Hypolipidaemic and antioxidant effects of fruits of *Musa* AAA (*Chenkadali*) in alloxan induced diabetic rats

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Hypolipidaemic and antioxidant effects of ethanol extract of mature green fruits of *Musa* AAA (*Chenkadali*) was evaluated in alloxan induced diabetic rats. The effect of extract at two doses, 500 mg/kg body weight and 1000 mg/kg body weight was analysed and compared with a standard drug, glibenclamide. Rats administered with alloxan showed significantly increased levels of serum triacylglycerol, total cholesterol and alanine amino transferase (ALT) activity. Lipid peroxides increased significantly while reduced glutathione (GSH) decreased considerably in liver and pancreas. Oral administration of the ethanol extract of fruits of *Musa* AAA (*Chenkadali*) significantly decreased the levels of serum triacylglycerol, cholesterol and ALT activity. Significant decrease was also observed in the level of lipid peroxides while GSH content increased substantially in liver and pancreas. The effect was dose independent and rats treated with 500 mg/kg body weight showed comparable levels of serum triacylglycerol, cholesterol, ALT activity and liver lipid peroxides to that of normal control and glibenclamide treated groups. Although, there was no significant difference, treatment with 500 mg/kg body weight of the extract showed a higher content of GSH and lower level of lipid peroxides in pancreas compared with glibenclamide. Histopathological examination of pancreas and liver revealed regeneration of islet cells and hepatocytes respectively, which correlate with the biochemical findings. The present study shows that ethanol extract of mature green fruits of *Musa* AAA (*Chenkadali*) has antioxidant and hypolipidaemic properties and may be used for treating diabetes mellitus.

**Keywords:** Antioxidant, Diabetes mellitus, Hypolipidaemic, *Musa* AAA (*Chenkadali*), Rats

Diabetes mellitus is a major endocrine disease, involving metabolic disorders of carbohydrate, fat and protein. It is the 4th leading cause of global mortality. Around 250 million people worldwide are living with the disease. By the year 2025 the disease incidence is expected to increase to over 380 million and of this, 80% would be in developing countries. It is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism. Insufficient insulin and increased oxidative stress along with hyperlipidaemia has been suggested in the pathogenesis and progression of diabetic complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy, retinopathy, micro and macro vascular damage and poor wound healing. Living organisms use a great variety of antioxidant compounds and produce antioxidant enzymes responsible for deactivating reactive intermediaries of oxygen. But antioxidant substances and enzymes are not wholly effective in preventing oxidative damage especially in conditions like diabetes mellitus, where free radicals are produced in excess. Oxidative stress occurs when there is an imbalance between free radical production and antioxidant defenses, resulting in deregulation of cellular functions.

In Indian system of medicine, plants have been a major source of drugs for the treatment of diabetes mellitus. World Health Organisation has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where there is lack of safe modern drugs. Since, plants contain substantial amount of antioxidants such as, carotenoids, ascorbic acid, flavonoids and other polyphenols, tannins, terpenoids and α-tocopherol, they are believed to exert their antidiabetic effect, at least in part, through their anti-oxidant property.

Plants belonging to *Musa* spp. are reported to have many medicinal effects. Different parts of banana plant such as, ripe and unripe fruits, stem, flowers and

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root were shown to have medicinal effects. Banana fruits have high content of minerals—iron, potassium, calcium, magnesium, phosphorus, sulphur and copper, vitamins - A, C, B₆, and B₁₂, tryptophan, 5-hydroxy tryptamine, flavonoids and phenolic compounds. Due to the presence of these constituents, banana fruit is being used to treat anaemia, gastric ulcers, high blood pressure, depression and oxidative stress induced neuro-degenerative diseases. Banana fruits are also used to treat constipation due to its high fibre content. Stem juice of *Musa paradisiaca* is found to be effective in treating urolithiasis. Inflorescence, stem, flowers and roots have been used traditionally for the treatment of diabetes mellitus.

*Chenkadali* is a widely cultivated and an easily available variety of plantain. Ripe fruits are used in a variety of ayurvedic preparations due to its medicinal properties. The present study was carried out to evaluate the hypolipidaemic and antioxidant effect of ethanol extract of mature green fruits of *Musa AAA* (*Chenkadali*) in alloxan induced diabetic rats.

**Materials and Methods**

*Drugs and chemicals*—Alloxan was procured from Sd Fine chemicals Ltd, Mumbai. Dionil® (glibenclamide - 5 mg) was procured from a local medical shop. Thiobarbituric acid (TBA), 1, 1, 3, 3 Tetra methoxy propane (TMP), disodium hydrogen phosphate, monosodium dihydrogen phosphate and 5, 5’ Dithio bis-2-nitrobenzoic acid (DTNB) were purchased from Himedia Laboratories Pvt Ltd, Mumbai. Sodium dodecyl sulphate (SDS) and Trichloroacetic acid (TCA) were procured from Sigma–Aldrich India, Bangalore and Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai, respectively. All other chemicals were procured from Merck India Ltd, Mumbai.

Fresh mature green fruits of *Musa AAA* (*Chenkadali*) were procured from Banana Research Station, Kerala Agricultural University, Kannara, Thrissur and were identified and authenticated by Dr. Rema Menon, Professor, Banana Research Station. Fruits were pooled, cut into small pieces, dried under shade, coarsely powdered in a pulverizer and used for the preparation of ethanol extract. The extract was made in a soxhlet apparatus and concentrated in a rotary vacuum evaporator at 50°C under reduced pressure. Phytochemical screening of the extract was carried out by colour reactions to detect the presence of possible active components such as, flavonoids (Ferric chloride test and Lead acetate test) and other phenolic compounds (Ferric chloride test), alkaloids (Mayer’s test, Hager’s test and Dragendroff’s test) glycosides (Benedict’s test and Sodium hydroxide test), di and triterpenoids (Copper acetate test and Salkowski test respectively), steroids (Salkowski test and Lieberman Burchard test), tannins (Ferric chloride test), saponins (Foam test). The extract was reconstituted to a final concentration of 5% (w/v) using 5% aqueous solution of gum acacia. Alloxan was dissolved in deionised double distilled water to make 3% aqueous solution.

*Animals*—Female Sprague Dawley rats weighing 150-200 g were housed in appropriate cages in a well ventilated experimental animal room under 12: 12 hr LD cycle at 22 to 28°C and 45 to 55% relative humidity with free access to standard rat pellet diet and drinking water. Experiments were conducted with the approval of Institutional Animal Ethics Committee. Animals were randomly divided into 6 groups, each comprising 6 animals. Rats in groups, G1 and G3 were normal groups and the rats in groups G2, G4, G5 and G6 were made diabetic by subcutaneous injection of alloxan at the dose of 130 mg/kg body weight. Serum glucose in alloxan administered animals was monitored on 3rd day and again on 7th day to confirm maintenance of high blood glucose level. All alloxan administered rats showed a serum glucose level above 200 mg/dl.

The rats of various groups were treated for 30 consecutive days.

Normal control (G1) - aqueous solution of gum acacia (5%)

Diabetic control (G2) - aqueous solution of gum acacia (5%)

Normal animals (G3) - Ethanol extract of fruits of *Chenkadali* at the dose of 500 mg/kg body weight

Diabetic animals (G4) - Ethanol extract of fruits of *Chenkadali* at the dose of 500 mg/kg body weight

Diabetic animals (G5) - Ethanol extract of fruits of *Chenkadali* at the dose of 1000 mg/kg body weight

Diabetic animals (G6) - Glibenclamide at the dose of 0.5 mg/kg body weight

Blood samples were collected from the retro orbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes on days, -7 (7 days before alloxan injection), 0 (8th day after alloxan administration), 15 (15 days after treatment) and 30 (30 days after treatment) and centrifuged at 1000 ×g for 10 min. at
15°C to separate serum. On day 31 (after 30 days of treatment), the rats were euthanized, samples from liver and pancreas were collected for the preparation of tissue homogenates and also fixed in 10% formalin for histopathological examination.

Biochemical analysis—Serum triacylglycerol, cholesterol and ALT activity were estimated using Ecoline Kits (M/s E. Merck India, Ltd, Mumbai). Level of lipid peroxides in tissue homogenates of liver and pancreas was determined by the method of Ohkawa et al. Tissue homogenates (10% w/v) were prepared in 1.15% KCl solution using a glass homogenizer, centrifuged at 2750 × g for 5 min. and the supernatant was used for the estimation of lipid peroxides.

Level of GSH in tissue homogenates (10% w/v in 0.2 M phosphate buffer, pH 8) was determined by measuring the absorbance of yellow coloured complex formed by the reaction between GSH and DTNB, at 412 nm.

Statistical analysis—Data obtained were compared by analysis of variance (ANOVA) followed by Duncan multiple range test to determine the level of significance. Period wise comparison of means was done by paired t-test. The value of \( P<0.05 \) was considered statistically significant.

Histopathological examination—Liver and pancreas sections were prepared by standard procedures and stained with haematoxylin and eosin (H & E). The sections were examined in detail under light microscope.

Results and Discussion

Administration of alloxan produced hyperlipidemia in rats with a significant increase in the levels of serum triacylglycerol and cholesterol. Elevated levels of serum lipids are usually seen in diabetes mellitus and represent a risk factor for coronary heart disease. The present findings correlate with the reports of many other workers on alloxan diabetic rats. One of the important actions of insulin is the inhibition of lipolysis by inhibiting hormone sensitive lipase. Alloxan administration destroys the islet cells of pancreas causing insufficient production of insulin. Insulin deficiency relieves the inhibition on hormone sensitive lipase, which along with other hormones, glucagon and catecholamines, promote lipolysis leading to abnormally high concentration of serum lipids. The marked hyperlipidemia in the diabetic state might, therefore, be regarded as a consequence of the uninhibited actions of lipolytic enzymes on the fat depots. Increased β-oxidation of fatty acids, produce more acetyl CoA, which augments cholesterol synthesis.

Treatment with both doses (500 and 1000 mg/kg body weight) of Musa fruit extract for 15 days significantly decreased the level of triacylglycerol, which was comparable to that of glibenclamide treated group and after 30 days treatment a value similar to that of normal control was observed (Table 1). Compared with diabetic control rats, treatment with both the doses of the extract for 30 days showed a significant reduction in serum cholesterol level while the level observed in animals treated with 500 mg/kg dose of the extract was significantly lower than that of day 0 and did not exhibit any significant variation from that of normal control rats. However, treatment with 1000 mg/kg of the extract could only prevent a significant rise in the level of cholesterol compared to that observed on day 0 (Table 2).

Hypolipidaemic potential of various plant extracts have already been reported. Phytochemical analysis of the ethanol extract of Musa fruits revealed the presence of flavonoids and other phenolic compounds, di and triterpenoids, steroids and glycosides. The lipid lowering effect of the extract might be due to the action of these compounds either independently or in combination. Normalized rate of lipogenesis due to the insulin-like activity of

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<td>G1</td>
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Level of significance was determined column wise between G1, G2, G3, G4, G5 and G6 and row wise between day 0 and day 30 of each group. Values not bearing a common superscript letter (a, b and c) in a column and the presence of * in a row differ significantly.
triterpenoids26 or achieving normoglycemia by the insulinotropic effect of flavonoids24 or the lipid lowering property of phenolic compounds21. Glycoside mediated increased activity of AMP-activated protein kinase in muscles and adipose tissue for increased glucose uptake and fatty acid oxidation27 might have contributed to the hypolipidaemic effect of the extract. Inhibition of endogenous synthesis of cholesterol or enhancement of the degradation by increased formation of bile acids and its excretion through intestinal tract26 may probably be the mechanisms behind the hypocholesterolemic effect.

Alloxan administration caused a significant increase in serum ALT activity (Table 3). Level of lipid peroxides increased significantly while GSH content decreased drastically in liver and pancreas (Table 4). These observations, in alloxan induced diabetes mellitus, indicate the generation of reactive oxygen species (ROS) and increased oxidative stress in these tissues. Several immunomodulatory factors, presence of free radicals and chronic inflammatory state might have contributed to insulin resistance and liver injury. Nuclear Factor-κB (NF-κB)-dependent inflammatory mediators produced in hepatocytes, such as TNF-α, might have acted in a paracrine manner and down regulated insulin sensitivity in the liver and favoured liver injury23.

Hyperglycaemia and the reduction products of alloxan28 may have played a role in increasing the free radicals. It has been suggested that increased blood glucose concentration, which upon auto-oxidation generate free radicals and attenuate antioxidant mechanisms, creating a state of oxidative stress and endothelial injury23. Moreover, glucose is known to induce lipid peroxidation through activation of lipoxygenase enzymes29. In the redox cycle of alloxan, presence of a thiol is required to enable reduction and to generate the redox cycling partner, dialuric acid. Typically under biological circumstances, the tripeptide thiol, GSH, might have fulfilled this role and contributed to the significant reduction in GSH content.

Diabetic rats treated with 500 mg/kg of the extract for 15 days significantly decreased serum ALT activity while such a significant reduction was not observed after 15 days treatment with 1000 mg/kg of the extract and glibenclamide. Administration of the
extract (500 mg/kg) for 30 days further decreased the serum ALT level, which was comparable to that of rats treated with glibenclamide for 30 days and did not differ significantly from that of normal control rats. Moreover, in both the groups (G4 and G6) the activity decreased significantly when compared with that of day 0. However, treatment for 30 days with 1000 mg/kg of the extract did not reveal any significant variation from the level observed on day 0 but could prevent a significant rise in the level of serum ALT compared to diabetic control rats, which was similar to the observations made in serum cholesterol level. Investigations on mice with the administration of Soy sterols at 5 and 10 mg/kg body weight revealed dual effect. The lower dose significantly increased the serum insulin level and reduced serum glucose, hepatic glucose-6-phosphatase activity and liver lipid peroxidation with concomitant increase in superoxide dismutase, catalase and GSH while the higher dose exerted an opposite effect by increasing serum glucose and hepatic lipid peroxidation. Another study carried out in European Polecat showed that administration of phytosterols decreased liver lipase esterase activity and serum cholesterol level remained unchanged. These observations correlate with the present findings on cholesterol level and ALT activity in G5 rats administered with the higher dose of the extract. Presence of double the concentration of phytosterol in the higher dose of the extract might have opposed the hypocholesterolemic and antioxidant activities of other active components. Furthermore, absence of increase in the levels of ALT and serum cholesterol from day 0 to day 30 substantiate the fact that effect of even double the concentration of phytosterols could only partially antagonize the combined effect of other active components in the extract or else the levels of would have increased significantly by day 30. Our observations suggest that various compounds such as flavonoids and other polyphenols, terpenoids, glycosides and phytosterols present in the extract in the lower dose might have exerted a synergistic effect and protected hepatic tissue from oxidative injury associated with diabetes mellitus, either by fighting hyperglycaemia or by scavenging free radicals.

Treatment with both doses of the extract significantly decreased lipid peroxidation and replenished the GSH content in tissues of liver and pancreas. In both the tissues, the level of lipid peroxides and GSH content did not differ significantly from that of glibenclamide treated and normal control rats. Although, there was no significant difference, treatment with 500 mg/kg dose of the extract showed a better effect. Studies with aqueous-methanol (40:60) extract of roots of Musa paradisiaca in diabetic rats have shown antihyperglycaemic properties by increasing serum insulin level and activities of hepatic enzymes such as, hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase utilizes glucose through pentose phosphate pathway and generates NADPH. The active components present in the extract might have acted in a similar manner and activated pentose phosphate pathway leading to the generation of NADPH necessary for the formation of GSH and free radical scavenging.

Hypoglycaemic activity of the extract could contribute to the lowering of free radical formation and lipid peroxides in tissues. Flavonoids and other polyphenols, terpenoids and phytosterols in the extract might have acted as antioxidants and scavenged the free radicals generated during alloxan-induced diabetes mellitus. In addition to free radical scavenging, flavonoids can exert their antioxidant activity by mechanisms such as, chelating metal ions or inhibiting enzymatic systems responsible for free radical generation. Phenolics act as direct aqueous phase free radical scavenger. Being good electron donors, phenolic compounds show the reducing power and prevent the formation of lipid peroxides. It has been suggested that dietary polyphenolics play an important role in protecting the body against diabetes mellitus. Phytosterols activate the enzymes responsible for free radical scavenging and reduce level of lipid peroxides.

Normal rats administered with the extract did not show any change in the levels of serum triacylglycerol, cholesterol and ALT activity compared to normal control rats (Tables 1, 2 and 3). Though, there was no significant difference in the level of lipid peroxides and content of GSH in liver and pancreas between normal rats treated with the extract and normal control rats, the content of GSH was higher in pancreas of treated normal rats (Table 4), which shows that the extract is having a strong antioxidant property. Reduced glutathione is an important antioxidant, functions as potent free radical scavenger within the β cells of islets, which protects β cells from destruction.
Histopathological examination of pancreas (Fig. 1a) and liver (Fig. 2a) of G1 rats showed normal histological architecture. Scattered necrosis of islet cells, shrunken necrosed cells with pyknotic nuclei, reduction in cell number, loss of compactness of the structure and cellular debris in the zone were observed in pancreas of G2 (Fig. 1b) while G4 and G5 showed intact, well preserved islet cells and some of the cells appeared regenerating with hyperchromatic nuclei and abundant cytoplasm (Fig. 1c and d). In G6, the islet cell zones appeared normal except for a few necrosed and hyalinised cells. Regenerating islet cells with hyperchromatic nuclei were seen around the necrosed zones (Fig. 1e). Multifocal diffuse haemorrhage, necrosis, focal coagulation of hepatocytes and central venous congestion (Fig. 2b) were observed in liver of diabetic control whereas, in both the extract treated groups, regeneration of hepatocytes were indicated by the presence of binucleated cells, cells with distended nuclei and condensed chromatin with moderately basophilic cytoplasm (Fig. 2c and d). In G6, most of the liver lobules remained normal and in certain lobes, the hepatocytes in the midzonal regions appeared normal except for a few necrosed and hyalinised cells.

**Fig. 1** — Section of pancreas (H & E × 200): (a) Normal control group; (b) Diabetic control group; (c) Treated with ethanol extract of *Musa* fruit (500 mg/kg); (d) Treated with ethanol extract of *Musa* fruit (1000 mg/kg); (e) Treated with glibenclamide (0.5 mg/kg) [I – Intact islet cells; N – Necrosed islet cells, H – Haemorrhage; RIC – Regenerating islet cells with hyperchromatic nucleus].
regenerating (Fig. 2e). The cells were prominent, moderately crowded and contained large nuclei with condensed chromatin and pale to basophilic cytoplasm. Rats of group, G3 showed a normal histological architecture of both pancreas and liver (Figures not shown). Observations on histopathology of both the tissues correlate well with the biochemical findings.

The present study suggests that ethanol extract of fruits of *Musa* AAA (*chenkadali*) is capable of exerting antioxidant and hypolipidaemic effects in animals that are hyperlipidaemic and under oxidative stress. However, the lower dose (500 mg/kg body weight) was found to have a better effect in normalizing all the parameters under study to a value similar to that of normal control rats and was comparable to the effect of the reference drug, glibenclamide. It shows that the lower dose contains appropriate concentrations of the active components which acted synergistically in decreasing the levels of

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**Fig. 2** — Section of liver (H & E): (a) Normal control group (H & E ×100); (b) Diabetic control group (H & E ×100); (c) Treated with ethanol extract of *Musa* fruit (500 mg/kg) (H & E × 200); (d) Treated with ethanol extract of *Musa* fruit (1000 mg/kg) (H & E × 200); (e) Treated with glibenclamide (0.5mg/kg) (H & E × 200) [N- Necrosed zone, H- Haemorrhage; RH - Apparently normal regenerated hepatocytes; MC- Mild congestion; RC - Regenerating hepatocytes with hyperchromatic nucleus, CVC- Central venous congestion]
serum lipids and tissue lipid peroxides. Therefore, increasing the concentration of active components in the fruit extract by increasing the dose may not always be beneficial to bring about the desired biological effect, if any of the components exhibit a reverse effect at high concentration. Further studies are required to isolate active principles from the extract to specify the extent of activity of each one to assess its hypolipidaemic and antioxidant effect.

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