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In vitro antioxidant, hypoglycemic and oral glucose tolerance test of banana peels

V.V. Navghare, S.C. Dhawale *

Department of Pharmacology, School of Pharmacy, S.R.T.M. University, Nanded 431606, Maharashtra, India

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Abstract Banana fruit is claimed to have antidiabetic effects despite its high calorie content, and its peels also contain vital phytoconstituents including gallocatechin. Previously banana pulp has been studied for antihyperglycemic effects, and in the present investigation antihyperglycemic effect of ethanolic extract of inner peels of *Musa sapientum* (EMS), *Musa paradisiaca* (EMP), *Musa cavendish* (EMC) and *Musa acuminata* (EMA) fruit was evaluated using oral glucose tolerance test in normoglycemic rats. *In vitro* antioxidant study was conducted using DPPH, H_2O_2 radical scavenging assay and ferric reducing power assay. Wistar rats were divided into fourteen groups and twelve groups received different doses of aforementioned extracts, while control group received gum acacia solution and remaining group received standard drug, glimepiride. All the rats received glucose load at a dose of 2 g/kg body weight. Groups treated with EMC and EMA showed significant decrease in glucose level ($p < 0.01$) at 150 min as compared to control group. In hypoglycemic study, only EMP 500 mg/kg, p.o. treated group revealed a significant decrease ($p < 0.05$) in glucose level at 120 min, while other groups did not show any sign of hypoglycemia. In glucose tolerance test, animals treated with EMC and EMA depicted dose dependent antihyperglycemic effect at 150 min while EMS and EMP showed significant reduction in plasma glucose at higher doses. In a similar fashion, EMA i.e. *M. acuminata* demonstrated highest antioxidant activity followed by EMC against DPPH radical. In ferric reducing power and H_2O_2 scavenging assay, EMA demonstrated maximal antioxidant activity when compared with other extracts.

1. Introduction

Diabetes mellitus (DM) ranks highly with the top ten disorders which cause mortality throughout the world and is affecting approximately 30% of the population worldwide.[1–3] It is not a single disease entity, but a set of metabolic disorders with a common underlying feature of high blood glucose level. It is a systemic metabolic disease characterized by increased blood glucose, triglyceride and hypo insulinemia that may lead to decrease in both insulin action and insulin secretion.[4,5] The increased blood glucose is associated with reduced quality of life and high risk factors for mortality and morbidity.

Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin is released from the pancreas to normalize the glucose level.[6] It

* Correspondent author. Tel.: +91 2164 263030.
E-mail address: shashiprathmesh@gmail.com (S.C. Dhawale).

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lowers the post-prandial blood glucose level when it is raised (e.g. after eating food). Hyperglycemia in DM, results from defects in insulin secretion, insulin signaling pathway or most commonly both.[7]

Uncontrolled DM is often associated with complications which pose challenges to clinicians. These complications include development of micro- and macro-vascular complications such as neuropathy, nephropathy, retinopathy, cardiovascular and cerebrovascular diseases.[8,9]

Currently available antidiabetic agents possess potential side effects such as risk of hypoglycemia, anemia, cholestatic jaundice.[10] Many herbal constituents have varying degree of hypoglycemic and antihyperglycemic activity and until now no case of adverse effect is counted with herbal constituents. Among these are alkaloids, glycosides, galactomannan, gallocatechin, hypoglycans, guanidine, steroids, carbohydrates, terpenoids, glycopeptides, amino acids and inorganic ions.[11,12] *Musa* commonly known as banana, reported to possess therapeutic potential in treatment of hyperglycemia.[13,14]

In Indian system of folk medicine, the peel of banana is also used to treat various diseases and disorders such as for treatment of wound, hyperglycemia, ulcer, dysentery.[15] Some people have a habit of eating inner peel, in addition to the pulp of banana fruit. Several flavonoids and related compounds (leucocyanidin, quercetin and its 3-O-glucoside, 3-O-galactoside, and 3-O-rhamnosyl glucoside, gallocatechin) were isolated from the unripe pulp and peel of plantain.[14,16,17] The various antioxidant components identified in bananas includes tocopherol, ascorbic acid, beta carotene, dopamine, phenolic groups and gallocatechin.[18] Banana is also reported to be a rich source of calcium, vitamins A, B1, B2, B3, B6, C and minerals such as potassium and phosphorus.[19] These phytochemicals have shown protective action against diseases which involve oxidative stress. On the basis of traditional claim, reported activities and chemical constituents, the present study was aimed to evaluate antihyperglycemic, hypoglycemic and *in vitro* antioxidant potential of peels of Musa species.

2. Materials and methods

2.1. Experimental animals

Albino rats of Wistar strain weighing 160–200 g were obtained from National Institute of Nutrition (NIN), Hyderabad. Animals of either sex were housed under standard laboratory conditions of 22 ± 3 °C temperature and relative humidity 30% and 12 h light and dark cycle maintained, free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (1613/PO/a/12/CPCSEA).

2.2. Acute toxicity study

The LD50 of the peel extract was tested to determine the safety of the agent according to the guidelines set by the OECD (Organization for Economic Cooperation and development) No. 423.[20] The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per group and administered 100, 600 and 1000 mg/kg of the Musa species extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2000, 3000 and 5000 mg/kg were administered to another set of three mice per group and these animals were observed for signs of toxicity and mortality for 72 h.

2.3. Collection and authentication of the plant material

Fresh unripe fruits of *Musa sapientum*, *Musa paradisiaca*, *Musa cavendish*, *and Musa acuminata* (Musa species) Linn. (Museaceae) were collected from Nanded, Satara of Maharashtra, Goa and Kerala states of India. The specimens were authenticated at Botanical Survey of India, Pune (MUP-NAV2), and Science College Nanded (25-9/12.)

2.4. Preparation of extract

Banana pulp was removed and from the remaining peel inner fibrous part was removed by knife (we termed it as 'inner peel') and it was shed dried at room temperature and latter powdered using grinder. This powder was then defatted with petroleum ether. *M. sapientum*, *M. paradisiaca*, *M. cavendish*, *and M. acuminata* peel powder was extracted with ethanol using soxhlet extraction. It was then removed and the solvent was evaporated under vacuum and the residue was stored for further use.

The extract was not freely soluble in distilled water so the suspension of extract was prepared using 1% gum acacia (as a suspending agent).

2.5. Experimental design

The Wistar albino rats weighing 160–200 g were used. The overnight fasted animals were divided into fourteen groups ($n = 6$). Group 1 served as Control: The animals of this group received 1% gum acacia (1 ml/kg, p.o.). Group 2 served as Glim (Standard): The animals of this group received glimepiride (0.09 mg/kg, p.o.). Groups 3 to 5: Received ethanolic extract of *M. sapientum* (EMS) 50, 100 and 200 mg/kg, p.o. Groups 6 to 8: Received ethanolic extract of *M. paradisiaca* (EMP) 125, 250 and 500 mg/kg, p.o. Groups 9 to 11: Received ethanolic extract of *M. cavendish* (EMC) 250, 500 and 1000 mg/kg, p.o. Groups 12 to 14: Received ethanolic extract of *M. acuminata* (EMA) 100, 200 and 400 mg/kg, p.o.

2.5.1. Hypoglycemic study in non-diabetic rats[21]

The effect of various extracts of Musa species was studied in non-diabetic rats for the assessment of hypoglycemic effect if any. Animals were treated with ethanolic extract of different species of Musa and glimepiride was used as standard drug as per the experimental design. Blood samples were collected by puncturing retro-orbital plexus at the 0, 60, 120 and 180 min after drug administration.

2.5.2. Oral glucose tolerance test (OGTT) in non-diabetic rats[21,22]

Oral glucose tolerance test of different extracts of Musa species was conducted in non-diabetic rats. In this study, glucose solution (2 g/kg, p.o.) was administered 30 min after vehicle/drug administration. Blood samples were collected at the 0, 30, 90

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