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Research Article

Antihyperlipidemic activity of stamen of *Musa paradisiaca* in streptozotocin-induced diabetic rats.

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ABSTRACT

***Objective*:** *Musa paradisiaca* belonging to family Musaceae is a well-known herb having many pharmacological properties including anti-diabetic activity. In ancient text *Basawaraja,* the stamen of this plant was reported as anantidiabetic agent in a dietary recipe. The main aim of present study was to explore the *in-vitro* antidiabetic property of stamen as well as antioxidant potential.

***Methods*:** The aqueous extract of stamen of *Musa paradisiacal* (100, 200 and 400 mg/kg)has been used for the experimental purpose. Streptozotocin (65 mg/kg, i.p.) and Nicotinamide (110 mg/kg, i.p.) were used for induction of diabetes in rats while Glibenclamide (5 mg/kg, p.o.) used as standard drug. Different biochemical parameters such as total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) and liver glycogen content as well as antioxidant activity were estimated.

***Results*:** Treatment of rats with AqMP reversed plasma lipid profile near to normal values. It also significantly increased (p<0.05) liver glycogen level as compared to diabetic control rats. The drug also prevents rats from oxidative stress.

***Conclusion*:** The study shows that the drug can be helpful in the management of alterations in lipid profile in diabetic mellitus patients.

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1. Introduction

Diabetes Mellitus (DM) is a serious chronic and most important metabolic disorder of endocrine system which has a shocking impact on health, quality of life and life expectancy of a human being, especially in India. According to International Diabetes Federation (IDF), currently 382 million people are suffering from DM worldwide and this ratio might increases up to 600 million in the year of 2035 (Zimmet *et al*., 2014). It is expected that by the year 2025 India maybe known as the diabetic capital of the world (Singh and Verma, 2013). The major micro vascular and macro vascular problems like, failure of organs especially eyes (blindness, retinopathy), brain (neuropathies, cerebral stroke), kidneys (nephropathy, renal failure, increase in serum uric acid, urea, creatinine level), blood vessels and heart (myocardial infarction, congestive heart failure) etc. (Armstrong, 2015) are associated with DM. Dyslipidemia plays a leading role in the development of these cardiovascular complications in DM patients. The increased plasma free fatty acid level is one of the essential metabolic conditions in DM, mediated by insulin resistance (Tangvarasittichai, 2015). Chronic hyperglycemia state promotes glycation and oxidation of low-density lipoprotein (LDL) and increases atherogenicity of LDL (Vergès, 2015). Moreover, another component of lipid profile like elevated triglycerides (TG), low high-density lipoprotein (HDL) and elevated very low-density lipoprotein (VLDL) silently encourage the progress of coronary artery disease. The available pharmaco-therapeutic agents chiefly belong to statin group, but unfortunately, they fail to achieve the target of recommended lipid profile especially LDL and HDL level (Raymond, 2014). Also, it produces several side effects like liver diseases, myalgia, and myopathy (Fung, 2015).

In DM diseases, oxidative stress also plays a vital role in the pathogenesis of diabetic complications (Sarma et al., 2010). It is has been reported that utilization of antioxidant contributes a considerable role in the anticipation, cure, and management of some of the degenerative health problem includes diabetes mellitus (Tiwari, 2015). Some hypothesis indicates the delayed development of type 2 diabetes by consumption of antioxidants rich diet (Porter, 2012).

Many efforts have been made in search of a plant/herbal product from medicinal plants having anti-diabetic activity along with antihyperlipidemic and antioxidant potential. In the traditional system of medicines approximately 1200 species of plants have been used for the management and cure of diabetes around the world (Hsu et al., 2009). More than 400 plants and its compounds have been scientifically evaluated for anti-diabetic activity (Singh et al., 2011). The utility of medicinal plants for management and treatment of DM is widespread all over India (Alamina, 2015). The proper use of resources of traditional medicinal herbs and its scientific validation remains an essential prospect for the therapeutic strategies and development of new drugs (Alamina, 2015).

Musa paradisiaca (Musaceae), commonly known as Banana is a perennial tree like herb, usually grown indigenously in the tropics and subtropics regions. Several parts of this plant have been traditionally used in India as antidiarrhoeal, antidysentery, antihelminthic, anti-ulcerative, antimicrobial agent (Imam and Akter, 2011). Previously we have reported that stamen from *M. paradisiaca*possess anti-diabetic activity as mentioned in classical text "*Basavarajiyam*". In the present study stamen from *M. paradisiaca* was screened for antihyperlipidemic activity in streptozotocin-induced diabetic in rats.

2. Materials and methods

2.1. Plant material

The banana inflorescence of *M. paradisiaca*was collected in the month of June- July 2015 from Mondh, District Bhadohi (Uttar Pradesh) (25° 24' N, 82° 38' E longitude and altitude, 85 m ASL) and its stamen were separated from it. Authentication of plant material was done at Ayurvedic Pharmacy Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur and a voucher specimen number (APRL/HERB/2015-16/04) was kept for further support in future.

2.2. Drugs and Chemicals

Streptozotocin (STZ), Glibenclamide and Nicotinamide (NDA) were purchased from (Sigma–Aldrich Co. LLC., New Delhi, India). All the solvents and reagents of analytical grade were used in all experiments.

2.3. Animals

Charles Foster albino rats of 160–200 g weight were used in the study. The animals were kept in separate cages in standard laboratory conditions (12 h light/12h dark cycle, 50-60% relative humidity and at 22±2°C). The animals were fed with animal feed and water *ad libitum*. Prior approval was obtained from animal ethical committee of Banaras Hindu University (Dean/2015/CAEC/1431).

2.4. Experimental protocol

Streptozotocin (65 mg/kg, i.p.) and Nicotinamide (110 mg/kg, i.p.) (Jadhav and Punchakayala, 2012) was used for induction of diabetes in rats. Animals with fasting blood Glucose > 250 mg/dL after 72h of drug administration were used for the study. Animals were divided into five groups (n=6) as follows and six non-diabetic animals serve as normal control (Group I). Group-II (Diabetic control rats), Group-III [Diabetic rats administered once with Glibenclamide (5 mg/kg, b.w. p.o.) (Sabitha et al., 2011) as the reference drug and Group-IV to VI: Diabetic rats were administered with the test drug.

Treatment was continued for 21 days. Blood was collected from the tail vein, and blood glucose level was measured before dosing (day 0) as well as regular intervals of 7th, 14th and 21st days respectively during the treatment in all groups. On the 22nd day, the rats were sacrificed by cervical dislocation and blood was collected by adirect cardiac puncture and further, it was evaluated for various biochemical parameters.

2.5. Biochemical analysis

Biochemical estimation kits were purchased from Span Diagnostic Surat, Gujarat, India which was used for the determination of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

2.6. Assay of Antioxidant activity

The isolated liver was weighed accurately and washed with ice-cold saline to remove traces of blood. Liver tissue was cut into small pieces and homogenized by Glass Teflon homogenizer (Thomas Scientific, Swedesboro, USA) in Tris–HCl buffer (0.025 M, pH 7.5). This liver homogenate was centrifuged at 10,000g for 10 min at 41°C temperature. The supernatant was separated and used for the estimations of antioxidant enzymes like glutathione peroxidase (GSH), superoxide dismutase (SOD) and lipid peroxidase (LPO). The level of LPO was estimated and expressed regarding malondialdehyde (MDA) as per the method of Ohkawa et al., 1979. The activity of SOD was estimated by following the procedure of Kakkar et al., 1984; based on the reduction of NBT to blue coloured formazan in the presence of phenazine methosulphate (PMS). GSH was estimated as per the method of Sedlak and Lindsay, 1968 and expressed in term of µmol/g of wet tissue.

2.7. Estimation of glycogen content

Glycogen content in Liver was determined by homogenization of 1 gm of the liver with phosphate buffer and the addition of 6mL of 0.6N hydrochloric acid in the test tube which was covered with a glass bulb. This reaction mixture was heated on a water bath for 2–2.5 hour to make sure that the sample hydrolyzed. The sample solution was kept for self-cooling, and it was neutralized with 0.5N sodium hydroxide with an indicator, phenol red. Then the whole sample was transferred into a volumetric flask and diluted. The amount of glucose in the solution was determined. On calculation, 0.93 was taken as a conversion factor for determination of glycogen from the glucose in the hydrolyzed glycogen sample (Sinnathambi, 2010).

2.8. Statistical analysis

All data were expressed as mean ±SEM. One-way ANOVA followed by Tukey's multiple comparison tests was performed for the statistical analysis using Software GraphPad Prism, Version 5.0.1. $p<0.05$ was taken as statistically significant.

3. Result

3.1 Effect on plasma lipid profile

Effect of AqMP on plasma lipid profile i.e. total cholesterol (TC), triglycerides (TG) and lipoproteins (LDL and HDL) are shown in Table 2. The levels of plasma total cholesterol triglycerides and LDL were significantly increased ($p< 0.05$), whereas the level of HDL was significantly decreased ($p< 0.05$), in diabetic rats as compared to normal control. Treatment with AqMP reversed plasma lipid profile near to normal values. It shows that treatment with AqMP significantly improved the lipid profile in diabetic animals. The effect of AqMP(200 and 400 mg/kg, p.o.) was found comparable with that of glibenclamide (5 mg/kg, p.o.).

3.2. Effect of AqMP on the lipid profile of STZ-NDA induced diabetic rats.

Values are mean±S.E.M of 6 animals in each group. $^{a}p< 0.05$ compared to normal control; $^{b}p< 0.05$ compared to diabetic control; (One-way ANOVA followed by Tukey's Multiple Comparison test). [Abbreviation: Glib-Glibenclamide; AqMP-Aqueous extract of *M. paradisiaca*]

3.3. Effect on glycogen content

The effect of treatment of AqMP in rats on liver glycogen is shown in Figure 1. A significant decline ($p< 0.05$) in liver glycogen content was observed in the diabetic control group as compared to normal control group. Rats treated with AqMP100 mg/kg p.o., did not show any significant increase in liver glycogen level. However, rats treated with 200 and 400 mg/kg, p.o., showed pronounced increases in liver glycogen level. Glibenclamide treatment also significantly increased ($p< 0.05$) liver glycogen level as compared to diabetic control rats.

3.4. Effect on antioxidant enzyme activity

Table 2 represents changes in oxidative stress in liver samples of normal and experimental rats. There was a significant elevation in tissue TBARS in animals during diabetes as compared to the normal group. Administration of AqMP (200 & 400 mg/kg, p.o.) significantly decreased the lipid peroxidation in diabetic rats. The effect of AqMP at the dose level of 400 mg/kg, p.o., was found comparable to glibenclamide. Statistical analysis by one-way ANOVA on the effect of AqMP on the activity of SOD and CAT showed a significant effect of treatment with AqMP. The activity of SOD and catalase were found significantly lower in diabetic rats as compared with their values in normal control rats. Treatment with AQMP in diabetic rats significantly restored the enzyme levels as compared to untreated diabetes animals.

4. Discussion

The key markers of dyslipidemia are the elevated plasma levels of triglyceride, total cholesterol, VLDL, LDL and decrease the level of HDL (Bitzur et al., 2009) which commonly occurs with the non-alcoholic fatty liver disease in diabetic patients (Firneisz et al., 2014). Insulin resistance condition and malfunction of β-cells are responsible for marked hyperlipidemia. The insulin has an inhibitory effect on the key enzyme (3-hydroxy-3-methyl-methylglutaryl) which is responsible for biosynthesis of cholesterol (Ghoul et al., 2012). In diabetic condition, high cholesterol leads to severe retinopathy complication which further leads to blockage of blood vessels and causes complete blindness (Chen et al., 2016).

Cardiovascular diseases like angina, congestive heart failure, stroke, myocardial infarction and peripheral artery disease associated with diabetes which are the leading cause of mortality and it can be improved by the AqMP treatment (IDF, 2013). Elevated levels of TG, TC, LDL, VLDL and decrease HDL might increase the risk of coronary heart diseases (Anwar *et al*., 2013) which is the secondary complications of diabetes. In diabetic conditions, the AqMP has the potency to minimize the long-term cardiovascular complications. The lowering of lipid level also reduces the risk of any vascular complication occur in DM (Movahedian et al., 2010). HDL promotes the efflux of

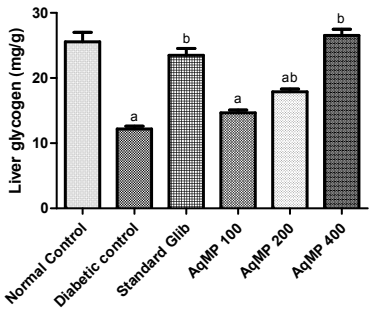

Figure 1.Effect of AqMP on Liver glycogen in STZ-NDA induced diabetic rats. Values are mean ± SEM of 6 animals in each group. $^{a}p< 0.05$ compared to normal control; $^{b}p< 0.05$ compared to diabetic control; (One-way ANOVA followed by Tukey's Multiple Comparison tests). (Abbreviation: Glib-Glibenclamide; AqMP-aqueous extract of *M. paradisiaca*)

Table 1. Effect of AqMP on Lipid profile of STZ-NDA induced diabetic rats

	LDL (mg/dL)	HDL (mg/dL)	TC (mg/dL)	TG (mg/dL)
Normal	19.04±0.25	45.31±1.66	73.54±0.91	65.67±1.00
Diabetic Control	126.96±0.72^{a}	24.07±0.52^{a}	185.46±0.78^{a}	158.16±0.75^{a}
Standard Glib	26.25±1.51^{b}	44.83±0.53^{b}	77.97±4.26^{b}	70.84 ± 3.09^{b}
AqMP (100)	81.58±2.22^{ab}	39.31±0.38^{ab}	114.47±1.21^{ab}	149.81±2.82^{a}
AqMP (200)	60.51±2.88^{ab}	42.91±1.15^{b}	91.96±0.79^{ab}	83.94±1.11^{ab}
AqMP (400)	23.61±1.76^{b}	51.15±0.77^{ab}	75.11±2.97^{b}	69.86±2.92^{b}

Table 2. Effect of AqMP on oxidative stress STZ-NDA induced diabetic rats

	LPO (MDA, nmol/g tissue)	SOD (units/g tissue)	GSH(µg GSH/g tissue)
Normal Control	12.46±2.43	33.45±0.36	3838.22±155.88
Diabetic Control	94.65±7.69^{a}	14.74±0.89^{a}	1096.43±121.39^{a}
Standard Glib	16.71±0.94^{b}	32.46±0.78^{b}	3684.69±124.92^{b}
AqMP 100	82.46±1.11^{a}	21.17±2.16^{ab}	1745.31±117.27^{a}
AqMP 200	50.32±7.89^{ab}	29.97±0.13^{b}	2248.62±167.67^{ab}
AqMP 400	17.07±2.53^{b}	31.24±1.25^{b}	3405.88±247.03^{b}

cholesterol from the peripheral tissues to the liver via. reverse cholesterol transport pathway and act through free radical scavenging and anti-inflammatory action which minimizes the risk of cardiovascular diseases (CVD)(Eren et al., 2012).

Hepatic glycolysis influenced the activity and amount of glucokinase and phospho-fructokinase (Berg et al., 2002). Increased glycogenesis is responsible for the elevation of hepatic glycogen content. Insulin promotes the deposition of glycogen *via* regulating glycogen synthase and inhibiting glycogen phosphorylase. The AqMP protect the streptozotocin toxified β-cells through free radical scavenging activity which is responsible for increased insulin secretion. Thus it controls the renovation of hepatic glycogen. It was observed that in diabetic control rats, the liver glycogen levels were decreased. It may be due to the inactive glycogen synthetase systems or amplified activity of glycogen phosphorylase (Renjith and Rajamohan, 2012). In our study, it was observed that the test drug significantly increases the liver glycogen in diabetic rats might be due to reactivation of glycogen synthetase system which is the key marker for improvement of liver glycogen synthesis.

Due to chronic hyperglycemia, several reactive oxidative species (ROS) such as superoxides were generated and leads to oxidative stress. The oxidative stress is considered as an important causative factor in the development of complications of DM (Naudi et al., 2012). In hyperglycemia, the superoxide radicals ($O_2\bullet^-$ and H_2O_2) are responsible for microvascular complications which are form when blood glucose is elevated (Nauseef,2014). Dialuric acid is formed due to the autoxidation of streptozotocin which further generates superoxide radicals and hydroxyl radical (OH•). Superoxide dismutase (SOD) converted into superoxide radical ($O_2\bullet^-$) to H_2O_2, which further turned into O_2 and H_2O due to CAT activity. Therefore, antioxidant properties of an antidiabetic drug enhances its activity by many folds in the prevention of complications.

The present findings shows that AqMP can protect the body from dyslipidemia in STZ-NDA induced diabetic rats. The antioxidant potential also contributes to the antidiabetic and antihyperlipidemic activity of *M. paradisiaca*.

Conflict of Interest

Authors report no conflict of interest.

Reference

Alamina MA, Yagib AI, Yagia SM. Evaluation of antidiabetic activity of plants used in Western Sudan. Asian Pac J Trop Biomed. 2015; 5: 395–402.

Anwar M, Shousha WG, El–mezayen HA, Raafat AW, Maha El–Wassef, Naglaa MN, *et al*. Antiatherogenic effect of almond oil in streptozotocin induced diabetic rats. J Applied Pharma Sci. 2013; 3: 59–65.

Armstrong AW, Guérin A, Sundaram M, Wu EQ, Faust ES, Ionescu–Ittu R, Mulani P. Psoriasis and risk of diabetes–associated microvascular and macrovascular complications. J Am Acad Dermatol. 2015; 72: 968–977.

Berg JM, Tymoczko JL, Stryer L. Freeman WH. The Glycolytic Pathway Is Tightly Controlled. Biochemistry, 5th ed., New York. 2002.

Bitzur R, Cohen H, Kamari Y, Shaish A, Harats D. Triglycerides and HDL Cholesterol:Stars or second leads in diabetes? Diabetes Care. 2009; 32(Suppl2): S373–S377.

Chen L, Cheng CY, Choi H, Ikram MK, Sabanayagam C, Tan GS, et al. Plasma metabonomic profiling of diabetic retinopathy. Diabetes. 2016; 65:1099-1108.

Eren E, Yilmaz N, Aydin O. High density lipoprotein and it's dysfunction. Open Biochem J. 2012; 6: 78–93.

Firneisz G. Non–alcoholic fatty liver disease and type 2 diabetes mellitus: The liver disease of our age? World J Gastroenterol. 2014; 20: 9072–9089.

Fung CSC, Wan EYF, Jiao F, Lam CLK. Five–year change of clinical and complications profile of diabetic patients under primary care: a population–based longitudinal study on 127,977 diabetic patients. Diabetol Metab Syndr. 2015; 7: 79.

Ghoul JE, Smiri M, Ghrab S, Boughattas NA, Ben–Attia M. Antihyperglycemic, antihyperlipidemic and antioxidant activities of traditional aqueous extract of *Zygophyllum album* in streptozotocin diabetic mice. Pathophysiol. 2012; 19: 35–42.

Hsu YJ, Lee TH, Chang CL, Huang YT, Yang WC. Anti–hyperglycemic effects and mechanism of *Bidenspilosa* water extract. J Ethnopharmacol. 2009; 18: 379–383.

Imam M.Z, Akter S. *Musa paradisiaca* L. and*Musa sapientum* L.:A Phytochemical and Pharmacological Review. J Applied Pharma Sci. 2011; 01: 14–20.

International Diabetes Federation, IDF Diabetes Atlas, 6th ed. Brussels, Belgium, 2013. http://www.idf.org/complications–diabetes. [Last assess: 08 February 2016]

Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocinnicotinamide induced type 2 diabetic rats. Int J Pharm Pharma Sci. 2012; 4: 251–256.

Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21: 130–132.

Movahedian A, Zolfaghari B, Sajjadi SE, Moknatjou R. Antihyperlipidemic effect of *Peucedanumpastinacifolium* extract in streptozotocin–induced diabetic rats. Clinics. (Sao Paulo). 2010; 65: 629–633.

Naudi A, Jove M, Ayala V, Cassanye A, Serrano J, Gonzalo H, Boada J, Prat J, Otin MP, Pamplona R. Cellular Dysfunction in Diabetes as Maladaptive Response to Mitochondrial Oxidative Stress. Exp Diabetes Res. 2012: 696215.

Nauseef WM. Detection of superoxide anion and hydrogen peroxide production by cellular NADPH oxidases. Biochimica Et Biophysica Acta. 2014; 1840: 10–16.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides.in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351–358.

Porter Y. Antioxidant properties of green broccoli and purple–sprouting broccoli under different cooking conditions. Bioscience Horizons. 2012; 5: 1–11.

Raymond C, Cho L, Rocco M, Hazen SL. New guidelines for reduction of blood cholesterol: Was it worth the wait? Cleve Clin J Med. 2014; 81: 11–19.

Renjith RS, Rajamohan T. Protective and curative effects of *Cocosnucifera* inflorescence on alloxan–induced pancreatic cytotoxicity in rats. Indian J Pharmacol. 2012; 44: 555–559.

Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench.instreptozotocin–induced diabetic rats. J Pharm Bioall Sci. 2011; 3: 397–402.

Sanjukta D, Ghosh S. *In vitro* effect on the antioxidative properties of crude extract of *Chenopodium album*in presence of the organophosphate, acephate. Int Food Res J. 2012; 19: 1033–1039.

Sarma DA, Mallick RA, Ghosh KA. Free radicals and their role in different clinical conditions: An overview. Int J Pharma Sci Res. 2010; 1: 185–192.

Sedlak J, Lindsay RH. Estimation of total, protein–bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25: 192–205.

Singh J, Cumming E, Manoharan G, Kalasz H, Adeghate E. Medicinal chemistry of the anti–diabetic effects of *Momordica charantia:* Active constituents and modes of actions. Open Med Chem J. 2011; 5(Supple 2–M2): 70–77.

Singh K, Verma B. Role of ayurvedic herbs on madhumeha (Diabetes Mellitus). Int J Ayurvedic Herb Med. 2013; 3: 1136–1140.

Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015; 6: 456–480.

Tiwari P, Recent Trends in Therapeutic Approaches for Diabetes Management: A Comprehensive Update. J Diabetes Res. 2015: 340838.

Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia. 2015; 58: 886–899.

Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: A 21st century challenge. Lancet Diabetes Endocrinol. 2014; 2: 56–64.

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