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Effect of Supplementation of Acacia Honey and Pioglitazone on Blood Glucose Levels in Streptozotocin-Induced Type 2 Diabetic Rats

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ABSTRACT

Objective: To determine the effect of combined supplementation of Pioglitazone and Acacia Honey on blood glucose levels in Streptozotocin-induced type 2 diabetic rats.

Study Design: Laboratory-based animal study.

Place and Duration of study: Al-Nafees Medical College, in collaboration with the National Institute of Health, Islamabad Pakistan, from Jan 2016 to Jun 2017.

Methodology: The study was conducted on 150 male Sprague Dawley rats, divided into five groups with 30 rats. Group-I was the control Group. Groups II-V were given Streptozotocin 65 mg Streptozotocin intraperitoneally to make them diabetic. Group II was diabetic control. Group-III was given acacia honey for three weeks. Group-IV received an intraperitoneal injection of Pioglitazone (oral hypoglycemic) 15 mg/kg body weight per day plus Acacia honey, and Group-V was given Pioglitazone intraperitoneally for the same dose and duration. Fasting blood glucose levels were measured after three weeks of supplementation.

Results: After three weeks of supplementation, the blood glucose levels were significantly (p-value <0.001) lower in all Groups (Group- III: 228.33±40.60 mg/dl, Group-IV: 132.50±22.67 mg/dl and Group- V: 120.73±14.19 mg/dl) as compared to Group-II (Diabetic Controls) (290.13±52.60 mg/dl).

Conclusion: Combined supplementation of Acacia honey and Pioglitazone reduces blood glucose levels in Streptozotocin-induced type 2 diabetic rats.

Keywords: Blood glucose, Diabetes mellitus, Honey.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease affecting multiple organs, potentially leading to irreversible complications if untreated. Its prevalence in Pakistan is high statistically, which was 7.6% in 2011, and it is estimated to reach 15% (14 million) by 2030. This has put Pakistan up the order at number 07 in the countries with a higher prevalence of DM. If this situation continues, it might move up to 4th place hence, presenting a challenge for health care professionals and policymakers in Pakistan.[1]

The management of DM involves pharmacological (use of oral hypoglycemic drugs and/or insulin) and non-pharmacological approaches.[2] The oral hypoglycemic drugs include Sulfonylureas, Biguanides and Thiazolidinediones. The Thiazolidinediones like Pioglitazone are selective ligands for peroxisome proliferator-activated receptors (PPAR-γ). When activated by a ligand such as Glitazones, PPAR-γ binds to the retinoid X receptor to form a heterodimer. This binds to DNA to regulate the transcription and translation of different proteins involved in glucose and lipid metabolism.[3]

The non-pharmacological approaches include healthy eating, exercise, herbal preparations and other natural products such as Bitter Melon and Honey.[4] There is a growing trend toward the alternative non-pharmacological approach to diabetes therapy. Functional foods-based research has gained much interest in this area. Honey is an example of functional food which promotes health and protects against diseases.[5] Studies suggest that Honey has antibacterial, antihypertensive, anti-inflammatory and antioxidant effects along with hepatoprotective and hypoglycemic activity, so an increasing trend regarding the use of Honey as a therapeutic agent is considered.[6-8]

This study uses Acacia Honey, a type produced by bees from the Acacia flowers. It is a supersaturated solution in sugars, mainly fructose and glucose, composed of around 300 compounds. Its antioxidant role is reflected by its composition of many minor components, including phenolic acids and flavonoids, enzymes, carotenoids, organic acids, proteins,

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vitamins, antioxidants, and hydrogen peroxide.[9] As per Islamic education, Honey is considered to be one of the food items which are considered to have 'Shifa' like dates and fig. DM is a serious health problem for the nation, and it is well established that poor control of diabetes results in markedly increased risk for heart disease, stroke, blindness, kidney failure and early death. Scientific evidence has demonstrated that most diabetes-related complications are avoidable if optimum glycemic control is achieved. Diabetic patients often have difficulty maintaining and/or achieving the recommended blood glucose control. Nowadays, the medical world is turning more and more on the health benefits of natural products in managing this illness. Acacia honey is easily available in Pakistan throughout the year at a reasonable cost and is known for its antioxidant potential. Pioglitazone is a well-known oral hypoglycemic drug controlling various aspects of lipid and carbohydrate metabolism. In the present study, we aimed to study the effect of acacia honey and Pioglitazone on glycemic control, lipid profile, oxidative stress and inflammatory mediators in STZ-induced diabetic rats. Our results would benefit further investigations regarding this type of natural Honey in diabetes treatment.

In Pakistan, no study has been done to determine the effect of Acacia honey on blood glucose levels. Therefore, this project was planned to evaluate the effects of Acacia honey as an adjunct to Pioglitazone on blood glucose in streptozotocin (STZ) induced diabetic rats.

METHODOLOGY

The study was a laboratory-based animal study conducted at the Department of Physiology, Al Nafees Medical College and Hospital, Islamabad Pakistan in collaboration with the National Institute of Health (NIH), Islamabad. The study duration was 18 months, from January 2016 to June 2017. Ethical approval was taken from the Ethical Review Board of Al Nafees medical college (ERC No: F.2/IUIC-ANMC/EC-142/2017).

Inclusion Criteria: Sprague Dawley rats of 3-4 months of age and weighing 200.00±50.00 grams were included in the study,

Exclusion Criteria: Rats showing any change in eating habits, behaviour and blood glucose levels of < 200mg/dl on the fourth day of STZ induction were excluded. One hundred and fifty male adult Sprague Dawley rats (n=150) were divided into five groups (Groups I-V), having 30 rats each. The rats were kept in the animal house of NIH, Islamabad. Rats were fed ad libitum on a standard diet prepared by NIH, Islamabad, according to the standards approved by the Universities Federation for Animals Welfare.[10]

Out of 150 rats on day-1 after taking baseline measurements, 30 rats were grouped as normal control, and 120 rats were injected with a single dose of 65mg/kg Streptozotocin (STZ) to induce DM. On day-4, diabetes was confirmed in 120 rats injected with STZ by measuring fasting blood glucose levels > 220mg/dl.[11] Group-II was taken as Diabetic Control and was given a normal standard rat diet and distilled water. Group-III was given Acacia Honey (1.0 g/kg body weight),[11] freshly dissolved in distilled water (2gm Acacia Honey in 200 ml of distilled water) orally as a single daily dose for three weeks. Group-IV also received an intra-peritoneal injection of Pioglitazone 15 mg/kg body weight per day,[12] and Acacia Honey orally for three weeks. Finally, for three weeks, Group-V was given intra peritoneal injection of Pioglitazone 15 mg/kg. The cages were labelled both for Group and Type of agent given.

Rats were segregated for the study according to the mentioned inclusion and exclusion criteria. The early morning (7.00 am) blood samples were drawn from the rat tail vein on days one and fourth to measure fasting blood glucose. It was estimated using an 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 to estimate plasma glucose levels.

Statistical analysis was done with the help of Statistical Package for the social sciences version 20.0. Mean±SD was calculated for the continuous variable. Student t-test and one-way analysis of variance (ANOVA) were used for multiple comparisons. When ANOVA showed a significant difference, Post-Hoc analysis was performed. The *p*-value of ≤ 0.05 was taken as significant.

RESULTS

Out of 150 rats on day-0 after taking baseline measurements, 30 rats were grouped as normal control and 120 rats were injected with STZ to induce DM. On day-4, diabetes was confirmed in 120 rats injected with STZ by measuring fasting blood glucose levels > 200mg/dl. The mean fasting blood glucose on day one (normal rates) was 107.51±9.30 mg/dl and on day four (Diabetic rates) was 298.81±51.77 mg/dl, as shown in Table-I. There was a highly significant (*p*=0.001) rise in

blood glucose levels and a decrease in body weight on day-4 compared to day-1.

Table-I: Comparison of Fasting Blood Glucose Levels of Sprague Dawley Rats at Day-1 and Day 4

Parameter	Day-1 Normal Rats (n=120)	Day-4 Diabetic Rats (n=120)	*p*-value
Fasting blood glucose (mg/dl)	107.51±9.30	298.81±51.77	0.001

The 120 diabetic rats were divided into five Groups (Group I-V, n=30). There was a highly significant (p = 0.001) rise in blood glucose levels in Group-II on day-25 after treatment with Acacia Honey and Pioglitazone, as compared to Group-III, IV and V, shown in Table-II.

Table- II: Comparison of Mean Blood Glucose of Group II with Group III, IV and V on day 25 after treatment with Acacia Honey and Pioglitazone

Parameter	Study Group (Mean±SD)				*p*-value
	Group-II Diabetic Control (n=30)	Group-III Honey (n=30)	Group-IV Honey±Pioglitazone (n=30)	Group-V Pioglitazone (n=30)	
Blood glucose(mg/dl)	290.13±52.60	228.33±40.86	132.50±22.67	120.73± 14.19	0.001

Intergroup comparison (Table-III) showed that significant difference among all groups (Group II vs III, IV and V).

Table-III: Inter-Group Comparison of Mean Blood Glucose of Group II with Group III, IV and V on day 25 after treatment with Acacia Honey and Pioglitazone

Parameter	Group-II Vs Group-III	Group-II Vs Group-IV	Group-II Vs Group-V
Blood glucose (mg/dl)	0.001	0.001	0.001

The comparison of mean blood glucose in Groups-III, IV and V on day-25 after treatment with Acacia Honey and Pioglitazone was shown in Table-IV. Furthermore, intergroup comparison of mean blood glucose on day-25 after treatment with Acacia honey and Pioglitazone in Group-II vs Group- III (p<0.001) and Group-II vs Group-III (p=0.001) and Group-II vs Group-IV (p= 0.062) shown in Table-V.

Table-IV: Comparison of means of Blood Glucose in Groups III, IV & V on day 25 after treatment with Acacia Honey and Pioglitazone

Parameter	Study Groups (Mean ± SD)			*p*-value
	Group-III Honey (n=30)	Group-IV Honey±Pioglitazone (n=30)	Group-V Pioglitazone (n=30)	
Blood glucose (mg/dl)	228.33±40.86	132.50±22.67	120.73± 14.19	0.001

Table-V: Inter-Group Comparison table of Blood Glucose In Groups III, IV & V on day 25 after treatment with Acacia Honey and Pioglitazone

Parameter	Group-III Vs Group-IV	Group-III Vs Group-V	Group-IV Vs Group-V
Blood glucose (mg/dl)	< 0.001	0.001	0.062

DISCUSSION

DM is a metabolic disorder with heterogeneous etiologies and various risk factors.[13] To find new management options, the global epidemic of DM has been extensively studied. The American Diabetes Association treatment guidelines have focused on nutritional therapy, lifestyle modification, pharmacologic therapy and the prevention and management of diabetes-related complications.[14] Effective management of DM requires sustained glycemic control to reduce the risk of complications associated with DM.[15] Nowadays, there is a growing trend regarding using natural products like ginger, bitter melon, and various herbs to maintain health. This has raised interest in inquiring about the health benefits of natural Honey in managing DM.[16]

To test our hypothesis, we used an animal model of Sprague Dawley rats, which develop a disease state that closely mimics the human metabolic syndrome. Most experimental models include rodents (especially rats and mice) for ethical, economical and practical reasons. Investigators have used several animal models like Pancreatectomized dogs, Zucker diabetic fatty rats and DB/DB mice to study the pathophysiology of DM according to their objectives.[17] STZ was used for induction of diabetes, a naturally occurring chemical used to produce type- 1 and type- 2 DM in animal models, depending on the dosage. The cytotoxic effects of STZ on β cells are believed to be due to the alkylation of DNA and the formation of ROS. The agent of choice to induce Diabetes Mellitus in experimental animals is STZ because of its chemical properties and stability.[18,19]

In the present study, on day 25, the mean blood glucose levels of the normal and diabetic control rats were 104.60±6.56 mg/dl and 290.13±52.60 mg/dl,

respectively, and this was comparable to the study done by Nasrolahi *et al.* 10 on male Wistar rats, where the blood glucose levels of normal and diabetic control rats (72.4±2.2 mg/dl, 252.2±4.1 mg/dl) were found to be lower. In contrast, the study on Sprague Dawley rats by Erejuwa *et al.*[20] showed higher blood glucose levels in diabetic control rats (403±1.0 mg/dl). The difference in the blood glucose levels could be due to different breeds of rats, the method used to determine blood glucose levels and the prolonged duration (04 weeks instead of 03 weeks) of the study.

In a study by Nasrolahi and Erejuwa on STZ-induced diabetic rats, they treated diabetic rats with Honey (Ilam, Tualang) and hypoglycemic agent (Metformin, Glibenclamide), in combination and alone.[10,20] The results of combined therapy of this study were in accordance with our results where Acacia honey and Pioglitazone decreased blood glucose levels when given in combination (Group-IV). However, the results of their study in which Honey alone was given also significantly decreased the blood glucose levels. This was inconsistent with our results, where Acacia honey caused a non-significant decrease in blood glucose levels compared to treated Groups IV and V, which were given combination therapy (Acacia and Pioglitazone) and Pioglitazone alone. This inconsistency may be due to different fructose-to-glucose ratios of acacia honey and environmental differences.

The blood glucose lowering effect of Acacia honey seen in our study was contradictory to the results of a study carried out on STZ-induced diabetic Sprague Dawley rats taking Gelam Honey, reporting no decrease in blood glucose levels in treated rats as compared to diabetic controls at the end of three weeks of Honey supplementation 20 which may be because of different dose of Honey (2.0 g/kg body weight) used for the same duration.

The major constituent of Acacia Honey, i.e. fructose, may be responsible for its hypoglycemic effect as it does not raise the concentration of plasma glucose, and insulin is not required for its metabolism. Fructose is absorbed slowly compared to glucose from the gastrointestinal tract and swiftly removed by the liver, causing a slight increase in blood sugar. So, it can be used as a sweetener by diabetic patients.

Glucokinase is a key enzyme for the intracellular metabolism of glucose which is known to be activated by dietary fructose. The conversion of glucose to glucose-6-phosphate is catalyzed by it, thereby decreasing blood glucose.[16] The studies also suggest the role of other bioactive constituents like oligosaccharides in mediating the hypoglycemic effect of Honey. Oligosaccharides act as prebiotics which enhance the activity and growth of gut microorganisms, and their anti-diabetic effect may be related to their ability to enhance fructose absorption and modulate the gut microflora ecosystem.[21] Erejuwa *et al.* also suggested the role of minerals in promoting the hypoglycemic effect of Honey.[18] Honey contains elements such as zinc, selenium, copper, calcium, potassium, chromium, and manganese that may be important in maintaining insulin secretion and normal glucose tolerance. In contrast, other ions such as copper and zinc may be involved in glucose and insulin metabolism. Although Honey contains small amounts of these minerals, when diabetic rats are supplemented daily with Honey might attain sufficient concentrations of these minerals to elicit pharmacological responses contributing to the hypoglycemic effect.[22]

Optimum glycemic control is now regarded as one of the most important therapeutic goals to prevent diabetic complications, as hyperglycemia appears to be the primary cause of microvascular complications like diabetic retinopathy, nephropathy and neuropathy.[23]

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LIMITATION OF THE STUDY

The cellular changes in the pancreatic tissue and end-organ level could have been studied along with the laboratory investigations which could not be done due to limited resources.

CONCLUSION

Combined supplementation of Acacia honey along with Pioglitazone leads to a reduction in blood glucose levels in STZ-induced diabetic rats. Thus, a controlled dose of Acacia honey as an adjunct to the hypoglycemic agent may be useful in preventing diabetic complications caused by poor glycemic control.

Conflicts of Interest: None.

Author's Contribution

MK:, UAK: Substantial contributions to the conception, Drafting, Final approval, MS:, MA: Study design, Data analysis, Final approval, BR: Conception, Data analysis, Final approval.

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