ORIGINAL ARTICLE

EFFECT OF SUPPLEMENTATION OF ACACIA HONEY AND PIOGLITAZONE ON LIPID PROFILE IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Background: There is growing interest in the use of alternative approaches like the use of herbs and natural products in the management of diabetes. The objective of this study was to determine the effect of combined supplementation of Pioglitazone and Acacia honey on lipid profile in streptozotocin induced diabetic rats. **Methodology:** This study was carried out on 150 male Sprague Dawley rats. The rats were divided into 5 groups with 30 rats in each group. Group I was normal control. Groups II–V, were given streptozotocin, intraperitoneally to make them diabetic. Group II was diabetic control. Group III was given acacia honey, group IV received intraperitoneal injection of pioglitazone (oral hypoglycaemic) along with Acacia honey, and group V was given pioglitazone intraperitoneally for same dose and duration. After 3 weeks of supplementation serum total cholesterol, low density lipoprotein, high density lipoprotein, very low density lipoprotein and triglycerides were measured. Statistical analysis was done on SPSS-20. All values were expressed as Mean±SEM. The differences were compared using one way analysis of variance followed by Tukey tests and $p<0.05$ were taken as significant. **Results:** After 3 weeks of supplementation serum triglycerides levels were significantly reduced in all groups while serum total cholesterol and low density lipoprotein levels were reduced in group IV only. **Conclusion:** Acacia honey along with Pioglitazone was able to improve lipid profile as indicated by decreased levels of total cholesterol, low density lipoproteins, and triglycerides in streptozotocin induced diabetic rats.

Keywords: Honey, Lipid profile, Diabetes mellitus, HDL, LDL, VLDL, Sprague Dawley
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INTRODUCTION

Diabetes mellitus (DM) has become one of the major threats to health all over the world. The current statistics of International Diabetes Federation (IDF) indicate that approximately 1 in 11 adults (415 million) has diabetes and is expected to reach 1 in 10 adults (642 million) by year 2040. In Pakistan the prevalence of DM is very high; ranging from 7.6% (5.2 million populace) to 11% in 2011, it is estimated to reach 15% (14 million) by 2030.[1]

Diabetic dyslipidemia includes abnormality of lipids and lipoproteins which are metabolically related to each other. The lipids include cholesterol, cholesterol esters, phospholipids and triglycerides (TG) which are transported in the blood as lipoproteins. The abnormalities commonly seen in diabetes are reduced high density lipoprotein (HDL) cholesterol levels and elevated TG. Glucotoxicity and lipotoxicity are related to each other in the way that chronic hyperglycaemia causes lipotoxicity. The raised levels of free fatty acids (FFA) are seen in diabetic patients so lipotoxicity is a diabetogenic outcome resulting from raised levels of FFA. The increased levels of circulating glucose, fat or both act on various cells and tissues to oppose insulin-mediated glucose uptake, hepatic output of glucose and insulin secretion.[2,3]

The oxidation of glucose is impaired by excess FFA. The excess of FFA affect glycolytic pathways and entry of glucose into cells in many ways. The formation of acetyl-Co A, which is a product of glucose and fat metabolism and is utilized in the tricarboxylic acid cycle by mitochondria for oxidative phosphorylation. In conditions of excess FFA, acetyl-Co A is converted to malonyl-Co A, which is the first step in fat synthesis. The malonyl-Co A is an inhibitor of carrier mediated fatty acid transport into mitochondria and it mediates glucose inhibition competitively to that of fat oxidation. The fatty acids also decrease glucose storage by inhibiting its conversion into glycogen.[3]

DM is a serious health problem for the nation and it is known that poor control of diabetes results in increased risk for cardiovascular and renal diseases, blindness and early death.[4] Nowadays, the medical world is turning more and more towards the health benefits of natural products in the management of this illness.[5] Nowadays there is a growing trend regarding the use of honey for medicinal purposes. As per Islamic education honey is considered to be one of the food items which are considered to have '*shifa*' (شفاء) like dates and fig. Acacia honey is easily available in Pakistan throughout the year at reasonable cost and is known for its antioxidant potential. Pioglitazone is a well-known oral hypoglycaemic drug controlling

various aspects of lipid and carbohydrate metabolism.[6] We aimed to study the effect of acacia honey and Pioglitazone on lipid profile in streptozotocin-induced diabetic rats.

MATERIAL AND METHODS

This study was carried out in the Department of Physiology, Al-Nafees Medical College in collaboration with National Institute of Health (NIH), Islamabad, from January 2016 to June 2017 after approval of synopsis. One hundred and fifty male, adult Sprague dawley rats were divided into 5 groups (groups I–V) with 30 rats in each group. It was a non-randomized control trial. The rats were kept in animal house of NIH, Islamabad. Rats were fed *ad libitum* on standard diet prepared by NIH, Islamabad according to the standards approved by the Universities Federation for Animals Welfare.[7]

Out of 150 rats on day 1 after taking baseline measurements, 30 rats were grouped as normal control and 120 rats were injected with single dose of 65 mg/Kg streptozotocin (STZ)[8] to induce DM. On day 4, diabetes was confirmed in 120 rats injected with STZ by fasting blood glucose levels >220 mg/dl.[8]

Group I was taken as normal control and given standard rat diet and distilled water. Group II was taken as diabetic control and given standard rat diet and distilled water. Group III was given Acacia honey (1.0 g/Kg body weight) freshly dissolved in distilled water orally as a single daily dose for a period of 3 weeks. Group IV also received intra-peritoneal injection of pioglitazone 15 mg/Kg body weight per day along with Acacia honey for 3 weeks.[9] Group V was given intraperitoneal injection of pioglitazone 15 mg/Kg body weight per day for 3 weeks.

The early morning (7:00 AM) blood samples were drawn from the rat tail vein at day 1 and day 4th to measure fasting blood glucose using On-Call EZ II glucometer (Acon Laboratories, USA). The blood was drawn by cardiac puncture on day 25 and samples were sent to the laboratory for estimation of Lipid profile including serum total cholesterol (TC), serum TG and serum HDL. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated from the values of TG and HDL by using Friedwald equation.

RESULTS

Table-1 shows comparison of means (ANOVA) of Lipid profile, serum TC, TG, LDL, VLDL, and HDL of group II with group III, IV and V on day 25.

Mean serum TC levels in group IV (120.86±3.92 mg/dl) were highly significantly (p=0.003) decreased as compared to group II (125.06±4.66 mg/dl).

Mean serum LDL levels in group IV (64.03±4.57 mg/dl) were also highly significantly (p=0.010) decreased as compared to group II (68.3±4.61 mg/dl). Mean serum VLDL levels in group III (16.5±0.77 mg/dl, p=0.012) and group V (16.4±0.72 mg/dl) were highly significantly (p=0.002) decreased as compared to group II (17.16±0.69 mg/dl).

Mean serum TG levels in group III (83.83±3.62 mg/dl, p=0.001), group IV (84.2±3.78 mg/dl, p=0.005) and group V (83.63±3.61 mg/dl, p=0.001) were also highly significantly decreased as compared to group II (87.6±3.60 mg/dl).

Table-2 shows comparison of means (ANOVA) of Lipid profile; serum TC, TG, LDL, VLDL and HDL in groups III, IV and V on day 25 after treatment with Acacia honey and Pioglitazone presenting no significant differences in the groups.

Table-1: Comparison of means of Lipid profile; serum TC, LDL, VLDL, HDL and TG of group II with group III, IV an V on day 25 after treatment with Acacia honey and pioglitazone (Mean±SD)

Variable	Group II Diabetic Control (n=30)	Group III Honey (n=30)	Group IV Honey+Pioglitazone (n=30)	Group V Pioglitazone (n=30)	*p*
Serum TC (mg/dl)	125.06±4.66	123.56±4.36	120.86±3.92	122.03±3.84	III: 0.676 IV: 0.003** V: 0.062
Serum LDL (mg/dl)	68.3±4.61	67.03±4.90	64.03±4.57	65.83±5.13	III: 0.863 IV: 0.010** V: 0.315
Serum VLDL (mg/dl)	17.16±0.69	16.5±0.77	16.6±0.85	16.4±0.72	III: 0.012** IV: 0.050 V: 0.002**
Serum HDL (mg/dl)	39.1±1.91	39.46±2.67	39.93±2.63	39.23±2.72	III: 0.978 IV: 0.682 V: 1.000
Serum TG (mg/dl)	87.6±3.60	83.83±3.62	84.2±3.78	83.63±3.61	III: 0.001** IV: 0.005** V: 0.001**

**$p\leq0.01$ is taken as highly significant

Table-2: Comparison of means of Lipid profile; serum TC, TG, LDL, VLDL, HDL and TG in groups III, IV and V on day 25 after treatment with Acacia honey and pioglitazone (Mean±SD)

Variable	Group III Honey n=30	Group IV Honey+Pioglitazone n=30	Group V Pioglitazone n=30	*p*
Serum TC (mg/dl)	123.56±4.36	120.86±3.92	122.03±3.84	III & IV: 0.125 III & V: 0.657 IV & V: 0.841
Serum LDL (mg/dl)	67.03±4.90	64.03±4.57	65.83±5.13	III & IV: 0.142 III & V: 0.884 IV & V: 0.631
Serum VLDL (mg/dl)	16.5±0.77	16.6±0.85	16.4±0.72	III & IV: 0.988 III & V: 0.988 IV & V: 0.866
Serum HDL (mg/dl)	39.46±2.67	39.93±2.63	39.23±2.72	III & IV: 0.948 III & V: 0.996 IV & V: 0.804
Serum TG (mg/dl)	83.83±3.62	84.2±3.78	83.63±3.61	III & IV: 0.996 III & V: 1.000 IV & V: 0.977

DISCUSSION

The diabetic dyslipidemia leading to abnormalities in serum lipoproteins is an important causative factor for macro vascular dysfunction resulting from arteriosclerosis in the larger blood vessels causing coronary, cerebral, and peripheral vascular disease, which may increase the risk of ischemic heart diseases.[10] There is a growing trend towards the non-pharmacological approach to diabetes therapy which includes the use of different herbs and natural products like honey.

The diabetic dyslipidemia resulting from decreased levels of insulin, causes increased lipolysis and entry of free fatty acids in the liver. There is increased synthesis of triglycerides in the liver due to rise in circulating FFA which causes decline in the protein content of lipoproteins like VLDL and LDL and at the same time increasing the triglyceride content. There is decreased uptake of these lipoproteins due to change in the protein and triglyceride concentrations causing dyslipidemia.[4] In the present study the diabetic control rats showed significant increase in serum TG and VLDL levels as compared to normal control rats on day 25.

The mean serum TG and VLDL levels of normal control were comparable with serum TG and VLDL levels of male albino rats in a study carried out to determine the reference values for the serum lipid profile; however, the mean serum TC, LDL levels were found to be higher and the mean serum HDL levels were lower.[11] The higher levels of mean serum TC, TG, LDL, VLDL and HDL were observed in diabetic controls as compared to the levels of STZ induced diabetic control wistar rats[8] which might be due to the difference in the biochemical analysis of lipid profile, different strain of rats and prolonged duration of that study. The results of our study revealing significant increase in TG and VLDL levels with no significant change in serum TC, HDL and LDL levels were similar to previous studies by Sani *et al*[4], Nasrolahi *et al*[8] and Erejuwa *et al*[12] reporting both significant and insignificant changes in lipid profile of STZ induced diabetic rats.

The significantly lower levels of serum TC, LDL, and TG levels observed in STZ-induced diabetic rats that received a combination of Pioglitazone with Acacia honey (Group IV) demonstrate that Acacia honey combined with this hypoglycaemic agent produced synergistic effect in reducing TC, LDL, and TG levels. This has been reported that honey causes modulation of lipoprotein lipase activity which hydrolyses triglycerides in chylomicrons thus affecting lipid metabolism. It has been shown that honey improves LDL metabolism by acting on LDL receptors at hepatic level. Honey also contains flavonoids such as quercetin which has been known to cause reduction in the synthesis of cholesterol, triglycerides, fatty acids, LDL and VLDL. It is also suggested that quercetin increases the faecal excretion of cholesterol causing decrease in cholesterol in rats.[13,14]

The results of our study showing beneficial effects of acacia honey supplementation in improving lipid profile are supported by previous data reporting significant reduction in serum TC, TG, LDL, VLDL levels with Tualang and Ilam honey in STZ induced diabetic rats.[4,8] The beneficial effects of honey supplementation in correcting dyslipidemia are also evident from the results of a study[13] carried out on smokers showing significant reduction in TC and LDL levels after 12 weeks of honey supplementation.

The diabetic dyslipidemia causing abnormalities in lipid profile is an important causative factor for macro-vascular dysfunction resulting from arteriosclerosis in the larger blood vessels, which may increase the risk of cardiovascular diseases.[10] The beneficial effects of Acacia honey along with pioglitazone, as shown by the results of our study, may delay the onset of diabetic complications by reducing the levels of serum lipoproteins.

CONCLUSION

Combined supplementation of Acacia honey along with pioglitazone improves lipid profile in STZ-induced diabetic rats indicated by decreased levels of TC, LDL and TG. Effect of Acacia honey in combination with pioglitazone may be helpful in reducing micro- and macro-vascular complications of DM.

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