal Endosulfan; S.L.F - Sublethal Fenvalerate, L.F – Lethal Fenvalerate

Table 4: Changes in the Lactate dehydrogenase of *Labeo rohita* (µ moles of formazan/mg protein/h) on exposure to sublethal concentrations of endosulfan and fenvalerate for 15 days

Organs	Control	Endosulfan		Fenvalerate	
		Sub-Lethal	% Change	Sub- Lethal	% Change
Brain	0.29± 0.01	0.26± 0.02	-10.34	0.23± 0.01	-20.68
Gill	0.39± 0.04	0.33± 0.01	-15.38	0.30± 0.01	-23.07
Kidney	0.27± 0.01	0.24± 0.03	-11.11	0.21± 0.01	-22.22
Liver	0.51± 0.03	0.40± 0.02	-21.56	0.36± 0.02	-29.41
Muscle	0.48± 0.04	0.39± 0.01	-18.75	0.36± 0.02	-25.0

Values are the means of five observations: (±) indicates the standard deviation values are significant at P > 0.05

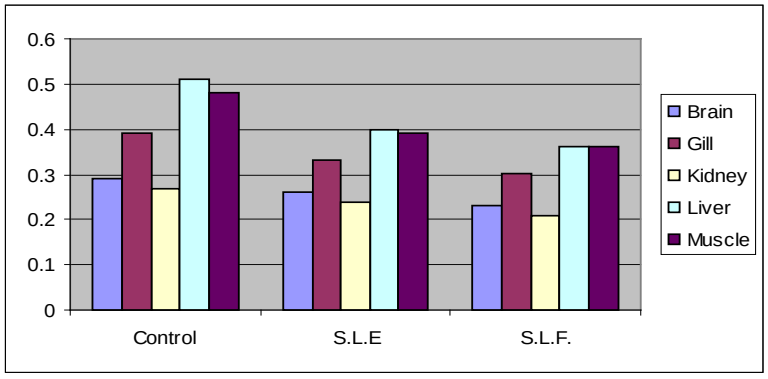

Fig. 4: Changes in the Lactate dehydrogenase of *Labeo rohita* (µ moles of formazan/mg protein/h) on exposure to sublethal concentrations of endosulfan and fenvalerate for 15 days

S. L. E – Sublethal Endosulfan; S. L. F - Sublethal Fenvalerate.

LDH mediates inter-converson of lactate to pyruvate depending on the availability of co-enzyme NAD[27]. In the present study, it was observed that the activity of LDH was decreased following sublethal and lethal exposures of endosulfan and fenvalerate in all the tissues of *Labeo rohita* for 24 hrs and 15 days. The decrease in lactate dehydrogenase activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis[27].

Decrease in lactate dehydrogenase (LDH) activity in *channa punctatus* after exposure to monocroophos was observed[28]. Significant decrease in LDH activity levels were observed in the tissues of *Channa Punctatus* exposed to *Euphorbia royeleana latex*[29]. Decrease in LDH activities was observed after exposure to endosulfan and fenvalerate on fresh water fish *Clarias Batrachus*, which indicates decrease in aerobic and anaerobic capacity of fish[30].

Succinate Dehydrogenase (SDH)

SDH is a vital enzyme of citric acid cycle catalyses the reversible oxidation of succinate to fumarate. In the present investigation it can be visualized that there is a rapid depletion of SDH activity in all tissues of fish *Labeo rohita* treated with lethal and sublethal doses of Endosulfan and Fenvalerate when compared to their respective controls. In control fish, SDH activity was more in liver followed by muscle, gill tissues and minimum in kidney. The higher activity of SDH in liver and muscle suggests higher distribution of mitochondria in these tissues, since SDH is mitochondrially localized [12].

Table 5: Changes in the SDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

Organs	Control	Endosulfan – 24 hrs				Fenvalerate- 24 hrs			
		Sub –Lethal	%Change	Lethal	%Change	Sub-Lethal	%Change	Lethal	%Change
Brain	0.69± 0.03	0.64± 0.01	-7.24	0.60± 0.02	-13.04	0.62± 0.01	-10.14	0.56± 0.01	-18.84
Gill	0.78± 0.02	0.68± 0.01	-12.82	0.65± 0.01	-15.38	0.61± 0.02	-15.38	0.59± 0.02	-24.35
Kidney	0.76± 0.02	0.67± 0.03	-11.84	0.65± 0.03	-14.47	0.65± 0.03	-14.47	0.60± 0.01	-21.05
Liver	0.92± 0.01	0.78± 0.01	-15.21	0.71± 0.04	-22.82	0.76± 0.01	-17.39	0.65± 0.04	-29.34
Muscle	0.83± 0.003	0.72± 0.02	-13.25	0.67± 0.04	-19.27	0.69± 0.02	-16.86	0.60± 0.03	-27.71

Values are the means of five observations: (±) indicates the standard deviation values are significant at P > 0.05

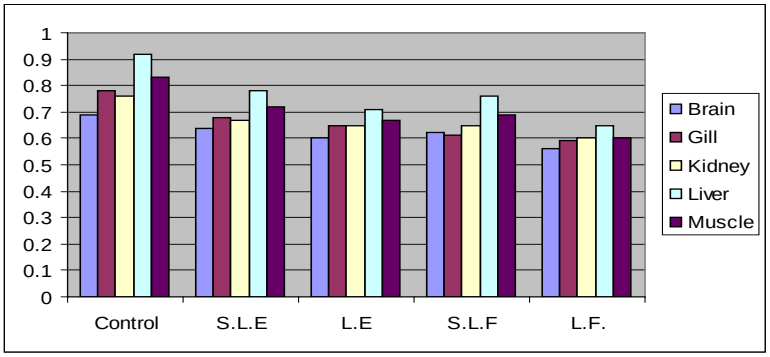

Fig. 5: Changes in the SDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

S.L.E – Sublethal Endosulfan, L.E- Lethal Endosulfan; S.L.F - Sublethal Fenvalerate, L.F - Lethal Fenvalerate.

Table 6: Changes in the SDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days

Organs	Control	Endosulfan		Fenvalerate	
		Sub-Lethal	% Change	Sub- Lethal	% Change
Brain	0.60± 0.01	0.55	-8.33	0.50± 0.03	-16.66
Gill	0.75± 0.02	0.64	-14.66	0.55± 0.01	-26.66
Kidney	0.63± 0.03	0.55	-12.69	0.52± 0.03	-17.46
liver	0.85± 0.04	0.61	-28.23	0.55± 0.02	-35.29
Muscle	0.78± 0.03	0.60	-23.07	0.54± 0.01	-30.76

Values are the means of five observations: (±) indicates the standard deviation values are significant at P > 0.05

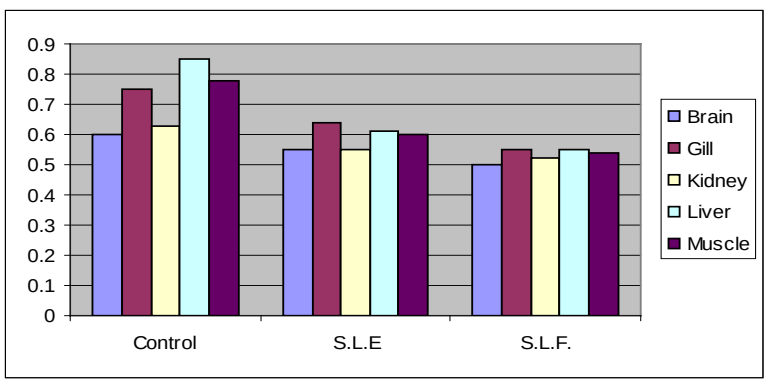

Fig. 6: Changes in the SDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days

S.L.E – Sublethal Endosulfan; S.L.F - Sublethal Fenvalerate,

Similar decrement in the SDH activity was also observed by the various workers in different species of the fish exposed to different pesticides. Decrease in activities of LDH and SDH in fish *Colisa fasciatus* after exposure to Cypemethrin[31]. The inhibition in LDH and SDH activities were observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum mill latex*[32].

The general decrease in SDH activity during pesticides stress was associated with the inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, architectural integrity and permeability of mitochondria[33].This prevents the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis[34].

Malate Dehydrogenase (MDH)

Malate dehydrogenase is an NAD dependent enzyme which converts malate to oxaloacetate and reversible oxidation of fumarate to malate. It exists in two isozymic forms (a) mitochondrial (b) cytosolic. This enzyme not only converts malate to oxaloacetate but also plays a significant role in CO_2 fixation and in gluconeogenesis[11].

Malate + NAD^+ → Oxaloacetate + $NADH^+$ + H^+

Table 7: Changes in the MDH (µ moles of formazon/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs

Organs	Control	Endosulfan – 24 hrs				Fenvalerate- 24 hrs			
		Sub –Lethal	%Change	Lethal	%Change	Sub-Lethal	%Change	Lethal	%Change
Brain	0.43± 0.01	0.40± 0.03	-6.97	0.39± 0.02	-9.30	0.38± 0.01	-11.62	0.36± 0.01	-16.27
Gill	0.55± 0.02	0.51±0.03	-7.27	0.48±0.01	-12.72	0.48±0.04	-12.72	0.42±0.02	-23.63
Kidney	0.47± 0.01	0.42± 0.02	-10.63	0.39± 0.04	-17.02	0.39± 0.01	-17.02	0.35± 0.03	-25.53
Liver	0.78± 0.01	0.69± 0.01	-11.53	0.61± 0.03	-21.79	0.63± 0.02	-19.23	0.55± 0.01	-29.48
Muscle	0.62± 0.04	0.55± 0.03	-11.29	0.50± 0.01	-19.35	0.51± 0.03	-17.74	0.45± 0.04	-27.41

Values are the means of five observations: (±) indicates the standard deviation values are significant at $P > 0.05$

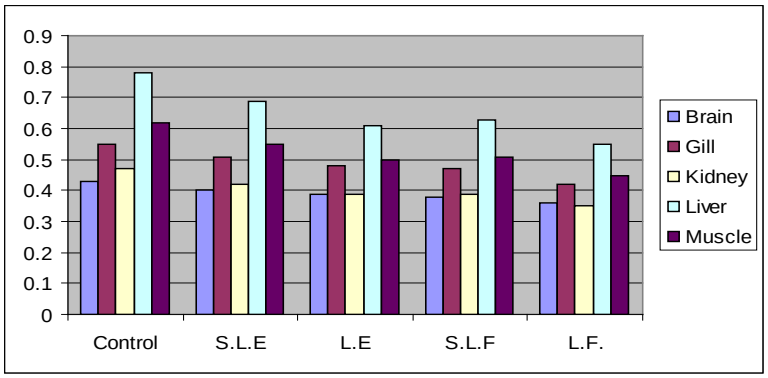

Fig. 7: Changes in the MDH (µ moles of formazon/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 24 hrs.

S.L.E – Sublethal Endosulfan, L.E- Lethal Endosulfan; S.L.F - Sublethal Fenvalerate, L.F - Lethal Fenvalerate.

Table 8: Changes in the MDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days

Organs	Control	Endosulfan		Fenvalerate	
		Sub-Lethal	% Change	Sub- Lethal	% Change
Brain	0.39± 0.02	0.36± 0.01	-7.69	0.33± 0.01	-15.38
Gill	0.50± 0.02	0.44± 0.02	-12.0	0.41± 0.03	-18.0
Kidney	0.42± 0.01	0.38± 0.04	-9.50	0.35± 0.02	-16.66
Liver	0.70± 0.03	0.60± 0.01	-14.28	0.55± 0.02	-21.42
Muscle	0.58± 0.01	0.50± 0.02	-13.79	0.46± 0.01	-20.68

Values are the means of five observations: (±) indicates the standard deviation values are significant at P> 0.05

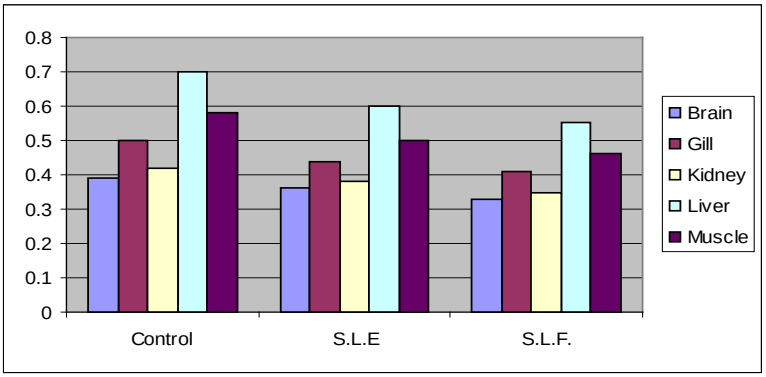

Fig. 8: Changes in the MDH (µ moles of formazan/mg protein/h) in different tissues of *Labeo rohita* on exposure to lethal and sublethal concentrations of endosulfan and fenvalerate for 15 days

S.L.E – Sublethal Endosulfan; S.L.F - Sublethal Fenvalerate

Decrease in MDH values were observed in tissues of *Clarias batrachus* on exposure to endosulfan [35]. A reduction in MDH activity was observed in matrinxa, *Brycon cephalus* after exposure to Folidol 600[36].

CONCLUSION

The present work indicates that both endosulfan and fenvalerate caused alterations in the carbohydrate metabolism of fish *Labeo rohita*, but comparatively fenvalerate treated fish tissues showed more decrement in glycogen values and inhibition in the activities of carbohydrate metabolic enzymes this may be due to more pesticidal stress. Total depletion of glycogen would result in the disruption of enzymes associated with carbohydrate metabolism[37]. Inhibition of LDH, SDH and MDH indicates that pesticides significantly inhibits aerobic, as well as anaerobic metabolism in exposed animals[31].

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