BIOCHEMICAL STUDIES ON ANTIDIABETIC EFFECT OF CENTRAL PART OF STEM OF *MUSA SAPIENTUM*.

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BY
PIYUSH DIKSHIT

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY COLLEGE OF MEDICAL SCIENCES
(UNIVERSITY OF DELHI)
DILSHAD GARDEN
DELHI-110095
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ABSTRACT

Thesis title—“Biochemical studies on antidiabetic effect of central part of stem of *Musa sapientum*”.

Candidate- Piyush Dikshit
Supervisor- Dr. Rimi Shukla
Co-supervisors- i) Dr. JK Gambhir; ii) Dr. Vibha Tandon
Place of work- University college of Medical Sciences and GTB hospital Delhi-110095

**Introduction** – Diabetes for the whole world is not an epidemic anymore but has turned into pandemic. Global prevalence of diabetes in 2010 was 284 million and it is expected to rise to 439 million in 2030. Plants have been major sources of drugs for the treatment of diseases including diabetes in India and other countries, *Musa sapientum* is one such plant. Antidiabetic action of flower, fruits and root have been reported by earlier researchers. Literature survey has revealed that central stem of this plant has not been evaluated for it’s antidiabetic potential.

**Aim and objectives**- This study was aimed to evaluate the antidiabetic effect of aqueous extract of central stem of *Musa sapientum*, to isolate active hypoglycemic component from it, study mode of action of this component and perform limited toxicity studies to evaluate its safety.

**Methods**- Male wistar rats and rabbits were used in this study. Diabetes was induced in rats by streptozotocin (45mg/kg i.p.) and in rabbits by alloxan injection, (120mg/kg i.v.), respectively. The stem of *Musa sapientum* was collected from local area and authenticated by NISCAIR. Aqueous extract was prepared by cutting stem into tiny pieces, grinding with water and filtering through cloth. Biochemical parameters- Fasting glucose (FBG) and postprandial blood glucose (PPG), glycosylated hemoglobin (GHB), serum insulin, C-peptide, tyrosine kinase activity of insulin receptors, *in-vitro* insulin release, expression of Glucose transporter-4 (GLUT-4), total cholesterol (TC), triacylglycerol (TAG), LDL+VLDL-cholesterol (LDL+VLDL-C),
HDL-cholesterol (HDL-C), glucokinase (GK), phosphofructokinase (PFK), glucose-6-phosphatase (G-6-pase), hydroxymethyl glutaryl Co-A reductase (HMG-Co A reductase), acetyl Co A carboxylase, malon dialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) catalase (CAT), kidney Function test, liver function test. Haemotological parameters and histopathology of pancreas, liver and kidney were also done. Toxicity and safety profile of MSH-3 were established according to guidelines of OECD 420. Statistical analyses were performed using ANOVA followed by Tukey’s test at 5%.

**Results**: In preliminary study, the most effective dose of AqMS was found to be 50 mg/kg. One month treatment with AqMS significantly decreased FBG, PPG and GHb in diabetic rats and rabbits (p<0.01), where as insulin levels were significantly increased (p<0.01). Lipid profile restored to near normal. AqMS showed significant hypocholesterolemic and antioxidant effect in cholesterol fed rats. It decreased TC, TAG, LDL+VLDL-C and increased HDL-C. MDA decreased and SOD and CAT increased after treatment. Phytochemical analysis showed the presence of phenols, tannins, terpenoids and sterols. Methanolic fraction prepared from lyophilized extract has significant hypoglycemic activity. It was further purified by column chromatography which gave three fractions, out of which one fraction (FIII) was active. TLC of this fraction with petroleum ether : ethyl acetate (1:9) gave 3 bands. Out of these bands, the band with Rf value 0.30 (FIIIc) was active in animals. It was named as MSH-3 and showed a single peak in gas chromatogram at retention time 27.06. NMR analysis of MSH-3 showed that it is a sterol with molecular weight of 414. MSH-3 exerted significant glycemic control in diabetic animals. It did not produce hypoglycemia in healthy rats. Treatment with MSH-3 has insulinotropic effect, as it increased the insulin as well as C-peptide levels significantly in diabetic rats (p<0.01). **In-vitro studies** clearly showed that MSH-3 increases release of insulin.
from β islets of pancreas. It showed increased insulin sensitivity by increasing tyrosine kinase activity of insulin receptor in RBCs and hepatocytes in diabetic rats. Treatment with MSH-3 in diabetic rats showed improvement in GLUT4 protein expression in gastrocnemius skeletal muscle as shown by immunohistochemical analysis and western blot analysis of GLUT-4. It also increased activity of enzymes of carbohydrate metabolism i.e. GK and PFK in liver. (P<0.01). It decreased the activity of G-6-P.(p<0.01). It also restored activities of enzymes of lipid metabolism; HMG Co-A reductase, acetyl co-A carboxylase (p<0.01). MSH-3 increased the muscle and liver glycogen content by 92% and 83% respectively. Treatment decreased total lipid content in muscle by 36% and liver by 30%. Histopathological examination of pancreatic tissue from MSH-3 treated rats revealed improvement in the number of β islets. Acute Toxicity study of MSH-3 suggested that ‘Limit Test Dose’ of MSH-3 was 500 mg/ kg. In subchronic toxicity study, there was no adverse effect on histopathological, hematological and biochemical parameters in rats treated even with 5 and 10 times ED doses.

**Conclusion**-Aqueous extract of stem of *Musa sapientum* (50 mg/kg b.wt.) has potent antihyperglycemic activity in diabetic animal. It has shown hypocholesterolemic and antioxidant effect in cholesterol fed rats. The isolated active compound, MSH-3 is a sterol derivative with molecular weight- 414. The effective dose of MSH-3 is found to be 2 mg/kg b.wt. MSH-3 treatment improved glycemic control and serum lipid profile in diabetic rats. Antidiabetic action of MSH-3 is by both mechanisms- pancreatic as well as extrapancreatic. It increases insulin release from pancreas. It increases insulin sensitivity by increasing tyrosine kinase activity. It increases glucose transporter GLUT4 expression and its translocation and has beneficial effect on enzymes of carbohydrate and lipid metabolism. MSH-3 is safe even at a high dose at 500 mg/kg and is non toxic. It may be used for treatment of diabetes in humans.