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Effects of Honey Supplementation on Hepatic and Cardiovascular Disease (CVD) Marker in Streptozotocin-Induced Diabetic Rats

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Abstract

Introduction: Honey is not just a sweetener it is also a nature's gift to mankind. Natural honey has various ingredients in it that contribute to its incredible properties. Honey contain health-beneficial effects such as gastro protective, hepatoprotective, reproductive, hypoglycemic, antioxidant, antibacterial, and anti-inflammatory effects. For that reason, honey could be considered as a potential remedy for diabetes as well as Cardiovascular Disease (CVD). The objective of this study was to determine the phytochemical activity of honey and its role on hypoglycemic and hypolipidemic condition in Streptozotocin- induced diabetic rats.

Material and method: 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.

Result: Honey bee-treatment significantly decreases blood glucose level in diabetic rats. TC, TG, LDL, VLDL are significantly ($p<0.05$) decrease whereas HDL significantly increase ($p<0.001$). The SGPT, SGOT and CRP were significantly decrease ($p<0.05$). The total phenolic and flavonoid were determined by Folin-Ciocalteu and colorimetric assay method where some flavonoid decreases diabetic condition as well as CVD. Honey has also effect of hepatoprotective activity.

Conclusion: On the basis of above findings, it can be concluded that, supplementation of honey could significantly contribute to control blood glucose level as well as lipid profile in diabetic subjects.

Keywords: Honey; CVD; Diabetes; Glucose; Lipid profile; CRP

Introduction

Diabetes mellitus a metabolic disorder characterized by hyperglycaemia, secretion insufficiency and receptor insensitivity to endogenous insulin. Another serious pathogenesis of diabetes is an abnormal lipid profile indicated by low levels of HDL and high concentration of triglyceride and LDL. Hence the potential remedy for diabetes not only needs the blood glucose levels lowering action, but also lipid regulating effect. Diabetes mellitus remains an incurable disorder which is associated with poor quality of life, cardiovascular complications, increased mortality and morbidity [1]. The recent statistics shows that the global prevalence of this disorder continues to rise unabated and thus becoming an epidemic [2]. 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 [3]. At the moment, the management of this disorder entails increased physical activity, healthy eating or diet and administration of anti-diabetic drugs and/or insulin. However, the currently available anti-diabetic drugs are far from being satisfactory. This may partly be attributed to the fact that diabetes is a disorder with multifactorial and heterogeneous etiologies. Besides, these agents are costly and, in some cases, not readily available. As a result of these limitations and unmet goals, a large percentage of the population is resorting to CAM [4]. This alternative approach to diabetes therapy includes the use of herbal preparations, dietary components or supplements and other natural products such as honey [5]. Honey is a natural substance produced by bees from nectar. In the last few years, there has been an increased interest in the therapeutic uses of honey. This is largely due to an increase in the availability of evidence-based findings demonstrating the health beneficial effects of honey in treating diverse disease conditions including diabetes mellitus and cardiovascular disease (CVD). Honey has hepatoprotective activity which reductions in the size of enlarged hepatocytes and edema. Major component of honey are melittin and phospholipase A2, a polypeptide and an enzyme that increase insulin secretion from pancreatic β-cells via depolarization of beta cell membrane [6]. Another potential mechanism is lowering blood glucose and cholesterol reducing action is lipolytic properties of Honey. The components partially lyse cell membrane which increases glucose transport and lipid take up into adipose tissue. Based on the above properties, Honey could be considered as a therapeutic agent for diabetes and Cardiovascular Disease (CVD). There is no systematic data available on the physicochemical and phytochemical properties of Bangladeshi honey. This study was undertaken to analyse Bangladeshi

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honey for better understanding of its medicinal properties. This study aims to investigate the effect of Honey on blood glucose, Total cholesterol (TC),Triglycerides (TG), Low density lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and High density lipoprotein (HDL) levels in Diabetic and Cardiovascular Disease (CVD) subjects.

Materials and Method

Sample collection and preparation

Honey sample were collected freshly in sterile containers from the Sundarbon, Khulna, Bangladesh. All samples were collected freshly in sterile containers (level 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.

Determination of total phenolic and total flavonoid content

Total phenolic content: Phenolic compounds from honey samples were detected by a modified spectrophotometric Folin-Ciocalteu method [7]. Briefly, 1 mL honey solution (0.5 g/ml) was mixed with 1 mL Folin and Ciocalteu'sphenol reagent. After 3 min, 1 mL 10% Na_2CO_3 solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm by a T 60 UV/VIS spectrophotometer (PG Instruments Ltd, UK). Ascorbic acid was used to calculate the standard curve (5, 10, 15, 20 and 25 µg/mL, r^2=0.9767). The estimation of the amount of phenolic compounds was carried out in triplicate. The results were reported as the mean ± standard deviations and were expressed as mg of Ascorbic acid equivalents per gm honey.

Total flavonoid content: Total flavonoids the total flavonoid concentration of each honey sample was determined according to the colorimetric assay developed by Zhishen [8]. 1 mL honey solution (0.5 g/ml) was mixed with 4 mL distilled water. At baseline, 0.3 mL $NaNO_2$ (5%, w/v) was added. After five min, 0.3 mL AlCl3 (10% w/v) was added, followed by the addition of 2 mL NaOH (1 M) six min later. The volume was immediately increased to 10 mL by the addition of 2.4 mL distilled water. The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 510 nm. A calibration curve was prepared by using a standard solution of quercetine (50,100,150,200 and 250 µg/mL, r^2=0.9966). The results were also expressed as mg quercetine equivalents (CEQ) per gm honey.

Animals care

Test animals were collected from International Cholera and Dysentery Disease Research, in Bangladesh (icddr,b). Albino rats (wistar strain) of both sexes weighing 175 g (average) were used for the study and also recruited both gender. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions. Feeding of animals was done a libitum, along with drinking water and maintained at natural day night cycle.

Induction of diabetics

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). The age-matched control rats received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after streptozotocin (STZ) administration. The development of hyperglycaemia in rats was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 48 hours after STZ administration, with a Portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level ≥ 11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and included in the study.

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 rat's marginal ear vein by 26 G needle and syringe [9]. "Humylazer 2000" analyser (Human, Germany) determined Blood glucose levels, plasma cholesterol levels, triglyceride levels, LDL and HDL levels. The values were expressed as mean ± S.E.M, Statistical analyses were performed by SPSS-16one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test for multiple comparisons. P<0.05 was considered as significant.

Experimental animals grouping and treatment

The animals were randomly divided into six 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: control rats feed with standard pellet diet and water.

Group-2: The rats were made diabetic by an intra-peritoneal injection of single dose of 110 mg/kg body weight followed by 65 mg/kg body weight Streptozotocin. Animals whose blood glucose level exceeded 11.0 mmol/L at 72 hafter treatment were considered diabetic. These animals served as untreated diabetic control.

Group-3: The diabetic rats treated with Honey at a dose of 1.0 g/kg body weight for 21 days.

Group-4: Hyper Cholesterol rats were given cholesterol (1.5%) and cholic acid (0.5%) mix with diet.

Group-5: Hyper cholesterol rats were treated with Honey at a dose of 1.0 g/kg body weight for 21 days.

Group-6: Diabetic rats were treated by Glibenclamide at a dose of 0.5 mg/kg b.wt.

Measurement of blood parameters

Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, SGPT, SGOT and CRP were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by automatic Bioanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan).

Results

Total phenolic content was determinates as a standard curve used Ascorbic acid (Figure 1) and the Flavonoid was determinates as a standard curve used quercetine (Figure 2). Figure 3 shows that the total content of Phenolic and Flavonoid compound. The Flavonoid content is higher than the total Phenolic content.

Comparing the blood sugar level in Streptozotocin induced diabetic rats, honey administered subject showed significant reduction of blood glucose level which is as near as glibenclamide administered subject at (P<0.001) (Table 1). 3rd, 6th, 9th, 12th, 15th, 18th, and 21th 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).

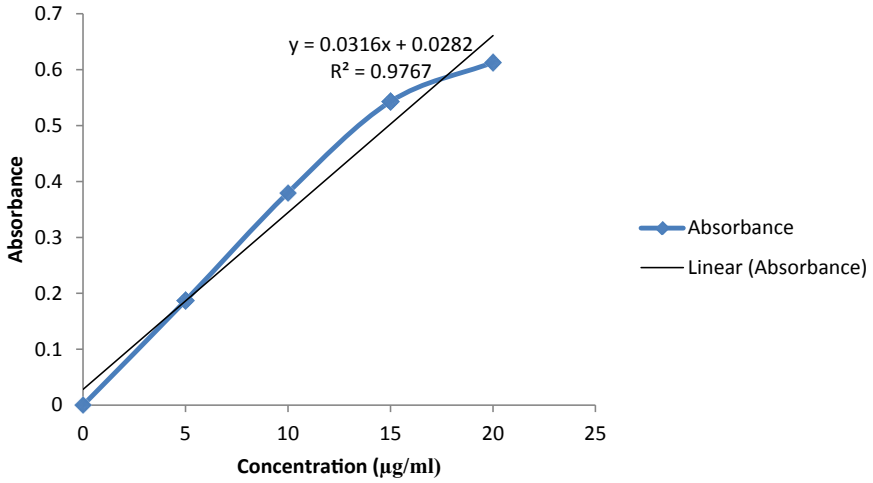

Figure 1: Standard curve of Ascorbic Acid for the determination of total phenolic compounds.

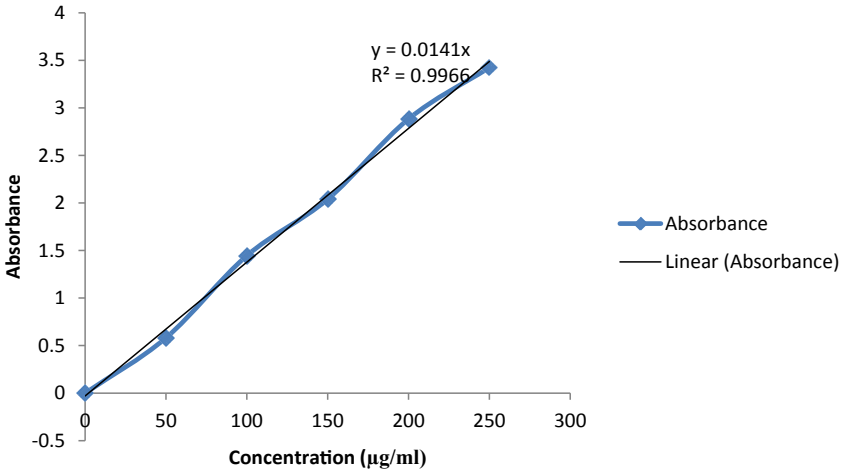

Figure 2: Standard curve of quercetine for the determination of total flavonoids compounds.

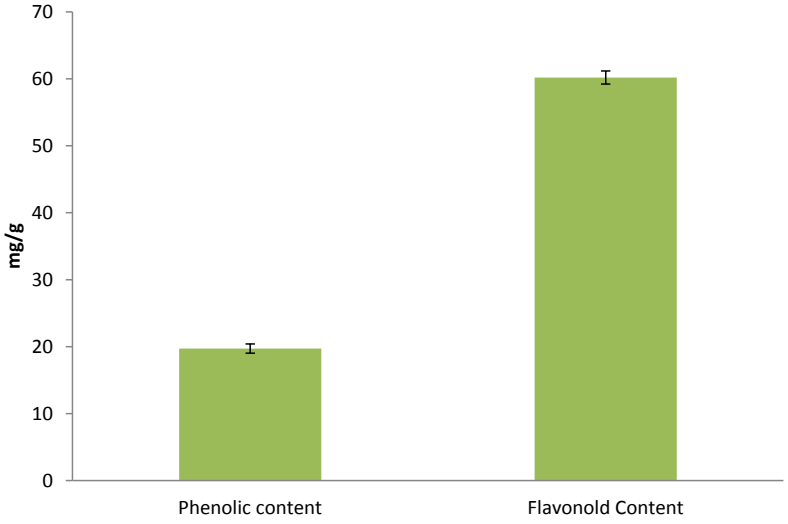

Figure 3: Phytochemical Activity of Honey.

Group	Blood sample drawing days							
	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Normal (control)	5.8 ± 0.2	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.2	5.8 ± 0.2	5.7 ± 0.1	5.8 ± 0.1
Diabetic control	19.4 ± 0.4^{a*}	20.0 ± 0.5^{a*}	20.3 ± 0.4^{a*}	20.6 ± 0.4^{a*}	21.0 ± 0.7^{a*}	21.6 ± 0.7^{a*}	23.3 ± 0.4^{a*}	24.5 ± 0.5^{a*}
Diabetic + Glibenclamide	22.8 ± 0.9	20.3 ± 0.9^{b*}	17.8 ± 0.8	15.5 ± 0.8^{b*}	13.5 ± 0.4^{b*}	11.5 ± 0.7^{b*}	10.4 ± 0.5^{b*}	9.4 ± 0.3^{b*}
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$^{b\#}$	12.6 ± 0.5$^{b\#}$
Body weight degreases with injection of STZ. Body weights were measure with 3 days interval for 21days. Each value is the mean ± SEM n=6. Blood glucose level in the treated rats were significantly different from normal and diabetic control groups at ^{a*}P<0.001, whereas^{b*}P<0.001 and $^{b\#}$p<0.001 indicated the significantly difference from diabetic control group								

Table 1: Effect of Honey on the blood glucose level of experimental rats (mmol/L).

Groups	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL (mmol/L)	VLDL(mmol/L)	HDL (mmol/L)
Normal (Control)	5.565 ± 0.119	1.423 ± 0.070	4.613 ± 0.143	0.646 ± 0.032	0.298 ± 0.011
STZ (Diabetic) control	6.556 ± 0.154^{a*}	1.965 ± 0.123^{a*}	5.427 ± 0.126^{a*}	0.892 ± 0.055^{a*}	0.231 ± 0.032^{a*}
Hypercholesterol control	8.126 ± 0.580	3.458 ± 0.142	6.102 ± 0.537	1.571 ± 0.064	0.445 ± 0.056
Diabetic+ Glibenclamide	5.603 ± 0.116^{b*}	1.725 ± 0.128^{b*}	4.610 ± 0.148^{b*}	0.783 ± 0.058^{b*}	0.205 ± 0.018^{b*}
Diabetic+Honey	5.045 ± 0.402$^{b\#}$	1.735 ± 0.171$^{b\#}$	3.943 ± 0.437$^{b\#}$	0.788 ± 0.077$^{b\#}$	0.308 ± 0.007$^{b\#}$
Hyper Cholesterol + Honey	4.346 ± 0.05^{**c}	0.613 ± 0.163	3.786 ± 0.109^{**c}	0.278 ± 0.074^{**c}	0.28 ± 0.014^{**c}
Total Cholesterol, Triglycerides, LDL, VLDL and HDL in the treated rats were significantly different from normal and diabetic control groups at^{a*}P < 0.05,whereas Glibenclamide ^{b*}p<0.001 and honey $^{b\#}$p<0.05 indicated the significantly difference from Diabetic control group and ^{**C} P<0.05 indicated significantly Difference from hypercholesterol group.					

Table 2: Effect of Honey on Total cholesterol, Triglyceride, LDL, VLDL, HDL, in experimental rats.

Group	SGPT (U/L)	SGOT (U/L)
Normal (control)	55.60 ± 2.30	41.02 ± 3.27
Diabetic control	67.80 ± 1.97*	87.00 ± 2.73*
Hypercholesterol Control	94.66 ± 2.86	124.16 ± 6.74
Diabetic+Glibenclamide	29.60 ± 3.71^{a*}	50.04 ± 3.33^{a*}
Diabetic+ Honey	62.00 ± 2.44^{b*}	21.08 ± 4.81^{b*}
Hypercholesterol+ Honey	82.16 ± 2.91**	85.83 ± 3.28**
Serum SGPT and SGOT in the treated rats were significantly different from normal and diabetic control groups at *P<0.001,^{a*}p<0.05 and^{b*}p<0.05 indicated the significantly difference from diabetic group with Glibenclamide and honey; whereas **p<0.05indicated significantly difference from hypercholesterol control group with honey.		

Table 3: Effect of Honey on serum SGPT and SGOT of experimental rats

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 was14.38%-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).

The effect of Honey treatment on serum triglyceride (TG) content in diabetic rats is illustrated in Table 2. The Levels of triglyceride in the diabetic and hyper cholesterol group on the 21 days increased. Compare with the diabetic and hyper cholesterol control group by the treatment of honey serum triglyceride was considerably lower 6.30% -11.73% whereas reduction of hypercholesterol group was 52.30%-82.31%. During the course of the experiment the significantly decrease diabetic and hyper cholesterol group (P<0.05).

The diabetic and hyper cholesterol group shows an increase in LDL levels higher than the (normal) control group. LDL level was significantly reduced (P<0.05) in diabetic group received honey bee-treatment 15.7%-27.30% and hypercholesterol 19.72%-38.03%. VLDL level was significantly reduced (P<0.05) for due to received honey bee-treatment 9.2%-12.35% whereas hypercholesterol 63.00%-82.80%. The HDL level was increased significantly (P<0.001) at diabetic 13.4%-30.43% whereas hypercholesterol increased 12.25%-37.40% (Table 2).

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).

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 significantly reduction of CRP 23.51% from 67.14% in hypercholesterol group (p<0.05), (Table 4).

Discussion

In this study, honey treatment showed blood glucose levels lowering activity in streptozotocin induced diabetic rats. STZ monohydrate induces type-2 diabetes in experimental rats through exclusive destruction of insulin producing beta cells in pancreas [10]. Glibenclamide or honey significantly reduced blood glucose concentrations in our study which is similar to findings from previous studies [11]. The 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 hypoglycaemic and hypolipidemic activity of honey in diabetic mice [12]. In another study, honey reduces glycaemia and cholesterolemia in healthy subjects depending on the inoculated dose [13]. One mechanism of honey

Group	CRP (mg/L)
Normal (control)	0.9 ± 0.07
Diabetic control	2.8 ± 0.79**
Hypercholesterol Control	3.4 ± 0.19
Diabetic+ Glibenclamide	1.6 ± 0.02*
Diabetic+ Honey	2.1 ± 0.11*
Hypercholesterol+ Honey	2.6 ± 0.32b*
Serum CRP in the treated rats were significantly difference from normal and diabetic control groups at **P<0.001,*p<0.05indicated the significantly difference from diabetic control group with Glibenclamide and honey, whereas b*p<0.05indicated the significantly difference from hypercholesterol control group with honey.	

Table 4: Effect of Honey on serum CRP of experimental rats.

to lower blood glucose levels is through the suppression of beta cell inflammation [14] and direct stimulation of insulin secretion [15]. Honey also contains elements such as zinc, selenium, copper, calcium, potassium, chromium, manganese, etc. [16]. 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 [17]. Other ions such as copper and zinc are also known to be involved in glucose and insulin metabolism [18]. According to Ginsberg, another possible strategy to treat diabetic dyslipidemia is to link glucose and fatty acid metabolism by improving insulin action in fat cells which result in lower LDL, triglyceride and in increased HDL levels [19]. The honey contain phospholipase A2 partially lyses cell membrane due to its enzymatic action on the plasmatic lipoproteins [13]. This activity increases glucose transport and lipid take-up into adipose tissue through partial lyses of adipocytes membrane and binding of higher number of insulin molecules [20].

Honey content flavonoid intake is associated with a reduced risk of cardiovascular diseases (CVD). In the coronary heart disease (CHD), the protective effects of flavonoids include mainly antithrombotic, antiischemic, anti-oxidant, and vasorelaxant. It is suggested that flavonoids decrease the risk of coronary heart disease by three major actions: (A) improving coronary vasodilatation, (B) decreasing the ability of platelets in the blood to clot and (C) preventing LDLs from oxidizing [21]. Oxidation of low density lipoproteins (LDLs) is believed to play an important role in the development of atherosclerosis. Based on the above discussion honey could be considered as a therapeutic agent for diabetes as well as cardiovascular diseases (CVD). Consistently, another study also confirmed that injection of flavonoid (quercetin) has a protective effect against pancreatic islet damage in STZ-induced diabetic rats [22]. The SGOT and SGPT level was increased in diabetic patient as an indication of the liver damage that back to their respective normal level after treatment with honey and glibenclamide. The low level of SGOT and SGPT in honey consuming diabetic rats comparing to control rats indicated the normal function of liver [23]. 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 (CVD) risk factor [24]. Based on the previous observation we designed to investigate the supplementation of Honey on lipid profile and CRP because this issue may have clinically important implication for high risk patient with coronary heart disease [25]. Similar hepatoprotective effect of honey was also reported in diabetic rats with obstruction of the common bile duct [26]. These findings, generally, suggest that oxidative stress in the liver may contribute to the hepatoprotective effect of honey.

Statistical analysis

All the data are expressed as the mean ± SEM. The statistical analysis was carried out by using Graph Pad Insat version 5. The obtained results are analyzed by ANOVA followed by post-test (Bonferroni; one way and two way) and significant levels are $p>0.05$, $p>0.01$, $p>0.001$.

Conclusion

There is considerable evidence from experimental studies that honey provide benefits in the management of diabetes mellitus. The hypoglycaemic and hypolipidemic activity of honey on Streptozotocin induced diabetic rats through suppression of pancreatic beta cell inflammation, promotion of insulin secretion and promotion of glucose uptake in adipose tissue due to improvement of lipid uptake into adipose tissue and hydrolysis of triglyceride. Honey contain flavonoids are comparable in function to the clinically used anti-diabetic drugs and that novel anti-diabetic effects are continuously identified. The Supplementation of honey 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.

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