

Comparative Study of Anti-Hyperglycemic and Anti-Hyperlipidemic Effects of Honey, *Coccinia cordifolia* and Hilsha Fish Oil in Streptozotocin Induced Diabetic Rats

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Abstract

This study was aimed to evaluate the efficacy of Honey, *Coccinia cordifolia* (Locally known as Telakucha) leaves and Hilsha fish oil as hypoglycemic and hypolipidemic agents in diabetic condition. The leaves were initially under shade, grinded to powder, extracted with ethanol and filtered through Whatmann filter paper. The filtrate was concentrated by rotatory evaporator and stored at 4°C. The experimental rats were divided into six groups (n=6). Diabetes mellitus (DM) was induced by single intraperitoneal injection (65 mg/kg BW) of freshly prepared streptozotocin hydrate solution in 0.9% saline solution. Hyperlipidemic was induced by mixture of cholesterol (1.5 %) and cholic acid (0.5%) with diet of rats. At the end of the treatment, the blood glucose level and lipid profile was measured by using commercial kits. It was observed that honey, plant leaves extract and Hilsha fish oil (HFO) have potential hypoglycemic effect as it significantly (p<0.001) decrease blood glucose level compared to diabetic control (DC) group. The serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and C- reactive protein (CRP) were also decreased significantly (p<0.001). An indicative antilipidemic effect was also observed as total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) showed significant (p<0.05) decrease whereas HDL showed significant increase (p<0.001) by the treatment compared to DC group. From the above observations it can be concluded that the honey, *C. cordifolia* leaves extract and HFO have an effective therapeutic value in the treatment of DM and in the management of associated cardiovascular and hepatic complications.

Keywords: Honey; Hilsha fish oil; *Coccinia cordifolia*; CRP; Streptozotocin; Lipid profile; Anti-hyperglycemic; Anti-hyperlipidemic

Abbreviations:

EEC: Ethanol Extract of *Coccinia cordifolia*; C: *Coccinia*; STZ: Streptozotocin; CRP: C-reactive protein; HFO: Hilsha Fish Oil; DC: Diabetic Control

Introduction

Diabetes mellitus is a principal cause of morbidity and mortality in man population [1]. It is a complex progressive disorder characterized by impaired insulin sensitivity, reduced insulin secretion and progressive failure of β -cells. Diabetes currently affects an estimated 15.1 million people in North America, 18.5 million in Europe, 51.4 million in Asia, and just under 1 million in Oceania [2]. It is estimated that globally, the number of diabetic patients will rise from 151 million in the year 2000, [3] to 221 million by the year 2010, and to 300 million by 2025 [4]. The recent statistics shows that the global prevalence of this disorder continues to rise unabated and thus becoming an epidemic [5]. This is of public health concern due to its social and

economic burdens. Even though diabetes has no known cause, complex interplay of several factors including genetic, social, and environmental factors is implicated in its etiology [5,6]. The risks of diabetic complications are particularly cardiovascular diseases (CVD) and peripheral vascular disease (PVD) [7]. Complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations, blindness etc. are known to be associated with DM [8]. Epidemiological reports have shown that the effect of postprandial hyperglycemia on cardiovascular risk is greater than the effect of fasting hyperglycemia. Despite excellent potencies, synthetic anti-diabetic drugs had offered adverse effects marked by fluid retention, drug-induced hypoglycemia, and increased rate of lactic acidosis, liver malfunctioning due to cirrhosis, weight gain and cardiac dysfunction [9]. The alternative approach to diabetes therapy includes the use of herbal preparations, dietary components or supplements and other natural products such as honey [10]. Since antique era, plants with medicinal properties are enormously used in treating diabetes throughout the world. Many recent scientific investigations have also confirmed the efficacy of plant preparations, few of which are remarkably effective [11]. Cucurbitaceae is a plant family well known to have about 125 extant genera including 960 species. It is considered one of the important families of plants with potent hypoglycemic

effects [12]. Hilsha (*Tenulosa ilisha*) 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 [13]. It has been documented that HFO may ameliorate the atherogenic lipid profile, platelet hyper aggregation and the anti-oxidative defense of STZ-diabetic rats [14]. Therefore the aim of this present study is to evaluate comparative antidiabetic efficacy of honey, plant leaves STZ-induced experimental rats.

Materials and Methods

Honey collection and preparation of sample

Honey sample were collected freshly in sterile containers from the Sundarban, Khulna, Bangladesh. All samples were collected freshly in sterile containers (label with numbers, place and date of collection) and stored at ambient temperature until analysed. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analysis.

Plant leaves collection and preparation of extract

The leaves of *C. cordifolia* were collected in December, 2014 from Rajshahi city in Bangladesh and authenticated by Botany Department, Rajshahi University. The leaves were initially dried under shade and grinded. The powder of *C. cordifolia* leaves (1 kg) was mixed with ethanol in a 600mL flask with mild shaking. The flask was closed with cotton plug and aluminum foil at 48 hours at room temperature. The extract was filtered through Whatman filter paper (No.1), concentrated using a rotary evaporator at low temperature (40-50°C). The extract was preserved in airtight container and kept at 4°C until further use.

Collection of Hilsha fishes and extraction

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

Animals care

Experimental animals were collected from International Cholera and Dysentery Disease Research, Bangladesh (icddr,b). Male albino rats weighing ranged (120-150 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, kept under maintained day night cycle. They were adapted for one week before the experiment.

Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin (65 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 >200 mg/dl (11.5 mmol/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 rats (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 rats fed with standard pellet diet and water.

Group 2: Diabetic control (DC) rats.

Group 3: Hypercholesterolemic control rats were given cholesterol (1.5%) and cholic acid (0.5%) mix with diet.

Group 4: The diabetic rats treated with Honey at a dose of 1.0 g/kg body weight.

Group 5: Diabetic rats were treated with ethanol extract of *Coccinia cordifolia* (200 mg/kg body weight).

Group 6: Hilsha fish oil (HFO) treated diabetic rats; supplementing 1% HFO with diet.

Group 7: Hyper cholesterol rats were treated with Honey at a dose of 1.0 g/kg body weight.

Group 8: Hypercholesterolemic rats were treated by ethanol extract of *Coccinia cordifolia* (200 mg/kg body weight)

Group 9: Treated rats; fed hypercholesterolemic (HC) diet supplemented with 5% hilsha fish oil.

Group 10: Glibenclamide treated rats; providing glibenclamide at a dose of 0.5 mg/kg body weight.

Blood collection

Blood samples from all groups were collected on days 1, 3, 6, 9, 12, 15, 18 and 21 in a fasting state from rats tail vein. At the end of experiment period (21 days), rats were sacrificed after overnight fasting. Rats 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 4000 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 analyzer (Hitachi 7180, Hitachi, Tokyo, Japan). Serum LDL was determined according to the Friedewald formula with use of HDL and total cholesterol value.

Histopathology

At 21 days all groups animal sacrificed under using mild anesthesia and isolated the different organ (heart and liver) of the animal for histopathology. The isolated organ (heart and liver) tissue fixed at 40% natural buffered formaldehyde (formalin), dehydrated by passing through a graded series of alcohol, and embedded in paraffin blocks and 5 mm sections were prepared using a semi-automated rotatory microtome. Hematoxylin and eosin were used for staining.

Results

Comparing the blood sugar level in Streptozotocin induced diabetic rats, honey administered animals showed significant reduction of blood glucose level which is as near as glibenclamide administered rats at (P<0.001) (Table 1). 3rd, 6th, 9th, 12th, 15th, 18th, and 21st days honey supplementation group's glucose levels maintained 16.50 % - 48.57% lower than the diabetic control group whereas in case of glibenclamide it was 14.31%- 61.63% lower than significantly diabetic control group (P<0.001).

The plant extract produced significant changes in the blood glucose level in Streptozotocin-induced diabetic rats. Comparing the blood sugar level in diabetic rats, plant extract administered animals showed

significant (P<0.001) reduction of blood glucose which was as near as glibenclamide administered animals (Table 1). In 3rd to 21st days, plant extract administration group's glucose levels maintained 6 % - 49% lower than the diabetic control group. In the 6, 9, 12 and 15th days of experiment with HFO glucose level was decreased by 17%, 20%, 27% and 30% respectively whereas by supplementing glibenclamide it was 17%, 26%, 34%, 41%, 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 36% and 40% in the 18 and 21st day. Glibenclamide reduced blood glucose level by, 47% and 52% in the last three intervals compared to DC group.

Groups	Initial day	3 day	6 day	9 day	12 day	15 day	18 day	21 day
Plasma glucose concentration (mmol/L)								
General control	5.86 ± 0.21	5.83 ± 0.11	5.83 ± 0.18	5.88 ± 0.17	5.8 ± 0.25	5.88 ± 0.27	5.7 ± 0.12	5.81 ± 0.13
STZ-Control	19.4 ± 0.47 ^a	20.05 ± 0.52 ^a	20.3 ± 0.47 ^a	20.6 ± 0.47 ^a	21 ± 0.58 ^a	21.68 ± 0.72 ^a	23.38 ± 0.44 ^a	24.53 ± 0.35 ^a
Diabetic + Honey	22.3 ± 1.0 ^{b#}	20.3 ± 0.9	18.4 ± 0.8 ^{b#}	17.2 ± 1.1	16.3 ± 1.3 ^{b#}	14.4 ± 1.1	13.5 ± 1.0 ^{b#}	2.6 ± 0.5 ^{b#}
Diabetic + EECc	21.55 ± 0.46 ^b	19.03 ± 0.43	16.91 ± 0.38 ^b	15.31 ± 0.24 ^b	14.13 ± 0.28 ^b	13.39 ± 0.31 ^b	12.8 ± 0.30 ^b	12.41 ± 0.26 ^b
Diabetic + HFO	19.5 ± 1.2a ^{**}	18.6 ± 1.0a ^{**}	17.6 ± 0.8 ^{**}	16.7 ± 1.2a ^{**}	15.9 ± 0.9a ^{**}	15.2 ± 1.1a ^{**}	14.6 ± 0.7 ^{**}	14.0 ± 1.0a ^{**}
Glibenclamide	23.1 ± 0.83 ^b	20.58 ± 0.81	18.08 ± 0.75 ^b	15.72 ± 0.77 ^b	13.52 ± 0.4b	11.6 ± 0.78 ^b	10.46 ± 0.54 ^b	9.4 ± 0.42 ^b

a indicates the statistically significant values from general control group at p<0.001. b# indicates the values are significantly different from diabetic control group at p<0.001. b and ** indicates the statistically significant values from Diabetic control group at p<0.001.

Table 1: Effects of honey, EECc leaves and HFO on serum glucose level in STZ-induced diabetes in rats. Values were expressed as mean ± SD.

Table 2 shows the serum levels of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL, HDL and hypercholesterol of control and streptozotocin-induced diabetic rats. Reduction of total Cholesterol (TC) level was 14.38%-23.05% observed by honey bee treatment respectively in diabetic rats whereas hyper cholesterol reduces 19.04%-46.55%. The 3 to 21 days Total Cholesterol levels in the honey treatment groups showed significant decrease compared with the diabetic control group and hyper cholesterol control group (P<0.05). Administration of the plant extract demonstrated significant (P<0.001), reduction of TC 21% in diabetic and 56% in hypercholesterolemic rats, respectively. The extract administration also demonstrated the reduction of TG 14% and 68%, respectively

(P<0.001). LDL level was significantly reduced (P<0.001) for extract treatment 26% in diabetic and 54% in hypercholesterolemic rats. The HDL level was increased significantly (P<0.001) 39% and 17.25% in diabetic and hypercholesterolemic rats, respectively. VLDL level was also reduced (P<0.001) for extract treatment 15% and 68% in diabetic and hypercholesterolemic rats respectively. TC, TG, VLDL and LDL-c level in HFO treated rats was decreased by 11%, 23%, 24% and 12% respectively whereas HDL-c increased by 12%. In contrast in glibenclamide supplemented rats Serum TC, TG, VLDL and LDL-c level were decreased by 14%, 31%, 31.42% and 13% respectively whereas HDL-c increased by 16%.

Groups	Parameters				
	Cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	VLDL (mmol/L)
General Control	5.56 ± 0.10	1.42 ± 0.06	4.61 ± 0.13	0.29 ± 0.01	0.65 ± 0.02
Diabetic control	6.55 ± 0.14 [*]	1.96 ± 0.11 [*]	5.49 ± 0.16 [*]	0.23 ± 0.02 [*]	0.89 ± 0.05 [*]
Hypercholesterolemic Control	8.12 ± 0.53	3.48 ± 0.20	6.09 ± 0.49	0.24 ± 0.02	1.57 ± 0.05
Diabetic + Honey	5.045 ± 0.402 ^{b#}	1.73 ± 0.17 ^{b#}	3.94 ± 0.43 ^{b#}	0.308 ± 0.007 ^{b#}	0.788 ± 0.077 ^{b#}
Diabetic + EECc	5.14 ± 0.32 ^{**}	1.68 ± 0.26	4.04 ± 0.36 ^{**}	0.32 ± 0.01 ^{**}	0.76 ± 0.11 ^{**}
Diabetic + HFO	5.96 ± 0.18 ^{a***}	1.50 ± 0.08 ^{a***}	4.90 ± 0.15 ^{a***}	0.28 ± 0.03 ^{a***}	0.68 ± 0.04 ^{a***}

Hyper Cholesterol + Honey	4.346 ± 0.05 ^{***c}	0.613 ± 0.163	3.78 ± 0.109 ^{***c}	0.28 ± 0.014 ^{***c}	0.278 ± 0.074 ^{***c}
Hyper Cholesterol + EECc	3.56 ± 0.17 ^b	1.08 ± 0.03 ^b	2.78 ± 0.19 ^b	0.28±0.02 ^b	0.49 ± 0.007 ^b
Hyper Cholesterol + HFO	6.25 ± 0.42 ^{a**}	1.20 ± 0.16 ^{a**}	5.82 ± 0.47 ^{a**}	0.28 ± 0.03 ^{a**}	0.91 ± 0.07 ^{a**}
Diabetic + Glibenclamide	3.51 ± 0.14 ^{**}	0.96 ± 0.03	2.76 ± 0.15 ^{**}	0.28 ± 0.02	0.44 ± 0.02 ^a

*denotes the statistically significant values from general control group at P<0.001. b# indicates the significant difference from diabetic control group at p<0.05. ** and *** denotes the statistically significant values from diabetic control group at P<0.001. a denotes the statistically significant value from diabetic control group at P<0.05. b denotes the statistically significant values from hypercholesterolemic control group at p<0.001. **C and a** indicates the significant difference from hypercholesterol group at P<0.05.

Table 2: Effects of honey, EECc and HFO on biochemical parameter in diabetic and hypercholesterolemic rats. Values were expressed as mean ± SD.

Increasing of SGPT and SGOT level after diabetes induction which was compensated by honey significantly (P<0.05). The reduction of SGPT by honey was 38.19% respectively whereas 23.59% for glibenclamide. The reduction of SGOT level was highly significant for honey 27.84% than glibenclamide 61.23% (Table 3). Administration of leaves extract demonstrated significant reduction of SGPT by 24% in diabetic and 30% in hypercholesterolemic rats and 57% for glibenclamide. The reduction of SGOT level was highly significant for extract treatment 13% and 35%, and 42% for glibenclamide, respectively. 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.

Group	SGPT (U/L)	SGOT (U/L)
General Control	55.60 ± 2.30	40 ± 3.28
Diabetic control	67.80 ± 1.97 [*]	87.00 ± 2.73 [*]
Hypercholesterolemia Control	93.60 ± 2.60	119.60 ± 3.65
Diabetic + Honey	62.00 ± 2.44 ^{b*}	21.08 ± 4.81 ^{b*}
Diabetic + HFO	73.0 ± 4.77 ^{a**}	56.67 ± 6.53 ^{a**}
Diabetic + EECc	51.00 ± 1.58 ^{**}	75.80 ± 3.20 ^{**}
Hypercholesterol + Honey	82.16 ± 2.91 ^{a*}	85.83 ± 3.28 ^{a*}
Hypercholesterolemic + EECc	65.40 ± 4.15 ^c	76.6 ± 2.87 ^c
Hypercholesterolemic + fish oil diet	72.16 ± 2.91 ^{**}	85.83 ± 3.28 ^{**}
Diabetic + Glibenclamide	29.60 ± 3.72 ^{**}	50.00 ± 2.23 ^{**}

*indicates the difference from normal group; whereas **indicates the difference from Diabetic control group. These values are statistically significant from hypercholesterolemia control group P<0.001, b* and a* indicates the values significantly different from diabetic group with Glibenclamide and honey at p<0.05.

Table 3: Effect of honey, EECc and HFO on serum SGPT and SGOT of experimental rats.

Serum SGPT and SGOT in the treated mice were significantly different from normal and diabetic control groups at P<0.001.

C - reactive protein (CRP) levels were higher in diabetic and hypercholesterol control group than the normal group. By the

treatment of honey CRP significantly reduces 25.06% from 61.32% diabetic group whereas glibenclamide reduce 42.0% from 61.32%. By the treatment of honey, there is a significant reduction of CRP 23.51% from 67.14% in hypercholesterol group (p<0.05) (Table 4). The administration of plant extract and glibenclamide reduced the CRP level significantly (P<0.05 and P<0.001) (Table 4). Administration of extract demonstrated that the significant reduction of CRP level 11% in diabetic and 26% in hypercholesterolemic rats compare with glibenclamide treatment rats (42%), respectively. C - Reactive protein (CRP) level was also higher in DC rats group than the normal rats which was declined significantly (P<0.001) by 36.70% with HFO and 45% with glibenclamide supplementations.

Group	CRP level (mg/l)
General Control	0.9 ± 0.2
Diabetic control	2.8 ± 0.5 [*]
Hypercholesterolemic Control	3.4 ± 0.37
Diabetic + Honey	2.1 ± 0.11 [*]
Diabetic + EECc	2.5 ± 0.35 ^{**}
Diabetic + HFO	1.88 ± 0.35 ^{**}
Hypercholesterol + Honey	2.6 ± 0.32 ^{**}
Hypercholesterolemic + EECc	2.5 ± 0.45 ^c
Hypercholesterolemic + fish oil diet	2.3 ± 0.32 ^{**}
Diabetic + Glibenclamide	1.6 ± 0.4 [*]

*represents the statistically significant values from control group at P< 0.001 and **represents the statistically significant values from diabetic control group at P<0.05. c represents the value was statistically significant from hypercholesterolemic control group at p<0.05.

Table 4: Effect of honey, EECc and HFO on CRP of experimental rats.

Histopathological study

The histopathological studies were shown in Figures 1 and 2.

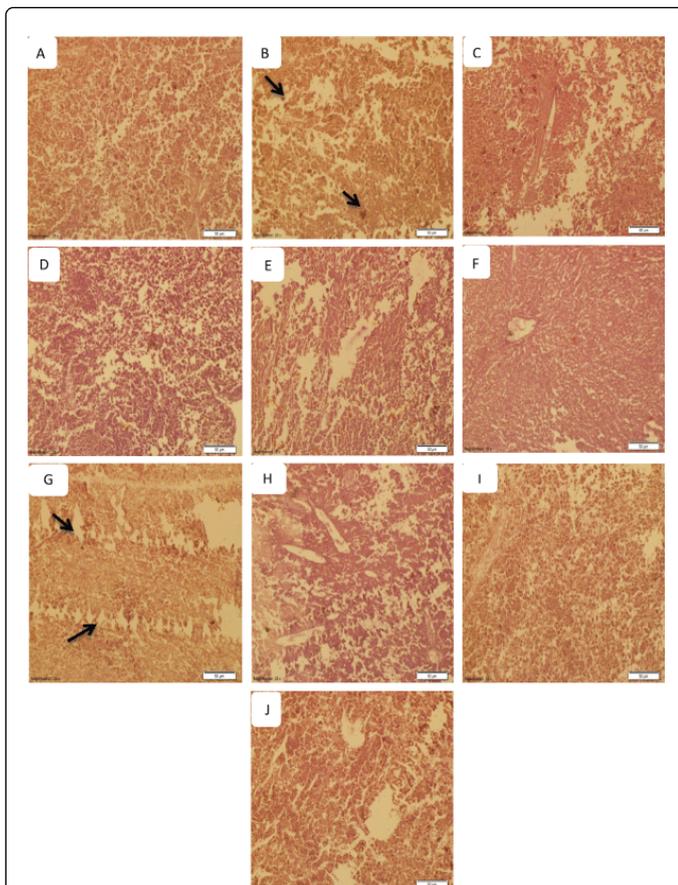


Figure 1: Fluorescence Microphotograph of Liver in different groups of rats. (A) Normal control: showing normal-appearing of hepatocytes, portal space (PS), sinusoids (arrows), and Kupffer cells, (B) Diabetic control: Diabetic control histopathology of rats liver shown the micro fat droplet deposition (Black arrow) and the onset of sinusoidal enlargement (arrows) and small amount of fatty vacuoles, respectively, (C) Honey showed apparently normal architecture. The structure was quite similar to that of the control group and tissue damage and necrosis were of less extent in these groups than the DC group, (D) *C. cordifolia* leaves extract (200 mg/kg): Tested liver histopathology there was only few micro fat droplet was present (yellow arrow). This histopathology similar to the glibenclamide treated group, (E) Treatment of HFO showed few micro fat droplets, (F) Glibenclamide (10 mg/kg): Standard drug treated group shown histopathology similar to the normal control group, (G) Liver from hypercholesterolemic rats sacrificed showing progressive worsening of sinusoidal enlargement (arrows) and liver fatty degeneration, (H) Honey treatment: Liver remained in apparently normal architecture with no deposition of cholesterol and triglyceride and no vesicular steatosis in the liver tissues, (I) Rats received treatment with *C. cordifolia* leaves xtract showed histopathology similar to the normal control group, (J) Treatment with HFO: Liver remained in apparently normal architecture.

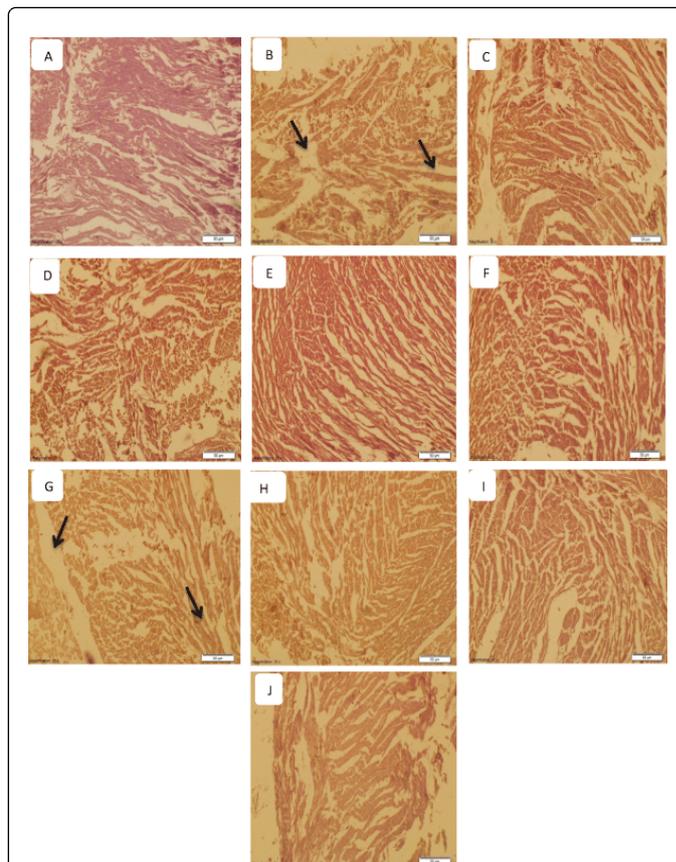


Figure 2: Fluorescence Microphotograph of Heart in different groups of rat. (A) Normal control: Normal control group shown normal histopathology of the heart, (B) Diabetic control: Diabetic control histopathology shown increased interstitial space and distort the intercalated disc (Black arrow), (C) Honey for Three weeks led to a normal histological organization in the heart cells and nuclei. The antioxidant activity may have prevented the oxidative damage at the myocardium in STZ-induced diabetic rats, (D) *C. cordifolia* leaves extract (200 mg/kg): Tested heart histopathology shown less interstitial space (Black arrow), as normal heart like the glibenclamide histopathology, (E) Heart intestinal space is reduced during the treatment with HFO in rats, (F) Glibenclamide (10 mg/kg): Glibenclamide treated drug shown the normal histopathology of heart. Photomicrograph showing longitudinal section of cardiac tissues in (G) Hypercholesterolemic control group heart histopathology showed degenerating muscle fibers and muscle fibers vacuolization, fibrosis, transverse striations and wide interfascicular spaces, (H) The heart cells and nuclei altered to a normal histological organization with honey treatment. Cardiac myofibres were arranged in normal structure, (I) *C. cordifolia* leaves extract (200 mg/kg): Tested heart histopathology showed normal heart like the glibenclamide histopathology, (J) Treatment with HFO affects abnormal organization to normal as done by glibenclamide. The samples were obtained from the same heart anatomical regions. For each group, 6 rats were examined and 50 pictures were taken. The above picture for each group was chosen randomly from the 80 pictures in this group. Original magnification, 25X.

Discussion

The observed significant increase in of blood glucose level in diabetes rats could be due to the destruction of pancreatic β -cells by STZ administration during diabetes. STZ monohydrate induces type-2 diabetes in experimental rats through exclusive destruction of insulin producing beta cells in pancreas [15]. Glibenclamide or honey significantly reduced blood glucose concentrations in our study which is similar to findings from previous studies [16]. Honey treatment lowered plasma glucose, cholesterol, triglyceride, and LDL levels and increased HDL levels in diabetic rats compare to untreated diabetic group. Our results were consistent with findings of Mousavi et al. which also confirmed hypoglycemic and hypolipidemic activity of honey in diabetic mice [17]. The low level of SGOT and SGPT in honey consuming diabetic rats comparing to control rats indicated the normal function of liver [18]. C - reactive protein (CRP) is a simple cost 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 [19]. Cucurbitaceae is one of the important families of the plants with potent hypoglycemic effect [20-22] to be used as best choice of alternative medicine for treating diabetes. Oral administration of plant extract produced significant hypoglycemic effect in streptozotocin induced diabetic rats compared with streptozotocin induced diabetic control rats. Our results were consistent with findings [23] which also confirmed hypoglycemic and hypolipidemic activity of the plant extract in diabetic rats. HDL-C level was significantly increased ($P < 0.001$) following a significant decrease in LDL-C level ($P < 0.001$) which is an indication of the reduction of the risk of coronary heart diseases that are very common in diabetic patient [24]. Serum SGOT and SGPT level in EECs treated rats reduced significantly but the effect is slightly less than our previous study [25]. CRP level was significantly decreased along with the decrease of blood glucose and serum cholesterol during the treatment of the extract in diabetic as well as hypercholesterolemic rats.

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) [26]. EAA-to-NEAA ratio showed a positive correlation to IGF-I and insulin whereas an inverse correlated to IGFBP-1 [27]. Minerals and Vitamins content of hilsha fish was also studied by [28]. 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 [29]. Other ions such as copper and zinc are also known to be involved in glucose and insulin metabolism. 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 macrovesicular steatosis. From the above discussion, it might be concluded that honey, plant extract and HFO had significant effect on STZ- induced hyperglycemic and hyperlipidemic rats.

Conclusion

There is considerable evidence from experimental studies that honey, extract and fish oil has potential hypoglycemic and hypolipidemic activity in STZ induced diabetic rats. The Supplementation of honey, extract and fish oil showed lower level of SGPT, SGOT and CRP by enzymatic function in liver (hepatoprotective activity) and reduce blood glucose level as well as Cardiovascular Disease (CVD) in diabetic rats. They also help in the

improvement of DM associated hypercholesterolemia. Hence, honey, plant leaves extract and Hilsha fish oil might be used as a substitute for synthetic drugs in treating diabetes and cardiovascular diseases.

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.

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Compliances and Ethics

Conflict of Interests: All authors declare that they have no conflict of interests.

Experimental Ethics (Animal): All institutional and national guidelines for the care and use of experimental laboratory animals were followed.

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References

1. Alwayn IP, Andersson C, Zauscher B, Gura K, Nose V, et al. (2005) Omega-3 fatty acids improve hepatic steatosis in a murine model: potential implications for the marginal steatotic liver donor. *Transplantation* 79: 606-608.
2. Amos AF, McCarty DJ, Zimmet P (1997) The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 14 Suppl 5: S1-85.
3. Arquilla ER, Packer S, Tarmas W, Miyamoto S (1978) The effect of zinc on insulin metabolism. *Endocrinology* 103: 1440-1449.
4. Azad Khan AK, Akhtar S, Mahtab H (1979) *Coccinia indica* in the treatment of patients with diabetes mellitus. *Bangladesh Med Res Counc Bull* 5: 60-66.
5. Omotayo O Erejuwa (2014) Effect of honey in diabetes mellitus: matters arising. *J Diabetes Metab Disord* 13: 23.
6. Tan BK, Tan CH, Pushparaj PN (2005) Anti-diabetic activity of the semi-purified fractions of *Averrhoa bilimbi* in high fat diet fed-streptozotocin-induced diabetic rats. *Life Sci* 76: 2827-2839.
7. Bnouham M, Ziyat A, Mekhfi H, Tahri A, Legssyer A (2006) Medicinal plants with potential antidiabetic activity - a review of ten years of herbal medicine research (1990-2000). *Int J Diabetes Metabol* 14: 1-25.
8. Chawla R, Thakur P, Chowdhry A, Jaiswal S, Sharma A, et al. (2013) Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. *J Diabetes Metab Disord* 12: 35.
9. Nathan DM (1996) The pathophysiology of diabetic complications: how much does the glucose hypothesis explain? *Ann Intern Med* 124: 86-89.
10. Erejuwa OO, Sulaiman SA, AbWahab MS (2009) Effects of Malaysian tualang honey supplementation on glycemia free radical scavenging enzymes and markers of oxidative stress in kidneys of normal and streptozotocin-induced diabetic rats. *Int J Cardio* 137: S45.

11. Filho JC, Hazel SJ, Anderstam B, Bergström J, Lewitt M, et al. (1999) Effect of protein intake on plasma and erythrocyte free amino acids and serum IGF-I and IGFBP-1 levels in rats. *Am J Physiol* 277: E693-701.
12. Ghosh R, Sharatohandra KH, Rita S, Thokchom IS (2004) Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. *Indian J. Pharmacol* 36: 222-225.
13. Imam K (2012) Clinical features, diagnostic criteria and pathogenesis of diabetes mellitus. *Adv Exp Med Biol* 771: 340-355.
14. Kar A, Choudhary BK, Bandyopadhyay NG (1999) Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. *J Ethnopharmacol* 64: 179-184.
15. King H, Aubert RE, Herman WH (1998) Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21: 1414-1431.
16. Khurseed J, Mosharaff (1998) Seasonal changes on biochemical composition of fresh water murrel *Ophiocephalus punctatus* (Bloch). *Hydrobiologia* 32: 206-213.
17. Lam DW, LeRoith D (2012) The worldwide diabetes epidemic. *Curr Opin Endocrinol Diabetes Obes* 19: 93-96.
18. Laws A, Reaven GM (1992) Evidence for an independent relationship between insulin resistance and fasting plasma HDL-cholesterol, triglyceride and insulin concentrations. *J Intern Med* 231: 25-30.
19. Mahmud I, Hossain A, Hossain S, Hannan A, Ali L, et al. (2004) Effects of Hilsa ilisa fish oil on the atherogenic lipid profile and glycaemic status of streptozotocin-treated type 1 diabetic rats. *Clin Exp Pharmacol Physiol* 31: 76-81.
20. Marles RJ, Farnsworth NR (1995) Antidiabetic plants and their active constituents. *Phytomed* 2: 133-169.
21. Minarul I, Manirujjaman M, Khan MMH, Matiar R, Ziaul AM (2014) Effects of Conjugated Linoleic Acid and Hilsha Fish Oil (Tenualosallisha) on Alloxan Induced Diabetes Mellitus in Sprague Dawley Rats. *J Diabetes Metab* 5: 412.
22. Mohanty BP, Ganguly S, Mahanty A (2012) Micronutrient profile of the Indian shad, *Tenulosa ilisha* (Hamilton, 1822), hilsa from the river Ganga, India. *Natl Acad Sci Lett* 35: 1-10.
23. Mohanty BP, Das S, Bhaumik U, Sharma AP (2011) *Tenulosa ilisha* - A rich source of omega-3 fatty acids. Central Inland Fisheries Research Institute (ICAR), Barrackpore, India.
24. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336: 973-979.
25. Mukherjee K, Ghosh NC, Datta T (1972) *Coccinia indica* Linn. as potential hypoglycaemic agent. *Indian J Exp Biol* 10: 347-349.
26. Filho JC, Hazel SJ, Anderstam B, Bergström J, Lewitt M, et al. (1999) Effect of protein intake on plasma and erythrocyte free amino acids and serum IGF-I and IGFBP-1 levels in rats. *Am J Physiol* 277: E693-701.
27. Kar A, Choudhary BK, Bandyopadhyay NG (1999) Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. *J Ethnopharmacol* 64: 179-184.
28. Arquilla ER, Packer S, Tarmas W, Miyamoto S (1978) The effect of zinc on insulin metabolism. *Endocrinology* 103: 1440-1449.
29. Alwayn IP, Andersson C, Zauscher B, Gura K, Nosé V, et al. (2005) Omega-3 fatty acids improve hepatic steatosis in a murine model: potential implications for the marginal steatotic liver donor. *Transplantation* 79: 606-608.