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Original Research Paper

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A CASE STUDY OF LIPID CONTENT IN THE BRAIN OF *CIRRHINA MRIGALA* AND *LABEO ROHITA* FROM RAJARAM TANK NEAR SHIVAJI UNIVERSITY KOLHAPUR, MAHARASHTRA, INDIA.

Dr. Sambhajirao Hindurao Bhosale*

Associate Professor, Department of Zoology And Research Centre, Shankarrao Mohite Mahavidyalaya, Akluj. Dist. Solapur. PIN: 413 101. *Corresponding Author

Dr. Rajendra Vishnu Salunkhe

Associate Professor, Department of Zoology Arts, Science And Commerce College, Indapur, Dist. Pune, Maharashtra, India PIN-413106.

ABSTRACT

Neutral lipids (NL) and phospholipids (PL) with their constituents were studied in brain of *Cirrhina mrigala* and *Labeo rohita,* by employing TLC and Bioassay technique . The total lipid in the brain of *Labeo rohita* is 24.14 mg lipids / gm , increase to 127.9 mg lipids / gm in the brain of *Cirrhina mrigala* . The ratio of cholesterol and phosphotidylcholine in the same species is 5:1 and 4:1 respectively .

Neutral lipids consists of six components , triacylglycerol being main component . Cholesterol (CHO) , Diacylglycerol (DG) , Cholesterol-ester (CE) , and Monoacylglycerol (MG) were moderate in concentration . Free fatty acids (FFA) was low in quantity . Phospholipids exhibits seven constituents , Phosphatidylcholine (PCL) and phophatidyl - ethanolamine (PE) were major constituents , sphingomyelin (SPG) , phosphatidyl - inositol (PI) and phosphatidyl- serine (PS) were moderate in concentration . Lysophosphatidylcholine (LPC) were low in quantity .

KEYWORDS : Neutral Lipid, Phospholipid, TLC technique, Bioassay technique.

INTRODUCTION

The brain is amongst the most complex and highly evolved organ, involved with special function which has given man his unique place on the ladder of evolution. It is remarkable for its state of continuous activity as may be evidenced both by experience as well as by electrophysiological monitoring. Since it is an important organ of body it has been studied for many aspects, including the biochemical information.

From the critical review of literature on the lipids of fish brain, the following significant facts emerges especially in *Cirrhina mrigala* and *Labeo rohita*. The lipid contents in the various organs like liver, testis , ovary and accessory reproductive organs of fishes including mammals, seems to have received greater attention from the workers in the fields. However, the most important organ systems like brain are overlooked due to which there is a paucity in the information of brain lipids particularly in lower vertebrates. Some research workers studied parameters like lipid peroxidation in vivo and in vitro studies.

From the above literature indicates very scanty information is available on lipids of fish, especially in the above species. Hence in the present case study the attempt was made to find out the content and composition of lipids in the brain of *Cirrhina mrigala* and *Labeo rohita* . A few research workers have made their contribution in reporting the brain lipids in some fishes. Pravdina and Chebotareva (1974) eel, Dasiatis Pastinaca, Kreps et.al. (1976), teleost and elasmobranch fishes; Malkhede et.al. (1981), Clarias batrachus, Vadhva and Mahdi (1987) fish, Hollander (1970) goldfish and Ushkolova and Ioanidis (1985) white fish.

MATERIALS AND METHODS

For the present study two Vertebrates were selected, each one representing a class of sub phylum vertebrata, While selecting the animals, care was taken to see their evolutionary states, they are as follows;

Two species of fishes *Cirrhina mrigala* and *Labeo rohita* were collected from Rankala tank in Kolhapur city (Maharashtra, India). Fish species of each type were collected at a time and brought to the laboratory, where they were kept in plastic containers for about six hours for acclimatization. The average weight of fish was about 250gm. The fish were sacrificed to take out the brain for further studies.

METHODS

Thin layer chromatographic technique for the analysis of lipids in fish brain was used. This gives a good separation of both neutral and phospholipid components for quantification studies bioassay method is used.

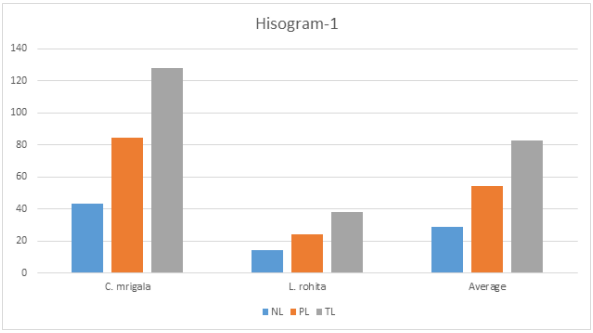
Extraction of lipids

The brain tissue were homogenized with 20 volumes of chloroform methanol (2:1, v/v) at room temperature. The homogenates were allowed to stand for 4 to 6 hours at 4°C and then filtered through the sinctered funnel into glass-stoppered container. The precipitate was rehomogenised with 10ml of chloroform methanol mixture (2:1, v/v) and then filtered through the sinctered glass funnel. Both the filtrates were pooled together and the resultant mixture was shaken well with 0.2 volume of glass distilled water. Extract were allowed to partitioned into two distinct phases. The upper phase while generally contained the major part of the non-lipid contaminants were removed as completely as possible with a fine tipped pipette. The lower phase which mainly contained lipid fraction was transferred quantitatively through sodium sulphate to remove water completely from the lipid sample. The more chloroform was added to remove any lipid fraction from the sodium sulphate. Then it was transferred quantitatively into a glass stoppered container and evaporated under vacuum at 40°C.The lipid sample was weighed accurately and preserved by desiccation under vacuum at 20°C for further use.

The NL and PL were separated by thin layer chromatography (TLC) using silica gel G (about 200 mesh containing CaSO4 as a binder E Merck Germany), the TLC plates (20*20cm) were prepared according to Wagner et. al. The known quantities of sample dissolved in chloroform were applied with Hamilton's micro syringe (Number 8206.B) 2.5 to 3.0 cm from the bottom edge of the plates. For neutral lipids the plates were developed in Hexane (b.p.65° to 70°C): diethyl ether: acetic acid (85:15:2,v/v) as recommended by Gloster and Flecter. The phospholipids plates were developed in chloroform: methanol: ammonia (115:45:5,v/v) as recommended by Barval and Kalra. The standards of neutral lipid and phospholipid (Sigma, USA) were co- chromatographed in each respective run and then plates were kept in Iodine chamber for identification of individual spots of lipids.

Estimation of neutral lipid and phospholipid

The Iodin was allowed to evaporate and the silica gel from the individual spots of glycerides was scraped and eluted in 1 ml of diethyl ether and assayed according toViogue and Holman. The Cholesterol and its ester were estimated according to Abell et,al. The rest of NL components were assayed titrimetrically (Skipskiet et,al.).The PL was determined by the method of Marinetti.



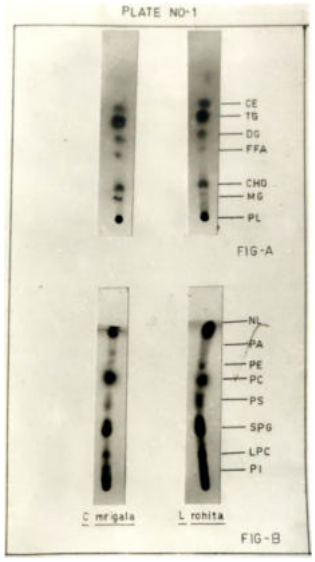


Table No.1 Composition of total and neutral lipids from the brain of fishes

Animals	Total lipids	Neutral lipids	MG	CHO	FFA	DG	TG	CE
C. mrigala	127.87 ± 8.31	43.20± 2.59	5.20± 0.36	9.84± 0.78	0.720 ± 0.04	8.67± 0.67	13.87± 0.97	5.62± 0.4
L. rohita	24.14 ± 1.56	13.99± 0.99	2.71± 0.2	2.19± 0.17	0.450 ± 0.02	3.19± 0.25	4.06± 0.29	1.64± 0.12

Note: The values for the total lipids and its constituents are expressed as mg/gm wet weight of tissue (brain).

Table No.2 Composition of total and phospholipids from the brain of fishes

Animals	Total Phospho lipids	PI	LPC	SPG	PS	PC	PE	PA
C. mrigala	84.67 ± 5.67	9.6± 0.6	5.48± 0.4	12.09 ± 0.87	8.06 ± 0.04	25.8 ± 1.87	18.13 ± 1.22	6.05 ± 0.4
L. rohita	24.14 ± 1.7	3.23 ± 0.23	1.13 ± 0.23	4.25 ± 0.31	2.83± 0.22	6.66± 0.45	4.63± 0.32	1.41 ± 0.098

Note: The values for the total PL are expressed as mg/gm wet weight of tissue, whereas PL constituents are expressed as mg-p/gm wet of tissue of brain.

RESULTS AND DISCUSSION

I) Neutral lipid:

The TLC separation and qualitative changes in the individual components of the NL are illustrated in plate no. 1. The values with statistical variations of total lipid, neutral lipids and its individual components from the brain of *Cirrhina mrigala* and *Labeo rohita* and similar information for total phospholipid and its individual constituents from the brain of the *Cirrhina mrigala* and *Labeo rohita* are givenin table no.2.

The TLC separation NL revealed the following components. Such as monoacyglycerol (MG), diacyglycerol (DG), triacyglycerol (TG), cholesterol (CHO),ester (CE) and free fatty acid (FFA) among the NL components TG occurred in higher concentration CHO & DG were moderate in occurrence. The CE,MG & FFA were found in low concentration.

II) Phospholipid:

The TLC separation and quantitative changes in the individual constituents of phospholipid are illustrated in plate No.1 fig-B; whereas the Table No.2 remaining information exhibit the quantitative variation in the phospholipid constituents. The TLC separation of PL indicated the following constituents. The TLC separation of PL indicated the following constituents such as PL,LPC,SPG,PS,PC,PE and PA in the PL constituents, PC and PE were predominants, SPG & PI were moderate in their values and the remaining two constituents such as LPC, PS and PA occurred in small amounts.

The significant findings of the above studies are as follows:

i) Neutral lipid percentage of the total lipids in *C.mrigala* is 37.78, enhanced to 57.95% in *L.rohita*.The difference in values of percentage of the neutral lipids might be due to species specific.

ii) The percent value of TG with the neutral lipid in *L. rohita*, raising to 29.0 increased to 32.12 in *C.mrigala*. Similar pattern is followed by MG and DG.

iii) The percent value of CHO is 15.66 in *L.rohita*,raising to22.78 in *C.mrigala*. The raising in percent values of CHO could be due to species variation. The same pattern is followed by CE.On the contrary, the percent values of FFA in *C.mrigala* is 1.66, increased to 3.2 in *L.rohita* again the reason is not known.

iv) In *C. mrigala* the quantity of phospholipids is double than the neutral lipid. Similar pattern of phospholipids is also observed in *L. rohita* .Phospholipids are mainly membrane lipids, hence in the brain the quantity of membrane lipid is increased ,concomitantly increase the phospholipids.

v) The phospholipids percentage of the total lipids in *C.mrigala* is 66.20, but a significant decrease to 13.38 in *L.rohita*. The cause remain unknown, further research is required.

vi) The phospholipids percentage of the total lipids in *C,mrigala* is 66.20 but a significant decrease to 13.38 in the phospholipids percent value in *L.rohita*. The causes remain unknown, further research is necessary.

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