Issn No: 0976-3090 www.pharmanest.in

RESEARCH ARTICLE

ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF STEM JUICE OF *MUSA PARADISIACA* ON ALLOXAN INDUCED DIABETIC RATS

B SUNEETHA [1*], D SUJATHA[2] and K V S R G PRASAD[1]

[1]Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupathi (A.P), India.

[2] Sri Padmavathi School of Pharmacy, Tirupathi(A.P), India.

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis. Plants contain a wide array of free radical scavenging molecules which posses both hypoglycemic and antioxidant activities. So, the present study was planned to evaluate the stem juice of *Musa paradisiaca* for its antidiabetic and antioxidant activities at two different doses (1 and 2 g/kg, b.wt, p.o).The antidiabetic and antioxidant activity was evaluated by using alloxan-induced diabetic rat model in which the rats were treated with alloxan (150 mg/kg, i.p). The parameters monitored in the present study were serum glucose, triglycerides, cholesterol, SGOT, SGPT, *in vitro* antioxidant parameters (NO scavenging and DPPH), *in vivo* antioxidant parameters like catalase, lipid peroxidation and reduced glutathione in pancreas.Our study clearly indicated a significant antidiabetic activity with the stem juice of *M. paradisiaca* at both the dose as significant decrease in serum glucose, triglycerides, cholesterol, SGOT and SGPT was observed, thus supporting the traditional usage of stem juice by the Ayurvedic physicians for the control of diabetes.

KEYWORDS: *Alloxan, antidiabetic activity, antioxidant activity, Musa paradisiaca*

Introduction

Diabetes mellitus is a group of metabolic disorders with the common manifestation, hyperglycemia. The prevalence of diabetes throughout the world has increased dramatically during the last few decades affecting nearly 10% of the world population[1]. Diabetes mellitus relates to the development of micro and macro vascular complications, which contribute greatly to the morbidity and mortality associated with the disease. There is a high level of treatment failures, unpleasant side effects and enormous cost associated with oral antidiabetic drugs generating an urgent need and desire for alternative treatments[2].

The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity. Over half these have been used ethnopharmacologically in traditional medicine as antidiabetics, and some 50% of these traditional remedies have been studied experimentally[3].

Musa paradisiaca of family *Musaceae* is widely distributed throughout the tropical regions. It is a plantain and a tall herb with aerial pseudostem dying after flowering, leaves oblong, narrowed to the base. Flowers are unisexual in spikes, dropping, females at the bottom and males at the top, bracts conspicuous, dull brown, falling off in succession, fruit berries in several clusters, golden yellow colour on ripening.

The fruits are sweet and used as tonic, depurative, astringent, emollient, antihelmintic and

aphrodiasic. The roots are used as antihelmintic, depurative, scabies, leprosy, and skin disease and fresh root juice has antidiabetic activity[4]. The leaves have been studied as treatment for bronchitis,cold and eye infections[5]. Plaintain juice was used as an antidote for snake bite in the east[6]. In animal studies, the extract of *Musa paradisiaca* green fruits reduced hyperglycemia in normal and diabetic mice[7], and protected the gastric mucosa from aspirin induced erosion stimulating gastric and colonic mucosa[8] and had vasodilation effect[9], nonspecific relaxing and inhibiting effect on aortic and portal smooth muscles and the stemjuice of *Musa paradisiaca* had antilithiatic activity[10]. There was evidence in vivo antimicrobial activity on *Musa paradisiaca* root extract[11]. The root extracts show in vitro anti microbial activity.

The plant contains Diglycosides - delphidin and cyanides, leucodelphinidin, flavonoids, leucocyanidin, triterpenoids, tannins. High tannin content in the plant and unripe fruits has antibiotic activity. Serotonin, levarterenol, and dopamine are available in the ripe fruit and peel. Other chemical constituents are alkaloids, steroidal lactones, and iron[12].

The stem juices of *Musa paradisiaca* (SEM)have been used in treatment of diabetes mellitus as claimed in literature[13]. Yet to systemic pharmacological studies have not been reported to support the claim. So, this study has been taken up to evaluate the antidiabetic and antioxidant activity of SEM on alloxan-induced diabetic rats.

$$\text{Scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

Materials and Methods

Plant Material:

The plant material was procured daily from the market. The stem is cut into pieces, crushed and the juice was obtained by squeezing through muslin cloth. The plant material is authenticated by S.R.K Prasad, PG Director, J K C College, Guntur.

Preliminary phyto chemical screening:

The SEM were subjected to various qualitative tests for the identification of various plant constituents present in this species[14].

In vitro antioxidant studies:

The antioxidant activities of SEM was determined by using Diphenyl picryl hydrazyl(DPPH) radical scavenging, Nitric oxide (NO) radical scavenging methods.

Nitricoxide radical scavenging activity[15]: Sodium nitroprusside (10 µM) in phosphate buffer pH 7.7 was incubated with 25, 50, 75, 100 and 125µM concentrations of drug dissolved in a suitable solvent (methanol) and tubes were incubated at 25°C for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess reagent. Positive control ascorbic acid was used. The absorbance of the chromophore was measured at 546nm. Results were expressed as means of triplicates and percentage scavenging activity was calculated as follows.

DPPH radical scavenging activity[16]: Solutions of drugs at different concentrations of 25, 50, 75, 100 and 125 µM were added to 100 µm DPPH in ethanol and tubes were kept at an ambient temperature for 20 minutes and absorbance were measured at 517 nm. Positive control ascorbic acid was used. Results were expressed as means of

triplicates and was calculated using the same formula as described above.

Animals:

Male Wistar albino rats weighing 150-200g were used in the study. They were housed in individual polypropylene cage under standard laboratory conditions of light, temperature and relative humidity. Animals were given standard rat pellets (Gold Mohor Ltd) and drinking water *ad libitum*. Ethical committee approval number is 1016/A/06-CPCSEA/1/2007

Acute toxicity studies:

Healthy adult male albino rats were fasted overnight with free access to drinking water. They were divided into five groups each consisting of six animals. Group -1 animals were treated with distilled water (2ml/kg/p.o) and Group-2 to group-4 animals received 1, 2, 4, 8 g/kg/p.o of fresh SEM respectively. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles . Then intermittently and at the end of 24 hours, the number of deaths were noted to calculate LD50[17].

Anti diabetic study:

Diabetes was induced by single Intra peritoneal injection of alloxan (150mg/kg). The alloxan was freshly prepared by dissolving 150mg of alloxan in 1ml of normal saline solution. Two days after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of >140mg/dl, were included in the study[18].

Animals were divided into five groups consisting of six rats each. First group of animals were received only vehicle and served as normal group. Second group of animals received Alloxan (150mg/kg i.p.) and served as diabetic control. Third group animals received Glibenclamide (10 mg/kg orally once daily). Fourth group animals received *Musa paradisiaca* ((1g/kg, b.wt, p.o). Fifth group received *Musa paradisiaca* (2g/kg, b.wt, p.o).

Estimation of biochemical parameters:

The blood samples were drawn on 7th, 14th, 21st day from the retro orbital plexus into the centrifuge vials. Later serum was separated by centrifugation of blood at a speed of 2000rpm for 10 minutes. The serum was collected and quantitatively analyzed for blood glucose[19], triglycerides (TG) [20], cholesterol[21], Serum glutamate oxaloacetate transaminase (SGOT)[22]and Serum glutamate pyruvate transaminase (SGPT)[22].

Estimation of antioxidant parameters

Preparation of Post Mitochondrial Supernatant (PMS):

Pancreas was, perfused with ice cold saline(0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 minutes at4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 minutes at4°C to get the post mitochondrial supernatant which was used to assay catalase and lipid peroxidation and reduced glutathione activity.

Estimation of lipid peroxidation (LPO) from PMS[23]: 0.5 ml of PMS was taken and to it 0.5 ml of Tris hydrogen chloride buffer was added and incubated at 37 °c for 2h and then 1ml of ice cold trichloro acetic acid was added, centrifuged at 1000 rpm for 10 min. from the above, 1ml of distilled water was added. Absorbance was measured at 532 nm by using a spectrophotometer. Blank was prepared with out tissue homogenate.The result was calculated by using the formula.

$$\frac{3\times \text{absorbance of sample}}{\text{50.156 mg of tissue taken)}} = \mu m/mg \text{ tissue}$$

Table-1: Effect of stem juice of *Musa paradisiaca* on serum glucose levels

Group	Serum glucose mg/dL on		
	7th day	14th day	21st day
	Mean ±SEM	Mean±SEM	Mean±SEM
Normal	55.612±3.725	61.267±4.494	55.217±2.623
Diabetic control Alloxan (150mg/kg)	215.13±13.777[a]	237.13±12.207[a]	239.13±6.940[a]
Glibenclamide (10mg/kg)	103.97±7.101[b]	90.105±5.804[b]	65.667±4.255[b]
Musa paradisiaca (2ml)	142.32±11.327[b]	140.13±10.203[b]	70.067±7.572[b]
Musa paradisiaca (4ml)	88.250±9.591[b]	79.533±7.796[b]	63.967±8.070[b]

a = p < 0.001 considered statistically significant as compared to normal group

b = p < 0.001 considered statistically significant as compared to diabetic group

Estimation of reduced glutathione (GSH) from PMS[24]: 1.0 ml of PMS (10%) was precipitated with 1.0ml of sulphosalicylic acid (4%). The samples were kept at 4°C for atleast 1hour and then subjected to centrifugation at 1200 g for 15 mins at 4°C. the assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH7.4) in a total volume of 3.0ml. the yellow color developed was read immediately at 412 nm on a spectrophotometer using the same formula as described above. Blank was prepared with out tissue homogenate.

Table-2: Effect of stem juice of *Musa paradisiaca* on serum Triglycerides levels

Group	Serum triglycerides (mg/dL) on		
	7th day	14th day	21st day
	Mean ±SEM	Mean±SEM	Mean±SEM
Normal	35.740±3.234	38.050±2.066	39.017±2.835
Diabetic control Alloxan (150mg/kg)	117.18±12.221[a]	135.32±12.948[a]	140.42±13.154[a]
Glibenclamide (10mg/kg)	60.550±9.213[b]	50.100±3.401[b]	51.417±3.467[b]
Musa paradisiaca (2ml)	71.367±6.997[c]	56.267±3.388[b]	61.067±3.876[b]
Musa paradisiaca (4ml)	57.717±5.244[b]	47.433±1.678[b]	53.217±2.793[b]

a = p < 0.001 considered statistically significant as compared to normal group

b = p < 0.001 considered statistically significant as compared to diabetic group

c = p < 0.01 considered statistically significant as compared to diabetic group

Estimation of catalase (CAT) from PMS[25]:

The assay mixture consisted of 1.95 mlphosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10%) in a final volumeof 3.0 ml. Changes in absorbance were recorded at240

nm. Catalase activity was calculated in terms of k minutes-1.

Statistical analysis:

All the data were represented as means ±SEM. Statistical analysis was performed by ANOVA test for multiple comparisons followed by Tukey-Kramer test. The statistical significance was set accordingly.

Results :

Phyto chemical screening

Preliminary phytochemical screening of the SEM revealed the presence of tannins, alkaloids, flavonoids, terpenoids.

Acute toxicity studies

All rats treated with SEM showed no discernible behavioural changes upto 4g/kg by oral route. No mortality was observed at this dose during 72 hours of administration.

Table-3: Effect of stem juice of *Musa paradisiaca* on serum cholesterol

Group	Serum cholesterol (mg/dL) on		
	7th day	14th day	21st day
	Mean ±SEM	Mean±SEM	Mean±SEM
Normal	89.992±1.450	93.570±2.785	95.118±2.609
Diabetic control Alloxan (150mg/kg)	453.98±19.794[a]	465.50±9.302[a]	456.38±10.929[a]
Glibenclamide (10mg/kg)	386.12±9.333[b]	331.67±7.447[b]	120.58±13.839[b]
Musa paradisiaca (2ml)	394.68±4.828[c]	355.87±2.874[b]	140.62±6.461[b]
Musa paradisiaca (4ml)	370.48±11.828[b]	324.80±9.706[b]	130.07±5.638[b]

a = $p < 0.001$ compared to normal group; b = $p < 0.001$ compared to diabetic group

c = $p < 0.01$ compared to diabetic group

Biochemical parameters:

Effect of SEM on alloxan induced diabetes in rats with reference to biochemical changes in serum are shown in a tabulated form. All the Values are expressed as mean ±SEM for six animals

Antioxidant studies:

The results of *in vitro* and *in vivo* antioxidant studies are shown in a tabulated form. All the Values are expressed as mean ±SEM for six animals.

Discussion

For evaluating antidiabetic agents, alloxan rat model has several advantages over the other models like less cost and more reproducibility. On administration of alloxan, liver glycogen depletes faster, severity of hypoglycemia is less and reversible action of alloxan can be seen after three months[26], hence it is considered as one of the suitable experimental animal model for type-2 diabetes mellitus. The cytotoxic action of alloxan is mediated by ROS. Alloxan and the product of its reduction, dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to H_2O_2. Thereafter, hydroxyl radicals are formed by the fenton reaction. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of ß-cells of islets of Langerhans resulting in reduced synthesis and release of insulin[27,28,29].

Table-4: Effect of stem juice of *Musa paradisiaca* on serum SGOT

Group	SERUM SGOT (IU/L)		
	7th day	14th day	21st day
	Mean ±SEM	Mean±SEM	Mean±SEM
Normal	48.96 ±0.64	47.91±0.96	48.70±1.00
Diabetic control;Alloxan (150mg/kg)	160.53±2.32[a]	161.36 ±2.12[a]	160.55±2.16[a]
Glibenclamide (10mg/kg)	62.45±1.09[b]	60.95±1.48 [b]	57.57±1.67[b]
Musa paradisiaca (2ml)	66.16±1.83[b]	64.19±1.81[b]	58.79±0.93[b]
Musa paradisiaca (4ml)	65.66±1.27[b]	62.22±1.25 [b]	58.20±1.30[b]

a = p < 0.001 compared to normal group; b = p < 0.001 compared to diabetic group

In our study, administration of alloxan increased serum glucose levels when compared to normal animals and also induced persistent diabetes mellitus in rats. In the present study, the standard drug used was glibenclamide. Glibenclamide produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes and inactive in intense alloxan-induced diabetes[30, 31] (nearly all ß-cells are destroyed).

The result of phytochemical screening on the stem juice of *M. paradisiaca* reveals that the extract contained various pharmacologically active compounds such as tannins and alkaloids. In alloxan-treated diabetic rats receiving SEM showed significant (p< 0.001) decrease of blood glucose levels in comparision to diabetic control and this could be due to the possibility that some ß-cells are still surviving to act upon by *M. paradisiaca* to exert its insulin releasing effect. This suggests that the mode of action of the active ingredients of *M. paradisiaca* is probably mediated by an enhanced secretion of insulin, like sulphonyl ureas[32]. Significant (p<0.001 and p<0.01) dose dependent reduction in blood glucose, triglycerides and cholesterol levels was also observed. The results are shown in Table-1,2,3. Increase in levels of liver enzymes like SGOT and SGPT is an indication of liver damage and so from the results obtained in this study, it was proved that the stem juice of *M. paradisiaca* had significant (p<0.001 and p<0.01) protective effects in the liver also. All effects shown are dose dependent only. The results are shown in Table-4 and Table-5.

Table-5: Effect of stem juice of *Musa paradisiaca* on serum SGPT

Group	Serum SGPT (IU/L)		
	7th day	14th day	21st day
	Mean ±SEM	Mean±SEM	Mean±SEM
Normal	36.10±1.18	37.934±0.66	37.31±0.35
Diabetic control; Alloxan (150mg/kg)	76.39±6.07[a]	74.77±5.41[a]	58.90±3.30[a]
Glibenclamide (10mg/kg)	52.34±2.14[b]	50.24±0.72[b]	42.79± 1.89[b]
Musa paradisiaca (2ml)	54.11±1.94[b]	49.35±2.61[b]	47.75±1.49[c]
Musa paradisiaca (4ml)	43.46±1.59[b]	42.20±1.68[b]	38.82±0.45

a = p < 0.001 compared to normal group; b = p < 0.001 compared to diabetic group
c = p < 0.01 compared to diabetic group

Oxidative stress occurs at an early stage in diabetes, leading to the appearance of complications. Hyperglycemia aggravates endothelial ROS generation by a variety of mechanisms such as activation of protein kinase-C isoform[33], increased formation of advanced glycation end products [34]and increased glucose flux through aldose reductase pathways. These are some of the known biochemical mechanisms of hyperglycemia-induced tissue/organ damage. Reactive species can be eliminated by a number of enzymatic and non enzymatic antioxidants and thus protecting tissue/organ damage from oxidative stress. In the present study, we estimated both enzymatic and non-enzymatic antioxidants in pancreas *in vivo.*

In the present study, SEM was tested for *in vitro* antioxidant activities in two different models. It was observed that SEM scavenged free radicals in a concentration dependent manner. The values were expressed as mean ±SEM and given in Table-7.

In our investigations, the levels of enzymatic antioxidants (catalase, reduced GSH) declined in diabetic animals were significantly ((p<0.001 and p<0.01) restored on treatment with stem juice of *M. paradisiaca*. The levels of non-enzymatic antioxidant (MDA) is significantly (p<0.001) increased in diabetic animals and significantly (p<0.001) decreased in diabetic animals treated with glibenclamide and stem juice of *M. paradisiaca.* This implies that this potential antioxidant defence is reactivated by the active principles of *M. paradisiaca*, with an increase in the capacity for detoxification through enhanced scavenging of oxy radicals. The result is shown in Table-6.

In nitric oxide scavenging the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°c was reduced by stem juice of *M. paradisiaca*.

Table-6: Effect of stem juice of *Musa paradisiaca* on in vivo Antioxidant Activities

Group	Catalase	Reduced Glutathione (GSH)	Lipid Peroxidation (LPO)
Normal	0.052±0.0074	0.167±0.0164	0.052±0.0073
Diabetic control Alloxan (150mg/kg)	0.004±0.0006[a]	0.005±0.0009[a]	0.407±0.0669[a]
Glibenclamide (10mg/kg)	0.080±0.0047[b]	0.076±0.0077[b]	0.058± 0.0044[b]
Musa paradisiac (2ml)	0.037±0.0043[b]	0.059±0.0047[c]	0.086±0.0039[b]
Musa paradisiaca (4ml)	0.067±0.0041[b]	0.073±0.0068[b]	0.057 ±0.0053[b]

a = p < 0.001 compared to normal group;
b = p < 0.001 compared to diabetic group
c = p < 0.01 compared to diabetic group

Maximum percentage inhibition of nitric oxide radicals by SEM was about 44% at 100µg/ml. Ascorbic acid at 100 µg caused about 75%.This may be due to the antioxidant principles in the stem juice, which compete with oxygen to react with nitric oxide there by inhibiting the generation of nitrite indicating, the SEM has greater inhibition than ascorbic acid in scavenging NO.

Table-7: *In vitro* antioxidant activity of SEM by NO method and DPPH

% INHIBITION (MEAN±SEM)				
Concentration (µg/ml)	NO Standard (µg/ml)	NO test (µg/ml)	DPPH Standard (µg/ml)	DPPH (µg/ml)
10	3.6±0.001	21.55±0.07	5.9±0.002	22.15±0.07
20	12.7±0.002	23.0±0.06	13.5±0.001	28.6±0.07
40	30.8±0.001	26.75±0.07	29.51±0.001	35.5±0.08
80	61.25±0.005	36.85±0.12	62.15±0.001	39.6±0.13
100	75.1±0.002	44.2±0.18	78.12±0.001	48.3±0.17

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction of the DPPH radical is determined by its decrease in absorbance at 517 nm. The extent of reduction of DPPH free radical is visualized as a discoloration from purple to yellow[35]. Maximum percentage inhibition of DPPH radicals by SEM was about 48% at 100µg/ml. Standard drug ascorbic acid about 78% of inhibition at 100 µg. The present investigation indicated that SEM has radical scavenging capacity.

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ADDRESS FOR CORRESPONDENCE
balusu_sunitha2001@yahoo.com