Thesis for the Degree of Master of Fisheries Science

The correlation between histopathological findings of liver and blood biochemical parameters in black rock fish, *Sebastes schlegeli*

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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조피볼락(*Sebastes schlegeli*) 에서 간장의 조직병리학적소견과 혈액학적 분석결과와의 상관관계에 대한 고찰

Advisor: Prof. Min Do Huh

by

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Abstract

This study was carred out to examine the correlation among the blood biochemical parameters and the histological health in Korean black rock fish *(Sebastes schlegeli)*.Fish mixed sex averaging 103.01±2.79g (mean ± SD) obtained from the farm in Tong-young city in Kyonganm province of korea, and acclimated for one week.Then, fish were separated into three groups(normal liver , abnormal(hypertrophic and fatty liver) and atrophic liver). Glass aquariums with external biofilters and artificial heaters were used. Fish were euthanized,blood samples were taken from each fish, blood were analyzed and measured some parameters such as Ht, Hb, TP, GOT and GPT. Fish were dissected at three time intervals. Tissues were fixed, re-fixed, processed, embedded with paraffin, sectioned and stained with hematoxylin& eosin for light microcopy. Morphological changes obsereved occurred were explained semi-quantitatively using indices. Significant

differences ($p<0.05$) of indices were found between different liver alterations groups. Livers with normal yellow color were observed from normal livers and atrophic liver groups, respectively. and whitish-yellow color were observed from hypertrophic and fatty liver. Normal hepatocytes with circular, conspicuous and centrally located nuclei were found from normal livers group. Hypertrophic and fatty change were observed with large size hepatocytes and nuclei dislocated to the periphery of the cell by large sized lipid vacuoles. Atrophic hepatocytes were found with minute lipid vacuoles and inconspicuous nuclei. LRI and HSI indices were shown significant different between different liver alterations and giver real fact about health status for fish, while GOT were given opposite result in some liver alterations that didn't give us the real information about health fish. Hence, the results in this study suggested that there was no obvious correlation between blood biochemical parameters and histological health of liver.

Key words: *Sebastes schlegeli,* Liver, Histology, blood biochemical.

Introduction

The Korean black rockfish (*Sebastes schlegeli*) is a mariculture fish belonging to the family of the *Scorpaenidae*. It is distributed along the coasts of Japan,Korea,and China (George and Boehlert, 1991).

This species is one of the most valuable species in the Korean aquaculture industry. Culture system for this species has expanded rapidly since 1987 with a production reaching 40.875 tons in 2012, amounting to 30.2% of the total finfish mariculture production in Korea (MIFAFF, 2012). On the otherhand, farming technique for this species has been rapidly developed since the last decade, and its production is the second one next to flounder *(Paralichthys olivaceus*),that known as the highest among marine fish aquaculture in Korea(Lee *et al*., 2000).

In general, this species is of great interest for fish culture as it is has different desirable qualities as:

1. Good tolerance to low water temperature.
2. Ease of seeding production due to the viviparous reproduction style

Korean rock fish with high economic value, are asked for a high amount in sea fishing, owing to the great value of Korean rock fish both in economic and academic .

The fish liver must be considered a target organ for many biological and environmental parameters that can alter liver structure and metabolism,food pollutants,toxins,parasites and microorganisms (Munshi and Hiran., 1996).

Indeed, fish blood is widely used in toxicological research and environmental monitoring as a promising indicator of physiological and pathological change (Mulcahy, 1975).Moreover, haematological and bio-chemical parameters are used as health indicators to detect the structural and functional status of fish understressful conditions (Saravanan *et al*., 2010). The evaluation of biochemical andhistological changes in fish liver has become an importanttool for monitoring environmental exposure of fish to contaminantsin experimental studies(Matus *et al*., 2007).Ingeneral ,measure of blood biochemistry parameters is commonly used diagnostic tool in biomonitoring according to (Coz-Rakovac *et al*., 2008), it presents good indicator of the artificial feeding effects. Inorder to document and better understand variability and dynamics of blood chemistry parameters(J.Ferri *et al*., 2011).

While the advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization, histological changes appear as a medium term response to sub-lethal

stressors,and histology provides a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Berent *el at* ., 1999).

The liver appears, similar as to the liver of other vertebrates, as a key organ which controls many life functions and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates), catabolism (nitrogen, glycogenolysis, detoxification), and important role in vitellogenesis (Munshi and Hiran., 1996).

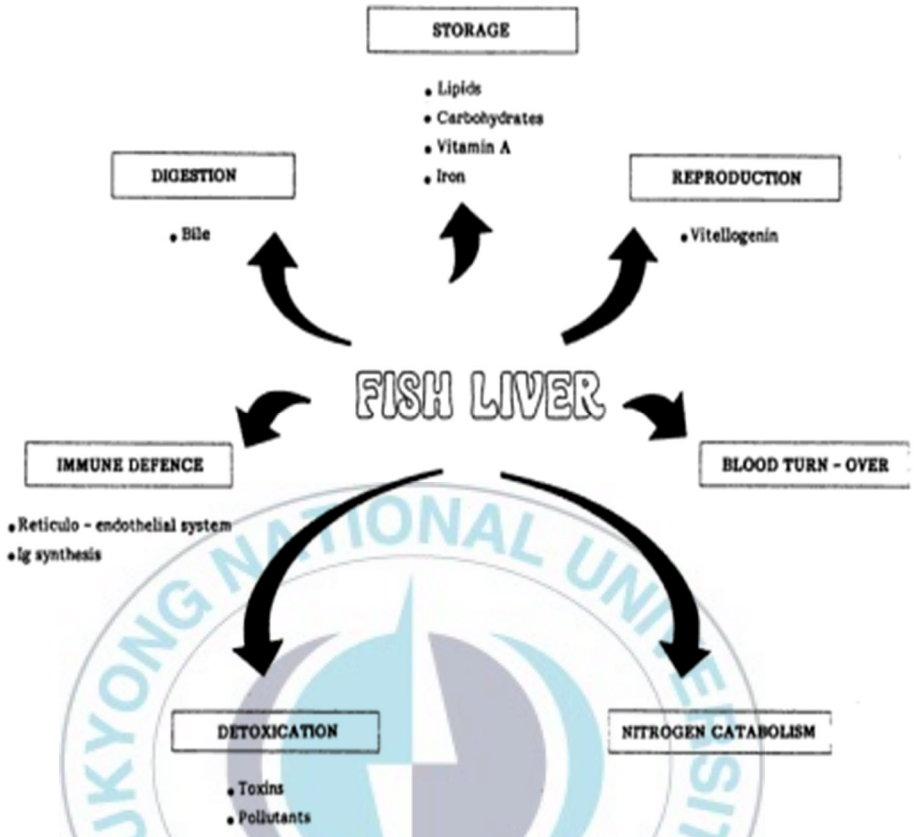

Fig. 1. Key functions of the fish liver (Munshi and Hiran, 1996).

It is a dense organ ventrally located in the cranial region of the general cavity, itscolour is generally reddish-brown because of its rich vascular-ization,tending towards yellow when fat storage is high (Ronald and Roberts, 2012).

Liver of yellow colour has been seen in *Anguilla anguilla*,*Dicentrarchus labrax,*and*Sparus aurata*(Bai *et al*., 1983), fed on artificial food responsible for lipid accumulation (Munshi and Hiran , 1996).

Studies of fish liver related to data obtained from field experiments mostly concern its functions in the detoxification of xenobiotic, specific responses to planar compound its susceptibility to cellular lesions visible for morphological examinations(Andrew and Hemingway, 2003).

Objectives

The main objective of this study is to know whether the correlation between some blood biochemical parameters and the histological health of the liver of Korean black rockfish (*Sebastes schlegeli*) would exist or not.

The specific objectives include:

1. To describe liver morphological alterations histologically.
2. Also to quantify liver structural alterations using semi quantitive methods.
3. To determine hepatosomatic index (HSI) ,and
4. To determine blood biochemical parameters GOT(glutamate oxaloacetate transaminase),GPT(glutamate pyruvate transaminase) and TP(total protein).

Materials and Methods

The study was carried out at Laboratory of Fish and Shellfish Pathology (LFSP), Department of Aquatic Life Medicine, Pukyong National University, Korea. The whole study had two major components namely, blood biochemical analysis and histopathological investigation.

Fish Source

Mixed sex population of *Sebastes schlegeli*, with average 103.01±2.79g, were obtained from a farm in Tong-young area, South Korea.

Acclimation

The rest 45 fish were acclimated to the experimental condition for about two week after arrival.

Aquarium Set Up

A recirculating system which comprises three170 liter glass aquariums was used. External biofilters (PhilGreen Ef-1300, China) was used to

recycle the water. Water temperature was maintained and monitored by submerged water heaters and thermometers. Twin water proof compact pH meter (B-212, Horiba, Japan) and Dissolved Oxygen meter (Oxyguard, Denmark) were used to measure pH and dissolved oxygen, respectively. Water was replaced one timeper two day for the whole period. Intensively aerated water at a temperature of 21±1 °C was used for replacement.

Scoop net was used for mechanical removal of faecal matter immediately after release. Regular cleaning of biofilters and checkups were done to prevent clogging and to maintain filtering efficiency. All optimum water quality requirements of *S.schlegeli* were maintained, besides, 12D and 12L hours were kept. Water temperature of aquariums was maintained at 21±1°C .Dissolved oxygen was 5-7 mg/l and pH was within the range of 7.2- 7.5.

Histological Methods

Dissection

Benzocaine was used at over dose concentration to euthanize the fish before dissection. Body weight and length measurements were taken prior to dissection. Dissection was done using scissors and forceps. Individual livers were weighed and images of internal organs were taken using digital camera(Olympus E-P2-Japan) during dissection. Blood samples were taken immediately after euthanizing the fish.

Fixation and Re-fixation

Subsequently after dissection, liver tissue samples were fixed in Bouin's solution. After 24 hours samples were cut into suitable pieces with sharp blade and put in labelled cassettes after being categorized as organ and individuals. Then, these samples were re-fixed in 10% buffered formalin solution.

Tissue processing

All fixed tissues were passed through a series of solvents before finally being embedded fully with paraffin wax. Tissues were washed and

dehydrated through ethyl alcoholic grades (70%, 80%, 90%, 95%, 100%, 100%, and 100%) and cleaned in xylene.

Embedding

Tissues were embedded with paraffin wax at 58- 62 °C.

Sectioning

A portion of embedded blocks of each tissue sample were sliced into 5micro meter sections using a rotary type microtome (Reichert –Jung 820, Leica,Germany). The sectioned ribbon was floated on warm water bath (540C) to flatten out the section. The sections were carefully collected on to a glass slide and allowed to dry fully before proceeding to H&E staining.

Staining

Hematoxylin and eosin (H&E) staining method was followed to stain the tissue sections. The following consecutive steps were followed to perform:

1. Xylene (1)-3minutes
2. Xylene (2) - 3 minutes

3. Xylene (3) -3 minutes
4. Ethyl alcohol100% - 1 minute
5. Ethyl alcohol95%- 1 minute
6. Ethyl alcohol 90%- 1 minute
7. Ethyl alcohol 80%- 1 minute
8. Ethyl alcohol70%-1minute

   Washing with flowing tap water -10 minutes
9. Hematoxylin -3 minutes
10. Washing with tap water – 1minute
11. HCL (Acid alcohol)- 2 times dipping
12. Washing with tap water – 1minute
13. Ammonia water – 4 times dipping
14. Washing with flowing tap water – 15 minutes
15. Eosin- 2 minutes
16. Ethyl alcohol70-80% - 4 times dipping
17. Ethyl alcohol 90%- 1 minute
18. Ethyl alcohol 95%- 1minute
19. Ethyl alcohol100%- 1minute
20. Ethyl alcohol100%-1minute

21. Xylene +Alcohol – 3minutes
22. Xylene (1) - 3 minutes
23. Xylene (2) -3 minutes
24. Xylene (3) - 3minutes

Mounting

The stained samples were mounted with malinol for permanent preservation.

Photography

Histological examination of liver structures was done using light microscope (U-MDOB, Olympus optical Co. Ltd., Japan). Images of the prepared slides of different organs were taken using the DP2-BSW (Olympus, Japan)software.

Hematological analysis

Blood sample and hematological assay

Blood samples were taken from each fish by puncturing the caudal vessel using heparinized syringes, haematocrit (Ht) was determined by the micro-Ht techniqueusing capillary tubes and centrifugation at (1500rpm)for5min. Heamoglobin (Hb) determined by spectrophtometrically (540 nm) using the cyanomethemoglobin method.

Serum chemistry analysis

Serum samples were analysed for total protein by the Biuret and Greenberg method.

Serum enzyme activity

Estimation of activities of GOT& GPT was done following Reitman and Frankel's method (1957).

Histological analysis

Hepatosomatic Index (HSI) Determination

The HSI was determined(HTUN-HAN, 1978;Fasil *et al.*, 2014)as:

$$\text{Hepatosomatic Index (HSI)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

Hepatohypertrophic Index (HHI) Determination

The HHI was calculated using the following formula as it described in (LEE, 2008;Fasil *et al.*, 2014). It has been used so far for histopathological health evaluation of fish and shellfish.

$$\text{Hepatohypertrophic Index (HHI)} = \frac{1}{\log(n)}$$

Where:

n = number of nucleus in 1000μm^2 of hepatic tissue

Determination of Liver Reaction Index (LRI)

Captured images of prepared slides were noticed and examined on Image-pro plus software version 6.0(Media Cybernetics, Inc., USA),

descriptions liver alterations were identified and described by (Bernet,1999;Hadi and Alwan, 2012;Takashima and Hibiya, 1995).

Histopathological liver alterations were evaluated semiquantitatively using a modifiedversion of the protocol described by (Bernet, 1999).

Liver alterations were described and termed into four alterations depends on(Bernet, 1999;Fasil *et al*., 2014).

Table1.Description of liver alterations (Bernet, 1999;Fasil *et al* ., 2014).

Alteration	**Description**
Plasma alterations	Change in cellular plasma caused by degenerative fatty vacuolization, granular degeneration, glycogen degeneration, deposits and intracellular accumulation of substances caused by degenerative process
Nuclear alterations	Changes in the nuclear shape and structure
Atrophy	Reduction in number or volume of cells and/or decreasing of intracellular substances
Hypertrophy	Enlargement of cell volume or tissue

Histological evaluation:

Importance factor (w)

The relevance of a lesion depends on its pathological importance, i.e. how it affects organ function and the ability of the fish to survive. This is taken into account by an importance factor assigned to every alteration listed in the histological description.

The alterations are classified into three importance factors according this table(Bernet, 1999).

Table2.Importance factor description modified from(Bernet, 1999;Fasil *et al* ., 2014)

Value	Description
1	Easily reversible- if exposure to stressor (overfeeding) ends
2	Moderately reversible- reasonably changed cell and assumed to take longer time to regenerate than the easily reversible
3*	Reversible- severely altered cell and will take longer time to regenerate than the other types

* This importance factor was not used in this study.

Table3.Assigned importance factor (w) for selected alterations(Bernet, 1999;Fasil *et al*., 2014)

Importance factor (w)	Alterations
1	Plasma alterations and deposits
2	Nuclear alterations
2	Atrophy
1	Hypertrophy

Score value (a):

Liver alterations are assessed using a score ranging from 0 to 6,depending on the degree and extent of occurrence of the alterations (Table5).

Table4.Score Value Description adopted from(Bernet, 1999;Fasil *et al*., 2014).

Value	Description
0	Unchanged
2	Mild occurrence
4	Moderate occurrence
6	Severe occurrence

Multiplication of importance factor (w)and score value (a) was used to quantify the specific alteration of liver .All four selected liver alterations were quantified for every individual fish (Table4).the sum of multiplication of importance (w) and score value (a) was used to calculate the liver reaction index (LRI) of liver for every individual fish.(Bernet, 1999;Fasil *et al*., 2014).

According to the formula below (Bernet, 1999;Fasil *et al*., 2014)LRI determined as sum of multiplied importance factors and score values of the alterations to assess the quality of liver morphological changes in response to overfeeding.

$$\text{Liver reaction index (LRI)} = \sum_{\text{alt}} (a \times w)$$

Where:

a= score value

w= importance factor

alt= alteration

Data Analysis

Histopathological description of morphological alterations, blood biochemical parameters and statistical results were used to present the research findings. Comparisons were made between liver alterations and blood parameters. One way ANOVA was used on SPSS version 16 (SPSS Inc. Chicago, USA) to detect the significant differences among all groups.

Results

Gross Liver Observation

All normal fish livers appeared normal (Fig. 2. A&B). No external abnormality was observed. Normal livers with yellowcolour were observedwith normal size.

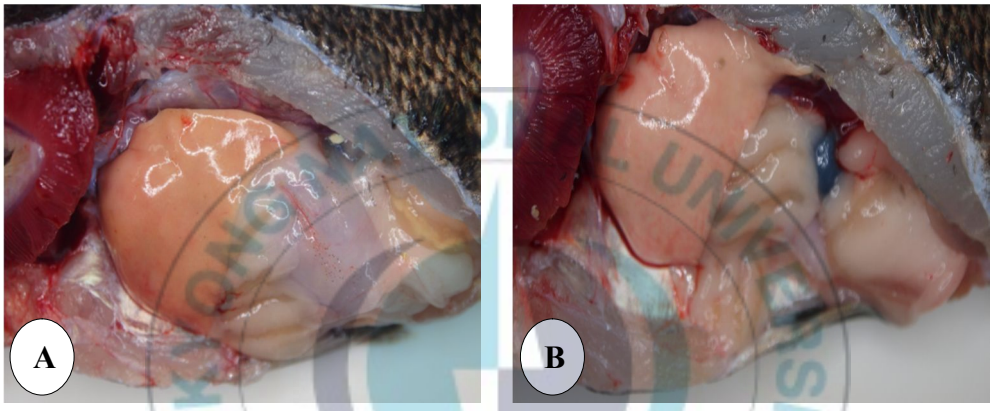

Fig. 2. Gross appearance of normal liver. **A.** Normal yellow colour **B.** Normal size.

While, fish were have large size livers with whitish-yellow colour and sometimes shiny and oily were also seen .those abnormalities were found in different shapes.(Fig. 3.A&B&C).

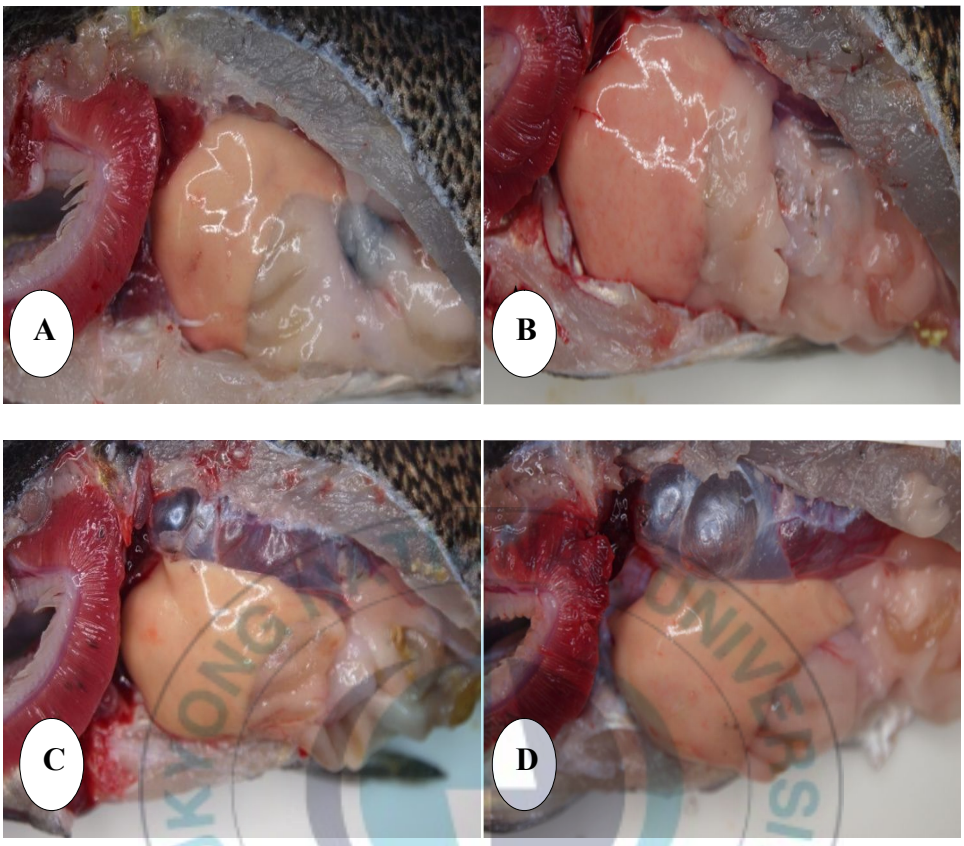

Fig. 3. Gross appearance of abnormal liver. **A and C** Abnormal colour whitish-yellow colour. **B** and **D** Abnormal size of liver (large size).

On otherhand,fish liver were found small size but with same norml color

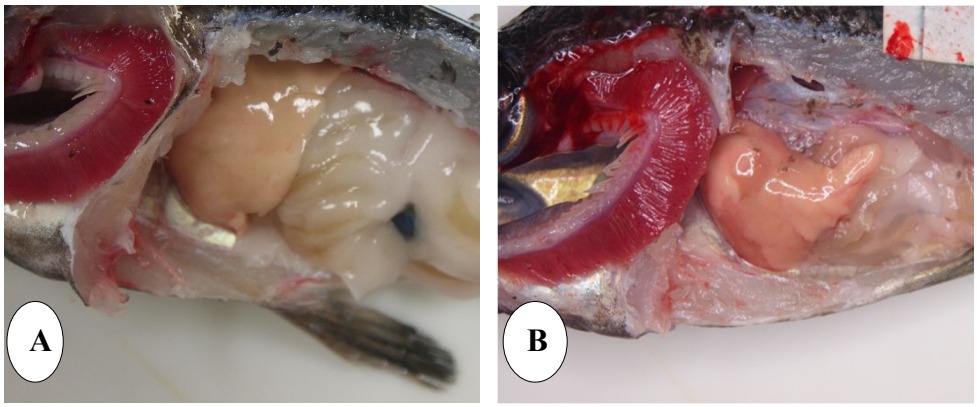

Fig. 4. Gross appearance of atrophic liver.**A** and **B** Normal colour with small size.

Microscopic Liver Observation

Normal regular shaped hepatocytes with conspicuous centrally located nuclei were found from the fish which were having normal characterized (Fig.5.A and B), while increase in size of the hepatocytes (hepatocellular hypertrophy) with nuclei dislocated to cells border(Fig.6.AandB). Moreover, circular lipid vacuoles were also present togetherwith large sized hepatocytes with nuclei located cell periphery(Fig.6.Cand D).Whereas, highly decreased hepatocytes volume and sever hepatocyte vacuolization were found from atrophic liver(Fig.7.AandB).

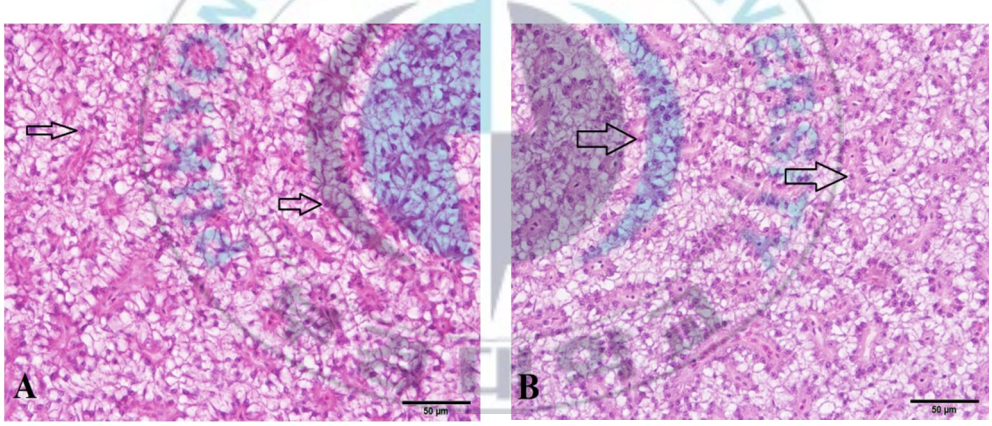

Fig. 5. Microscopic structureof normal liver (**A and B**) .Normal hepatocytes with conspicuous, circular and centrally located nuclei (arrows). (**H&E**; X400; Scale bars - 50 µm).

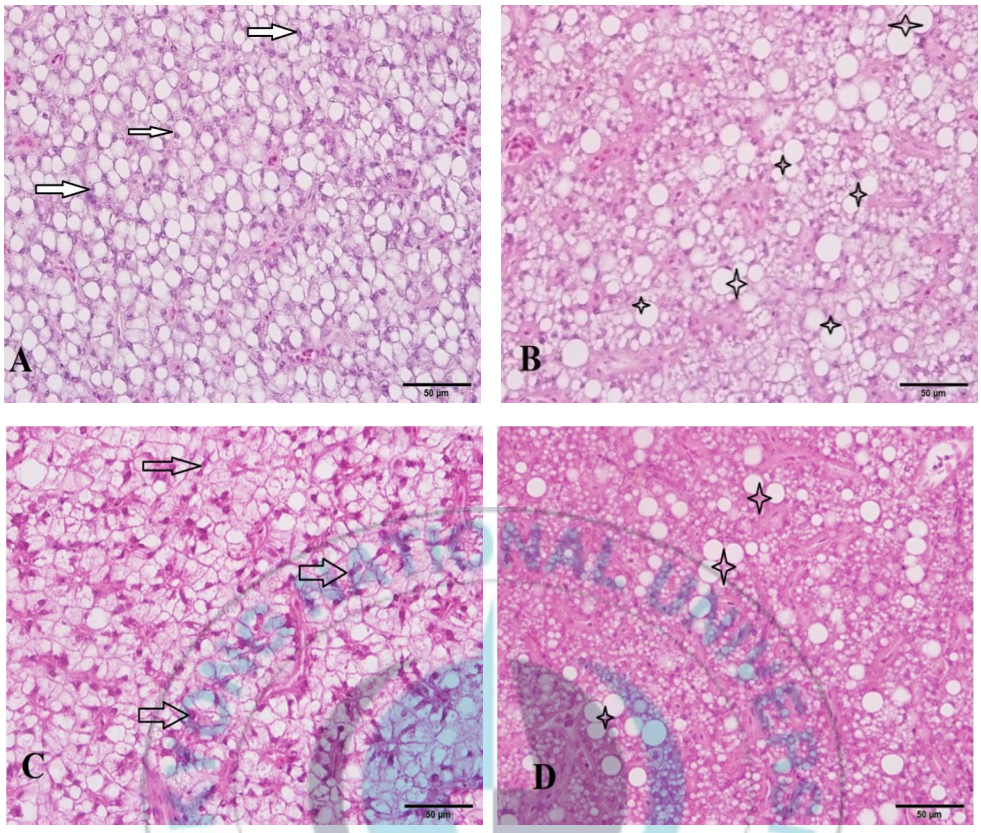

Fig. 6. Hypertrophic and fatty liver A.B.C hepatic microscopic structure. **A**, **B**,**C**&**D. A** and C Large hypertrophic hepatocytes (arrows) with nuclei dislocated to cell periphery (arrows),B and D lipid vacuoles (four point stars). (**H**&**E**; X400; Scale bars - 50µm).

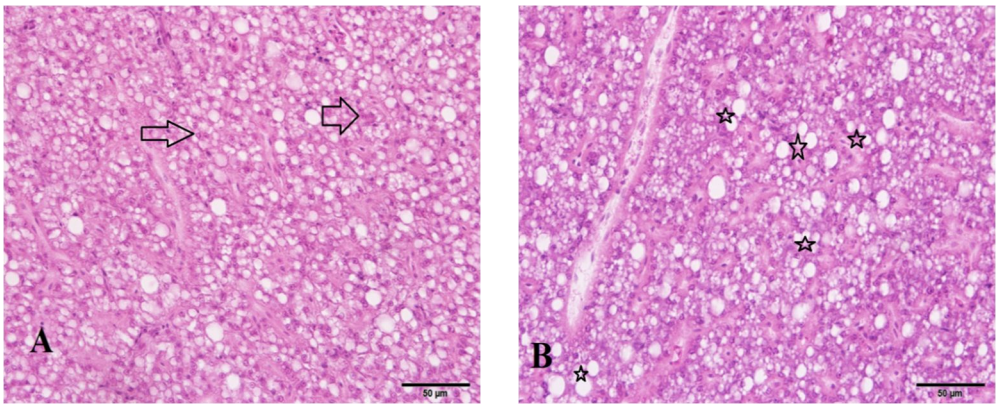

Fig. 7. Microscopic structure of atrophic liver (AandB). Shrunken hepatocytes with inconspicuous dislocated nuclei (arrow) and circular lipid vacuoles(five point stars) (**H&E**; X400; Scale bars - 50μm).

Liver Reaction Index (LRI)

The liver which were found as atrophic status the highest mean liver reaction index (Table 5), while the lowest mean liver reaction index was scored from fish that have normal status. Significant variation ($p<0.05$)was observed between different liver status (Fig. 8.).

Table 5.Mean±SE of liver reaction index (*P<0.05*); mean values with different status are significantly different.

Liver Status	N	Liver Reaction Index
Normal	2	0±0
Hypertrophy	9	6±0
Fatty change A	6	10±0
Fatty change B	11	20±0
Fatty change C	10	27±0
Atrophy	7	30±0

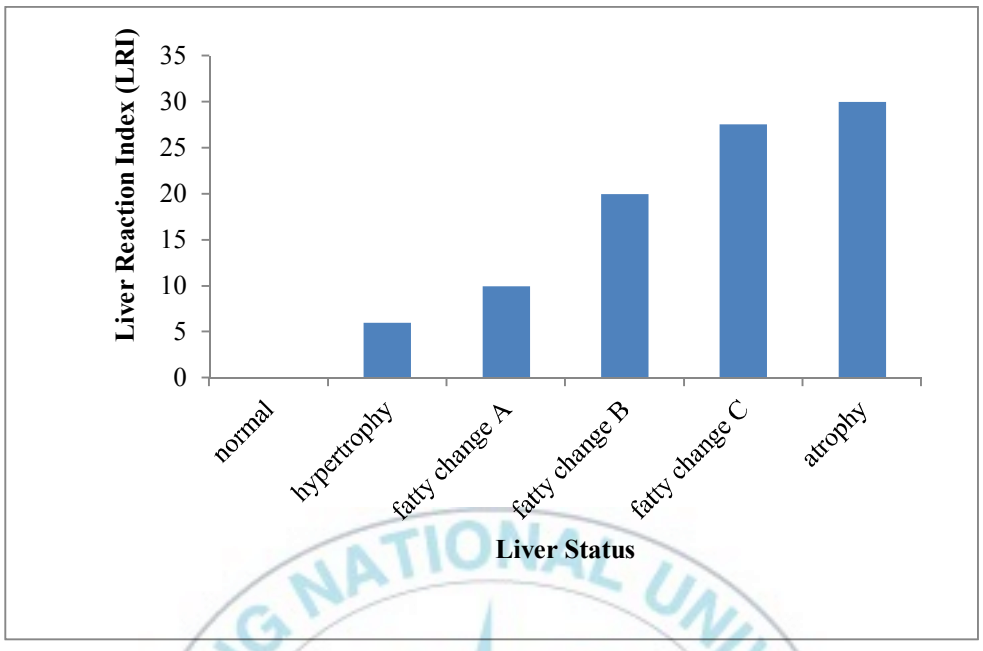

Fig. 8. Mean of Liver reaction index (LRI) of all groups (*P<0.05*).

Hepatosomatic Index (HSI)

The Hepatosomatic index for hypertrophic liver were extraordinarily (Table6) higher than other group. Significant variation (*p<0.05)* between groups were observed between different groups. The mean hepatosomatic index for different liver status (Fig.9).

Table 6. Mean±SE of hepatosomatic index ($P<0.05$); mean values with different letters are significantly different.

Liver status	N	Hepatosomatic index
Normal	2	1.85±0.254[a]
Hypertrophy	9	2.59±.14[d]
Fatty change A	6	2.39±.083[c]
Fatty change B	11	2.11±.11[b]
Fatty change C	10	1.75±.088[a]
Atrophy	7	1.80±.16[a]

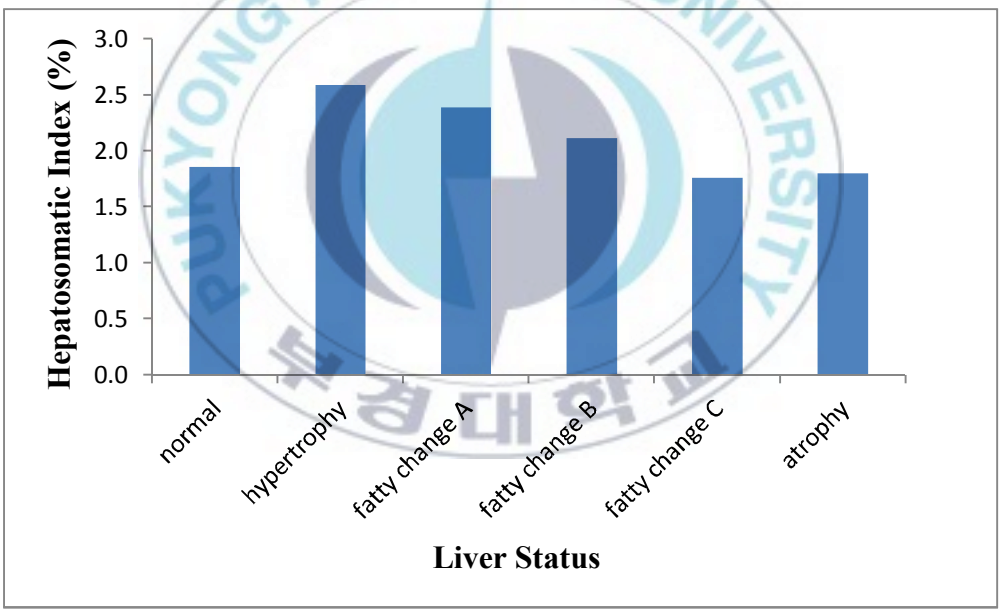

Fig. 9. Men of Hepatosomatic index (HSI) of different groups.

Hepatohypertrophic Index (HHI)

Mean hepatohypertrophic index for fish that have fatty changes B status show higher mean than other status(Fig 10). No significant difference (*p>0.05)*between groups. (Table 7) show mean values of HHI.

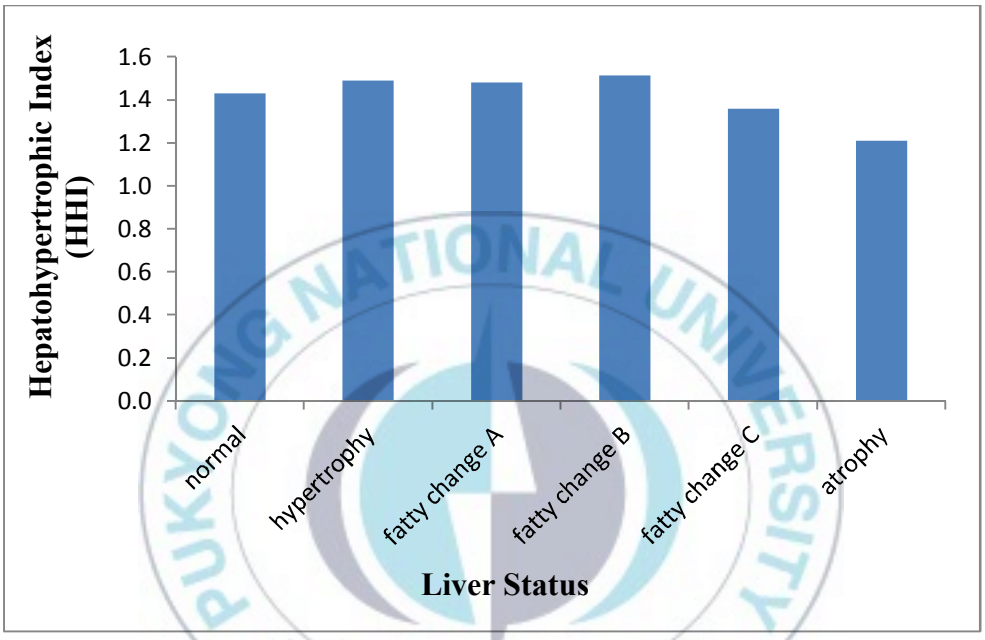

Fig. 10. Mean of Hepatohypertrophic index (HHI) of all groups (P>*0.05*).

Table 7. Mean±SE of hepatohypertrophic index (*P>0.05*).

Liver Status	N	Hepatohypertrophic Index(HHI)
Normal	2	1.43±0.000[a]
Hypertrophy	9	1.49±0.045[d]
Fatty change A	6	1.48±0.060[d]
Fatty change B	11	1.51±0.095[c]
Fatty change C	10	1.35±0.100[b]
Atrophy	7	1.21±0.149[a]

Haematocrit and Haemoglobin

Mean haematocrit and hemoglobin values for all groups (Table 8).No significant variation (p>0.05) was found between group (Fig.11).Mean hemoglobin values (Fig.12) were not significantly different between groups.

Table 8. Mean±SE of haematocrit and haemoglobin(*P>0.05*).

Liver status	N	Hematocrit(%)	Hemoglobin (g/dl)
Normal	2	27.5±7.5	7.42±.08[a]
Hypertrophy	9	30±2.05	7.12±.48[d]
FattychangeA	6	28.3±4.23	26.18±10.83[c]
FattychangeB	11	29.9±1.87	16.59±9.93[b]
FattychangeC	10	28±2.64	6.45±.45[a]
Atrophy	7	32.28±1.67	20.61±12.69[a]

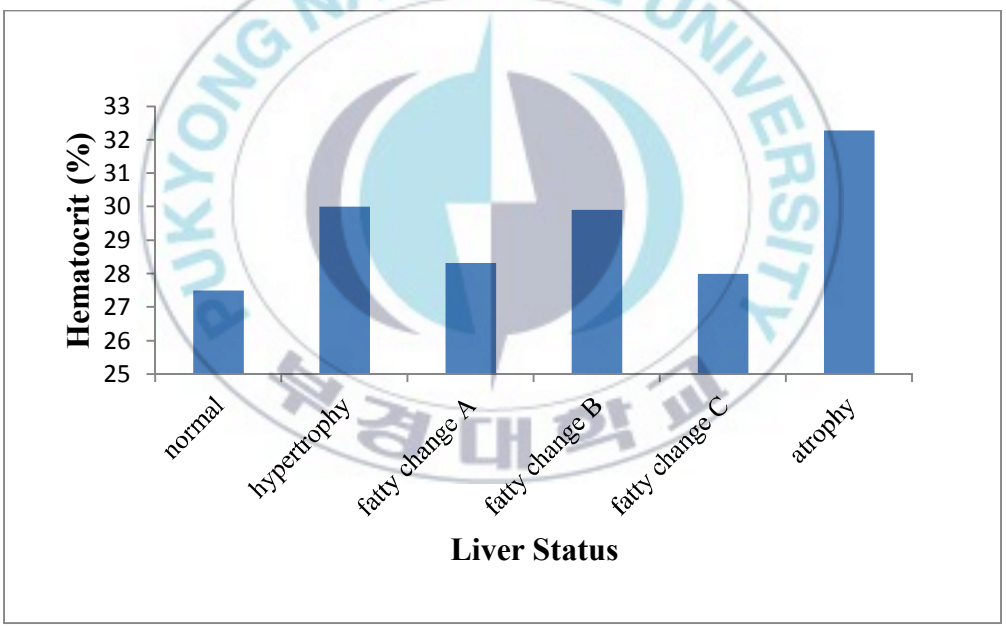

Fig. 11. Mean of Hematocrit (Ht) valuesbetween groups.

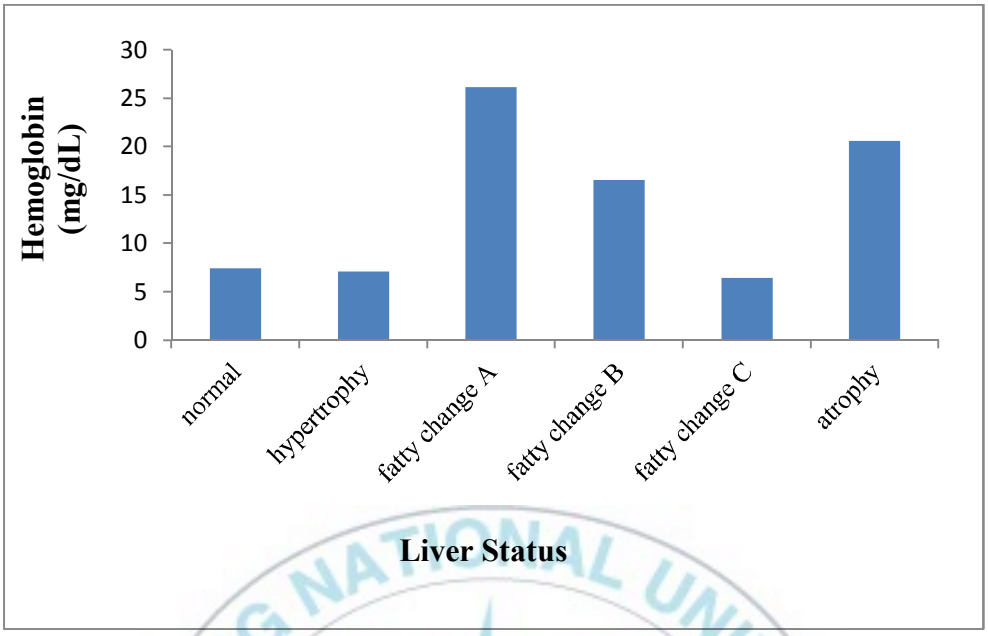

Fig. 12. Hemoglobin (Hb) values between groups .

GOT (glutamate oxaloacetate transaminase)

Mean GOT values for all fish liver status (Table 9).Significant variation ($p<0.05$) was found between groups(Fig. 13).

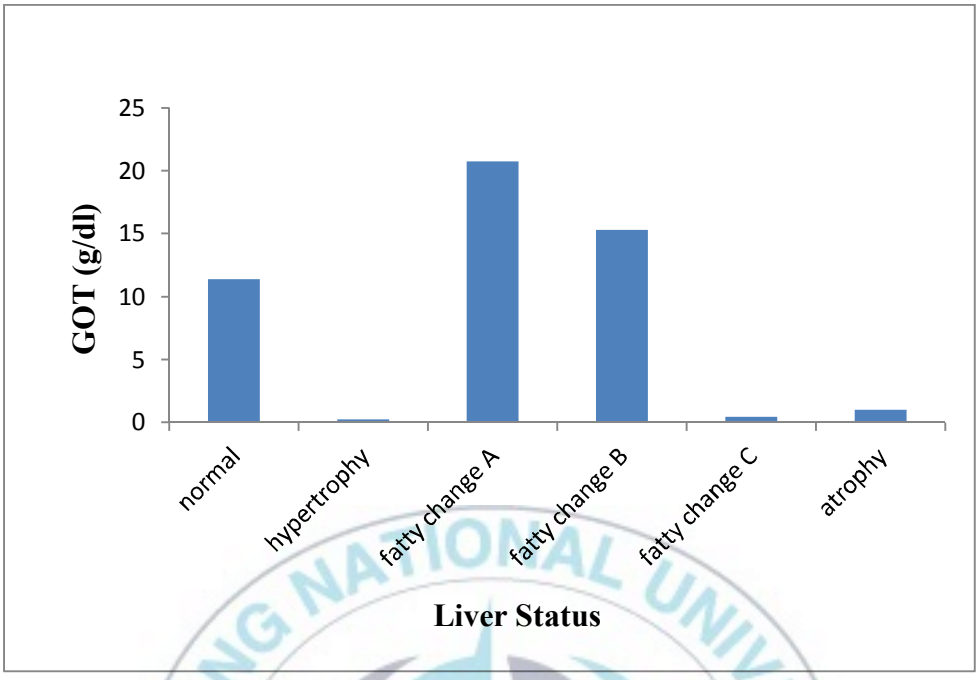

Fig. 13. GOT values between groups.

Table 9. Mean±SE of GOT ($P<0.05$).

Liver status	N	GOT
Normal	2	1.85±0.254[a]
Hypertrophy	9	2.59±0.140[d]
Fatty change A	6	2.39±0.083[c]
Fatty change B	11	2.11±0.110[b]
Fatty change C	10	1.75±0.088[a]
Atrophy	7	1.80±0.160[a]

GPT (glutamate pyruvate transaminase)

No significant variation ($p>0.05$) was found between different groups (Fig.14). Mean GPT values of all groups(Table 10).

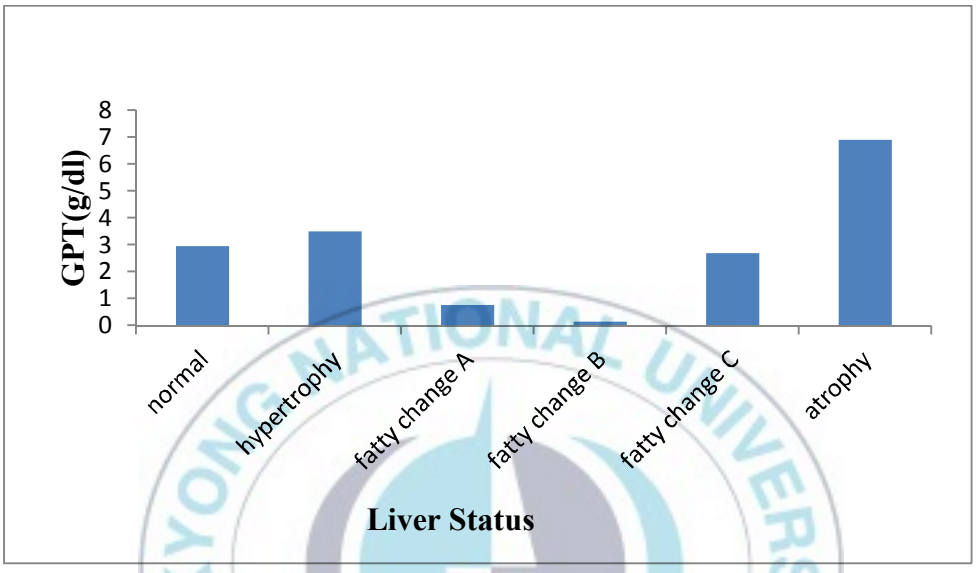

Fig. 14. GPT values between groups. Significant varaiation ($p>0.05$).

Table 10. Mean±SE of GPT (*P>0.05*).

Liver Status	N	Glutamate Pyruvate Transaminase(GPT)
Normal	2	2.95±12.73
Hypertrophy	9	3.50±2.22
Fatty change A	6	0.77±2.07
Fatty change B	11	0.14±1.67
Fatty change C	10	2.69±2.11
Atrophy	7	6.91±4.41

Total Protein(TP)

No significant variation($p>0.05$) between groups(Fig.15).Mean TP values (Table 11).

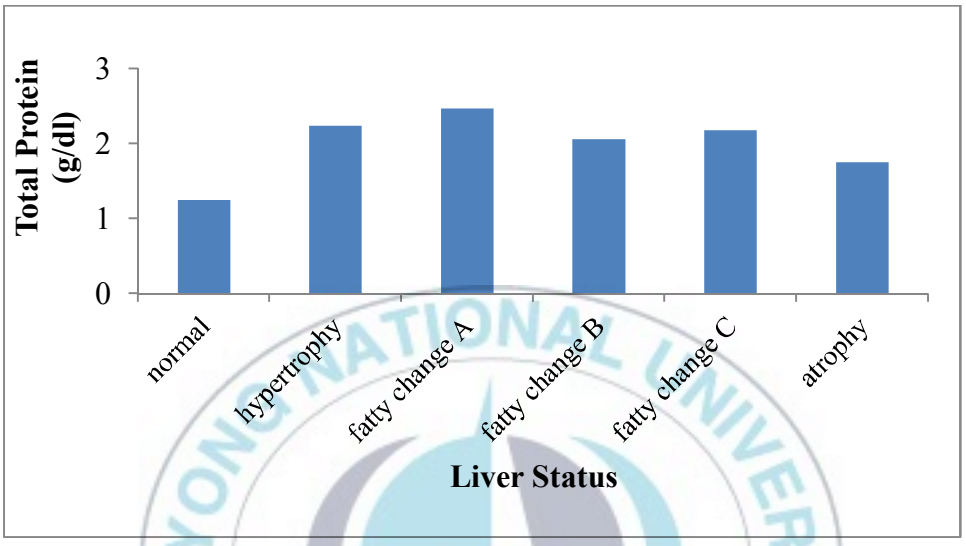

Fig. 15. TP values between groups.No Significant varaiation ($p>0.05$).

Table 11.Mean±SE of Total Protein (TP)(*P>0.05*).

Liver Status	N	Total Protein (TP)
Normal	2	1.25±0.12
Hypertrophy	9	2.24±0.24
Fatty change A	6	2.46±0.28
Fatty change B	11	2.06±0.28
Fatty change C	10	2.18±0.29
Atrophy	7	1.75±0.39

Discussion

As already well known, liver serves function similar to those in other vertebrates,as a key organ which controls many life functions and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates), catabolism (nitrogen, glycogenolysis ,detoxication), and important role in vitellogenesi(Hiran *et al*., 1996; Genten *et al*., 2009).

The liver is considered as a good indicator of nutritional pathology due to its function in metabolizing products coming from the digestive tract (Bozider *et al*., 2011). The intestine and liver are the most important organsin digestion and absorption of nutrients from food, and therefore monitoring ofthese organs is considered necessary (Takashima and Hibiya, 1982; Roberts, 1989). The most common changes observed in the liver are:hepatocytes vacuolization, fatty degeneration of the liver, changes in metabolicactivity, changes in liver parenchyma and necrosis (Roberts, 1989). In the present study, normal sized hepatocytes with no lipid vacuoles and with conspicuous,circular centrally located prominent nuclei (Fig.5) were observed in almost all livers from normal status.However,large hypertrophic hepatocytes with nuclei dislocated to cell periphery and lipid vacuoles were found in almost of all livers that have hypertrophy anddifferent degrees fatty

changes A,B,C status(Fig. 6), Mild liver alteration is might be caused by the difference between the poor farming from which the fish were obtained and the feeding condition.

Large hypertrophic hepatocytes as well as high mean HSI and HHI values were observed.hence, the HSI known as indicator of hepatomegaly or atrophy , the HSI increases with the increase of the number of lipid vacuoles in the liver values means, hypertrophy liver status(Fig. 9)indicating more accumulation of lipids in the liver. Whilst, high HHI refers a very small number of nuclei per 1000μm² of hepatic tissue, Nevertheless the hepatohypertrophic Index did not have significant difference between the different statuses of the liver(Table8),suggesting that the hepatohypertrophic index is not a parameter related to the health of fish.

Disrupt of microcirculation of the hepatic parenchyma occurred due to stressors to liver (temperature,diet less,starvation..etc),lead to increased the transport of nutrients, mainly free fatty acids, to liver cells from stomach and intestine via hepatic portal vein. Increased nutrient inflow means increased work load for these cells and this situation puts them on a hyperfunctional condition. It is known that free fatty acids from ingested food are normally transported into hepatocytes, where they are esterified to

triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies(Fasil *et al*., 2014) .

In cultured fish, hepatocytes are often swollen with glycogen(extensive irregular vacuolations) or neutral fat. When diet is less than ideal or duringcyclical starvation phases, the cells may be shrunken and contain varying amounts ofyellow ceroid pigments. Swelling of hepatocytes as a result of hypertrophy might have caused narrowing of sinusoids and disse spaces (perisinusoidal space located between hepatocytes and sinusoids),Hepatocytes get blood and oxygen via sinusoids from hepatic portal vein and artery,the blood reaches to hepatocytes from sinusoids through disse spaces. Narrowing of sinusoids and disse spaces as a result of swelling hepatocytes likely caused obstruction of blood and oxygen flow to hepatocytes reported by (Hwang, 2011), increased nutrient inflow and obstructed blood as well as oxygen supply could lower the metabolizing capacity of hepatocytes. This probably led to deposition of lipid in hepatocytes and fatty degeneration. Accumulation of lipid droplets in hepatocytes was inferred from the appearance of vacuoles as round, single or coalescing droplets after hematoxylin and eosin staining referred by (Takashima and Hibiya, 1995) .

In other hand, the livers were observed with shrunken hepatocytes with inconspicuous dislocated nuclei and circular lipid vacuoles(Fig.7).This was detected in one of indices used LRI.

Hepatocytes size reduction lead to atrophy which showed a higher LRI (Fig.8),In fact, this shows how histological description can be affirmed by the use of indices.

Hepatocytes become in a hypofunctional condtion because of decreased workload and low blood supply are known to cause atrophy to cells(Takashima and Hibiya, 1995; Kumar *et al*., 2007; Fasil *et al*., 2014).For this reason,the lipid vacuolation and atrophy observed in this study might have happened because of poor metabolizing capacity of hepatocytes as a result of obstruction of sinusoidal bloosd flow. In addition, hepatocytes vacuolization as aresult of lipid vacoules and atrophy were shown in LRI indice, in accord with the finding of previous study about overdrfeeding in tilapia(fasil *et al.*, 2014).

Histological changes in the liver are easily recognized if the food used is notadequate (Tacon, 1992). If food containing protein or fat is used, this can lead to histopathological changes in the liver (Caballero *et al*., 2004).

Hemoglobin and hematocrit mean values didn't show any significant ($p>0.05$) difference , mean haemoglobin values were detected (Fig. 12) between different liver status and fatty change A has shown higher mean value. While , Similar trend in hemoglobin was observed in Tilapia mossambica after mercury exposure (Cyriac *et al*., 1989) .The decrease in haemoglobin concentration may be because of either an increase in the rate at which haemoglobin is destroyed or decrease in the rate of hemoglobin synthesis (Jee *et al*., 2005).Hb value of fish *C. carpio* exposed to sublethal concentrationof lindane for 25 day was decreased up then elevated(Saravanan *el at*., 2011).

Mean hematocrit value of atrophy livers were found to be significantly ($p>0.05$) different higher than other status. Hence,the obereved values of haematocrit in atrophy case could be because of the age on hematological parameters of *Sebastes schlegeli*.while, decreased in Hct value of fish *C. carpio* exposed to sublethal concentration of lindane found by (Saravanan *el at*., 2011) .

Total Protein values of Hypertrophy and Fatty degeneration were found to be higher than other alterations, plasma protein level was found to

be lower in *C.carpio* treated with lindane during sublethal treatment (Saravanan *el at*., 2011).

Total Protein also one of the important biochemical parameters which have been used to understand the general state of health and biological mechanism of metabolism under pollutant stress.Fish under stress condition may also mobilize protein to meet energy demand to maintain increased physiological activity (Martineze *et al*., 2004).

GOT (glutamate oxaloacetate transaminase) value of fatty change A status has shown increased GOT, increase in the activities of blood transaminase indicated liver damage (Palanivlu *et al*., 2005). in addition, increased GOT in blood plasma of *Channa punctatus* (Bloch) exposed to both sublethel concentrations of momocrotophos indicated hepatic damage (Agrahari *et al*., 2007).

GPT activity was observed increase in atrophy liver status, in this study,there hasn't any explain to increase GPT in case of liver atrophy status.while, increase in GOT and GPT in the blood of *C. punctatus* following the treatment of mercuric chloride (Sastry and Sharma, 1980).

Histopathology together with other methods such as: biochemical, growth ,diseases diagnostic, are biomarkers used in assessing effects of both

internal(feed used) and external (aquatic) environmental conditions(Grund *et al*., 2010; Rajeshkumar andMunuswamy, 2011).

The biochemical parameters in fish are sensitive for detecting potential adverse effects and relatively early events of pollutant damage(Almeida *el at.,* 2002). The evaluation of biochemical and histological changes in fish liver has become an important tool for monitoring environmental exposure of fish to contaminants in experimental studies(Hinton and Lauren, 1990; Biagianti-Risbourg, 1997).

Direct comparison of the present study with the many previous ones is apparent difficulty. However,biochemical and morphological responses induced by carbaryl in the liver of Nile tilapia *Oreochromis niloticus*(Matos *el at*., 2007) ,the pesticide lindane caused alterations on haematological and biochemical parameters of *C. carpio* (Saravanan *el at*., 2011) and these alterations can be used as non specific biomarkers in pesticide contaminated aquatic ecosystem , diet induced liver alterations such as hepatocytic hypertrophy and fatty degeneration which agree with findings of the present study were reported for different fish species (Morais *et al*., 2001; Ruyter *et al*., 2006; Ferri *et al*., 2011; Hu *et al*., 2013).

Resemblance between liver morphological alterations and biochemical changes in this study and other studies. in response to toxic chemicals, feeding levels and feed quality was observed. Hypertrophic hepatocytes with disintegrated nuclei in *Tilapia zillii*was found after acute exposure to aluminum (Hadi and Alwan, 2012) and sub–chronic alachlor (Peebua *et al*., 2008). Fatty degeneration of liver of O.niloticus was also resulted after being exposed to extracts of *Ipomoea aquatic* leaf (Ayoola, 2011), various degress of hepatic degeneration focal necrosis and sever sinusoidal congestion resulted after acute exposure to heavy metals (Cd and Pb)(Hanan, 2007),increased lipid content of liver could be explained by rather increased deposition of lipid in exess of nutritional requirements or a failure to mobilize lipid stores during dietary Cu toxicitysuggested by(Handy, 2003),inotherhands, Zn induced severe fatty degenerative changes in liver, alterations on haematological and biochemical parameters of *C. carpio*(Gardner and Yevich, 1970).

Blood biochemical parameters GOT and GPT in plasma used to estimate the significance of intoxication in liver of grey mullet *Mugil auratus* (Krajnovic-Ozretic and Ozretic,1987). Considering hepatocyte structural changes as cellular responses to stressors(Jobling, 2012) , In fish caged in

the urban stream the most common lesions were hepatocytes with hypertrophy, cytoplasmic and nuclear degeneration , histopathology showed most severe lesions in liver (Marina *et al*., 2007). Moreover, hepatocyte hypertrophy and fatty degeneration induced by various toxic chemicals happened to be similar to the alterations found in the present study. It might be because of the fact that many stressors can provoke similar cellular responses. Vacuolation of hepatocytes was associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilization (Hinton and Laurén, 1990).Swelled and dissociated hepatocytes were also reported in arsenic exposed Zebrafish (Lam *et al*., 2006). Cellular swelling occurs either directly by denaturation of volume-regulating ATP ases or indirectly by disruption of the cellular energy transfer processes required for ionic regulation (Hinton and Laurén, 1990).

Conclusion

The outcome of this study claims the hepatohypertrophic index (HHI)) and the blood biochemical paramerters did not express any significant change to the alteration structural of liver.

From results obtained, it might be concluded that the histopathological assessment of the liver and the other organs could be used as fish health indicator. Liver structural changes observed in this study are believed to be a good enough to disrupt the normal functioning of the liver. Taking in consideration the important role the liver plays in physiological homeostasis of fish, it is quite reasonable to think that histologically affected liver can lower the fish resistance to a variety of disease entity.

The use of different methods in the evaluation of fish health on liver histology is a must. Methods of choice in many studies are semiquantitative methods, since they are not time-consuming, and are easy for evaluation of samples, but they are not objective as others.

Indeed no constant correlation between histopathological aspects of liver and blood biochemical paramerters that indicate for the health fish ,so that we required more studies in this feild

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