ORIGINAL ARTICLE

EFFECT OF SUPPLEMENTATION OF ACACIA HONEY AND PIOGLITAZONE ON INFLAMMATORY MEDIATORS AND OXIDATIVE STRESS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Background: Diabetes mellitus (DM) has emerged as a major health threat worldwide. There is an increasing advocacy of natural products in the treatment of DM and its complications. Objective of this study was to see the effect of supplementation of Pioglitazone and Acacia honey on inflammatory mediators and oxidative stress in streptozotocin (STZ) induced diabetic rats. **Methods:** The study was conducted in Al-Nafees Medical College in association with National Institute of Health, Islamabad. There were 150 male Sprague Dawley rats divided into 5 equal groups. Group-I was taken as normal control whereas Group-II was diabetic control. The Groups-II–V were given STZ through intraperitoneal route. Group-III received Acacia honey for 3 weeks. Group-IV was given intra-peritoneal injection of pioglitazone plus Acacia honey, and Group-V was administered pioglitazone through intraperitoneal route for same dose and duration. Serum catalase, hydrogen peroxide, CRP and TNF-α were measured after three weeks. Statistical analysis was done on SPSS-22. The values were presented as Mean±SEM and $p<0.05$ was considered significant. **Results:** On day 25 compared to Group-II, serum TNF-α and hydrogen peroxide levels were significantly decreased in Groups-III, IV and V. The mean serum CRP levels were significantly decreased in Group-III and IV while Group-II and III had significantly raised serum catalase levels. **Conclusion:** Acacia honey along with pioglitazone decreases oxidative stress as predicted by increase in levels of catalase. This combination also reduces inflammation as measured by decreased levels of CRP and TNF-α in STZ induced diabetic rats.

Keywords: Diabetes mellitus, Honey, Oxidative stress

Pak J Physiol 2024;24(3):18–22, **DOI:** https://doi.org/10.69656/pjp.v20i3.1536

INTRODUCTION

Diabetes mellitus (DM) is an incompletely understood clinical condition with a progressive course resulting in hyperglycaemia. The pathophysiology of Type 2 diabetes mellitus (T2DM) commonly involves impaired secretion of insulin, insulin resistance or a merger of these two gradually progressing to complete loss of insulin secretion by the beta cells of pancreas.[1]

T2DM has major contribution towards alarming rise in the incidence of non-communicable diseases all over the world.[1] The prevalence of DM is high in Pakistan; statistically it was 7.6% in 2011. It is expected to reach 15% by the year 2030.[2] DM has emerged as a major health concern for the healthcare professionals in our country.

The micro- and macro-vascular diabetic complications result from oxidative stress secondary to hyperglycaemia.[3] There is increased production of reactive oxygen species beyond the capacity of antioxidant defence mechanisms. The metabolic derangements and insulin resistance seen in T2DM is also strongly associated with chronic inflammation which leads to increased secretion of inflammatory factors, such as CRP, IL-6, TNF-α.[4]

The management of DM focuses on maintaining levels of glucose as close to normal by using pharmacological and non-pharmacological measures like use of hypoglycaemic agents, insulin and healthy lifestyle approaches.[5]

Pioglitazone is one of the oral hypoglycaemic drugs used that improves glycaemic control and lowers free fatty acid levels. The non-pharmacological approaches for diabetes therapy involving natural products like honey and herbal preparations are gaining popularity among diabetics because of no side-effects with health promoting effects. Researches based on functional foods (foods with nutritional values and health promoting and disease prevention potential) are also gaining interest of people nowadays.[6]

Different studies suggest a recent trend regarding use of honey as a therapeutic agent. Data already available shows its hypoglycaemic, anti-inflammatory and antioxidant effects. Acacia honey is a well-known honey in Pakistan and is easily available at affordable rates. The composition of acacia honey produced by bees from Acacia flowers includes several different compounds like fructose and glucose, with the antioxidant potential provided by flavonoids, phenolic compounds, and different enzymes etc.[7]

This study was planned to determine the effects of acacia honey and Pioglitazone on oxidative stress and inflammatory mediators in Streptozotocin

(STZ)-induced diabetic rats. The results of this study would be helpful for further research considering use of acacia honey in diabetes treatment.

METHODOLOGY

This experimental study was conducted in the Department of Physiology, Al-Nafees Medical College and Hospital in association with National Institute of Health, Islamabad after ethical approval (ERC No. F.2/IUIC-ANMC/EC-142/2017). The study was carried out from Jan 2017 to Jun 2020. The samples were collected through convenient sampling technique. Potential confounders were equally distributed by group allocation and randomization. Bias was reduced by day one baseline measurements, standardized housing and food. Consistency in the diabetic condition was guaranteed by uniform diabetes induction and confirmation.

Three to four months old healthy and active male Sprague Dawley rats weighing 200±50[8] grams were included. The rats exhibiting any kind of change in eating habits, behaviour, and showing blood glucose levels of <200 mg/dL[8] on 4th day of STZ induction were excluded. One hundred and fifty[9] male adult Sprague Dawley rats were divided into 5 groups (Groups I–V) with 30 rats in each group (n=30).

The rats were kept in animal house of NIH, Islamabad. They were fed *ad libitum* on standard diet prepared by NIH, Islamabad based on the standards of Universities Federation for Animals Welfare.[10]

The baseline measurements were done on day 1. Thirty rats were taken as normal control, and rest of the rats (120) were injected with single dose of 65 mg/Kg STZ[8] for induction of DM. The diabetes was confirmed on day 4 in 120 rats injected with STZ having fasting blood glucose levels >200 mg/dL[8].

Group-II was declared as diabetic control. Group-II was given normal standard rat diet and distilled water. Acacia honey (acquired and certified by National Agriculture and Research Institute) dissolved in distilled water (1.0 g/Kg body weight) was given to Group-III (2 gm acacia honey in 200 mL of distilled water)[8], orally as a single daily dose for a period of 3 weeks.

Intra-peritoneal injection of pioglitazone 15 mg/Kg body weight per day[11] was given to Group-IV along with Acacia honey orally for 3 weeks whereas intra peritoneal injection of pioglitazone 15 mg/Kg body weight for 3 weeks was administered to Group-V. Cages were labelled for the type of agent given and the group number.

The samples were taken from the rat tail vein on day 1 and day 4 to determine fasting blood glucose at 7.00 AM. On day 25 cardiac puncture was done and blood samples were collected to estimate serum catalase and hydrogen peroxide (oxidative stress indicators) (K-Assay. No. KT-711, Sigma Hydrogen Peroxide Assay Kit CS0270), serum CRP and TNF alpha (inflammatory mediators Abcam's C-reactive protein PTX1 Rat ELISA Kit ab108827, Abcam's TNF alpha Rat ELISA Kit ab46070) through calorimetric detection method.

SPSS-20 was used for statistical analysis. Calculation of means and standard deviations for the data was done. Analysis was based on ANOVA and $p \leq 0.05$ was considered as significant.

RESULTS

There was highly significant increase in the serum CRP (p=0.004) and TNF-α (p=0.046) levels in diabetic rats on day 4 after the administration of STZ, as compared to day 1 when the rats were healthy while there was no significant difference in the values of serum catalase (p=0.128) and hydrogen peroxide (p=0.308) in diabetic rats on day 4 as compared to day one.

The means of serum CRP and TNF-α, serum catalase and hydrogen peroxide of Groups-II, III, IV and V, on day 25 after treatment with acacia honey and pioglitazone are shown in Table-1.

Table-2 shows the inter-group comparison ANOVA (Post Hoc analysis) of means of serum CRP and TNF-α, serum catalase and hydrogen peroxide of Group-II, III, IV and V on day 25 after treatment with acacia honey and pioglitazone.

There was highly significant decrease in the mean serum CRP levels in the Group-III and Group-IV as compared to Group-II. The mean serum CRP levels were significantly lower in Group-III and IV as compared to Group-V.

The mean serum TNF-α level in Group-III, IV and V showed highly significant decrease as compared to Group-II. There was significant decrease in mean serum TNF- α levels in Group-III as compared to Group-IV.

The mean serum catalase levels in Group-IV and V were also significantly decreased as compared to Group-II. There was significant increase in serum catalase levels in Group-III as compared to Group-IV and V.

Regarding serum hydrogen peroxide levels the mean of Group-II was significantly high as compared to Group-III, IV and V.

Table-1: Means of serum CRP and TNF-α, serum catalase and hydrogen peroxide of Group-II, III, IV, and V on day 25 after treatment with acacia honey and pioglitazone (n=30)

Parameter	Group-II	Group-III	Group-IV	Group-V
CRP (mg/L)	0.23±0.02	0.21±0.02	0.22±0.02	0.22±0.01
TNF-α (pg/mL)	47.23±3.57	40.16± 2.27	42.90±3.92	40.83±2.26
Catalase (U/mL)	17.05±2.77	16.41±2.75	12.98±2.43	12.13±2.62
H_2O_2 (ng/mL)	10.11±1.33	9.01±0.67	9.19±0.58	9.14±0.50

Table-2: Inter-group comparison (Post Hoc analysis) showing *p* of serum CRP and TNF-α, serum catalase and hydrogen peroxide of all groups 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	Group-III *vs* Group-IV	Group-III *vs* Group-V	Group-IV *vs* Group-V
Serum CRP (mg/L)	0.001*	0.019*	0.051	0.860	0.666	0.997
Serum TNF-alpha (pg/mL)	0.000*	0.000*	0.000*	0.019*	0.942	0.134
Serum Catalase (U/mL)	0.889	0.000*	0.000*	0.000*	0.000*	0.745
Serum Hydrogen peroxide (ng/mL)	0.001*	0.011*	0.007*	1.000	1.000	1.000

*Significant

DISCUSSION

Diabetes Mellitus as a metabolic disease with an immense range of aetiologies and factors is recognized worldwide. New evidence is currently being gathered to introduce advanced and effective therapeutic options.[12] For management and deterrence of diabetes-related complications, the emphasis has been laid by The American Diabetes Association treatment guidelines on physical activity, nutritional adjustments, pharmacologic options and prevention methods.[12] A constant glycaemic control is necessary for alleviation of DM associated complications. Researchers are now shifting their focus to study natural products such as acacia honey for its benefits in therapeutic management of DM.[13]

The animal model of STZ administration to Sprague Dawley rats was utilized as it induces a metabolic disturbance closely resembling that in humans. It is accessible and cost effective, so most of the investigative animal models use rodents.[14] It acts by DNA alkylation and reactive oxygen species (ROS) formation.[15]

Oxidative stress is the predecessor of diabetic complications.[3] The off-balanced ROS production in this metabolic state of hydrogen peroxide, hydroxyl radicals and superoxide anions cannot be compensated by antioxidant enzymes such as catalase whose production is raised during oxidative stress.[3,16] In this study significantly higher catalase and hydrogen peroxide levels were noted in diabetic rats as compared to normal controls. This is in accordance with the results of Qujeq and Rezvani reporting significant rise in catalase activity in diabetic rats.[16] In a project conducted by Krishna A. Adeshara[17] on the relationship of plasma glycation products and isoforms association with oxidative stress in diabetic vascular complications, intracellular ROS generation was found to be increased in patients with such complications particularly diabetic nephropathy. This phenomenon can be attributed to several interconnected pathophysiological mechanisms like hyperglycaemia-induced oxidative stress, mitochondrial dysfunction, inflammatory processes stimulating the production of ROS, and upregulation of NADPH activity.

It was found that honey treated group (Group-III) with significantly higher blood glucose levels among the treated groups reflected significantly raised mean serum catalase levels on day 25 as compared to other groups taking pioglitazone alone and in combination (Group-IV and V) with no significant change in serum hydrogen peroxide assay in all the treated groups. The increase in catalase levels corroborates with the results of honey supplementation in STZ diabetic rats reported by Erejuwa *et al*[18] suggesting that hypoglycaemic tendency of honey reduces oxidative stress in kidney of diabetic rats. This most likely to have occurred because in conditions of increased oxidative stress, such as diabetes, the body often responds by enhancing the expression and activity of antioxidant enzymes like catalase to counteract the harmful effects of ROS. The increased activity of catalase after honey supplementation may suggest the increased bio-availability of catalase to scavenge hydrogen peroxide preventing rise in the hydrogen peroxide levels in treated groups in the present study. A systematic review by Ugusman A *et al*[19] on the role of honey in obesity management states that acacia honey is more effective than orlistat in controlling obesity in diabetic Sprague Dawley rats. This emphasizes the need of adding such natural remedial methods in management of diabetes.[19]

Hyperglycaemia exerts toxic effects on the pancreatic β-cells.[20] The antioxidant potential of acacia honey is believed to be related to its ability to lower blood glucose levels however studies also suggest its ability to alter antioxidant enzymes activities in DM due to its phenolic antioxidants content or through the action of catalase activity in reducing hydrogen peroxide.[19] Exogenous antioxidants such as honey may play a role in reducing the large amount of ROS in DM by reducing blood glucose levels thus acting synergistically with the hypoglycaemic agents.

Inflammation brought about by raised glucose levels is notorious for enhancing oxidative stress as it increases the synthesis of advanced glycation end products and increases TNF.[21] In this study significantly higher mean serum CRP and TNF alpha levels on day 25 were observed in diabetic control rats as compared to normal rats. This is in accordance with the results of Sunarti *et al*[22] where the mean serum CRP levels of normal and diabetic control rats were comparable to that of male Wistar rats, also showing significant increase in serum CRP levels of diabetic rats compared to normal healthy rats. The rise in serum TNF-α was also in agreement with the results of the study carried out on

rats by Sunarti *et al*[22]. The raised levels of serum CRP and TNF- α may suggest that hyperglycaemia leading to oxidative stress may induce the production of inflammatory cytokines. The most likely reason could be the body's response to the physiological stress caused by high blood sugar levels. Hyperglycaemia is known to contribute to oxidative stress, which in turn may trigger the release of inflammatory mediators such as CRP and TNF-α as part of the body's inflammatory response. Raised levels of pro-inflammatory cytokines like CRP, are targeted for intervention in type 2 diabetes as abnormal CRP values are significantly related to increased risk of DM.[22]

The hypoglycaemic effect of treatment given to diabetic rats in the present study resulting in decreased blood glucose levels on day 25, hence demonstrated significantly lower serum CRP levels in acacia honey treated groups (Group-III and IV) and TNF-α levels in all treated groups (Group-III, IV, and V) than diabetic control rats. This is in accordance with the result of study by Asaduzzaman *et al*[23] on supplementation of bee honey (1.0 g/Kg body weight for 21 days), where they noted significant reduction in CRP levels of STZ induced diabetic rats. The most likely reason could be honey's anti-inflammatory properties, characterized by its rich antioxidant content.

The hypoglycaemic potential of acacia honey and pioglitazone resulted in decreased levels of inflammatory mediators in STZ induced diabetic rats as hyperglycaemia increases circulating cytokine concentrations by an oxidative mechanism leading to inflammatory response.[24] The pathways mainly responsible for the inflammatory response are mitogen-activated protein kinase and nuclear factor kappa β in the cells, as their activation generates inflammatory cytokines. Previous studies also suggest that flavonoids in honey help to reduce expression of mitogen-activated protein kinase and nuclear factor kappa β in cells to prevent the release of IL-1β, IL-6, TNF-α and COX-2.[25] Thus controlled dose of acacia honey as prescribed by physician, as an adjuvant with Pioglitazone may be useful in the prevention of macro vascular diabetic complications like coronary atherosclerosis by reducing the levels of these inflammatory biomarkers.

CONCLUSION

The supplementation of acacia honey with pioglitazone decreases oxidative stress as predicted by increased levels of catalase and reduced inflammation as measured by decreased levels of CRP and TNF-α in streptozotocin induced diabetic rats.

LIMITATIONS OF THE STUDY

The cellular changes in the pancreatic tissue and end organ level could have been studied along with the laboratory investigations.

ACKNOWLEDGEMENTS

The authors are thankful to the animal handlers for taking good care of the rats according to the protocol provided. We are also grateful to the laboratory technicians for all the technical support provided whenever needed.

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Received: 6 Mar 2023 **Reviewed:** 20 Jul 2024 **Accepted:** 4 Aug 2024

Contribution of Authors:
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Conflict of Interest: None
Funding: None