A PROJECT REPORT ON:

"EFFECT OF *WEDELIA FRUCTICOSA* LEAVES POWDER ON LIPID PEROXIDATION IN FISH *CIRRHINUS MRIGLA*"

SUBITTED TO:

VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

KOLHAPUR

IN THE PARTIAL FULFILLMENT OF BACHELOR OF SCIENCE IN ZOOLOGY

IN THE YEAR: 2023-2024

By

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UNDER THE GUIDANCE OF

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DECLARATION

We the undersigned students, declare that the project entitled, **"Effect of *Wedelia fructicosa* leaves powder on lipid peroxidation in fish *Cirrhinus mrigla"*** is submitted by us under the supervision of **Dr. Tekchand. C. Gaupale,** Assistant Professor, Vivekanand College, Kolhapur (Empowered Autonomous). It is our original work. The empirical findings in this project are based on the data collected by us and it is authenticable to the best of our knowledge. The presented matteris not copied from any other source.

Place: Kolhapur

Date: 21-3-24

Student sign

(Alisha Tanaji Kothavale)

CERTIFICATE

This is to certify that the project entitled, **"Effect of *Wedelia fructicosa* leaves powder on lipid peroxidation in fish *Cirrhinus mrigla"*** being submitted herewith for the Degree of **Bachelors of Zoology**, Vivekanand college, Kolhapur (Empowered Autonomous) Affiliated to Shivaji University, Kolhapur, under the faculty of Science is the result of the original work completed by **Alisha Tanaji Kothavale** under my supervision and guidance and to the best of my knowledge and belief, the work embodied in this project has not formed earlier.

Place: KolhapurDate: 21-3-24

Project Supervisor

Dr. G. K. Sontakke
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Examined by
22/3/2024

Examiner

ACKNOWLEDGEMENT

I wish to express my deepest sense of gratitude to my project supervisor **Dr. Tekchand C. Gaupale,** for his/ her continuous help and valuable guidance during the entire period of my Project work. It is a genuine pleasure to express my deep sense of thanks to **Dr. G. K. Sontakke,** Head, Department of Zoology, Vivekanand College Kolhapur (Empowered Autonomous) for administrative help and encouragement during the Project work. I express my profound thanks to **Dr. R. R. Kumbhar** Principal, Vivekanand College Kolhapur (Empowered Autonomous) for his constant support and inspiration.

I am also thankful to other faculties of Department of Zoology who have directly or indirectly guided and helped me in the Project work.

I am also thankful to non-teaching staff for their constant co-operation during project work.

Alisha Tanaji Kothavale

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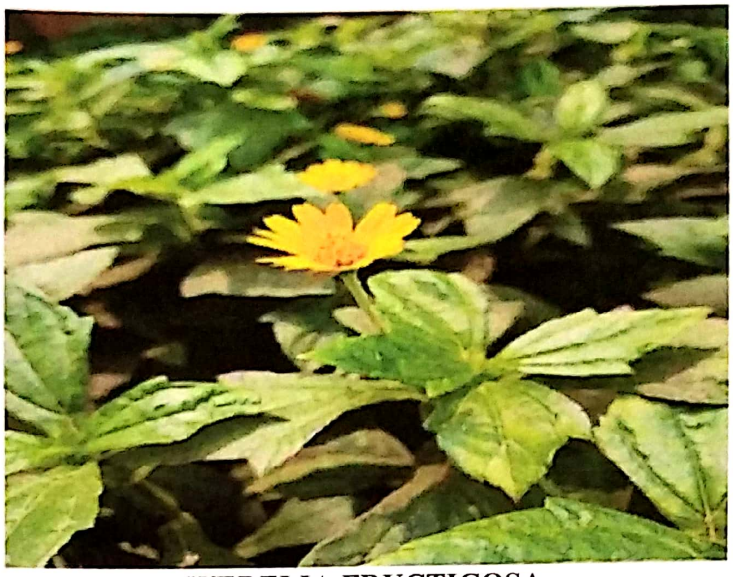
ABSTRACT

The aim of study was to study effect of *Wedelia fructicosa* plant leaves powder on fish *Cirrhinus mrigla* on lipid peroxidation. *Wedelia* is a notorious invasive plant species that exhibits superior tolerance to adapt to environmental stresses. The *Wedelia fructicosa* is not edible to the plant and may be toxic. We investigated the effect plant leaves power that may induce the stress in aquatic animals or fishes. Mrigal fish was exposed to 50mg/Liter and 100mg/Liter of water along with the control group. Fish is treated in 50mg/Liter and 100mg/Liter for 96 hours and lipid peroxidation was checked on liver, gills muscles and intestine. The lipid peroxidation was recorded in In that time of investigation we can observed effected organs such as muscle, gills, liver & intestine of given fish. The lipid peroxidation was increase in all the organs as compared to the control. The highest lipid peroxidation was reported in the liver in 50mg/Liter group and muscles in the 100mg/Liter for acute treatment. From this data it was concluded that this weed induce oxidative stress in fishes. Therefore, it is necessary to study the effect of weeds on aquatic sensitive to this stress.

INTRODUCTION

Wedelia is a genus of flowering plants belongs to the family *Asteraceae*. They are one of the genera commonly called "creeping oxeyes". This type of species is *Wedelia fructicosa Jacq.* The genus is named in honor of German botanist and physician Georg wolfgang wedel 1646-1721. Many species were considered part of *Wedelia* but have been now transferred to other genera. This plants bear yellow flowers and are usually used as ground covers in landscaping. It can grow up to 10 inches tall. It requires full sunlight to partial shade for its optimum growth. Wedelia is also used to cure Hepatitis and Infections.

Perennial herb or shrub, 0.4-3 m tall, erect; stems are much branched and hispid. Leaf blades are elliptic to ovate, chartaceous, hirsute and stipitate glandular on both surfaces, and pinnately above base, the apex acute to attenuate. The base is acute to rounded, attenuate onto petioles, the margins subentire to coarsely dentate; petioles 2-20 mm long, those of a node inconspicuously connate. Inflorescence of 1-3 terminal heads on peduncles 0.5-4 cm long. Heads radiate, ca. 28-40-flowered; involucre bell-shaped, 7-15 x 6-12 mm, 2- seriate; involucral bracts green, lanceolate, the outer series wholly or partly herbaceous, hispid, the inner series scarious herbaceously tipped, hispidulous to nearly glabrous; receptacle weakly convex, ca. 2 mm broad; palea lanceolate, 6 mm long. Ray flowers 8-12; corolla tubes 2 mm long, the limb 7-13 x 3-4 mm , the lower surface of the limb hispidulous on the 2 larger veins, apically bilobed to deeply so, the lobes 1-3 mm deep. Disk flowers 20- 30; corollas 4.5-5.5 mm long. Achenes obconic to obovoid at maturity, 3-4 mm long; pappus an irregularly cut crown 0.5-1 mm tall.



WEDELIA FRUCTICOSA

Classification:

Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Eudicots
Clade : Asterids
Order : Asterales
Family : Asteraceae
Subfamily : Asteroideae
Tribe : Heliantheae
Subtribe : Ecliptinae
Genus : Wedelia

India is the second largest producer of fresh water fish next only to Japan. Fresh water fish accounted for 55% of total production of 6.57 MMT of fish and carps contribute more than 87% of the total production in India. Fish eggs (roes) are available in large quantities at low prices during the spawning period, which accounts for about one-third of the weight of total fish. The quality and quantity of roe proteins differ depending on the variety of fish (Mukhopadhyay et al. 1981). *Mrigal* (*Cirrhinus mrigala*) commonly known as the Indian major freshwater carp is found to be a good source of protein. *Mrigal* is an abundantly available in India which is also utilized in other Asian countries. The fish forms a unique protein source for application in food products to combat protein calorie malnutrition. Fish egg biomass is one of

the most valuable food by-products from fishery sources for human consumption (*Eun et. al.*, 1994). Literature on proximate composition and other biochemical characteristics is available on fishes (*Bledsoe et. al.*, 2003, *Al-Holy and Rasco* 2006). The chemical composition of fishes were reported and the protein content was estimated to be in the range of 11.5–30.2% (*Iwasaki and Harada* 1985). Protein content of fishes from sturgeon species was alos reported to be in the range of 26.2 to 31.1% (Wirth et al. 2000).

Mrigal is a Indian major carp endemic to Indo-Gangetic riverine systems. Cultivated widely in Southeast Asian countries. The traditional culture of the species was restricted to eastern parts of India until the 1950s. Later the species was transplanted in the peninsular riverine systems of India. Subsequently, it is found all overs India. In addition, *Mrigal* play an important roles in the fish culture systems of Bangladesh , Pakistan, Burma etc. It is also famous carp and commonly known as *Mrigal* l. Body is length reaches up to 66cm and weight upto 1.1-2.8kg. Colour of the body is silvery, dark grey and sometime with coppery tinge. Body is covered by large cycloid scales. The scales are absent on head . Head is small and the slightly sub-terminal mouth is situated on the lower side of snout. Barbles small in fold lips. It is bottom feeder and feeds on algae, rotten plant material and phytoplankton.

Sexual maturity is obtained in 2 years .This fish breed during in the months of June to August and lays eggs at the rate of 1 to 3 lakh per kg body weight. This fish found in northern part of India and apart from India also Pakistan, Bangladesh, Myanmar, Nepal. Today, *Mrigal* fish is highly popular for several reasons . It is mainly popular for its fast growth rate, compatibility to raise with other fish species, culture adaptation to different aquaculture systems and also for the assured supply of seeds.

Mrigal Carp fish

Classification

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Cypriniformes
Family	:	Cyprinidae
Subfamily	:	Labeoninae
Genus	:	*Cirrhinus*
Species	:	*Cirrhinus mrigala*

Mrigal fish is one of the 3 Indian major carp fish species, cultivated widely in India. It has long been important in aquaculture with other native fish species. *Mrigal* is very popular Fish and has very good local demand and value. Commercial *Mrigal* fish farming can be a great employment source for the unemployed people, and it's a great business for making high profits in relatively less investment. *Mrigal* is a very important fish both in small scale and commercial fish farming business. The *Mrigal* fish is not just tasty, it's very nutritious and good for health. It is very good source of protein, essential vitamins and minerals. It contains calcium, phosphorus and vitamin A. Protein content in the range of 39.1-43%, fat content in the range of 14.1–14.8% and elements like Fe, Cu, and Zn were reported in dried and salted roes of hake (Merluccius merluccis) and ling (Molva molva L) (Rodrigo et al. 1998). Fish egg protein concentrate (FEPC) and pickle from rohu (Labeorohita) roe (*Balaswamy et al.* 2007, *Balaswamy et al.* 2010) and protein hydrolysates from mrigal roe (*Chalamaiah et al.* 2010) were prepared and their proximate composition and functional properties have been studied.

Fish protein concentrate from whole fish prepared by isopropyl alcohol extraction has been described, which exhibited a decreased emulsifying capacity and solubility (Sikorski and Nacl 1981).Solubility is one of the most important characteristic of proteins which have pronounced effects on functional properties such as emulsification and foaming capacities. The solubility of proteins is also influenced by NaCl concentration wherein ion-dipole interactions

between water, polar groups on bio-molecules and salt occur. Hydrophobicity plays an important positive role in determining emulsifying properties. Literature is available in abundance on fish proteins as a whole but only meager information is available on the functional properties of fish roes. The objective of the present research work was to prepare protein concentrates from *Mrigal* carp roe, study their chemical composition and functional properties and their application in pasta preparation.

Materials and methods :

Plant - *Wedelia fructicosa* (leaf) powder. Fish - *Mrigal carp*, water bath, Aerator , fish tank, cloth, test tube, test tube stand, test tube holders, petri dish, scissor , forcep, ice pack , micro tube, morter pestle , centrifuge machine , conical flask, spectrophotometer Ascorbic acidferic chloride, distilled water, Lipid peroxide buffer, Thiochloroacetic acid, TBA clove oil etc

Method :

***Wedelia fructicosa* leaves were collected and separate** from its plant, washed with tap water followed by distilled water. The leaves were dried for 7 to 8 days at room temperature in shade. Leaves were crushed, grinded and sieved with cloth t make fine power. This powder colour looks like blakish dark green.

Plant leaves

Plant leaf Powder

1. Collecting and Cleaning

Mature and healthy wedelia plants were carefully selected. Leaves were harvested, and any extraneous materials were removed. Subsequently, the leaves were thoroughly cleaned under running water to eliminate any contaminants.

2. Drying and Grinding

The cleaned wedelia leaves were subjected to a controlled drying process using an oven at temperature of 40 °C for a duration of 48 hours. Following the drying process, the leaves were finely ground into a powder using a blender.

3. Sieving

3. The powder material then carefully sieved using a 16 mesh sieve to obtain a uniform particle size. This step ensures consistency in the size of the plant material used in subsequent analyzes and extraction.

Procedure of making Plant leaf Powder

Procedure:

Lipid peroxidation was measured in the homogenates of liver, gills, muscles and intestine. These organs were homogenized in a reaction mixture containing 75mM potassium phosphate buffer (pH 7.04) containing lmM ascorbic acid, lmM $FeCl_3$ & 0.0l ml Chlorotetracycline. After preparation of homogenate in reaction mixture 0.2ml sample was taken in a tube labeled as sample, 1.8ml distilled water was added in it 1ml of 20% Trichloroacetic acid (TCA) was added followed by 2ml of 0.6% thiobarbitarin acid (TBA). In a blank tube 2ml of distilled water was taken and 1ml of 20% TCA and 2ml of 0.67% TBA water added tubes were placed in boiling water bath for 10min and cooled. After centrifugation of sample it was read at 532 nm against blank.

Calculation :

Lipid peroxidation / mg tissue= O.D OF SAMPLE/ (0.156)0.4

Where ,

0. D. of sample = Optical dencity of sample

0.156= the absorbance for lnm solution of malondialdehyde in a1cm think cell at 532 nm

0.4 = amount of tissue taken in mg present in 0.2 ml of sample.

Procedure for lipid peroxidation

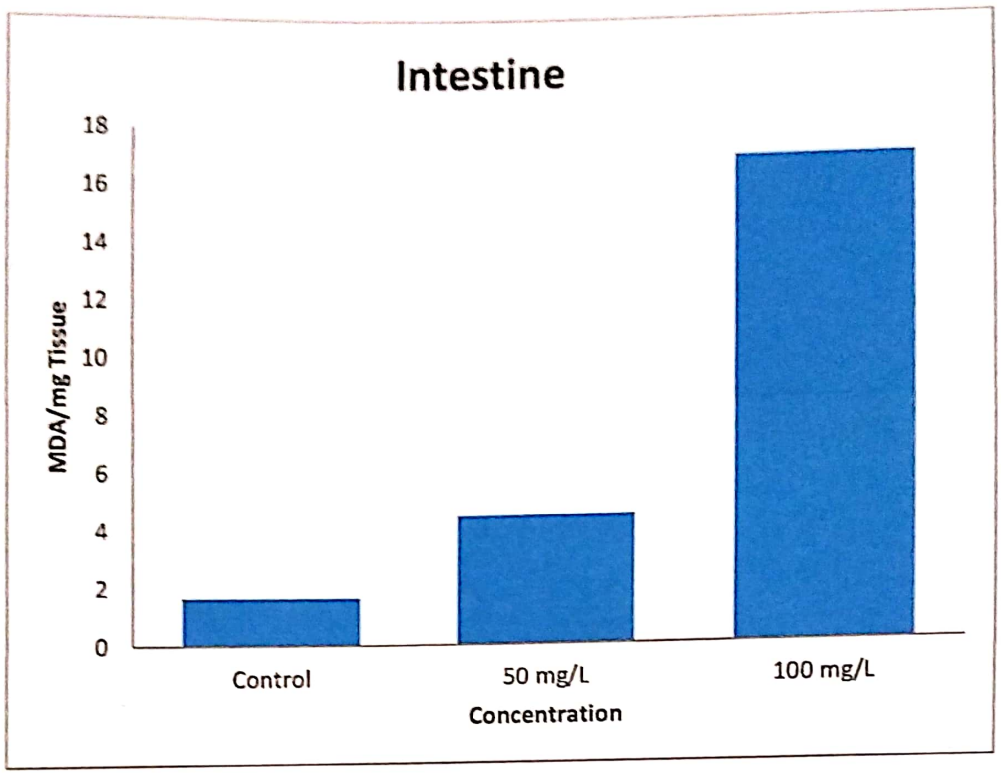
OBSERVATION AND RESULTS

The Lipid peroxidation was observed in fishes treated with leaves powder. The higher level of lipid peroxidation was reported in Liver (5.5271MDA/mg tissue) and low activity was noted in muscles (3.7499 MDA/mg tissue) in 50mg/Liter group for 96 hours as compared to the control. The lipid peroxidation was observed in response to leaves powder at 100mg/lit. High lipid peroxidation was reported in muscles (17.5248MDA/mg tissue) and low activity was noted in liver (13.0875MDA/mg tissue) in 100mg/Liter group for 96 hours. The Lipid peroxidation was more in 100mg/Liter exposed tissue as compared to50mg/ L.

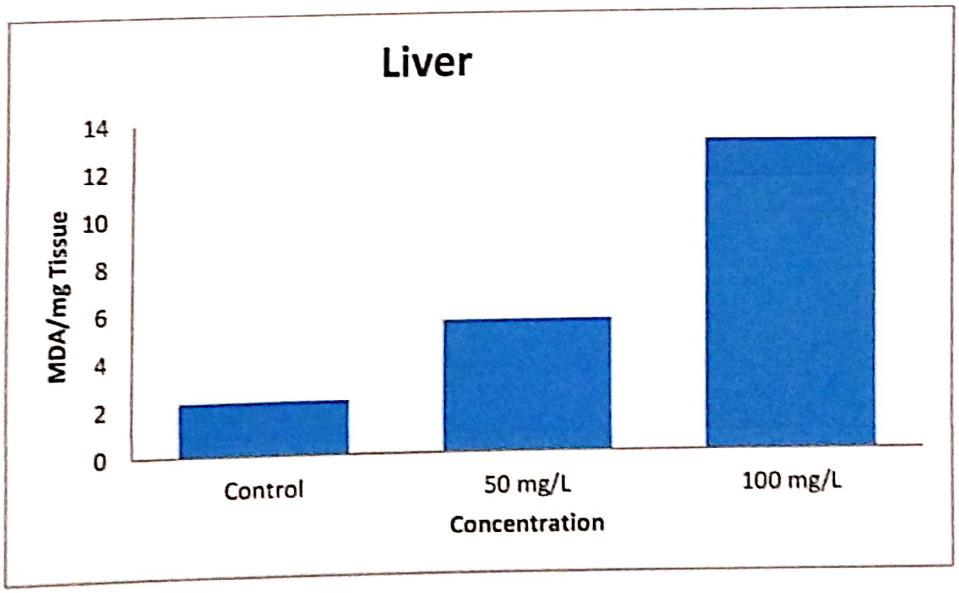
Lipid peroxidation/mg tissue

Sample Fish	Control	50 mg/L	100 mg/L
Intestine	1.6655	4.4817	16.8781
Liver	2.2523	5.5271	13.0875
Muscles	1.5564	3.7499	17.5248
Gills	1.6151	4.5365	14.0704

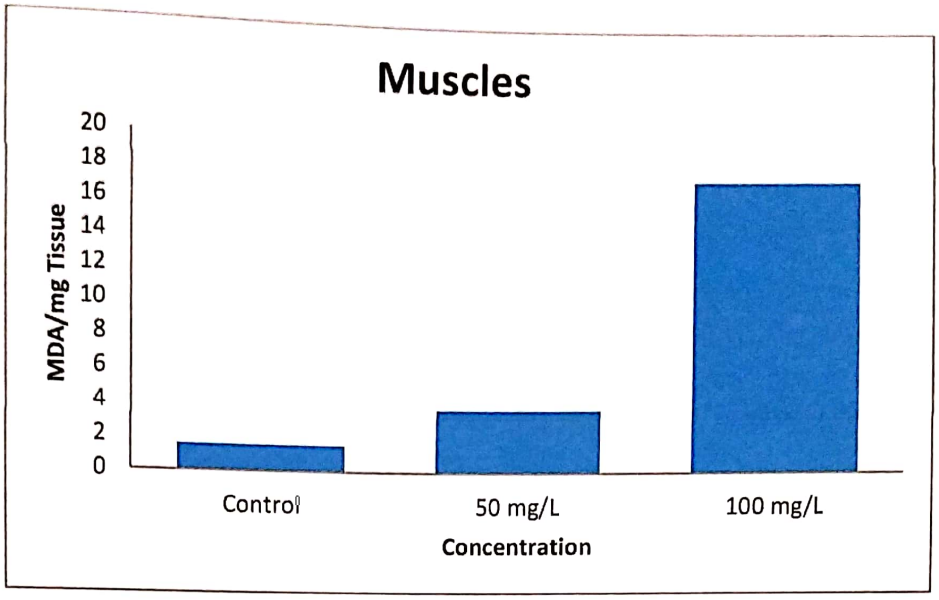
Lipid peroxidation/mg tissue (Unit is MDA/mg tissues)



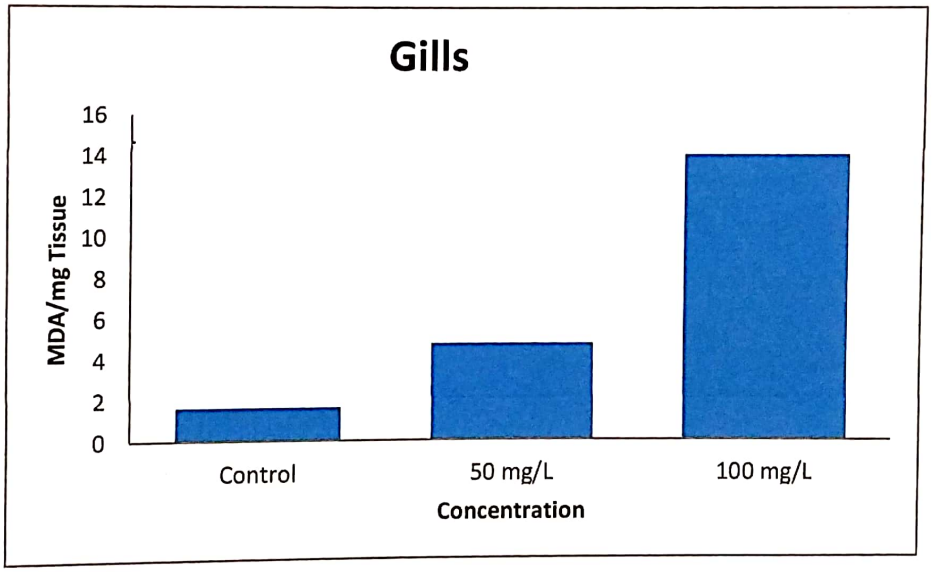
Graph 1. Lipid peroxidation in intestine



Graph 2. Lipid peroxidation in Liver



Graph 3. Lipid peroxidation in Muscles



Graph 4. Lipid peroxidation in Gills

DISCUSSION:

Fish are used as indicator organisms for ecotoxicological studies because which play number of roles in the ecosystem. Therefore, fish used as biomarkers and in early detection of aquatic environmental problems. Acute toxicity data has been used for water quality and the result of subethal concentration for leaves powder were described in the present study at 96h. The results show that the toxicity of leaves powder *mrigal* is both time and concentration.

The elevated level of lipid peroxidation in the liver in response to the exposure to *Wedelia fructicosa* leaves powder was observed in the present study suggests that there is increased production of ROS (Reactive oxygen species). Increased ROS production may, thus, be associated with the metabolism of toxic compounds leading to the peroxidation of membrane lipids of the liver. The liver is factory of oxidative reactions and maximal free radical generation. Previous have been reported the induction of LPO by pesticides such as deltamethrin, alachlor, Malathion, and butachlor in fish. The different LOP responses to *Wedelia fructicosa* leaves powder in different organs may be because of functions of organs, state of metabolic activity, exposure time, and may be of concentration of stressors. The observed LPO resulting from ROS generated by the *Wedelia fructicosa* leaves powder may lead to cell death. ROS and oxidative stress triggers of apoptosis in various tissues. Oxidative stress results depletion of antioxidant enzymes. Every organism organisms are equipped with enzymes to cope up withs oxidative stress and repair damaged molecules, produced during normal metabolism or due to exposure to xenobiotics.

In this experiment we studied the effect of leaves powder on lipid peroxidation in fish. The toxicity of various plant extract has been reported earlier. The toxicity induces the oxidative stress in fishes is well known. Here we retorted in the induction of lipid peroxidation in response to weed powder. Results noted that toxicity effect is present in this plant for this fish and how can they affect internally. Then this plant is also known as poisonous plant for aquatic animals or fishes. The plants directly affected of fish but we can observed one more thing and this is this plants leaf powder fish can eat and death after 96 hours in both set which are 50mg/L as well as 100mg/L. And concluded that in this powder of given plant effects of fish Gills , Muscles , Liver and also Intestine. After that studying we got the result the stress of *Wedelia Fructicosa* plants leaf powder is toxic as well as it was the study by using method of lipid peroxidation. The *Wedelia Fructicosa* plant is harmful or toxic for aquatic animals or fishes. *Wedelia fructicosa* leaves powder potentially could have affected the biochemical

measurements in exposed *mrigal fish*. Thus further studies are required to determine these biochemical changes after the exposure of fish to *Wedelia fructicosa* leaves powder and identification of its toxic compound

CONCLUSION :

This study alerts *Wedelia fructicosa* plant leaves powder is very toxic for fish and how toxicity induced in given plant is to be further investigation. It was done by oxidative lipid peroxidation method to identify the toxicity of plant for fish.

It is important to all of them for alive the fishes for without given plant toxicity and we cannot live this plant in most of aquatic areas.

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