Effect of insecticide such as cypermethrin on protein content, mortality and cod liver oil concentration of fish Labeo rohita.

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ABSTRACT

It was the industrial revolution that gave birth to environmental pollution as we know it today. The emergence of great factories and consumption of immense quantities of coal and other fossil fuels gave rise to unprecedented air pollution and the large volume of industrial chemical discharges added to the growing load of untreated human waste. With the introduction of insecticides in farming techniques, though has helped to improve the yield of crops, but simultaneously, had a cryptic effect that has surfaced recently. The insecticide seeps in through soil and though flowing water reaches water bodies, where it plays a drastic role in not only contaminating the water, but also its inmates such as fishes and other animals. These fishes are eaten by humans and which ultimately harms human civilization. This phenomenon is called as Biological Magnification. Thus through this study, we would investigate the harmful effect of pollutants that contaminate the water bodies, mainly insecticide cypermethrin, which is abundantly used throughout the globe.

1. INTRODUCTION

Pollution is the introduction of contaminants into an environment that causes instability, disorder, harm or discomfort to the ecosystem i.e. physical systems or living organisms. Pollution can take the form of chemical substances or energy, such as noise, heat, or light. Pollutants, the elements of pollution, can be foreign substances or energies, or naturally occurring; when naturally occurring, they are considered contaminants when they exceed natural levels. The contaminants that causes pollution of water bodies, leads to a deadly issue of water pollution is the contamination of water bodies, rivers, oceans, groundwater occurs .Water pollution affects plants and organisms living in these bodies of water; and, in almost all cases the effect is damaging either to individual species and populations, but also to the natural biological communities. Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without

adequate treatment to remove harmful compounds. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, including moth pests of cotton, fruit, and vegetable crops. It is also used for crack, crevice, and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses, apartment buildings, greenhouses, laboratories, and on ships, railcars, buses, trucks, and aircraft. It may also be used in non-food areas in schools, nursing homes, hospitals, restaurants, hotels, in food processing plants, and as a barrier treatment insect repellent for horses.

2. MATERIALS AND METHODS

2.1. Effect of cypermethrin on protein contents in heart, liver, kidney of Labeo rohita.

2.1.1 METHODOLOGY OF DISSECTION

Dissection of Labeo rohita is done and various tissues were isolated for the protein quantification.

GILLS

- Fish breath by gulping water through their mouth, then close their mouth and throat. The water is forced though the opening in the back of their throat that is lined with gills.
- Gills are very thin, they look like fine, branched structures, like a Christmas tree. This gives the greatest possible surface area to absorb oxygen from the water.
- Gills are red because they are filled with blood. Oxygen in the water passes into the blood and is carried through their body. Gills are more efficient than lungs at extracting oxygen.

Fig.1- Gills of fish Labeo rohita

THE VENT

- The vent opening on the underside of the fish. Eggs are laid from hereby females.
- Milt is released from here by males. As well, both males and females eliminate waste from the vent.

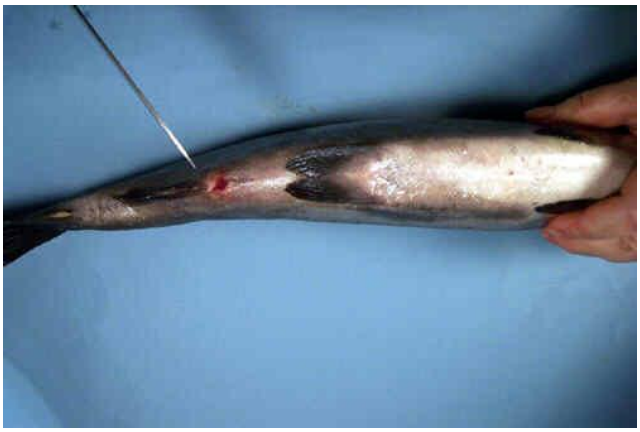


Fig.2- Vent of fish Labeo rohita

1. Cut the fish open beginning at the vent. Do not cut too deeply or the internal organs will be damaged
2. Open the fish from the vent to the throat.

LIVER

- The liver is the largest organ in the fish's body. It is part of the digestive system. As in humans, it is essential for maintaining the proper level of blood chemicals and sugars.

1. Remove the liver and gall bladder by gently cutting any small membranes that join it to the digestive system
2. Pull away from the stomach and remove

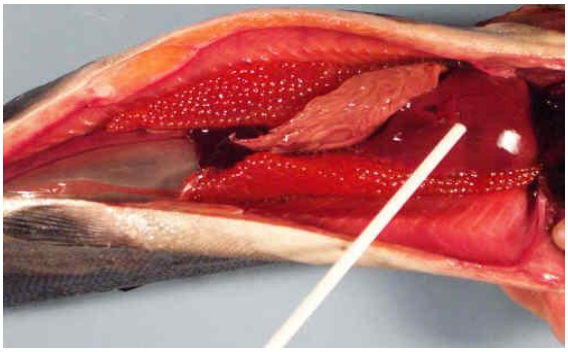


Fig.3- Liver of fish Labeo rohita

GALL BLADDER

- Turn the liver over to view the gall bladder. The gall bladder contains green bile which is used to help digest fats.

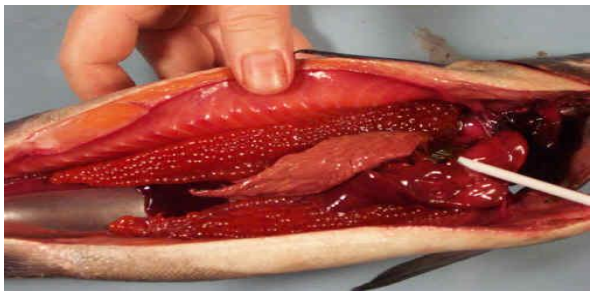


Fig.4- Gall Bladder of fish Labeo rohita

DIGESTIVE SYSTEM

- Observe the digestive system by gently pushing a probe (8" spoon handle or chopstick) through the mouth and into the stomach.
- The digestive system is shorter and simpler than in mammals. Because fish are cold-blooded they do not use as much energy to keep warm and do not need as much energy from their food so they expel it more.

Fig.5- The digestive system of fish Labeo rohita

1. Remove the stomach by cutting it away at the throat and gently pulling
2. Remove the complete digestive system and intestines, which end at the vent.
3. Most food is absorbed in the intestine, the tube-like section at the end of the digestive system

THE HEART

- The heart pumps blood through the body. It is very close to the gills where fresh oxygen enters the blood. In humans, the heart is close to the lungs to pump fresh oxygen through our bodies

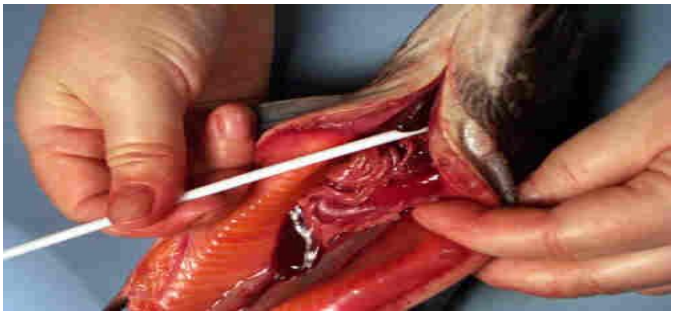


Fig.6- Heart of fish Labeo rohita

SWIM BLADDER

- Labeo rohita fill their swim bladder with air for the first time as swim-up fry. The air provides buoyancy, allowing them to float in the water.
- It can adjust the air in their swim bladder so they can hover at different levels in the water.

- Often the swim bladder remains full of air after the fish dies.

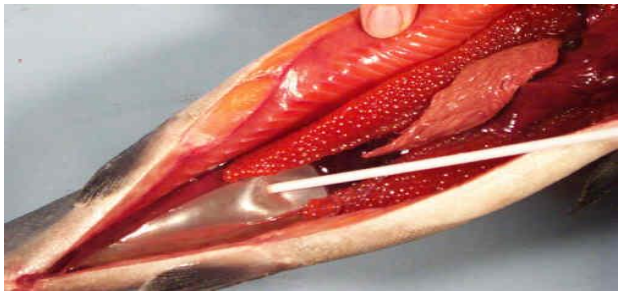


Fig.7- Swim Bladder of fish Labeo rohita

KIDNEY

- Labeo rohita have two kidneys joined together. The front kidney produces red blood cells and the back kidney cleans the blood. Urine is collected by ducts near the vent.

- The kidney is also critical in the smolting process (going from fresh to salt water) in a process called osmoregulation

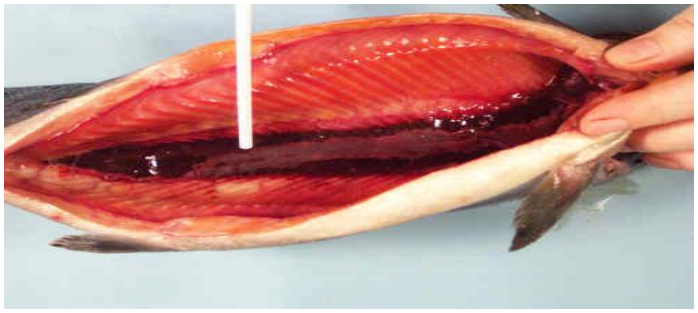


Fig.8- Kidney of fish Labeo rohita

1. Remove the kidney by cutting along each side.

2. Use a spoon to lift it out.

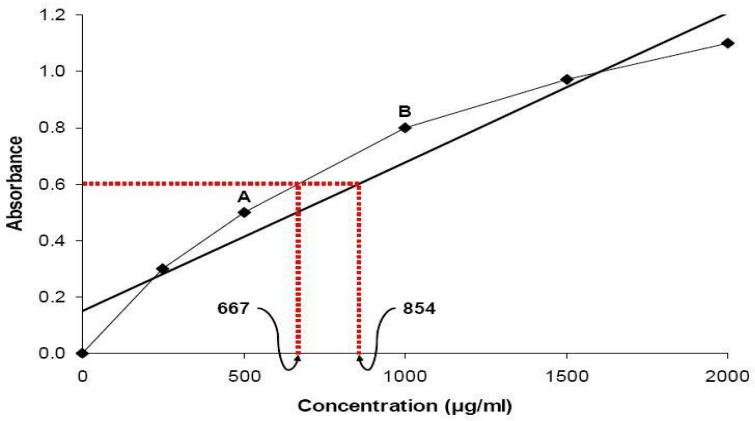
2.1.2. PROTEIN DETERMINATION - LOWRY METHOD

PROCEDURE

1. Set up eleven sets of three 16 x 150 mm test tubes in rack.
2. Add BSA [0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µl] to these tubes.

3. Add 2 ml of solution D to each test tube.
4. Incubate for 10 minutes at room temperature.
5. Add 0.2 ml of dilute Folin-phenol solution to each tube.
6. Vortex each tube immediately.
7. Incubate at room temperature for 30 minutes.
8. Determine absorbance of each sample at 600 nm.
9. Plot absorbance *vs* mg protein to obtain standard curve.
10. Set up triplicate assays for all "unknowns".

2.1.3. To calculate protein concentration from the absorbance by comparing with standard curve-Typical protein assays are used to determine protein concentration by comparing the assay response of a sample to that of a standard whose concentration is known. Protein samples and protein standards are processed in the same manner by mixing them with assay reagent and using a spectrophotometer to measure the absorbance (Graph.1).While protein quantification from absorbance values is straightforward, one common source of confusion is the assumption that dilution of the sample with assay reagent is a necessary consideration.



Graph.1- Absorbance values of protein estimation

2.2. EFFECT OF CYPERMETHRIN ON SURVIVAL RATE OF Labeo rohita.

Labeo rohita fingerlings weighing 3±0.5 g and average length of 6 cm were collected and acclimatized to laboratory condition in large cement tanks previously washed with potassium permanganate to free the walls from any microbial growth. Physico-chemical characters of water was maintained according to methods in APHA and found as follows: Temperature: 26±1°C, pH: 7.8±0.2 at 26°C.During acclimatization fish were fed with rice bran mixed with oil cake in the ratio 2:1 every day.

For the present investigation, technical grade cypermethrin (92.95%) was taken. The stock solution was prepared in acetone, which was found to be non-toxic to fish. Required quantity of cypermethrin was drawn from this stock solution for the further experiment.

Preliminary tests were carried out to find out the median tolerance limit (LC50) of the fish to cypermethrin for 96 h . The concentration of cypermethrin at which 50% mortality occurred was taken as the median lethal concentration (LC 50) for 96 h, which 50 was found to be 4.0 µg LG . One seventh of the LC 50 value 150 (0.57 µg) was selected for sub lethal concentration studies. The control and the exposed fish were aerated frequently to prevent hypoxic condition of the medium. The control and cypermethrin exposed fish were kept under continuous observation during the experiment period, 24, 48, 72 and 96 h for lethal concentration and 1, 5, 10 and 15 days for sub lethal concentration. During this experiment the behavioural changes were also critically observed.

2.3. EXTRACTION OF COD LIVER OIL

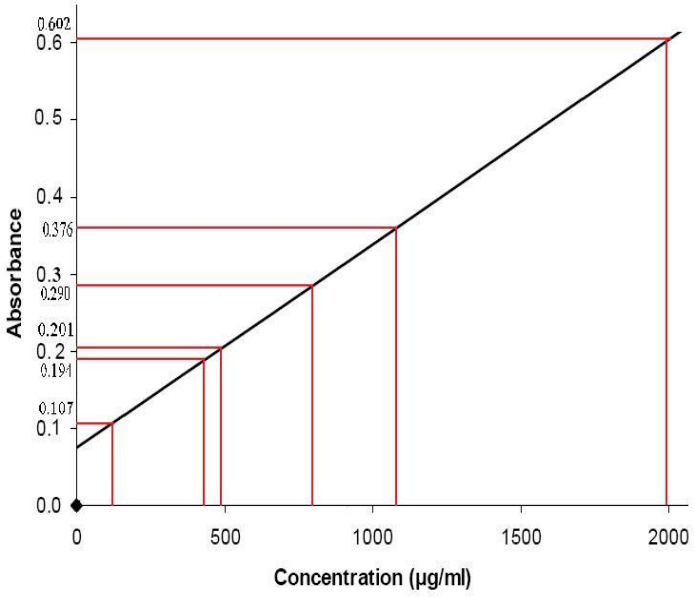
1. Cut up the fish and remove the head and the tail completely. The rest of the fish can be cut into strips. If you like, fillet the fish. However, this is not necessary since you will likely not be able to eat the fish once it has been pressed anyway.
2. Press the fish pieces in the hand-crank press until just a watery, oily mush is left in the bottom of the press. Turn the crank until the press is as far down as it will go. You can then empty the fish sludge into a bucket of distilled water before pressing more fish in the press.
3. Strain the distilled water and fish mush. You can use a sieve or pour the mixture through a screen held over another bucket. Either way, the fish flesh, bones and skin will remain in the strainer, while the oil and water will pass through.

4. Allow the water to evaporate. Since oil and water do not mix, you can simply let the water evaporate off the bucket of oil-and-water mix. This will leave a whitish, greasy substance that is fish oil. If you are in a hurry, you can skim the fish oil off the top, but you will still need to allow some time for evaporation. Depending on the volume of water, you may need 1 day to nearly a week for all the water to disappear.

3. OBSERVATION AND RESULTS

3.1. RESULTS OF EFFECT OF CYPERMETHRIN ON PROTEIN CONTENTS IN HEART, LIVER, KIDNEY OF Labeo rohita

Typical protein assays are used to determine protein concentration by comparing the assay response of a sample to that of a standard whose concentration is known. Protein samples and protein standards are processed in the same manner by mixing them with assay reagent and using a spectrophotometer to measure the absorbance (Graph.2).

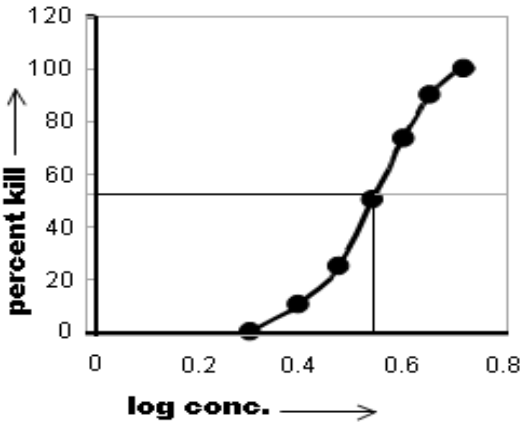


Graph.2- Absorbance value of protein wrt cypermethrin concentration

3.2. RESULT ON EFFECT OF CYPERMETHRIN ON SURVIVAL RATE OF Labeo rohita

The maximum concentration at which zero percent mortality and minimum concentration at which 100% mortality of Labeo rohita were observed at 2.5 and 5.0 µg LG respectively . Behavioural changes are physiological responses shown by the

animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it, consequently the behavioural changes were observed in control and exposed fish.



Graph.3- Mortality rate vs cypermethrin concentration

Control Fish: Control fishes maintained a fairly compact school, covering about one third of the bottom during the first five days of the 15 days experiment. By fifth day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were sensitive to light and moved to bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behaviour was observed (Table.1).

Exposed Fish- When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. The frequency of surfacing phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface (Table.1)

Concentration of cypermethrin ($\mu g\ L^{-1}$)	Log conc.	No. of fish alive out of ten	Percent kill (%)	Probit kill	Dragstedt- Behren's LC_{50} value ($\mu g\ L^{-1}$)
2.5	0.3979	10	0	----	3.97
3.0	0.4771	9	10	3.72	
3.5	0.5440	8	20	4.16	
4.0	0.6020	5	50	5.00	
4.5	0.6532	4	60	5.25	
5.0	0.6989	1	90	6.28	
5.5	0.7403	0	100	8.09	

Table.1- Fish mortality wrt cypermethrin concentration

3.3. RESULT OF EXTRACTION OF COD LIVER OIL FROM LIVER OF Labeo rohita AND QUANTIFICATION OF ACID VALUE

FORMULA USED:

$$\textbf{ACID VALUE} = \frac{56.1 \times N(KOH) \times V(KOH)}{\textbf{MASS}}$$

Volume of KOH used = 82ml

Mass of sample (Cod liver oil) used = 5gm

Normality of KOH used = 0.2N

Thus,

$$\textbf{Acid value} = \frac{56.1 \times 0.2 \times 82}{5}$$

$$= 184.008$$

For being used as a vegetable oil the Cod liver oil should have an acid value in the range of 178.0 to 189.0.

And the acid value calculated for the sample used is **184.008**.

Thus, it can be used as a vegetable oil.

3.3.1. QUANTIFICATION OF ACID VALUE OF COD LIVER OIL

REAGENTS:

1. Solvent mixture (95% ethanol/diethyl ether, 1/1, v/v)

2. 0.1 M KOH in ethanol accurately standardized with 0.1 M HCl (pure ethanol may be also used if aqueous samples are analyzed)

3. 1 % phenolphthalein in 95% ethanol.

PROCEDURE:

- Weigh 0.1 to 10 g of oil or fat (according to the expected acid value) in glass vial and dissolve in at least 50 ml of the solvent mixture (if necessary by gentle heating).

- Titrate, with shaking, with the KOH solution (in a 100 ml burette graduated in 0.1 ml) to the end point of the indicator (5 drops of indicator), the pink color persisting for at least 10 sec.

- The acid value is calculated by the formula: 56.1 x N x V / M where V is the number of ml of KOH solution used and N his exact normality, M is the mass in g of the sample.

4. DISCUSSIONS

4.1. EFFECT OF CYPERMETHRIN ON PROTEIN CONTENT OF Labeo rohita

S.NO.	TISSUE	PROTEIN QUANTITY(µg/ml)
1)	LIVER (Normal)	770
2)	LIVER (With Cypermethrin)	38

S.NO.	TISSUE	PROTEIN QUANTITY(µg/ml)
1)	HEART (Normal)	1000
2)	HEART (With Cypermethrin)	470

S.NO.	TISSUE	PROTEIN QUANTITY(µg/ml)
1)	KIDNEY (Normal)	1899
2)	KIDNEY(With Cypermethrin)	389

Table.2- Protein concentration decreases with presence of cypermethrin

This thus proved that the cypermethrin effects the protein concentration of various organs of fishes and is extremely harmful for both fishes and humans who consume it. The symptoms associated with acute lethality suggest that effects on the nervous system, respiratory surfaces and renal ion regulation are associated with the mechanism of lethal action in fish.

Qualitative structure - activity relationships indicate that the structural features required for good insecticidal activity and for lethality to fish are the same. Lethality also varies with biological (species, size) and environmental (temperature, sediment) factors. Some of these effects may be related to bioavailability and rates of pyrethroid biotransformation.

Chronic exposure studies indicate that newly hatched larvae or early juveniles are the life stages most sensitive to pyrethroids. Exposures of fish to sublethal concentrations of pyrethroids have resulted in decreased growth and impaired swimming performance. The effects on bioenergetics and energy metabolism are variable.

The present investigation shows that the significant reduction ($P< 0.01$) of the total number of eggs, total amount of egg (litre), total amount of egg (litre per kilogram of body weight), fertilization percentage, and expected fertilized egg number at the concentrations of 0.40 and 0.80 µl l^{-1} of cypermethrin. Also, the reduction in hatching percentage, expected number of hatchling, and expected number of hatched larvae were significantly different ($P<0.01$) between the treatment and the control at all cypermethrin concentrations. No significant differences for the 96 h survivability of hatched larvae were reported at 0.16 and 0.40 µl l^{-1} levels of cypermethrin, whereas significant differences ($P< 0.05$) were reported at 0.80 µl l^{-1}. Depending on the doses, Na value first decreased a little then increased. We found no study in our archiveabout the effects of chemicals on Na value, and so we were unable to discuss this parameter.

4.2. EFFECT OF CYPERMETHRIN ON SURVIVAL RATE OF Labeo rohita.

Cypermethrin toxicity is shown to increase with increased concentration. The observation is in consonance with earlier reports of Omoregie and Ufodike (1991), Gesamp, 1991). Neibor and Richardson (1980) reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reaction of the organism receiving the toxicant. The three physicochemical parameters of the test were fluctuated slightly
during the toxicity test. The values were normal for toxicity test (FAO, 1977). The water quality parameters may have probably contributed to the variation in behavioural pattern and the mortality of the test fish
during the study period. There was a significant negative correlation between pH and dissolved oxygen values. In case of dissolved oxygen, the treatments did not only show a dose dependent decline in concentration, but also rapid depletion of dissolved oxygen with time.
Warren (1977) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation.
It was also observed that the higher the concentration of the toxicant, the higher the mortality rate. This demonstrates the observation of Fryer (1977), that in all toxicant, a threshold is reached above which there is no
drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and mortality were observed from the record of the relative mortality time in different concentrations of cypermethrin for 96hours. The histopathological examination of the brain, gill, and kidney of the exposed fish indicated that the liver and kidney were the organ most affected. Damages of the gills indicated that the lethal concentrations of insecticide caused impairment in gaseous exchange efficiency of the gills.

4.3. EXTRACTION OF COD LIVER OIL FROM LIVER OF Labeo rohita

Cod liver oil is a nutritional supplement derived from liver of cod fish. It has high levels of the omega-3 fatty acids, EPA and DHA, and very high levels of vitamin A and vitamin D. It is widely taken to ease the symptoms of arthritis and for other health benefits. It was once commonly given to children, because the high levels of vitamin D in cod liver oil have been shown to prevent rickets. Webb and Brett, (1972, 1973) worked on cod

liver oil of *Labeo rohita* and Indian carp and discovered its beneficial effects on human health. They discovered that it is highly rich in vitamin content and can be administered to pregnant women. Their work extended and they found out that cod liver oil can be used as a vegetable oil for edible purposes. According to their experiments the acidic value of their sample was 182.98. Thus this values lies between the range (178-189) and is almost similar to our experimental value 184.008. Therefore cod liver oil from fish is highly beneficial and has several therapeutic properties and can thus be used for edible purposes. Lawrence, J. L. and Casida, J. E. (1982) also worked on cod liver oil and studied its properties that it supports the immune system and promotes hearth health and mentains cholesterol balance.

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