



Effect on Biomarkers of *Cirrhinus mrigala* due to *Aeromonas hydrophila* along with remedial effects of extract of *Curcuma longa*

Sabiha Khan¹, Sonia Patharia^{*2}

¹ Department of Zoology, Samrat Prithviraj Chouhan Government College Ajmer, Rajasthan, India.

^{2*} Department of Zoology, Samrat Prithviraj Chouhan Government College Ajmer, Rajasthan, India.

Abstract

The current study aimed to find effect of infection with *Aeromonas hydrophila* and treated with extract of *Curcuma longa* on some biochemical parameter in *Cirrhinus mrigala* fish namely acid and alkaline phosphatase enzyme (ACP & ALP), alanine and aspartate amino transpharase enzyme (ALT & AST), total protein, glucose, and cholesterol. To accomplish this, *C. mrigala* infected with *Aeromonas hydrophila* and treated with *Curcuma longa* for 20, 40, and 60 days. *Aeromonas hydrophila* were detected, isolated and identified from infected fish obtain from Anasagar Lake situated in Ajmer, Rajasthan (India). One year old *mrigala* carps obtained from the Aquaculture farm. Fishes acclimated for three days. For experiment 7 culture tanks (1 for control, 3 for infected, 3 for treated) containing 100 L aerated water were setup and divided in to 3 groups G1, G2, and G3. Further all groups divided in to 3 subgroups A, B, C (9 subgroups). 10 fishes in each tank for control, experimental and treated (20, 40, 60 days). Each fishes of G2 group injected intraperitoneally with 0.1ml concentration of 10^8 CFU/ml of *Aeromonas hydrophila*. Control fishes injected with 0.1ml of phosphate buffered saline. Fishes of G3 group injected with 0.1×10^8 CFU/ml *Aeromonas hydrophila* and treated with 5 mg/ml *Curcuma longa*. Regarding biochemical tests we noted significant decrease in the level of Total protein, Glucose, and cholesterol and significant increase in ACP [acid phosphatase] & ALP [alkaline phosphatase], ALT [alkaline transpharase] & AST [acid transpharase] in the infected group [G2] compared with a control group [G1] and significant increase in Total protein, Glucose, and cholesterol and significant decrease ACP [acid phosphatase] & ALP [alkaline phosphatase], ALT [alkaline transpharase] & AST [acid transpharase] in the treated group [G3] compared with infected group [G2]. The mean of groups of G1 & G2 and groups of G2 & G3 are significantly different at $p < 0.05$. In this study infection with *Aeromonas hydrophila* can induce alteration in the level of some biochemical parameters and causes economic losses in production of fish.

Keywords: *Aeromonas hydrophila*, ACP & ALP, ALT & AST, *Cirrhinus mrigala*, Cholesterol, Glucose, Total Protein.

1. Introduction:

Aeromonas hydrophila is a gram negative Bacteria. It is conceded that it is an opportunistic Bacteria that caused haemorrhagic septicemia, ulcer and red sore disease, fin rot, tail rot, exophthalmia, and abdominal swelling in fishes. Alternatively it may be affect internal organs. *Cirrhinus mrigala* is a species of ray fined fish in the genus *Cirrhinus*, it is the Indian major carp species cultivated widely in southeast Asian countries. It is mainly found in fresh water or northern India, Punjab, West-bangal, and Orissa. It resemble very much with *Labeo rohita* except that it has wider mouth and thinner lips. Body is dark grey on the dorsal side and white orange on the ventral side. It is a bottom feeder. It has the economic value that is quite important. ACP

and ALP are lysosomal enzymes, which catalyse the splitting of phosphoric acid from phosphoric esters and commonly found in most of tissues of the body. They are generally located on absorptive surface and secretory surface of cells as the membrane bound enzymes. ACP is composed of specialized proteins that catalyze, or stimulate, certain biological reactions. ACP is a hydrolase enzyme because it catalyze the hydrolysis the chemical bonds. Specifically Acid phosphatase breaks the molecular bonds of phosphate groups. ACP is hydrolysis the ester linkage of phosphate esters at acidic pH 5 to 6 and helps in autolysis of the degenerated cells [Khan S., et al., 2012] Alkaline Phosphatases (ALP) are a group of enzymes found primarily in the liver and bone. The small amount of ALP also produced by cells lining of intestines, placenta, and kidney (in the proximal convoluted tubules). ALP released from these tissues into the blood. ALP plays role in phosphate hydrolysis and in membrane transport and also a good bio-indicator of stress in biological systems, [Khan S., et al., 2012]. ALP splitting phosphorus esters at alkaline pH 10 and mediates membrane transport and is intimately associated in protein synthesis. Protein is also one of the biochemical parameters used to understand the state of fish health and mechanism of metabolism under stress. Cholesterol [CHL] is a vital component of cell membrane and important for maintaining the structural integrity of cell membrane and insuring proper physiological function. It also serves as a precursor for the synthesis of various hormones which essential for fish growth and development. Low level of cholesterol increase the skin haemorrhages and lesion morbidity it can lead to decreased growth and feed utilization. Decrease cholesterol value aggravated the inflammation response. The toxicity stress decreased value of cholesterol [Ganeshwade et al., 2012] The glucose [GLU] is the permanent and immediate source of energy. Its concentration in the blood acts as a indicator of stress and overall health, a significant rise in blood glucose can signals a stressful situation. Carbohydrates are important metabolic functional nutrients in fish and it is also a kind of relatively cheap energy source in aquatic feed. Several studies have shown the role of glucose is the state of stress in fish due bacterial infection. Curcuma longa [turmeric] [family Zingiberaceae] is most important medicinal plant. It contain curcumin, curcuminoids, termerone, arturmerone, zingiberene that have antioxidant activities [Ruby et al., 1995]. Turmeric exhibits pharmacological effects such as antioxidant, anti-inflammatory, hepatoprotective, antitumor, anticancer, antimutagenic, antiangiogenic, antimicrobial, and wound healing [Prasad et al., 2011].

2. Material and Method

The material used in this study were *Cirrhinus mrigal* as a test fish, isolates *Aeromonas hydrophila*, MacConkeys agar, blood agar, aquades, extract of Curcuma longa, clinical syringes and blood vials, test tubes, pipettes, required reagents, biochemical kits, spectrophotometer or colorimeter, incubator, Previously *Aeromonas hydrophila* isolated from diseased fish collected from Anasagar lake situated in Ajmer, Rajasthan (India) were sub-cultured onto MCA plates (Griffin et al 2013.) and incubated at 37°C (Abbott et al., 2010). *Cirrhinus mrigals* were collect from fish farm and acclimatized in aerated water for three days. The fish brought in the laboratory were humanely euthanized. 7 culture tanks (1 for control, 3 for infected, 3 for treated) containing 100 L aerated water were setup and divided in to 3 groups G1 (control group), G2 (infected group), and G3 (treated group). Further all groups divided in to 3 subgroups A, B, C (9 subgroups). Fishes fed with a commercial pellet diet at daily during the experiment. Water of the tank was exchanged partially 3 to 5 days to remove waste feed and fecal matter. Water temperature (26±1°C) water p^H (6.8±0.2) and oxygen concentration in the range of 5 to 6 mg/l were maintained. For the experiment an intraperitoneal injection method was use to observed the biomarkers of C. mrigala for the period of 20, 40 and 60 days. Sterile and disposable 1ml insulin syringes were use for the injection. Group G2 fishes were inject intraperitoneally with 0.1ml concentration of 10⁸ CFU/ml of A. hydrophila. Control fish (group G1) were injected with 0.1 ml of saline. Group G3 fishes were inject with 5 mg/ml of extract of Curcuma longa. The C. mrigal were monitor for 20, 40 and 60days after being infected and treated for biochemical changes. Blood samples collected from healthy, diseased and treated fishes. During the sampling utmost care was taken to avoid contamination and human infection. Every observed value was expressed in Mean±SD and Total variations present in the data is estimated using the unpaired T-test, on p<0.05 the differences are considered to be significant statistical.

Experimental groups:

G1A: Group of control fishes for 20 days.

G2A: Group of treated fishes with *Aeromonas hydrophila* for 20 days.

G3A: Group of treated fishes with *Aeromonas hydrophila* and extract of Curcuma longa for 20 days.

G1B: Group of control fishes for 40 days.

G2B: Group of infected fishes with *Aeromonas hydrophila* for 40 days.

G3B: Group of treated fishes with *Aeromonas hydrophila* and extract of *Curcuma longa* for 40 days.

G1C: Group of control fishes for 60 days.

G2C: Group of infected fishes with *Aeromonas hydrophila* for 60 days.

G3C: Group of treated fishes with *aeromonas hydrophila* and extract of *Curcuma longa* for 60 days.

Biochemical parameters determination:

1. Acid phosphatase enzyme (ACP) activity determined by using UV kinetic method 2008.
2. Alkaline phosphatase enzyme (ALP) activity determined by using UV kinetic method 2008.
3. Alanin amino transferase enzyme (ALT) activity determined by using IFCC method 1972.
4. Aspartate amino transferase enzyme (AST) activity determined by using IFCC method 1980.
5. Total protein level determined by using Flank and woolens, Biuret method (1984).
6. Glucose level determined by using Beach and Truner's enzymetic method 1958.
7. Cholesterol level determined by using Roeschlau method 1974.

3. Observation:

Table1.1: Biochemical parameters of groups G1A and G2A.

S. NO.	PARAMETERS	G1A	G2A	t-VALUE
1.	Total Protein	4.22±0.118	3.4± 0.109	11.38
2.	Glucose	48.61±0.774	32.16± 0.331	43.72
3.	Cholesterol	120.48±0.701	75.58 ±1.774	52.69
4.	AST	103.52±1.115	129.44± 0.726	41.94
5.	ALT	72.28±1.689	89.06± 0.549	21.16
6.	ACP	14.6±0.384	17.6± 0.593	9.49
7.	ALP	61.42±0.885	70.84± 0.428	21.45

The mean of G1A and G2A are significantly different at $p < 0.05$.

Graph 1.1: Biochemical parameters of G1A & G2A.

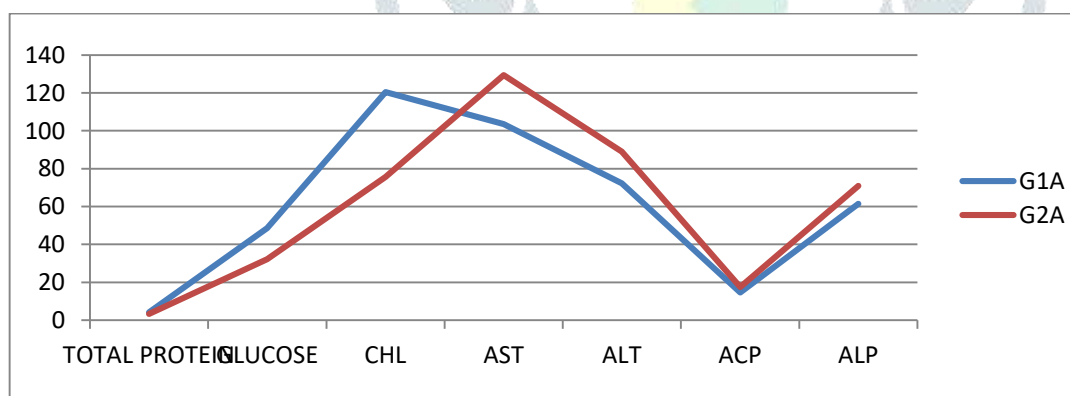
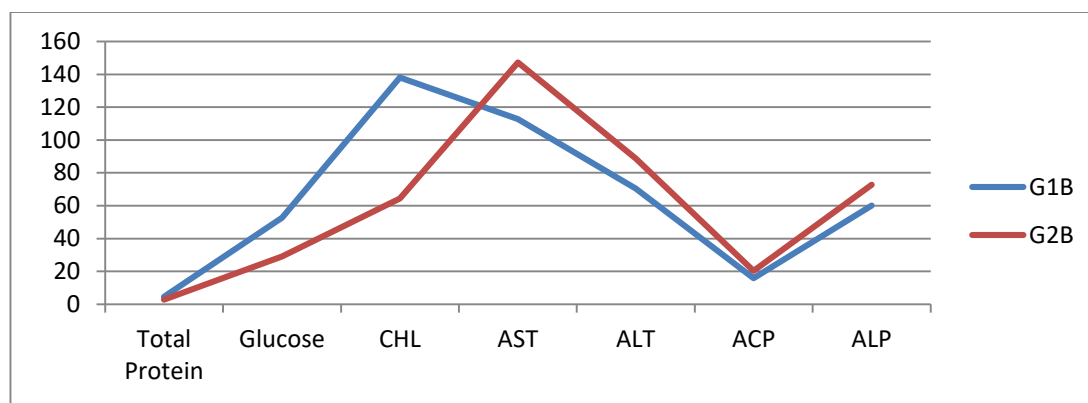


Table 1.2: Biochemical parameters of groups G1B and G2B.

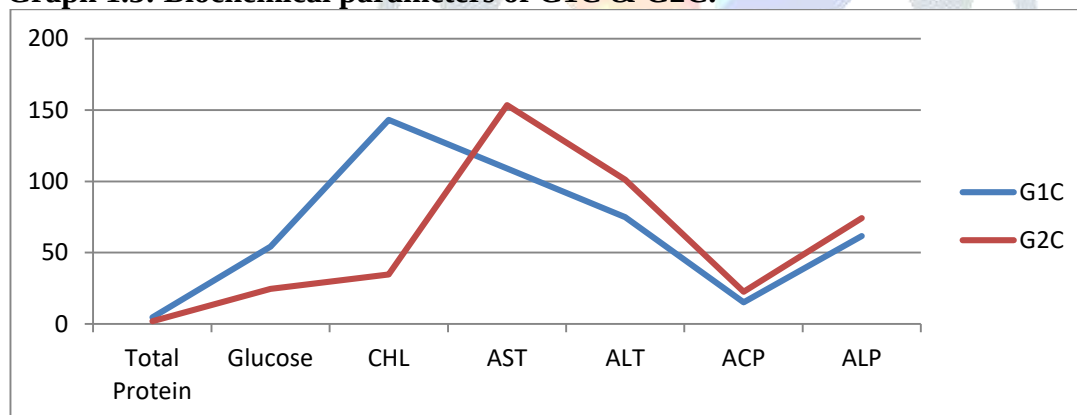
S. NO.	PARAMETERS	G1B	G2B	t-VALUE
1.	Total Protein	4.46± 0.148	2.78± 0.268	12.53
2.	Glucose	52.74± 0.69	29.06 ± 0.752	51.70
3.	Cholesterol	138.04 ±0.189	64.42 ±1.165	139.69
4.	AST	112.68 ±2.731	147.36 ± 2.49	20.96
5.	ALT	70.48 ± 1.309	88.78 ±0.985	25
6.	ACP	15.8 ± 0.384	20.38 ± 1.468	6.755
7.	ALP	60.16 ± 1.556	72.7 ± 0.660	16.60

The mean of G1B and G2B are significantly different at $p < 0.05$.

Graph 1.2: Biochemical parameters of G1B & G2B.**Table 1.3: Biochemical parameters of groups G1C and G2C.**

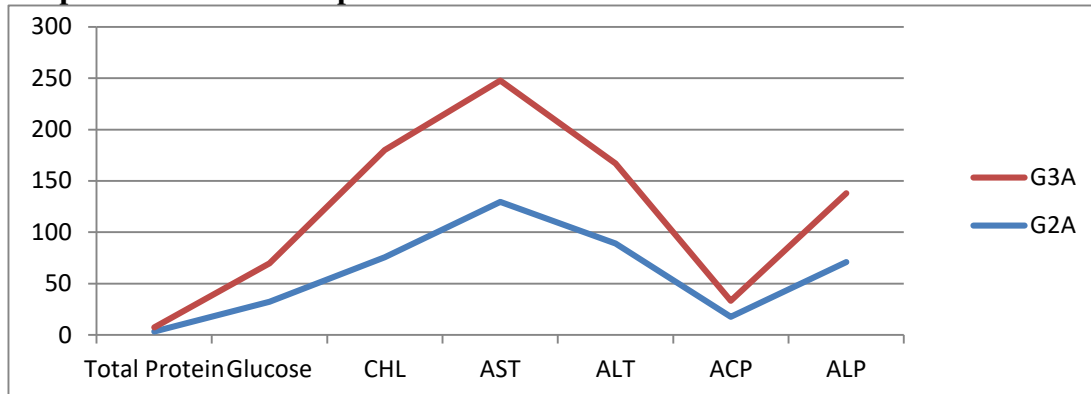
S. NO.	PARAMETERS	G1C	G2C	t-VALUE
1.	Total Protein	4.68 ± 0.141	1.9 ± 0.141	33.49
2.	Glucose	54.26± 0.649	24.46±0.444	84.65
3.	Cholesterol	143±0.629	37.6±0.509	291.96
4.	AST	108.96± 2.134	153.42±0.813	43.54
5.	ALT	74.94±0.689	101.2±1.811	30.32
6.	ACP	15.14±0.428	22.52±0.768	18.82
7.	ALP	61.5±0.927	74.24±1.196	18.84

The mean of G1C and G2C are significantly different at $p < 0.05$.

Graph 1.3: Biochemical parameters of G1C & G2C.**Table 1.4: Biochemical parameters of groups G2A and G3A.**

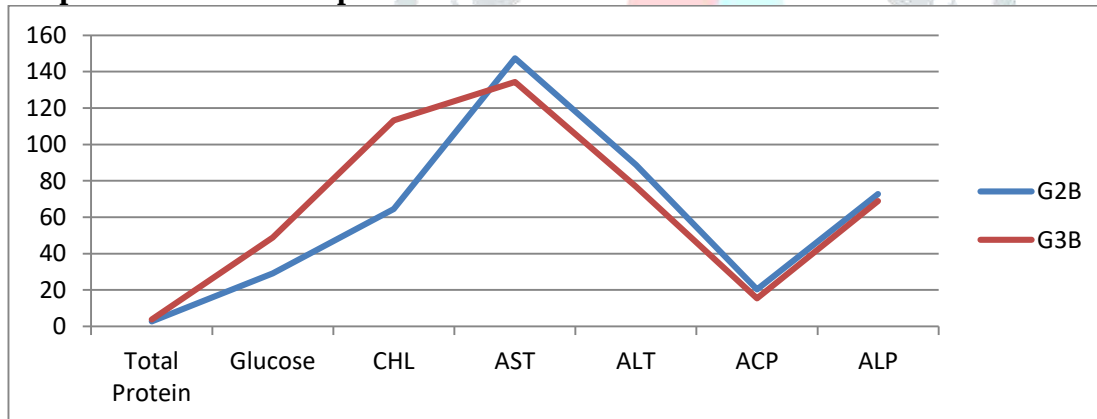
S. NO.	PARAMETERS	G2A	G3A	t-VALUE
1.	Total Protein	3.4±0.109	4.06±0.118	9.42
2.	Glucose	32.16±0.331	37.56±0.293	27.41
3.	Cholesterol	75.58±1.774	104.36±1.030	31.38
4.	AST	129.44±0.726	118.14±1.346	16.52
5.	ALT	89.06±0.549	78.02±1.458	15.86
6.	ACP	17.6±0.593	15.7±0.209	6.76
7.	ALP	70.84±0.428	67.14±0.807	9.06

The mean of G2A and G3A are significantly different at $p < 0.05$.

Graph 1.4: Biochemical parameters of G2A & G3A**Table1. 5: Biochemical parameters of groups G2B and G3B.**

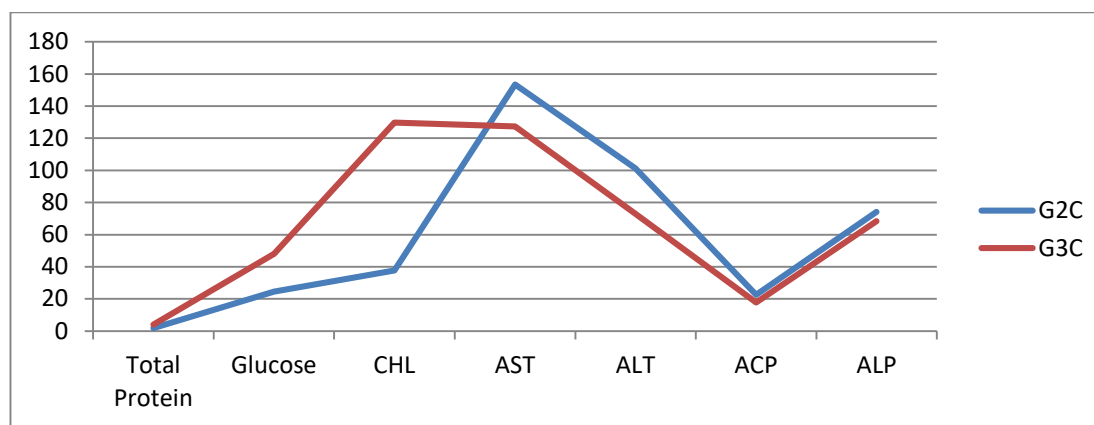
S. NO.	PARAMETERS	G2B	G3B	t-VALUE
1.	Total Protein	2.7±0.268	3.88±0.774	3.055
2.	Glucose	29.06±0.752	48.84±1.150	24.07
3.	Cholesterol	64.42±1.165	113.18±3.107	32.85
4.	AST	147.36±2.494	134.32±1.556	9.92
5.	ALT	88.78±0.985	76.76±0.635	22.93
6.	ACP	20.38±1.468	15.44±0.663	6.86
7.	ALP	72.7±0.660	68.84±0.493	10.48

The mean of G2B and G3B are significantly different at $p < 0.05$.

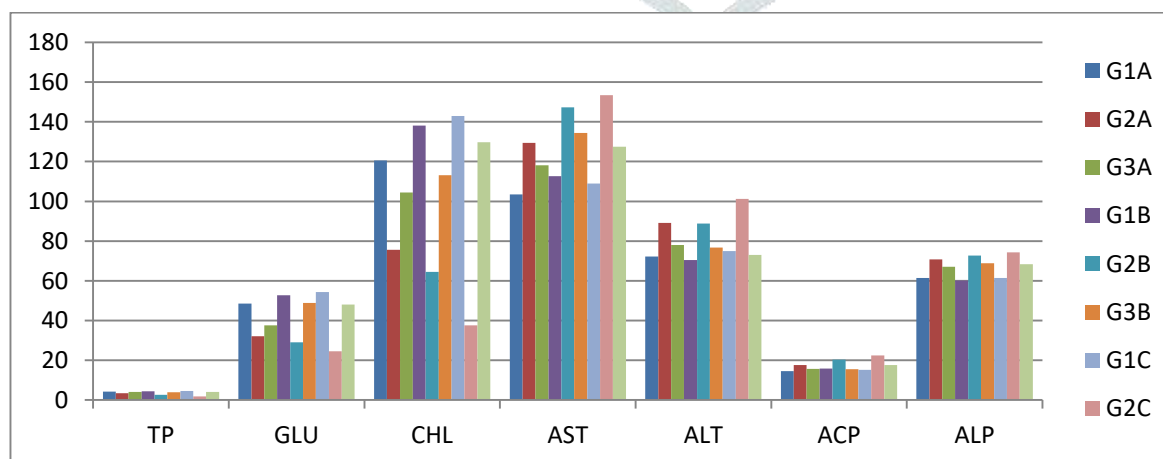
Graph 1.5: Biochemical parameters of G2B & G3B.**Table 1.6: Biochemical parameters of groups G2C and G3C.**

S. NO.	PARAMETERS	G2C	G3C	t-VALUE
1.	Total Protein	1.9±0.141	4.08±0.178	21
2.	Glucose	24.46±0.444	48.12±0.366	92.42
3.	Cholesterol	37.6±0.509	129.72±0.646	251
4.	AST	153.42±0.813	127.42±0.732	53.27
5.	ALT	101.2±1.811	73.02±0.331	34.24
6.	ACP	22.52±0.768	17.68±0.927	8.99
7.	ALP	74.24±1.196	68.38±1.263	7.54

The mean of G2C and G3C are significantly different at $p < 0.05$.

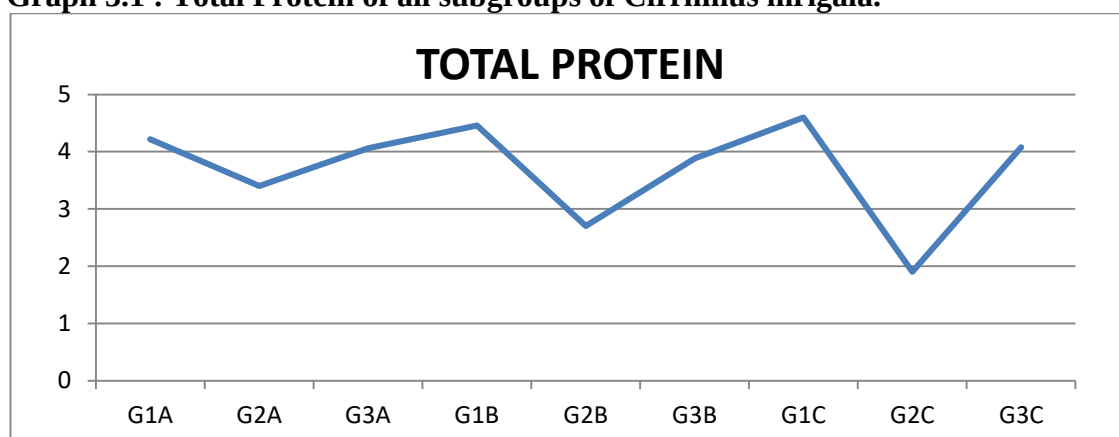
Graph 1.6: Biochemical parameters of G2C & G3C.**Table 2: Biomarkers of all experimental subgroups of *Cirrhinus mrigala* control, infected with *Aeromonas hydrophila* and treated with extract of *Curcuma longa*.**

S. NO	PARAM ETERS	G1A	G2A	G3A	G1B	G2B	G3B	G1C	G2C	G3C
1	TP	4.22±0.118	3.4±0.109	4.06±0.118	4.46±0.148	2.7±0.268	3.88±0.774	4.6±0.141	1.9±0.141	4.08±0.178
2	GLU	48.61±0.774	32.16±0.331	37.56±0.293	52.74±0.69	29.06±0.752	48.84±1.150	54.26±0.649	24.46±0.444	48.12±0.366
3	CHL	120.48±0.701	75.58±1.774	104.36±1.030	138.04±0.189	64.42±1.165	113.18±3.107	143±0.629	37.6±0.509	129.72±0.646
4	AST	103.52±1.115	129.44±0.726	118.14±1.346	112.68±2.731	147.36±2.494	134.32±1.556	108.96±2.134	153.42±0.813	127.42±0.732
5	ALT	72.28±1.689	89.06±0.549	78.02±1.458	70.48±1.309	88.78±0.985	76.76±0.635	74.94±0.689	101.2±1.811	73.02±0.331
6	ACP	14.6±0.384	17.6±0.593	15.7±0.209	15.8±0.384	20.38±1.468	15.44±0.663	15.14±0.428	22.52±0.768	17.68±0.927
7	ALP	61.42±0.885	70.84±0.428	67.14±0.807	60.16±1.556	72.7±0.660	68.84±0.493	61.5±0.927	74.24±1.196	68.38±1.263

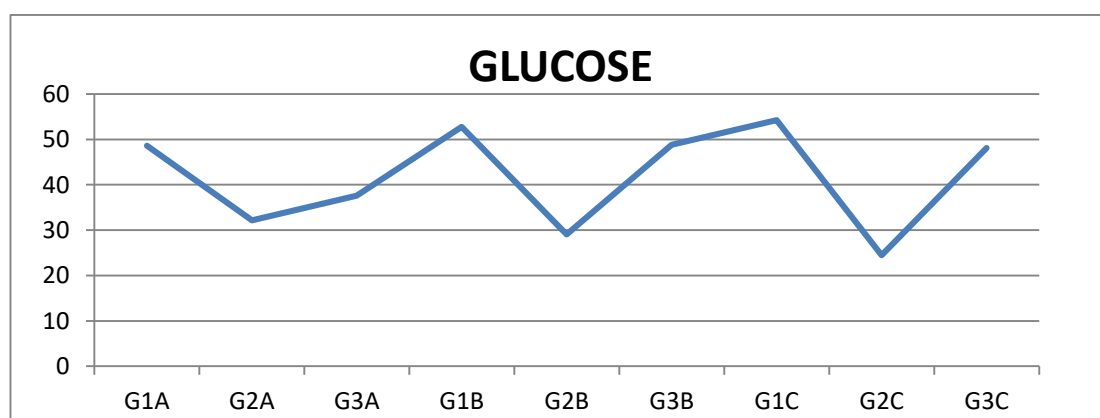
Graph 2: Biochemical parameters of all experimental subgroups.**Table 3.1 : Total Protein of all subgroups of *Cirrhinus mrigala*.**

S.NO.	SUBGROUPS	TOTAL PROTEIN
-------	-----------	---------------

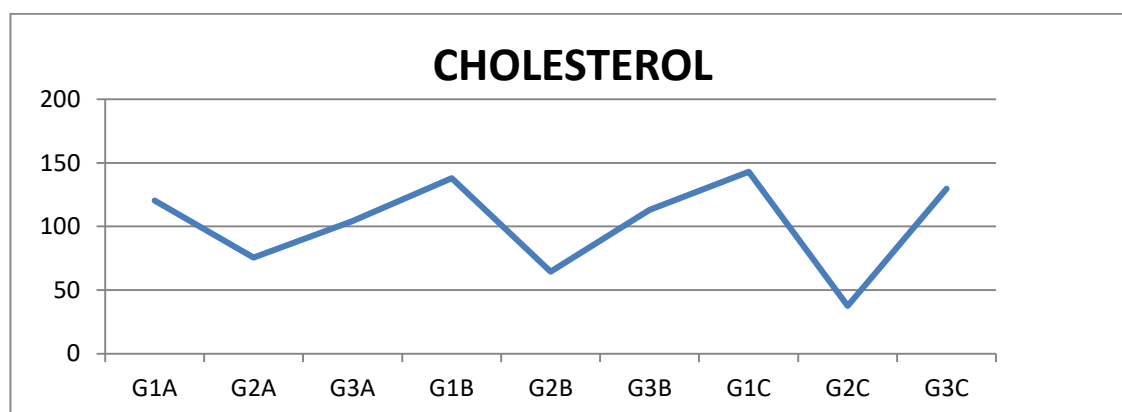
1.	G1A	4.22±0.118
2.	G2A	3.4±0.109
3.	G3A	4.06±0.118
4.	G1B	4.46±0.148
5.	G2B	2.7±0.268
6.	G3B	3.88±0.774
7.	G1C	4.6±0.141
8.	G2C	1.9±0.141
9.	G3C	4.08±0.178

Graph 3.1 : Total Protein of all subgroups of *Cirrhinus mrigala*.Table 3.2 : Glucose of all subgroups of *Cirrhinus mrigala*.

S.NO.	SUBGROUPS	GLUCOSE
1.	G1A	48.61±0.774
2.	G2A	32.16±0.331
3.	G3A	37.56±0.293
4.	G1B	52.74±0.69
5.	G2B	29.06±0.752
6.	G3B	48.84±1.150
7.	G1C	54.26±0.649
8.	G2C	24.46±0.444
9.	G3C	48.12±0.366

Graph 3.2 : Glucose of all subgroups of *Cirrhinus mrigala*.Table 3.3 : Cholesterol of all subgroups of *Cirrhinus mrigala*.

S.NO.	SUBGROUPS	CHOLESTEROL
1.	G1A	120.48±0.701
2.	G2A	75.58±1.774
3.	G3A	104.36±1.030
4.	G1B	138.04±0.189
5.	G2B	64.42±1.165
6.	G3B	113.18±3.107
7.	G1C	143±0.629
8.	G2C	37.6±0.509
9.	G3C	129.72±0.646

Graph 3.3 : Cholesterol of all subgroups of *Cirrhinus mrigala*.Table 3.4: AST of all subgroups of *Cirrhinus mrigala*.

S.NO	SUBGROUPS	AST
1.	G1A	103.52±1.115
2.	G2A	129.44±0.726
3.	G3A	118.14±1.346
4.	G1B	112.68±2.731
5.	G2B	147.36±2.494
6.	G3B	134.32±1.556
7.	G1C	108.96± 2.134
8.	G2C	153.42±0.813
9.	G3C	127.42±0.732

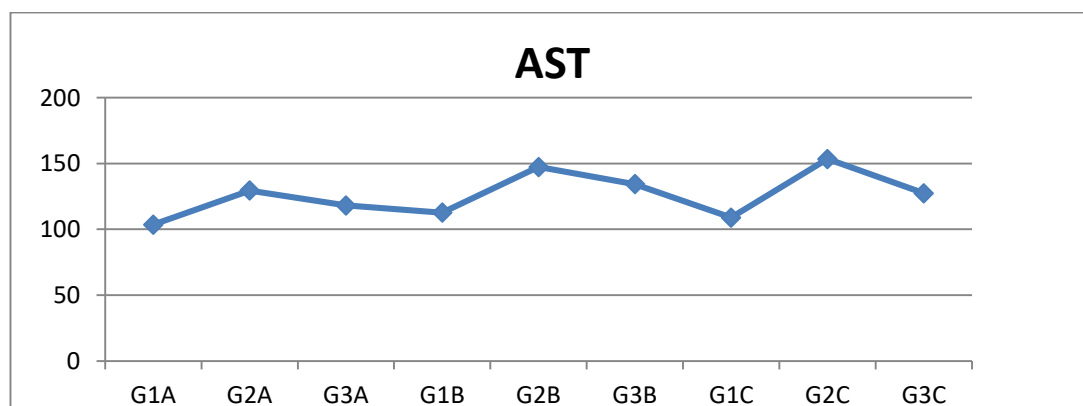
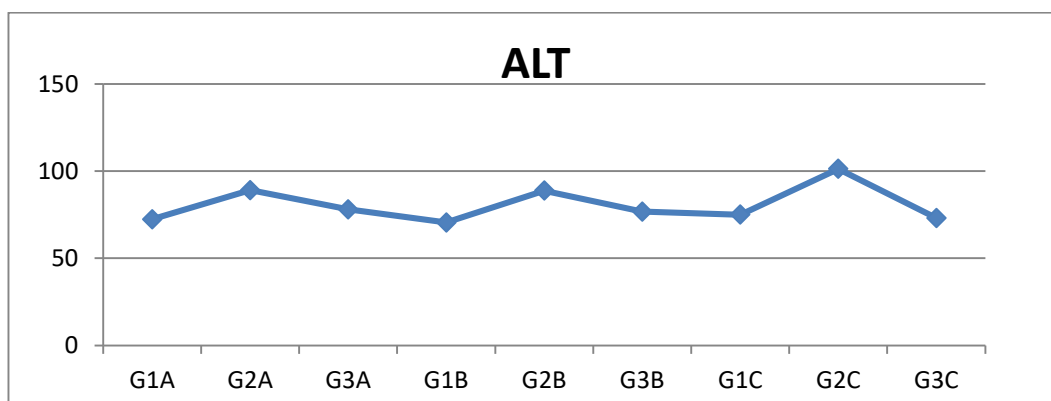
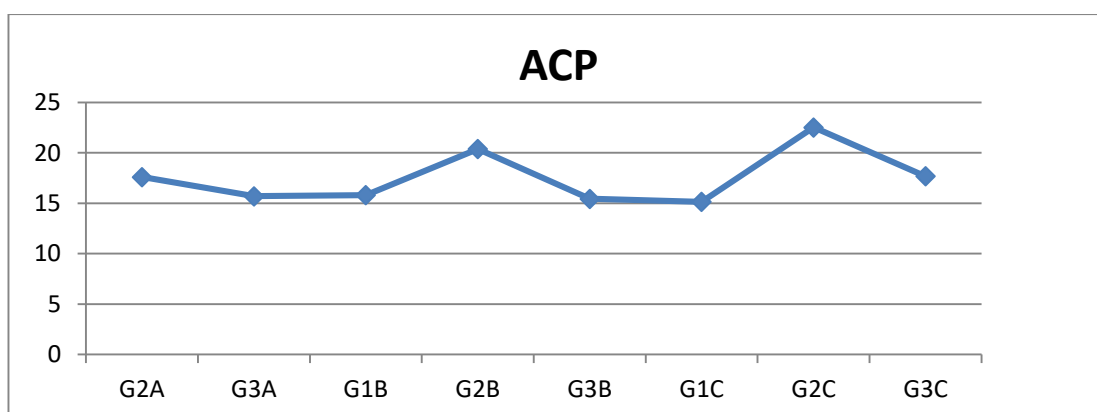
Graph 3.4: AST of all subgroups of *Cirrhinus mrigala*.

Table 3.4 : ALT of all subgroups of Cirrhinus mrigala.

S.NO.	SUBGROUPS	ALT
1.	G1A	72.28±1.689
2.	G2A	89.06±0.549
3.	G3A	78.02±1.458
4.	G1B	70.48 ±1.309
5.	G2B	88.78±0.985
6.	G3B	76.76±0.635
7.	G1C	74.94±0.689
8.	G2C	101.2±1.811
9.	G3C	73.02±0.331

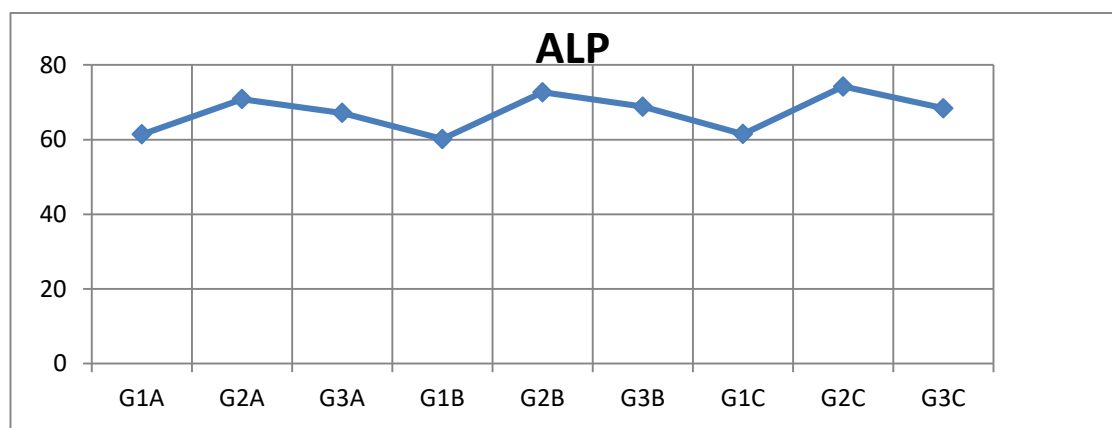
Graph 3.4 : ALT of all subgroups of Cirrhinus mrigala.**Table 3.5 : ACP of all subgroups of Cirrhinus mrigala.**

S.NO	SUBGROUPS	ACP
1.	G1A	14.6±0.384
2.	G2A	17.6±0.593
3.	G3A	15.7±0.209
4.	G1B	15.8 ±0.384
5.	G2B	20.38±1.468
6.	G3B	15.44±0.663
7.	G1C	15.14±0.428
8.	G2C	22.52±0.768
9.	G3C	17.68±0.927

Graph 3.5 : ACP of all subgroups of Cirrhinus mrigala.**Table 3.6 : ALP of all subgroups of Cirrhinus mrigala.**

S.NO	SUBGROUPS	ALP
1.	G1A	61.42±0.885
2.	G2A	70.84±0.428
3.	G3A	67.14±0.807
4.	G1B	60.16 ±1.556
5.	G2B	72.7±0.660
6.	G3B	68.84±0.493
7.	G1C	61.5±0.927
8.	G2C	74.24±1.196
9.	G3C	68.38±1.263

Graph 3.6 : ALP of all subgroups of *Cirrhinus mrigala*.



4. Result

Study of Biochemical Parameters of *Cirrhinus mrigala* after infected with *Aeromonas hydrophila*:

After the 20 days of infection of *Aeromonas hydrophila* with 0.1ml concentration of 10^8 CFU/ml compare with control group to *Cirrhinus mrigala* Group G1A & G2A [table 1.1] a significant decrease [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G1A & G2A [table 1.1] were 4.22 ± 0.118 , 3.4 ± 0.109 ; 48.61 ± 0.774 , 32.16 ± 0.331 ; 120.48 ± 0.701 , 75.58 ± 1.774 respectively. A significant increase [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP. The value of AST, ALT, ACP, & ALP in group G1A & G2A [table 1.1] were 103.52 ± 1.115 , 129.44 ± 0.726 ; 72.28 ± 1.689 , 89.06 ± 0.549 ; 14.6 ± 0.384 , 17.6 ± 0.593 ; 61.42 ± 0.885 , 70.84 ± 0.428 respectively.

After the 20 days of treated group with *Curcuma longa* and 0.1×10^8 CFU/ml *Aeromonas hydrophila* compare with infected group to *Cirrhinus mrigala* G2A & G3A [table 1.4] a significant increase [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G2A & G3A [table 1.4] were 3.4 ± 0.109 , 4.06 ± 0.118 ; 32.16 ± 0.331 , 37.56 ± 0.293 ; 75.58 ± 1.774 , 104.36 ± 1.030 respectively. A significant decrease [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP. The value of AST, ALT, ACP, & ALP in group G2A & G3A [table 1.4] were 129.44 ± 0.726 , 118.14 ± 1.346 ; 89.06 ± 0.549 , 78.02 ± 1.458 ; 17.6 ± 0.593 , 15.7 ± 0.209 ; 70.84 ± 0.428 , 67.14 ± 0.807 respectively.

After the 40 days of infection of *Aeromonas hydrophila* with 0.1ml concentration of 10^8 CFU/ml compare with control group to *Cirrhinus mrigala* Group G1B & G2B [table 1.2] a significant decrease [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G1B & G2B [table 1.2] were 4.46 ± 0.148 , 2.78 ± 0.268 ; 52.74 ± 0.69 , 29.06 ± 0.752 ; 138.04 ± 0.189 , 64.42 ± 1.165 respectively. A significant increase [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP. The value of AST, ALT, ACP, & ALP in group G1B & G2B [table 1.2] were 112.68 ± 2.731 , 147.36 ± 2.494 ; 70.48 ± 1.309 , 88.78 ± 0.985 ; 15.8 ± 0.384 , 20.38 ± 1.468 ; 60.16 ± 1.556 , 72.7 ± 0.660 respectively.

After the 40 days of treated group with *Curcuma longa* and 0.1×10^8 CFU/ml *Aeromonas hydrophila* compare with infected group to *Cirrhinus mrigala* G2B & G3B [table 1.5] a significant increase [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G2B & G3B [table 1.5] were 2.7 ± 0.268 , 3.88 ± 0.774 ; 29.06 ± 0.752 , 48.84 ± 1.150 ; 64.42 ± 1.165 , 113.18 ± 3.107 respectively. A significant decrease [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP.

The value of AST, ALT, ACP, & ALP in group G2B & G3B [table 1.5] were 147.36 ± 2.494 , 134.32 ± 1.556 ; 88.78 ± 0.985 , 76.76 ± 0.635 ; 20.38 ± 1.468 , 15.44 ± 0.663 ; 72.7 ± 0.660 , 68.84 ± 0.493 respectively.

After the 60 days of infection of *Aeromonas hydrophila* with 0.1ml concentration of 10^8 CFU/ml compare with control group to *Cirrhinus mrigala* Group G1C & G2C [table 1.3] a significant decrease [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G1C & G2C [table 1.3] were 4.68 ± 0.141 , 1.9 ± 0.141 ; 54.26 ± 0.649 , 24.46 ± 0.444 ; 143 ± 0.629 , 37.6 ± 0.509 respectively. A significant increase [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP. The value of AST, ALT, ACP, & ALP in group G1C & G2C [table 1.3] were 108.96 ± 2.134 , 153.42 ± 0.813 ; 74.94 ± 0.689 , 101.2 ± 1.811 ; 15.14 ± 0.428 , 22.52 ± 0.768 ; 61.5 ± 0.927 , 74.24 ± 1.196 respectively.

After the 60 days of treated group with *Curcuma longa* and 0.1×10^8 CFU/ml *Aeromonas hydrophila* compare with infected group to *Cirrhinus mrigala* G2C & G3C [table 1.6] a significant increase [$t > 2.26$ at $p = 0.05$] occurred in total protein, glucose and cholesterol. The value of total protein, glucose, cholesterol in group G2C & G3C [table 1.6] were 1.9 ± 0.141 , 4.08 ± 0.178 ; 24.46 ± 0.444 , 48.12 ± 0.366 ; 37.6 ± 0.509 , 129.72 ± 0.646 respectively. A significant decrease [$t > 2.26$ at $p = 0.05$] occurred in AST, ALT, ACP & ALP. The value of AST, ALT, ACP, & ALP in group G2C & G3C [table 1.6] were 153.42 ± 0.813 , 127.42 ± 0.730 ; 101.2 ± 1.811 , 73.02 ± 0.331 ; 22.52 ± 0.768 , 17.68 ± 0.927 ; 74.24 ± 1.196 , 68.38 ± 1.263 respectively.

5. Discussion

Total protein, glucose and cholesterol value were estimated. A significant changes were observed in total protein, glucose and cholesterol of *Cirrhinus mrigala* due to infection of *Aeromonas hydrophila* and treated with *Curcuma longa*. The value of total protein, glucose, and cholesterol were decrease due to infection with *A. hydrophila* and increase due to treated with *C. longa* that shown in table 2. Decrease value of cholesterol increase the skin haemorrhages and lesion morbidity it can lead to decreased growth and feed utilization. Low level of cholesterol aggravated the inflammation response. CHL is the steroid which required to formed cell membrane and hormone. 80% CHL is produced by liver and transported to different parts in the body. Decrease protein level can be caused by liver or kidney disease. Decrease level of protein lead to fluid leaking into tissue, reduce muscle mass and energy level, and also causing swelling. Decrease value of glucose causes difficulty in concentrating, dizziness, anxiety, irritability, weakness, trembling and loss of consciousness. *Aeromonas hydrophila* altered ACP and ALP activities. Acid Phosphatase enzyme hydrolysis of phosphate esters at an acidic pH. Its increased level is associate with the variety of hepatic conditions, disorders of bone and cellular leakage while decreased level is associate with several diseases like wilson's disease, aplastic anemia and pernicious anemia. Alkaline phosphatase enzyme hydrolysis of phosphate at alkaline pH. And plays role in membrane transport and also it is a good bio-indicator of stress. A significant increase in the value of ALP indicates disorder in tissue and cell membrane of fish. AST, and ALT enzymes were estimated. Significant changes were observed in enzyme levels of *C. mrigala* fish. ALT, and AST enzymes involved in amino acids metabolism and an increase in these enzymes indicate tissue damage, toxic effects in liver, cellular damage and increased permeability of plasma membrane.

6. Conclusion

This study suggests that freshwater fishes could always be under the threat of *Aeromonas* infection, since the species are common in freshwater environments. *Aeromonas* spp. might also pose a threat to public health. The study clearly shows that typical biochemical alterations seen in the blood of *C. mrigala*. We hypothesized that the bacterium could be major pathogens of carp farming. However, further studies are needed to demonstrate the molecular and biochemical characteristic of the organism. In addition, identification of virulence factors of *A. hydrophila* from carp also needs to be studied. An infection of *Aeromonas hydrophila* ultimately lead to the mortality of fishes. Therefore, the scope of this study is the proper management require of water quality and food health and the preventing and controlling *A. hydrophila* infection in fishes and avoiding its transmission to humans.

7. Acknowledgement :

I would like to thank Maharishi Dayanand Saraswati University, Ajmer for granting the Ph.D research work. The Department of Zoology, Prithviraj Chouhan Government College, Ajmer of the M D S University is highly appreciated for allowing the laboratory work. I am also grateful to the Microbiology Department of J L N Medical College Ajmer for laboratory work and also thank to Dr.Sabiha Khan from Department of Zoology S P C Government College Ajmer for her guidance in research.

Author Contributions

Dr. Sabiha Khan : Gave her guidance in paper.

Sonia Patharia : Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from commercial and financial funding agencies.

Data availability statement

Data included in article/ supplementary material/ referenced in article.

Informed consent statement

This study did not involve human participants therefore informed consent was not required.

Clinical trial registration

This research does not involve any clinical trial.

Permission to reproduce material from other sources

Not applicable.

Ethics statement

Not required.

Additional information

No additional information is available for this paper.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abbott S. L., Janda J. M. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin. Microbiol. 2010; Rev. 23(1): 35-73. Doi: 10.1128/CMR.00039-09.
2. Beach. E. F., Truner J. J. Enzymetic method for Glucose determination in body fluids. Clinical Chemistry. 1958; 4 (6): 462-475. PMID: 13608908.
3. Flack C. P., Woollen J. W. Biuret method for Total Protein. Clin Chem. 1984; 30(4): 559-561. PMID: 6200256
4. Grrifin M. J. et al. Quantitative detection of *Aeromonas hydrophila* strains associated with disease outbreaks in catfish aquaculture. 2013; 25(4) 473-481. Doi: 10. 1177/1040638713494210.
5. Ganeshwade R. M. Effect of dimethoate on the level of cholesterol in fresh water fish, *Puntius ticto* [Ham]. Sci. Res. Rep. 2012; 2(1): 26-29. ISSN: 2249-2321.
6. IFCC Scientific Committee. Method for the measurement of catalytic concentration of enzymes. Clin. Chem. Biochem., 1980; 18(8):521-534. ISSN: 0939-4974.
7. Khan S., Sharma N. A. Study on Enzymes Acid Phosphatase and Alkaline Phosphatase in the Liver & Kidney of fish *Gambusia affinis* Exposed to the Chlorpyrifos, An Organophosphate, International Journal of Pharmaceutical Sciences Review and Research, 2012; 13(1): 88-90.
8. Kinetic method for quantitative determination of acid phosphatase and prostatic phosphatase (P ACP) activity [EC 3.1.2] in human serum. www.biolabo.fr, Version: AT 82560 25 09 2008.
9. Kinetic method for quantitative determination of alkaline phosphatase activity [EC 3.1.3.1] in human serum and plasma. www.biolabo.fr, Version: AT 92214 11 07 2008.
10. Prasad S. Aggarwal B. B. Turmeric the golden spice from traditional medicine to modern medicine. 2011; Pages 263-288 in I. F. F Banzie and S. Wachtel-Galor editors. Herbal medicine: Biomolecular and Clinical aspects, 2nd edition CRC Press, Taylor and Francis group, Boca Raton, Florida, USA. PMID: 22593922, Bookshelf ID: NBK92752.
11. Ruby A. J., Kuttan G. Babu K. D. Rajasekharan K. N. Anti-tumour and Anti-oxident activity of natural curcuminoides. Cancer letters. 1995; 94:79-83. Doi: 10.1016/0304-3835(95)03827-j
12. Roeschlau P., Bernt E. Gruber F. Cholesterol estimation. Clin. Chem. Clin. Biochem. 1974; 12(5): 226. PMID: 4440114.