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EFFECT OF *MUSA PARADISIACA* FLOWERS ON ENZYMATIC AND NON- ENZYMATIC ACTIVITIES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

R. Kowsalya*[1] and V. Barathi[2]

[1*]Department of Biochemistry D.G. Government Arts College for Women Mayiladuthurai (Tamilnadu), India.
[2]Department of Biochemistry Government Arts College for Women Krishnagiri (Tamilnadu), India.

Abstract

Diabetes mellitus is a illness of ancient times which was earlier regarded as "sweet urine" and now it is defined as a group of complex metabolic disorders characterized by deficient insulin secretion, impaired insulin activity. *M.paradisiaca* flowers were dietary factors rich in nutraceuticals, antioxidants, dietary fiber, etc. This makes the use of experimental animal models for the disease imperative. Type-1 diabetes requires insulin treatment, whereas Type-2 diabetes, which is characterized by insulin resistance, can be treated using a variety of therapeutic approaches. The major organs active in the regulation of blood glucose are the pancreas, liver, tissue and kidney. To investigate the effect of *Musa paradisiaca* (220mg/kg/body.wt) on markers of in streptozotocin (40mg/kg body wt.) induced diabetic rats. The enzymatic antioxidant enzymes incorporate Superoxide dismutase SOD, Catalase CAT, glutathione peroxidase GPx, glutathione reductase GR. The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Hence the present study was aimed to examine the effect of banana (*Musa paradisiaca*) flower antioxidant of enzyme activities during diabetes. To investigation this *in vivo* antidiabetic and antioxidant potential of *M. paradisiaca* decreased oxidative stress as it is evidenced by improvement in antioxidants defence mechanisms. So the work have highly effect of *M. paradisiaca* compound produce from induced diabetic rats.

Key words: Diabetes Mellitus, streptozotocin, *Musa paradisiaca* flowers, Antioxidants.

Introduction

Blood sugar level, insulin is a hormone produced by the pancreas that allows your body to use glucose for energy, diabetes mellitus more a spectrum of metabolic disorder, which has become a major good health challenge worldwide (Girox *et al*., 2001). The World Health Organization WHO has predicted that the major burden will occur in the developing. The prediction for next 15 years shows that diabetes will become epidemic reaching a prevalence of 7.7% adults worldwide by 2030. Metabolic disorder, affecting about 170 million people worldwide. At present approximately 73 million adults are estimated to have diabetes in India with an average prevalence of 10.4% and this number is expected to rise to 151 million by the year 2045. (Ahuja *et al*., 2017) Epidemiological studies of diabetes mellitus have displayed that gender, age and ethnic background are important element when considering the development of diabetes mellitus and its difficulty (Stiegler et *al*., 2009). In normal therapy, type 1diabetes mellitus is treated with exogenous insulin and type 2 diabetes mellitus is oral hypoglycemic agents (sulphonylureas, biguanides (Pepato *et al*., 2005). The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and non-enzymatic. The antioxidant enzymes incorporate Superoxide dismutase SOD, Catalase CAT, glutathione peroxidase GPx, glutathione reductase GR (Young and Woodside, 2001). The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Different types of biological antioxidants include, for instance, Glutathione Vitamin C & vitamin E, cystine, etc. (Savita Khanna *et al*., 2006). ROS level elevation in diabetes may be due to decrease in destruction and increase in the production by catalase CAT–enzymatic/non-enzymatic, superoxide dismutase SOD and glutathione peroxidase (Pham-Huy, 2008). Streptozocin (also known as streptozotocin) is an agent with specific effects on pancreatic beta cells.

***Author for correspondence :** E-mail: kowsalyamouli@yahoo.com

Because pancreatic beta cells have high concentrations of glucose transporter 2 (GLUT2), streptozocin is selectively toxic to these cells. (Thames *et al*., 2016). Streptozocin has a rapid half-life in animals, but metabolites may be active *M. paradisiaca* is considered as an indigenous food and its consumption is limited due to the tedious preparation procedure. We assume that *M. paradisiaca* flowers are rich in antioxidant compounds. (Sheng *et al*., 2010). *M. paradisiaca* has tremendous nutritional value similar to banana fruits. They are outstanding source of vitamins, certain minerals, good source of fibre and protein. Besides *Musa paradisiaca* are also excellent source of certain phytochemicals which (Merchant *et al*., 2015). Such alkaloids, carotenoids, flavonoids, terpenoids, glycosides and can often have anti diabetic effect **(**Sheng *et al*., 2010). Along with dietary fibers, proteins and unsaturated fatty acids, are also rich in vitamin C and flavonoids. They are part of many cuisines in the world. They also possess immense medicinal values. The present study was undertaken to investigation this *in vivo* antidiabetic and antioxidant potential of *M. paradisiaca*. However isolated from *Musa paradisiaca* induced diabetic rats. The work highly the effect of *M. paradisiaca* compound produce from induced diabetic rats.

Materials and Methods

Materials

Chemicals and all Glassware's were obtained from the Department of Biochemistry, Government Arts College for women, Krishnagiri. All chemicals used were of analytical grade with 99% purity. All the chemicals and reagents used for screening test, quantitative analysis and antioxidants activity were of analytical grade obtained from various companies.

Collection of plant samples

The freshly and healthy of Musa paradisiaca flowers were collected from local market of panjaliyur village, Krishnagiri (DT), Tamilnadu, in India. The flower were cut into small pieces and dried under shade for three to four weeks. The dried materials were grind in to fine powder. The entire mixture was homogenized in blander than the homogenized mixture was left at room temperature for about 48 hr.

Preparation of flower extract

9g of *Musa paradisiaca* powder was extracted with 150 ml ethanol in a soxhlet apparatus for 48 hours. After extraction the solution was left out for 24 hrs was filtered and the clear filtrate was evaporated to dryness using water bath at 40°C. After completion of the reaction, the entire slurry was filtered to get blossom powder extract (Singh *et al*., 2017). All extracts obtained were stored in a refrigerator until required for use. Further Phytochemical screening of flower extract were analysed.

Animal purchase and maintenance

Albino (Wistar strain) male rats, weighing about 200-220g procured from Sri Vengateshwara Enterprise pvt. Bangalore. The animals were housed in polyacrylic cages with six (6) animals per cage and maintained under standard laboratory conditions (Temperature 25±2°C with dark and light cycle (12/12 h.) with a relative humidity of 45% to 65%. Animals ethical number (Approval No. MCAS /IAEC/2019/3/9.2.2019) were allowed to acclimatize for at least 7 days before each experiment; and were observed regularly for signs of ill health; body weights were noted daily. They were allowed free access, fed with standard dry pellets; containing protein-21%, lipds-5%, crude fibre-4%, ash-8%, calcium-1%, phosphours-0.6%, nitrogen free extract-55% and provided with metabolisable energy at 3600 Kcal/Kg and also enriched vitamins and minerals. It was supplied by the Hindustan Lever, Kolkata, India marketed under the name, gold Mohr and water *ad libitum*.

Experimental Induction

Streptozotocin (40mg/kg body wt.) was added to dissolve in citrate buffer and injected IP (Interperotional injection) for period of 24 hours for 2 days.

Experimental Design

The ethanolic extract was administrated at concentration of 10mg/kg/body weight dissolved in water given to rats through intragastric tube for a period of 15 days. In the experiment, a total of 24 rats were used in the study. The rats were divided into four groups of six rats in each group. Group I (Control rats): Normal rats fed with pellets & balanced diet and Water *ad libitum* Group II Normal rats were administered Streptozotocin (40mg/kg/bodywt) (IP) after 24 hours for 2 days (Zafar *et al*., 2009). Group III (ethanolic extract of *Musa paradisiaca*): Streptozotocin administered (40mg/kg/bodywt) rats were treated with ethanolic extract of *Musa paradisiaca* (220mg/kg/body.wt). Group IV (ethanolic extract of *Musa paradisiaca* administered rats): Animals were orally administered with ethanolic extract of *Musa paradisiaca* (220kg/body/wt) administered along with normal diet.

Preparation of the serum, tissue, liver and kidney from experimental animals

By cervical decapitation the rats were sacrificed after induction & treatment period. Serum to Plasma was

Table 1: Enzymatic antioxidant in serum, liver and kidney sample in control and diabetic rat.

Source	Experiment	Normal	Diabetic	Diabetic + *M. paradisiaca*	Normal + M. *paradisiaca*
SERUM	GR	5.74±0.43[a]	4.39±0.27[a]	3.85±0.26[c]	5.03±0.39[a]
	GPX	9.79±0.71[a]	7.63±0.39[b]	5.15±0.41[c]	9.61±0.62[a]
	SOD	5.99±0.39[a]	4.47±0.39[b]	3.55±0.27[c]	5.64±0.44[a]
	CAT	35.55±2.75[a]	27.66±2.17[b]	22.88±1.19[c]	34.68±2.62[a]
LIVER	GR	0.61±0.04[a]	0.42±0.16[b]	0.23±0.03[c]	0.56±0.04[a]
	GPX	8.94±0.86[a]	6.5 5 ±0.55[b]	5.24±0.39[c]	8.67±0.75[a]
	SOD	6.76±0.55[a]	4.54±0.35[b]	3.55±0.27[c]	6.03±0.46[a]
KIDNEY	CAT	70.58±5.42[a]	60.14±0.61[a]	5.64±53.61[c]	69.37±5.45[a]
	GR	7.23±2.41[a]	5.57±2.29[b]	3.78±1.71[c]	6.61±4.04[a]
	GPX	4.32±0.33[a]	3.01±0.23[b]	3.98±0.30[c]	4.05±0.25[a]
	SOD	5.44±0.41[a]	3.74±0.35[b]	5.66±0.55[a]	4.54±0.35[c]
	CAT	60.01±4.9	46.89±3.65	50.67±4.44	55.38±3.19

Values are mean ±SD of six samples in each group : a : comparison of Group I to Group II, b: comparison of Group II, Group IV and Group I, c: comparison of Group I and III. Significance: a, b, are significant at p<0.05, c- represent insignificant.

separated from heparinized and non-heparinized blood respectively after incubated for 15 minutes at room temperature and then centrifuged at 3000rpm for 30min. Supernatant serum separated, preserved in a sterile tube in an ice box and used for the estimation of various biochemical parameters.

Results and Discussion

Assay of Enzymatic antioxidants

Presents changes in the activities of enzymic antioxidants: GR, GPX, SOD and CAT. The activities of GR, GPX, SOD, CAT was found to be decreased significantly (P<0.05) in serum, liver and kidney of group II (intoxicated) rats, when compared to control (group I) rats. While a concomitant significant decrease (P<0.05) in the above enzymic antioxidants activity was found in serum, liver and kidney of group II rats. These activities was reversed to near normal in, *Musa paradisiaca* ethanolic extract (group IV) treated rats when compared to group II rats at significant (P<0.05) level at the above mentioned sources. In *Musa paradisiaca* ethanolic extract administered control (group III) rats, activities of the enzyme was sustained.

Table 2: The Non-enzymatic antioxidant in serum, liver and kidney sample were control and diabetic rat.

Source	Experiment	Normal	Diabetic	Diabetic + *M. paradisiaca*	Normal + M. *paradisiaca*
SERUM	VIT C	1.98±0.16[a]	1.55±0.13[a]	1.25±0.08[b]	1.93±0.15[a]
	GSH	23.17±1.58[a]	17.34±1.18[b]	16.36±1.35[c]	22.66±1.82[a]
LIVER	VIT C	44.43±7.646	36.43±12.01[a]	23.76±7.696	41.9±4.09
	GSH	5.04±0.37[a]	4.49±0.34[b]	4.49±0.29[c]	5.73±0.44[a]
KIDNEY	VIT C	1.47±0.12[a]	0.79±0.06[b]	1.20±0.09[c]	1.45±0.11[a]
	GSH	5.04±0.33[a]	3.02±0.24[b]	3.99±0.31[c]	4.49±0.37[a]

Values are mean ±SD of six samples in each group :a : comparison of Group I to Group II, b: comparison of Group II, Group IV and, c: comparison of Group I and III. Significance: a, b, are significant at p<0.05, c- represent insignificant. Unit 1 represent Vit C expressed as µg/mg or ml of serum, liver and kidney tissue. 2 represent GSH expressed as µg protein or ml of serum, liver and kidney tissue.

Assay of Non enzymes antioxidants

Presents the changes in the levels of Serum, liver and Kidney. Non-Enzymic antioxidant: VIT-C, GSH, in the control and streptozotocin induced rats. The VIT-C, GSH, (Non-enzymic antioxidant) levels were found to be decreased significantly (P<0.05) in serum, liver and kidney of group II (intoxicated) rats, when compared to control (group I) rats. While a concomitant significant increase (P<0.05) were found in serum of group II rats. Vitamin C and GSH levels were reversed to near normal in, *Musa paradisiaca* ethanolic extract (group IV) treated rats when compared to group II rats at significant (P<0.05) level in all sources. In *Musa paradisiaca* administered control (group III) rats, levels of Vitamin C and GSH were sustained. Group IV results were found to be similar to that of group. Antioxidants constitute the foremost defence system that limit the toxicity associated with free radicals.Oxidative stress in diabetes is coupled with decrease in the antioxidant status, which can increase the effect of free radicals (Singh *et al*., 2017). Antioxidant enzymes form the first line of defence against reactive oxygen species in the organism include the enzymes SOD, CAT, GPX and GSH, which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are two major scavenging enzymes that remove radicals *in vivo*. The decrease in CAT activity could also result from inactivation by glycation of the enzyme. Treatment with *M. paradisiaca* increased the activity of SOD and CAT in diabetic rats when compared with diabetic control rats. The increased activities of antioxidant enzymes may act as an added compensation mechanism to maintain the cell integrity and protection against free radical

damage. GPX catalyzes the reaction of hydroperoxides with GSH to form glutathione disulfide. Vitamin C reacts with superoxide and inhibits the formation of nitrosamines during protein digestion and protects damages to DNA and cellular proteins (Das *et al*., 2008). The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals and removal of molecular oxygen (Richard *et al*., 1959). The decrease in GSH level during diabetes is probably due to its increased utilization by the hepatic cells which could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes.

Conclusion

Taken together with our findings augmented with literature survey, the current research showed that *M. paradisiaca* improves glucose homeostasis, modified the activities of key enzymatic and non- enzymatic antioxidants of the derangements in carbohydrate metabolism. *M. paradisiaca* administration decreased oxidative stress as it is evidenced by improvement in antioxidants defiance mechanisms. Further clinical trials are warranted before *M. paradisiaca* could be developed as a drug for the treatment of diabetes mellitus.

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