ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

IMPACT OF HILSHA FISH OIL ON TYPE-2 DIABETIC MARKER IN STREPTOZOTOCIN INDUCED EXPERIMENTAL MICE

Munira S[1], Asaduzzaman M[1], Rahman M. S[1], Rahman M. M[1], Hasan M[2], Khan M.H[1], Rahman M, Ha Y. L[3], Islam M. A[1]

[1] Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.
[2] Dept. of Biophysical Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-Ku, Kyoto 607-8414, Japan.
[3]Division of Applied Life Sciences, Graduate School, Gyeongsang National University, Jinju 660-701. Korea.
Email: maislam06@gmail.com

Received on 29-10-2015 *Accepted on 20-11-2015*

Abstract

Diabetes mellitus (DM) is an irremediable metabolic affliction which becomes an object of concern worldwide. This study attempts to find a remedy by supplementing hilsha fish oil (HFO) which can beat DM.DM was induced by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (55 mg/kg body weight) solution. Mice were divided into four groups: control, diabetic control (DC), HFO treated and glibenclamide treated. This study was conducted for 27 days and blood glucose level was measured at 3 days interval. After treatment period serum SGPT, SGOT and CRP level and lipid profile (total cholesterol-TC, low density lipoprotein-LDL, high density lipoprotein-HDL and triglyceride-TG) were determined by using commercial kits. It was observed that (HFO) has a potential hypoglycemic effect as it significantly ($p<0.001$) decrease blood glucose level compared to DC group. The SGPT, SGOT and CRP were also decreased significantly ($p<0.001$). Therefore HFO might be effective against liver malfunction. An indicative antilipidemic effect was also observed as TC, TG, LDL, VLDL showed significant ($p<0.001$) decrease whereas HDL showed significant increase ($p<0.001$) by HFO treatment compared to DC group. HFO is rich in omega 3 fatty acids which might increase insulin production and improve lipid index in treated mice. From the above observations it can be concluded that HFO have an effective therapeutic value in the treatment of DM and in the management of associated cardiovascular and hepatic complications.

Key words: Diabetes mellitus, Glucose, Glibenclamide, Streptozotocin, Lipid profile, CRP.

Abbreviations: Diabetes mellitus (DM), Hilsha fish oil (HFO), Streptozotocin (STZ), Diabetic control (DC), Cardiovascular disease (CVD),

Introduction

Diabetes mellitus, an epidemic with numerous devastating complications, has risen dramatically over the past two decades, with substantial variation worldwide [1]. It is a disease of carbohydrate metabolism, but it is a general metabolic disorder of all three of the energy nutrients fat, carbohydrate and protein. It is characterized by high levels of blood glucose resulting from defects in insulin production and/or receptor insensitivity. It can be also caused by progressive β cell loss and it is widely accepted that reactive oxygen species (ROS) contribute to pancreatic cell or tissue damage and dysfunction both in type 1 and 2 diabetes, even though the underlying mechanisms differ [2]. Although diabetes has no known cause, complex interplay of several factors including genetic, social, and environmental factors is implicated in its etiology [3]. The major risks of diabetic complications are particularly cardiovascular diseases (CVD) and peripheral vascular disease (PVD) [4]. The long term manifestation of this disease can result in the development of vascular disorders such as retinopathy, nephropathy, neuropathy, and angiopathy [5]. Diabetes is associated with the generation of reactive oxygen species (ROS) which cause oxidative damage, particularly to heart, kidney, eyes, nerves, liver, small and large blood vessels, and immunological and gastrointestinal system [6,7].Despite excellent potencies, several synthetic anti-diabetic drugs had offered unwanted therapeutic profiles marked by fluid retention, drug-induced hypoglycemia, and increased rate of lactic acidosis, liver malfunctioning due to cirrhosis, weight gain and cardiac dysfunction [8]. Therefore other measures such as dietary therapy with potential compounds to prevent DM and associated complications are still in race. Hilsha (*Tenualosailisha*) is locally known as "Macher Raja" in Bangladesh which means the king of fish due to its taste and flavour. It is also the national fish of Bangladesh. It is highly abundant in rivers, estuaries and marine area in Bangladesh. The proximate composition of hilsha include four basic ingredients in varying proportions major nutrients such as water (70 - 80%), protein (18 - 20%), fat (5%) and minerals (5%) and minor nutrients such as vitamin, carbohydrate [9]. Hilsha fish oil (HFO) is enriched in poly unsaturated fatty acids (PUFAs) especially EPA and DHA along with mono unsaturated fatty acid such as oleic acid (OA). It has been reported that oleic acid suppresses over expression of the oncoprotein (Her-2/*neu*-coded) p185Her-2/neu in vitro and a higher level of OA in breast tissue provide an effective means of influencing the outcome of breast cancer [10]. Is has been

documented that HFO may ameliorate the atherogenic lipid profile, platelet hyper aggregation and the anti-oxidative defense of STZ-diabetic rats [11].

Diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder and as the knowledge of the heterogeneity of this disorder increases so does the need for more appropriate therapies [6]. Therefore the aim of this present study is to evaluate the anti- diabetic effect of HFO on experimental mice.

Materials and methods

Sample Collection and Preparation

Hilsha fishes (*Tenualosailisha*) were collected from Padma River. Fish oil was extracted from the dried fish material with n-hexane by Saxhlet apparatus and the extracted oil was evaporated under reduced pressure in a rotary evaporator to obtain oil.

Determination of physical characteristics of HFO

Determination of Specific Gravity

Specific Gravity of the oil was determined by means of a specific gravity bottle using the following formula [12].

$$\text{Specific gravity} = \frac{(\text{Sp. Gr.})\ \text{Weight of oil in bottle}}{\text{Weight of distilled water in bottle}}$$

Determination of Refractive index: Refractive index of a medium is the ratio of the speed of light at a definite wavelength in vacuum to its speed in the medium.

Determination of Smoke point, Flash point and Fire point

The smoke point, flash point and fire point of the fish oil were determined according to the official methods of the American oil Chemist Society [13].

Determination of Pour point, Cloud point and Solidification point

The pour point, cloud point and solidification point of the fish oil were determined according to the ASTM standard methods for lubricating oils [14].

Study on chemical characteristics of HFO

Determination of Iodine Value

The iodine value of oil was determined by the Hanusmethod using the formula [15].

$$\text{Iodine value} = \frac{(V_1 - V_2)\ X\ S\ X\ 0.127\ X\ 100}{W}$$

Where

V_1 = Volume in mL of sodium thiosulphate solution required for blank experiment.

V_2 = Volume in mL of sodium thiosulphate solution used.

S = Strength of sodium thosulphate.

W = Weight of the oil in gram.

A blank experiment was performed exactly in the same manner without the oil.

Determination of Saponification Value and Saponiafication Equivalent

The saponification value of oil is the number of milligrams of potassium hydroxide (KOH) required saponifying completely 1 g of oil. Saponification value was calculated using the following formula [16].

$$\text{Saponification Value (SV)} = \frac{(V_1 - V_2) \times N \times 56.1}{W}$$

Where

V_1 = mL of acid used for blank test.

V_2 = mL of acid used for the test experiment.

N = Strength of acid used,

W = Weight of oil.

Saponification equivalent was calculated from the saponification value using the following formula [17].

$$\text{Saponification Equivalent (SE)} = \frac{56100}{\text{Saponification value of the oil}}$$

Determination of peroxide value

The peroxide value is expressed in terms of milli equivalents of active oxygen per kilogram of oil.Peroxide value was calculated using the following formula [18].

$$\text{Peroxide value} = \frac{(S\text{-}B) \times N \times 1000}{\text{Weight of sample}}$$

Where

S= mL of thiosulphate required in the test experiment.

B= mL of thiosulphate required in the blank experiment.

N= Strength of sodium thiosulphate.

Determination of Acid Value

The acid value of oil is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of oil. This is used for determining the rancidity due to the presence of free fatty acid.

The acid value was calculated using the following formula [19].

$$\text{Acid value} = \frac{56.1 \times N \times V}{W}$$

Where

N = Strength of alkali.

V = mL of sodium hydroxide solution used for the oil.

W = Weight of oil in gram.

Determination of Free Fatty Acid (% FFA) of the oil

The percentage of free fatty acid (as oleic acid) was calculated using the following formula [20].

$$\text{Percent of free fatty acid} = \frac{\text{Acid value}}{1.99}$$

Determination of unsaponifiable matter

The unsaponifiable matter (UM) is a fraction of oil that remains insoluble after saponification of the fat sample by alkali. This includes sterols, higher alcohols, pigment, and hydrocarbons. The amount of unsaponifiable matter present in the oil was determined using the method as described [21].

The quantity of unsaponifiable matter present in 100 gms of oil was calculated from the formula.

$$\text{U.M} = \frac{\text{Weight of unsaponifiable matter}}{\text{Weight of oil taken}} \times 100$$

Study on anti-diabetic effect of HFO

Animals care: Experimental animals were collected from International Cholera and Dysentery Disease Research, Bangladesh (icddr, b). Ethical permission was provided by Institute of Biological science (IBSc), Rajshahi University.

Male albino mice weighing ranged (25-27 g) were used for the study. They were individually housed in polypropylene cages in well-ventilated rooms under hygienic conditions, allowed free access to food (standard pellet diet) and water *ad libitum* and kept under maintainedday night cycle. They were adapted for one week before the experiment.

Induction of diabetics

Diabetes was induced in overnight fasted mice by a single intra-peritoneal injection of streptozotocin (55 mg/kg body weight) in a 0.1M sodium citrate buffer (pH-4.5). Food and water intake were closely monitored daily after streptozotocin (STZ) administration. The threshold level of fasting serum glucose to diagnose diabetes was taken as >150 mg/dl (8.35 m mol/dl) with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and only those animals were included in the study, rest are excluded from the study.

Grouping and treatment of animals

The animals were randomly divided into four groups. Each group contain six mice (n=6). The treatment of animals began on the initial day after STZ injection and this was considered as 1st day of treatment. The animals were treated for 3 weeks as follows:

Group-1: Normal mice fed with standard pellet diet and water.

Group-2: Diabetic control (DC) mice.

Group-3: Hilsha fish oil (HFO) treated mice; supplementing 1% HFO with diet.

Group-4: Glibenclamide treated mice; providing glibenclamide at a dose of 0.5mg/kg body weight.

Blood Collection

Blood samples from all groups were collected on days 1, 3, 6, 9,12,15,18, 21, 24 and 27 in a fasting state from mice tail vein.At the end of experiment period (27 days), mice were sacrificed after overnight fasting. Mice were anesthetized with diethyl ether and blood was collected from the heart. The serum was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 3000 rpm for 20 minutes, then kept in plastic vials at -20°C until analysis.

Biochemical Analysis: Fasting blood glucose level was measured by using commercial kit (Linear chemicals, Barcelona, Spain). Hepatic enzymes such as SGPT and SGOT as well as CRP were also measured using quantification

kits (Linear chemicals, Barcelona, Spain).Serum lipid profile such as triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), Very Low Density Lipoprotein (VLDL) were measured using kits by automatic Bio analyser (Hitachi 7180, Hitachi, Tokyo, Japan). Serum LDL was determined according to the Friedewald formula with use of HDL and total cholesterol value.

Statistical analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). Results were analyzed by using Scientific Package of Social Science (SPSS) version 17.0. Two different set of statistics, which is descriptive and analytical statistics was applied. The descriptive statistic was used to analyze mean, standard deviation (SD) whereby analytical statistics, one-way ANOVA was used to determine statistical significance ($p<0.05$) among the groups.

Results

Physical properties of hilsha fish oil (HFO) was illustrated in Table 1. Specific gravity and refractive index of HFO was 0.912 and 1.398 respectively. Other properties include Smoke point (217°C), Flash point (326°C), Fire point (346°C), Pour point (-6.2°C), Cloud point (1.8°C) and Solidification point (-9.2°C).

Table- 1: Physical characteristics of Hilsha fish oil.

Parameters	Values
Specific Gravity (at 25°C)	0.912
Refractive index (at 25°C)	1.398
Smoke point (°C)	217
Flash point (°C)	326
Fire point (°C)	346
Pour point (°C)	-6.2
Cloud point (°C)	1.8
Solidification point (°C)	-9.2

Table 2 showed the chemical characteristics of HFO. These characteristics include Iodine value (96.43 ± 0.437), Saponification value (182.76 ± 0.648), Saponification equivalent (276.32 ± 0.552), Peroxide value (8.20 ± 0.713), Acid value (4.07 ± 0.247), % of free fatty acid (2.32 ± 0.375) and % of unsaponifiable matter (1.78 ± 0.652).

Table- 2: Chemical characteristics of Hilsha fish oil.

Parameters	Values
Iodine value	96.43 ± 0.437
Saponification value	182.76 ± 0.648
Saponification equivalent	276.32 ± 0.552

Peroxide value	8.20 ± 0.713
Acid value	4.07 ± 0.247
% of free fatty acid	2.32 ± 0.375
Unsaponifiable matter (%)	1.78 ± 0.652

Changes in blood glucose level at 3 days interval was illustrated in Table 3. These data revealed that there was significant (P<0.001) increase in blood glucose level after injecting STZ which was gradually declined by HFO treatment. In the 6,9, 12, 15 and 18th days of experiment glucose level was decreased by 17% , 20% 27%, 30% and 36% respectively whereas by supplementing glibenclamide it was 17%, 26%, 34%, 41%, 47% in those respective days compared to diabetic control (DC) group. In the last half of the experiment a slow reduction of blood glucose level was seen by HFO supplementation and it was 40%, 44% and 47% in the 21, 24 and 27th day. Glibenclamide reduced blood glucose level by 52%, 58% and 62% in the last three intervals compared to DC group.

Table- 3: Effect of fish oil on the blood glucose level of experimental mice.

Group	Plasma glucose concentration (mmol/L)									
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27
Normal	5.8 ± 0.2	5.8 ± 0.5	5.9 ± 0.6	5.8 ± 0.1	5.8 ± 0.3	5.8 ± 0.2	5.8 ± 0.3	5.8 ± 0.2	5.7 ± 0.1	5.8 ± 0.8
Diabetic control (DC)	19.4 ± 0.3^{a*}	20.0 ± 0.7^{a*}	20.5 ± 0.6*	21.1 ± 0.2^{a*}	21.8 ± 0.4*	22.3 ± 0.8^{a*}	22.9 ± 0.4^{a*}	23.4 ± 0.7*	23.9 ± 0.5^{a*}	24.5 ± 0.2^{a*}
Diabetic + HFO	19.5 ± 1.2^{a**}	18.6 ± 1.0^{a**}	17.6 ± 0.8**	16.7 ± 1.2^{a**}	15.9 ± 0.9^{a**}	15.2 ± 1.1^{a**}	14.6 ± 0.7**	14.0 ± 1.0^{a**}	13.4 ± 1.2^{a**}	12.9 ± 0.8^{a**}
Diabetic +Glibenclamide	19.8 ± 0.2^{a**}	18.3 ± 0.4^{a**}	16.9 ± 0.7^{a**}	15.6 ± 0.8**	14.4 ± 0.3^{a**}	13.2 ± 0.7**	12.2 ± 1.1^{a**}	11.1 ± 0.9**	10.1 ± 0.6^{a**}	9.20 ± 0.4^{a**}

a: P<0.001; * vs Normal group, ** vs Diabetic control.

Data in Table 4 depicted the initial and final body weight as well as body weight change after the treatment period. These data viewed that induction of DM causes weight loss as body can't utilize glucose. On the other hand HFO treatment helped to gain weight in experimental mice as similar as glibenclamide. Changes in body weight were not so significantly happened.

Table – 4: Changes in body weight of different group of mice.

Groups	**IBW**	**FBW**	**BWC**
Normal	26.56 ± 1.32	41.07 ± 0.73	14.51± 0.73
Diabetic control (DC)	26.35 ± 1.02*	38.24 ± 1.14*	11.89 ± 0.73*
Diabetic + HFO	26.42 ± 1.66^{a**}	39.56 ± 1.52^{a**}	13.14± 0.73**
Diabetic + Glibenclamide	26.51 ± 1.26^{a**}	40.63 ± 1.33^{a**}	14.12± 0.73**

IBF: Initial body weight, FBW: Final body weight, BWC: Body weight change a: P<0.001; * vs Normal group, ** vs Diabetic control.

The serum SGPT and SGOT level was viewed in Table 5. These data showed a significant (P<0.001) increase in SGPT and SGOT level after STZ treatment. The SGPT level was declined by 15% with HFO supplementation and 24% by glibenclamide. SGOT level was also significantly reduced by 32% and 42% with HFO and glibenclamide treatment compared to DC group.

Table- 5: Effect of fish oil on the serum SGPT and SGOT levels.

Group	SGPT (U/L)	SGOT (U/L)
Normal	52.83±2.48	41.33±2.80
Diabetic control (DC)	86.5±6.09^{a*}	83.33±2.58^{a*}
Diabetic + HFO	73.0±4.77^{a**}	56.67±6.53^{a**}
Diabetic + Glibenclamide	65.5±4.59^{a**}	48.0±5.44^{a**}

a: P<0.001; * vs Normal group, ** vs Diabetic control.

Data in Table 6 showed the changes of lipids profile in different group of mice. These data clarified that due to STZ treatment serum TC, TG, VLDL and LDL-c concentration increased significantly (P<0.001) whereas the level of good cholesterol HDL-c decreased significantly compared to normal group. Serum TC, TG, VLDL and LDL-c level in HFO treated mice was decreased by 11%, 23%, 24% and 12% respectively whereas HDL-c increased by 12%. In contrast in glibenclamide supplemented mice Serum TC, TG, VLDL and LDL-c level were decreased by 14%, 31%, 31.42% and 13% respectively whereas HDL-c increased by 16%.

Table-6: Effect of fish oil on Total cholesterol, Triglyceride, LDL, VLDL, HDL level in experimental mice (mmol/L).

Groups	Total Cholesterol	HDL	LDL	Triglycerides	VLDL
Normal	5.50±0.08	0.30±0.03	4.52±0.11	1.47±0.11	0.67±0.05
Diabetic control (DC)	6.70±0.26^{a*}	0.25±0.03^{a*}	5.55±0.23^{a*}	1.96±0.12^{a*}	0.89±0.05^{a*}
Diabetic + HFO	5.96±0.18^{a**}	0.28±0.03^{a**}	4.90±0.15^{a**}	1.50±0.08^{a**}	0.68±0.04^{a**}
Diabetic + Glibenclamide	5.72±0.15^{a**}	0.29±0.03^{a**}	4.81±0.16^{a**}	1.35±0.08^{a**}	0.61±0.04^{a**}

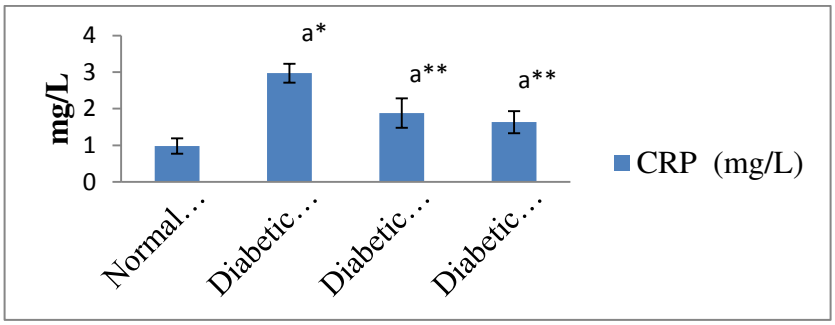
a: P<0.001; * vs Normal group, ** vs Diabetic control.

Discussion

There are estimated 246 million people worldwide sufferings from diabetes [22]. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 [23].

Therefore finding a proper remedy is still now a matter of concern. In this present study streptozotocin (STZ) was used to induce DM. STZ monohydrate induces type-2 diabetes in experimental rats through exclusive destruction of insulin producing beta cells in pancreas. [24]. The hypoglycemic effect of fish oil result from the enhancement of the production of insulin from the existing β cells of islets of langerhance and it may increase the peripheral uptake of glucose [25]. The plasma glucose lowering activity was compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β cells [26]. Previous studies on nutritional analysis of Hilsha fish showed that it is rich in various essential amino acids (EAA) and non-essential amino acids (NEAA). [27]. EAA-to-NEAA ratio showed a positive correlation to IGF-I and insulin whereas an inverse correlated to IGFBP-1 [28]. Minerals and Vitamins content of hilsha fish was also studied by [29]. Some of these minerals are reported to play vital roles in the maintenance of normal glucose tolerance and insulin secretion from the pancreatic β-cells [30]. Other ions such as copper and zinc are also known to be involved in glucose and insulin metabolism [31]. Hilsha fish oil is very much effective against hyperglycemia due to the presence of poly unsaturated fatty acids specially EPA and DHA. A medium size of hilsa contains 1.55 g EPA + DHA per 100 g of fish meat [32]. Comparing the fatty acid profile of hilsa with other marine fishes, we found that ω-3 and EPA + DHA content of hilsa (14.06 and 11.83 %) is higher than that of Indian Mackerel (11.2 and 6.42 %) [33]. HFO effectively reduced serum SGPT, SGOT and CRP level in diabetic mice. Omega-3 fatty acids also improve hepatic steatosis in mice and may be used to increase the pool of potential live liver donors that are currently excluded because of the presence of macrovesicularsteatosis [34].

Figure 1: Effect of hilsha fish oil on serum CRP of experimental mice.



Values are expressed as (Mean ± SD), a: P<0.001; * vs Normal group, ** vs Diabetic control.

C- Reactive Protein (CRP) is an effective test, which can predict the cardiovascular risk. The addition of CRP-testing to standard lipid screening appears to provide an important method to determine Cardiovascular Disease (CVD) risk factor [35]. Dietary supplementation of HFO decline CRP level significantly and thus reduced the risk of cardiovascular diseases in Diabetic mice.

Conclusion: From the above findings it can be conclude that hilsha fish oil has great significance in the management of Diabetes Mellitus in STZ induced mice. This indicative effect is due to the presence of omega 3 fatty acids, minerals and essential fatty acids that may help in the suppression of pancreatic beta cell inflammation, promotion of insulin secretion and promotion of glucose uptake in adipose tissue. HFO also reduced SGPT, SGOT and CRP level so it may have hepatoprotective activity. It also helps in the improvement of DM associated hypercholesterolemia.

Acknowledgements: The financial supported was given by The Ministry of National Science and Technology (NST) Bangladesh, University Grant Commission (UGC) Bangladesh and Faculty of Science Rajshahi University, Rajshahi-6205, Bangladesh.

References

1. King H.; Aubert, R. E. and Herman, W. H. Global burden of diabetes 1995–2025. Diabetes Care 1998;21:1414–1431.
2. M. Cnop, N. Welsh, J.C. Jonas, A.Jorns, S. Lenzen, D.L. Eizirik. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 2005;2:S97–S107
3. Imam K. Clinical features Diagnostic criteria and pathogenesis of diabetes mellitus. AdvExp Med Biol 2012; 771:340–355.
4. L Pirart. Diabetes mellitus and its degenerative complieations : a prospective study of 4400 patients observed between 1947 and 1973. Diabetes Care 1978; 168-172.
5. N. Orsolic and I. Basic, Honey bee products and their polyphenolic compounds in treatment of diabetes, in Phytopharmacology and Therapetutic Values IV, J.N.GovilandV.K.Singh,Eds.,2008;22:455–471.
6. Rosen CJ. Revisiting the rosiglitazone story–lessons learned. N Engl J Med. 2010; 363: 803-806.
7. Williams, K.A., Oils, Fats, Fatty Foods. 4thEdn., J and A Churchill Ltd. London. 1996; pp.124.

8. I.G.Obrosova, A.G.Minchenko, R.Vasupurametal."Aldose reductase inhibitor fidarestat prevents retinal oxidative stress and vascular endothelial growth factor overexpression in streptozotocin-diabetic rats". Diabetes 2003;52:864–871.
9. Khurseed J and Mosharaff. Seasonal changes on biochemical composition of fresh water murrel Ophiocephaluspunctatus (Bloch). Hydrobiologia 1998;32:206-213.
10. Menendez, J.A., Vellon, L., Colomer, R. and Lupu, R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erb B-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin) in breast cancer cells with Her-2/neu oncogene amplication. Ann Oncol 2005;16:359-371.
11. Mahmud I, Hossain A, Hossain MS, Hannan A, Ali L, Hashimoto M. Effects of Hilsailisa fish oil on the atherogenic lipid profile and glycemic status of streptozotocin-treated type I diabetic rats. ClinExpPharmacol Physio 2004;l31:76-81.
12. Hilditch,T.P.. The Industrial Chemistry of Fats and Waxes. 3rdEdn.BalliereTindall and Cox, London, 1949; pp.83.
13. Official and tentative Methods and Materials of the American Oil Chemist's Society, 3rdEdn., 1980; I, USA.
14. American Society for Testing and Materials, Method No.D.1952; pp.128-147.
15. IUPAC. Standard Methods for the Analysis of Oils, Fats and Derivatives. 6thEdn., Pergamon Press, Oxford, 1967;pp.56.
16. Jacobs, M.B. The Chemical Analysis of Foods and Foods Products. 3rdEdn.,1958;pp.393.
17. Augustus West, p. and O. Aurelio Cruz. The Phil. J.Sci., 1933; 54-52.
18. Device, J. and P.N.Williams. The Chemistry and Technology of Edible Oils and Fats, Pergamon Press Oxford, 1961; p.127.
19. Wild S, RoglicG, GreenA, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
20. Tunali, S., Yanardag, R. Effect of vanadylsulphate on the status of lipid parameters and on stomach and spleen tissues of streptozotocin-induced diabetic rats. Pharmacol. Res.2006;53:271-277.
21. Mohanty, B.P., Das, S., Bhaumik, U. and Sharma, A.P. *Tenualosailisha* - A rich source of n-3 fatty acids. Central Inland Fisheries Research Institute (ICAR), Barrackpore, India, 2011b; Bulletin No. 171; ISSN: 0970-616X.

22. Filho, J.C.D., Hazel, S.J., Anderstam, B., Bergstro, J., Lewitt, M.M. and Hall, K. Effect of protein intake on plasma and erythrocyte free amino acids and serum IGF-I and IGFBP-1 levels in rats. Am JPhysiolEndocrinolMetab 1999;277:693-701.
23. Mohanty, B.P., Ganguly, S. and Mahanty, A. Micronutrient profile of the Indian shad, *Tenualosailisha*, hilsa from the river Ganga, India. Natl. Acad. Sci. Lett. 2012c; 35:00-00.
24. Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. J Ethnopharmacol 1999;64:179-184.
25. Arquilla ER, Packer S, Tarmas W. The effect of zinc on insulin metabolism. Endocrinology 1978;103:1330-1449.
26. Mohanty, B.P., Paria, P., Mahanty, A., Behera, B.K., Mathew, S., Sankar, T.V. and Sharma, A.P. Fatty acid profile of Indian shad Tenualosailisha oil and its dietary significance. Natl. Acad. Sci. Lett. 2012a;35:263-269.
27. Marichamy, G., Raja, P.S., Veerasingam, S., Rajagopaland Venkatachalapathy, R. Fatty acids composition of Indian mackerel Rastrilligerkanagurta under different cooking methods. Curr. Res. J. Biol. Sci 2009;1:109–112.
28. Alwayn IP, Andersson C, Zauscher B, Gura K, Nose V, Puder M. Omega-3 fatty acids improve hepatic steatosis in a murine model: potential implications for the marginal steatotic liver donor. Transplantation 2005;79:606-608.
29. Ross R. Atherosclerosis an Inflammatory disease. N Eng. j med 1993;340:115-26.

Corresponding author:
Dr. Mohammad Amirul Islam*,
Email: *maislam06@gmail.com*