HYPOGLYCAEMIC EFFECT OF FRUITS OF *Musa* AAA (Chenkadali) ON DIABETIC RATS

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DECLARATION

I hereby declare that this thesis, entitled **"HYPOGLYCAEMIC EFFECT OF FRUITS OF *Musa* AAA (Chenkadali) ON DIABETIC RATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"HYPOGLYCAEMIC EFFECT OF FRUITS OF *Musa* AAA (Chenkadali) ON DIABETIC RATS"** is a record of research work done independently by **Smitha Kaimal,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Plates

Introduction

1. INTRODUCTION

Diabetes mellitus is the most common disorder of carbohydrate metabolism, which is characterized by high blood glucose level. It is the fourth leading cause of global death. Today, around 250 million people worldwide are living with the disease. By the year 2025 this total is expected to increase to over 380 million and of this, 80 % would be in developing countries (Roglic *et al.*, 2005). The global prevalence of diabetes mellitus is set to double over the next 25 years. The disease is increasing worldwide at an alarming rate due to population growth, obesity, sedentary lifestyle and ageing.

Two types of diabetes mellitus are prevalent, Type 1 and Type 2. Type 1 is autoimmune in nature, where antibodies are produced against insulin or β-cells of pancreas and such patients require insulin supplementation for survival. Type 2 diabetes mellitus is caused by insulin resistance, β-cell dysfunction or selective glucose unresponsiveness. Several modifiable risk factors play a role in the onset of Type 2 diabetes mellitus, which include obesity, physical inactivity, poor nutrition, genetic predisposition and ageing. A male predominance is found, with a male-to-female ratio of 1.8:1 for Type 1 diabetes mellitus and 1.3:1 for Type 2 diabetes mellitus.

Diabetes mellitus can lead to acute complications such as, ketoacidosis or non-ketotic hyperosmolar coma, if the disease is not adequately controlled. It can also lead to serious long-term complications such as, cardiovascular disease, chronic renal failure, retinal damage, nerve damage, micro and macro vascular damage and poor wound healing. Poor healing of wounds, particularly of the feet, can lead to gangrene, and possibly to amputation. In the developed world, diabetes mellitus is the most significant cause of adult blindness in the non-elderly and the leading cause of non-traumatic amputation in adults. Increased oxidative stress is seen in both Type 1 and Type 2 diabetes mellitus, which causes the destruction of insulin-producing cells of the pancreas and worsen the condition.

As in humans, diabetes mellitus also prevails in pets and large animals. Some breeds of dogs such as, the Standard Poodle, Golden Retriever and Miniature Schnauzers

are genetically more susceptible to the disease. Both Type 1 and 2 diabetes mellitus have an almost equal distribution in dogs. Most probable reasons for Type 1 diabetes mellitus in dogs are immune destruction of β-cells and extensive pancreatic damage, due to chronic pancreatitis or environmental factors such as high-fat diets. Type 2 diabetes mellitus is the most common form in cats. A transient form of the disease occasionally occurs in cats, in which sufficient insulin is produced, but the body does not respond properly (Rand, 2009). Diabetes mellitus has been reported in cattle, pigs, sheep, horses, elephant and bison. There are several possible concurrent predisposing diseases or risk factors for diabetes mellitus in large animals such as, fatty liver, fat cow syndrome and parturition, as well as viral diseases, especially bovine viral diarrhoea (Clark, 2003).

Treatment for diabetes mellitus includes administration of insulin and oral antihyperglycaemic drugs. Oral antihyperglycaemic drugs cause side effects such as weight gain, fluid retention, peripheral oedema, central nervous system disorders, skin reactions, aplastic/hemolytic anaemia, flatulence, bloating and nausea. Insulin and other medications are expensive, especially for poor patients. According to a recent study conducted in India, patients with diabetes mellitus pay about 4-7% of household income on medications alone.

Considering the adverse effects and cost factor of antihyperglycaemic drugs, herbal therapy is being tried to treat diabetes mellitus. In the indigenous system of medicine many plants have been claimed to be useful in the treatment of diabetes mellitus. There are about 45,000 plant species in India and several thousands have been used as medicine because of the purported medicinal properties. Though, the precise mechanism of action of many hypoglycaemic plants is not known, it is believed that these plants contain certain hypoglycaemic principles, which inhibit some of the enzymes in the production pathway of glucose, stimulate insulin production and release or make the receptors of target cells more sensitive to insulin. In addition to this, antidiabetic effect of many plants could be attributed to the presence of trace elements such as vanadium, zinc, chromium, copper, iron, potassium, sodium, and nickel, which might activate the β-cells of pancreas and play an important role in the maintenance of normoglycaemia.

Banana plant (*Musa* sp.) is reported to have many medicinal effects. Different parts of banana plant such as ripe and unripe fruits, stem, flowers and 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, B_6, and B_{12}, amino acid, tryptophan and phenolic compounds. It also has high fibre content. Due to the presence of these compounds, banana fruit is being used to treat anaemia, gastric ulcers, high blood pressure, constipation, oxidative stress induced neuro-degenerative diseases, depression and urolithiasis. Inflorescence, stem, flowers and roots have been used traditionally for the treatment of diabetes mellitus.

Though, different parts of *Musa* sp. have been shown to have antidiabetic effect, reports on the hypoglycaemic and hypolipidaemic effects of its fruits are scanty. The present study is aimed to explore the hypoglycaemic and hypolipidaemic effect of *Musa* AAA (Chenkadali), which is a widely cultivated species of banana plant. The study has been designed with the following objectives:

1. To evaluate the hypoglycaemic and hypolipidaemic effect of ethanol extract of mature unripe fruits of *Musa* AAA (Chenkadali)
2. To compare the hypoglycaemic effect of the fruit extract with a known antidiabetic drug

Review of Literature

2. REVIEW OF LITERATURE

2.1 Antidiabetic effect of various plants

2.1.1 Antidiabetic effect of whole plant

Latha and Pari (2004) reported that aqueous extract of whole plant of Sweet broom (*Scoparia dulcis)* at a dose of 200 mg/kg significantly reduced blood glucose, sorbitol dehydrogenase, glycosylated haemoglobin, Thiobarbituric acid reactive substances (TBARS) and hydro-peroxides in streptozotocin diabetic rats. It was also observed that the extract significantly increased the level of plasma insulin, activities of hepatic glutathione peroxidase, glutathione-S-transferase and level of reduced glutathione (GSH). Effect showed by the extract was comparable to that of glibenclamide.

Methanol, n-hexane and dichloromethane extracts (50, 100, 250 and 500 mg/kg of each extract separately) of whole plant of Western horse tail (*Equisetum arvense)* were evaluated for their hypoglycaemic activity in streptozotocin diabetic rats. The methanol (50, 250 and 500 mg/kg), dichloromethane (50 and 250 mg/kg) and n-hexane (50 mg/kg) extracts produced significant reduction in serum glucose level (Safiyeh *et al.*, 2007).

2.1.2 Antidiabetic effect of leaves

Ignacimuthu and Amalraj (1998) reported that rats allowed to drink aqueous extract of leaves of Common jujube (*Zizyphus jujuba) ad libitum* for 30 days exhibited significant antidiabetic effect by reducing the elevated blood glucose, total

cholesterol and triglycerides in alloxan diabetic rats. Antidiabetic effect was attributed to the presence of the alkaloid berberine and it was comparable to that of a standard hypoglycaemic drug, glibenclamide (500 µg /kg).

Ananthi *et al.* (2003) evaluated the hypoglycaemic effect of finely powdered leaves of False daisy (*Eclipta alba),* suspended in 2 per cent gum acacia, in alloxan diabetic rats. Oral administration of leaf suspension (2 g/kg and 4 g/kg) for a period of 60 days resulted in significant reduction in blood glucose, HbA1c, activities of serum glucose-6 phosphatase and fructose 1, 6-bisphosphatase. An increase was observed in the level of liver hexokinase activity. Between the two doses, 2 g/kg was found to be more effective.

Administration of methanol extract of leaves of Apple wood (*Aegle marmelos,* 100 mg/kg) for a period of 12 days significantly reduced blood glucose level in alloxan diabetic rats. Significant increase was observed in the activities of serum glutathione reductase, glutathione peroxidase and liver superoxide dismutase (SOD) and catalase. Lipid peroxidation, levels of hydroperoxides and conjugated dienes significantly decreased in the treated groups when compared with diabetic control rats (Sabu and Kuttan, 2004).

Oral feeding of hot water extract of leaves of Custard apple (*Annona squamosa,* 350 mg/kg) for a period of 15 days in alloxan diabetic rabbits resulted in 75.5 per cent reduction in blood glucose level. In the treated animals, total serum cholesterol, LDL cholesterol, triglycerides and glycated haemoglobin levels were reduced significantly where as, HDL cholesterol, haemoglobin and serum insulin levels increased (Gupta *et al.*, 2005).

Oral administration of aqueous extract of leaves of Custard apple (*A. squamosa,* 300 mg/kg) to streptozotocin diabetic rats for a period of 30 days significantly reduced the levels of blood glucose, lipids and lipid peroxidation, but increased the levels of plasma insulin, GSH and the antioxidant enzymes viz. catalase, SOD and glutathione peroxidase (Kaleem *et al.*, 2006).

Tedong *et al.* (2006) reported that hexane extract of leaves of Cashew (*Anacardium occidentale,* 300 mg/kg) showed significant reduction in blood glucose level and glycosuria in streptozotocin diabetic rats. They also observed a significant reduction in the accumulation of mucopolysaccharides in the kidneys of diabetic animals.

Akhthar *et al.* (2007) investigated the antihyperglycaemic and hypolipidaemic effects of ethanol extracts of the leaves of Ivy gourd (*Coccinia cordifolia)* and Madagascar periwinkle (*Catharanthus roseus)* in alloxan diabetic rats. They observed that administration of extracts of *C. cordifolia* (300 mg/kg) and *C. roseus* (150 mg/kg) independently as a single dose, significantly reduced blood glucose, serum total cholesterol and serum triglycerides. Their study also revealed that *C. cordifolia* exhibited a better effect in lowering blood glucose and serum triglycerides than *C. roseus*, but *C. roseus* showed a better cholesterol lowering effect.

Mostofa *et al.* (2007) screened the antihyperglycaemic effects of aqueous extracts of leaves of Madagascar periwinkle (*C. roseus,* 1g/kg), Neem (*Azadirachta indica,* 500 mg/kg) and seeds of Garlic (*Allium sativum,* 1 g/kg) and compared with a standard antidiabetic drug, glimepride, (100 mg/kg) in streptozotocin diabetic rats. Oral administration for a period of 14 days revealed that glimepride exhibited highest body weight gain and maximum blood glucose lowering effect followed by *A. indica, C. roseus* and *A. sativum.*

Aqueous extract of leaves of Coral bead (*Cocculus hirsutus)* produced significant reduction in serum glucose level at doses of 250, 500 and 1000 mg/kg in alloxan diabetic mice and maximum hypoglycaemic activity was showed by 1000 mg/kg (Badole *et al.*, 2008).

Ebong *et al.* (2008) reported that ethanol extracts of leaves of Neem (*A. indica,* 400 mg/ kg), leaves of Bitter leaf (*Vernonia amygdalina,* 400 mg/ kg) and the reference drug, chlorpropamide (14.286 mg/kg) when administered separately for 24 days produced 44.95 per cent, 88.63 per cent and 75.83 per cent reduction in blood glucose level in alloxan diabetic rats. They also noticed that *A. indica* could not reverse the hyperglycaemic state to normoglycaemic status where as, *V. amygdalina* showed a tendency towards hypoglycaemia, but a combination of the two extracts (200 mg/kg each) complimented each other thereby, produced the desired normoglycaemia.

Edwin *et al.* (2008) investigated the antidiabetic effect of ethanol extract of leaves of Banana passion fruit (*Passiflora molissima*) at 100 mg/kg and 200 mg/kg in alloxan diabetic rats for a period of five days. The extract significantly lowered the blood glucose level at both the doses.

Oral administration of ethanol extract of leaves of Neptune Grass (*Posidonia oceanica*) for 15 days at 50, 150 and 250 mg/kg doses resulted in a dose-dependent decrease in blood glucose level by 20.9 per cent, 48.5 per cent and 60.1 per cent respectively in alloxan diabetic rats. At 150 and 250 mg/kg doses, the extract significantly reduced the ALP and ALT activities and significantly enhanced the liver glycogen content (Gokce *et al.*, 2008).

Kumar *et al.* (2008) studied the hypoglycaemic potential of the petroleum ether extract and ethanol extract of leaves of Potato-bush (*Phyllanthus reticulatus*) in alloxan diabetic mice. They found that, separate administration of the two extracts, at 500 mg/kg, for a period of 21 days exhibited weak hypoglycaemic activity, while at 1000 mg/kg showed a sustained decrease in blood glucose level. Phytochemical screening of the extracts revealed the presence of terpenoids, glycosides and absence of alkaloids and steroids.

Noor *et al.* (2008) observed that streptozotocin diabetic rats when administered with ethanol extract of leaves of True aloe (*Aloe vera,* 300 mg/kg) for a period of 21 days showed normal blood glucose levels compared with the diabetic control rats. Histopathological examination of pancreas of diabetic control rats revealed damaged and shrunken islet cells infiltrated with lymphocytes where as, the islet cells appeared normal in the treated rats.

Veeramani *et al.* (2008) investigated the antihyperglycaemic effect of ethanol extract of leaves of Balloon vine *(Cardiospermum halicacabum)* on streptozotocin diabetic rats. Oral administration of the extract for 45 days showed an improvement in body weight, increased the level of haemoglobin, plasma insulin and decreased blood glucose level. They also observed an increase in the activity of glucokinase and decrease in the activities of glucose-6-phosphatase and fructose 1, 6-bisphosphatase in the liver.

2.1.3 Antidiabetic effect of fruits

The effect of aqueous decoction of Juniper berries (*Juniperus communis,* 250 mg/kg) was studied in streptozotocin diabetic rats. Significant reduction in blood

glucose level and gain in body weight were achieved by the oral administration of the extract (Medina *et al.*, 1994).

The lipid lowering and antioxidant potential of ethanol extract of fruits of Black false pepper *(Embelia ribes burm)* were investigated in streptozotocin diabetic rats. Feeding the extract (200 mg/kg) to diabetic rats for 20 days resulted in significant decrease in blood glucose, serum total cholesterol, triglycerides and an increase in HDL cholesterol. Further, the extract lowered the levels of liver and pancreas TBARS. The results obtained were comparable to gliclazide, a standard antihyperglycaemic agent (Bhandari *et al.*, 2002).

Fernandes *et al.* (2007) reported that Bitter Melon *(Momordica charantia)* fruit extract showed significant antihyperglycaemic activity by lowering blood glucose and glycosylated haemoglobin levels in alloxan diabetic rats. They also observed a significant reduction in the serum cholesterol and triglyceride levels. The effect of *M. charantia* extract at a dose of 300 mg/kg was more significant than that of 150 mg/kg and was comparable with that of glibenclamide at 4 mg/kg. The extract at 150 mg/kg produced 72.6 per cent increase in liver glycogen level, while 300 mg/kg resulted in 100 per cent increase as compared with the diabetic control.

2.1.4 Antidiabetic effect of seeds

The effect of Neem *(A. indica)* kernel powder (500 mg/kg) and a combination of neem kernel powder (250 mg/kg) and glibenclamide (0.25 mg/kg) were studied in alloxan diabetic rats. Significant decrease was observed in the levels of serum lipids, blood glucose and activities of serum enzymes such as ALP, ACP and LDH. Decreased activities were also noted in the levels of glucose 6-phosphatase and

hydroxy methyl glutaryl CoA reductase in liver and intestine, while the activity of liver hexokinase was increased (Bopanna *et al.*, 1997).

Raju *et al.* (2001) studied the effects of finely powdered Fenugreek (*Trigonella foenum graecum*) seeds, incorporated at 5 per cent level in rat diet, on glucose homeostasis in alloxan diabetic rats. A significant increase was observed in the activities of glycolytic enzymes, phosphofructokinase, pyruvate kinase, and lactate dehydrogenase when compared with diabetic control group and the activities were comparable to that of the normal control.

Sridhar *et al.* (2005) examined the antidiabetic potential of oral administration of Black plum (*Eugenia jambolana)* seed powder (500 mg/kg and 1000 mg/kg) suspended in 2 per cent gum acacia for a period of 21 days in streptozotocin diabetic rats. They observed a better antidiabetic effect with the lower dose as evidenced by increased mean body weight, reduced glucose levels and elevated liver glycogen values.

Chloroform extract of Myrobalan (*Terminalia chebula)* seed powder (300 mg/kg) produced significant reduction in blood glucose level when administered for eight weeks in streptozotocin diabetic rats. The effect was comparable to treatment with glibenclamide (Rao and Nammi, 2006).

Treatment of alloxan diabetic rats with barley, chromium and amino acids (L-leucine and L-glutamine) independently and a combination of all the three, reduced plasma glucose level by 69, 63, 64.5 and 86 %, respectively. Treated groups also showed a significant reduction in the levels of plasma total lipids, triglycerides, cholesterol, LDL and VLDL (Yousef *et al.*, 2006).

Streptozotocin diabetic rats when treated with alkaloid extract of dried seeds of Fenugreek (*Trigonella foenum-graecum,* 50 mg/kg) resulted in significant reduction in blood glucose, serum lipids, lipid peroxide and increase in serum insulin level. Histopathological examination of liver and pancreas of diabetic group revealed periportal necrosis and hyalinization of the tissues respectively; whereas those of treated group showed an apparently normal histological architecture (El-Soud *et al.*, 2007).

Pavana *et al.* (2007) assessed the antihyperglycaemic and antilipid peroxidative effects of ethanol extract of seeds of Wild Indigo *(Tephrosea purpurea,* 300 mg/kg) in streptozotocin diabetic rats. Hyperglycaemia, altered hexokinase and glucose-6-phosphatase activities, elevated lipid peroxidation, disturbed enzymatic and non-enzymatic antioxidants status observed in the diabetic group were reversed in the treated group by oral administration of the extract, which was similar to that of the standard drug, glibenclamide, treated group.

2.1.5 Antidiabetic effect of other aerial parts

Pari and Latha (2002) studied the effect of aqueous extract of Tanner's Cassia (*Cassia auriculata)* flowers in streptozotocin diabetic rats. They reported that administration of the extract (0.45 g/kg) brought the blood glucose, HbA1c, plasma insulin and total haemoglobin levels towards the level observed in the normal control. It was also noted that the activity of hexokinase in liver tissue increased, while that of glucose-6-phosphatase decreased. Similar observations were made in the animals administered with the standard antidiabetic drug, glibenclamide (600 μg/kg).

Raghavan and Krishnakumari (2006) reported that oral administration of ethanol extract of Arjuna herb (*Terminalia arjuna)* stem bark (250 mg/kg and 500 mg/kg) for 30 days significantly reduced the level of blood glucose in alloxan diabetic rats. It was also observed that the activities of hexokinase and phosphoglucoisomerase were significantly increased and the activity of aldolase was significantly decreased. The enzyme activities showed by the treated groups were similar to that of normal control group. According to their study, the effect was dose dependent.

Ene *et al.* (2007) studied the effect of different doses of Black caraway (*Nigella sativa)* oil on the body weight of alloxan diabetic rats. They reported that glucose threshold level, which was exceeded in the diabetic rats, was brought to normal with 10 mg/kg of Black caraway oil. The diabetic rats treated with 5, 20, 40 and 80 mg/kg Black caraway oil showed inconsistencies in their per cent mean weekly body weights, while those treated with 10 mg/kg showed progressive and steady increase in their per cent mean weekly body weights.

Mahesh and Brahatheeswaran (2007) studied the antihyperglycaemic and antihyperlipidaemic effects of aqueous and ethanol extracts of aerial parts of Bermuda grass (*Cyanodon dactylon*) in streptozotocin diabetic rats. They observed that administration of the two extracts separately at 400 mg/kg showed a significant increase in liver glycogen and HDL cholesterol while significant decrease was observed in fasting blood glucose, glycosylated haemoglobin, serum total cholesterol and triglyceride levels.

Mai *et al.* (2007) screened the antihyperglycaemic effect of an aqueous extract of flower buds of Cleistocalyx *(Cleistocalyx operculatus)* in streptozotocin diabetic rats. They observed a marked reduction in blood glucose level, when the extract was

administered at 500 mg/kg. It was also observed that the body weight of the treated rats remained stable while that of diabetic control decreased.

Ghosh *et al.* (2008) assessed the hypoglycaemic activity of ethanol extract of aerial parts of Thyme leaved gratiola (*Bacopa monnieri)* in alloxan diabetic rats. Administration of the extract (300 mg/kg) for a period of ten days caused significant reduction in blood glucose level and increased body weight. Liver tissue showed significant decrease in the level of TBARS, increased GSH content and activities of SOD and catalase.

Selvan *et al.* (2008) reported that administration of methanol extract of aerial parts of Artanema (*Artanema sesamoides*) at doses of 200 and 400 mg/kg for a period of 14 days produced significant reduction in the levels of blood glucose, total cholesterol, LDL cholesterol, triglycerides, ALT and increase in liver glycogen and serum HDL cholesterol of streptozotocin diabetic rats. The treated groups also showed an increase in the level of GSH and activities of SOD and catalase in liver and kidney tissues.

2.1.6 Antidiabetic effect of roots

Oral administration of dichloromethane extract (300 mg/kg) of the root of Wereki (*Ibervillea sonorae)* for a period of 41 days reduced blood glucose, triglycerides and ALT levels in alloxan diabetic rats (Aguilar *et al.*, 2005).

Rao (2006) reported that administration of chloroform extract of Indian Echinacea (*Andrographis paniculata)* roots (150 mg/kg) for a period of four weeks produced significant reduction in the level of blood glucose in alloxan diabetic rats

and the effect was similar to that observed in rats treated with the standard antidiabetic drug, glibenclamide.

The antihyperglycaemic efficacy of ethanol extract of Black musale *(Curculigo orchioides Gaertn)* rhizome was evaluated in normal, glucose loaded and alloxan diabetic rats. The extract at a dose of 100 mg/kg exhibited significant hypoglycaemic activity in all the three animal models when compared with the diabetic control group. The hypoglycaemic and antihyperglycaemic effect produced by the extract might be due to the increased uptake of glucose at tissue level, increase in pancreatic ß cell function or due to inhibition of intestinal absorption of glucose (Chauhan and Dixit, 2007).

Okokon *et al.* (2007) reported the antidiabetic activity of ethanol extract of roots of Elephant's knee (*Homalium letestui,* 500, 750 and 1000 mg/kg) in streptozotocin diabetic rats. Administration of the extract at all doses for a period of 14 days significantly decreased the serum glucose level and the effect of the extract at 1000 mg/kg was similar to that of glibenclamide (10 mg/kg) treated group.

Thomson *et al.* (2007) investigated the hypoglycaemic and hypolipidemic effects of aqueous extract of bulbs of garlic (*Allium sativum*) at 500 mg/kg intra-peritoneally for a period of seven weeks in streptozotocin diabetic rats. Compared to diabetic control group, garlic-treated group showed 57 per cent less serum glucose, 40 per cent less serum cholesterol and 35 per cent less serum triglycerides.

2.2 Composition and medicinal properties of Musa sp.

2.2.1 Composition of the fruit

Vanderslice and Higgs (1991) evaluated the vitamin C content of banana fruit using chromatographic procedures. According to their studies, ascorbic acid and dehydro ascorbic acid content of banana fruit was 4.5 ± 1.0 and 7.7 ± 2.6 mg/ 100 g respectively.

Someya *et al.* (2002) isolated the anti-oxidant gallocatechin from banana (*Musa* AAA, Cavendish) peel and fruit using High Performance Liquid Chromatography. They reported that gallocatechin was more abundant in peel (158 mg/100 g dry weight) than in pulp (29.6 mg/ 100 g dry weight).

Englberger *et al.* (2003) reported that the different cultivars of ripe Micronesian banana had a β-carotene content ranging from 40-4960 µg/100 g as analyzed in different laboratories.

Wall (2006) evaluated the vitamin C, provitamin A and mineral composition of ripe fruits of banana (*Musa* sp.) cultivars grown in Hawaii. The average vitamin C content for Dwarf Brazilian banana (Santa Catarina Prata, *Musa* sp., AAB) and Williams fruit (Cavendish subgroup, *Musa* sp., AAA) were 12.7 mg and 4.5 mg/100 g respectively. Dwarf Brazilian bananas had 96.9 mg β-carotene, 104.9 mg α-carotene and 154.9 µg lutein/100 g whereas, Williams fruit averaged 55.7 mg β -carotene, 84.0 mg α –carotene and 108.3 µg lutein/100 g. Potassium content of Dwarf Brazilian banana and Williams fruit ranged from 288.5 to 485 mg and 287.1 to 355.2 mg/100 g fresh weight respectively.

The chemical composition of the lipophilic extracts of unripe pulp and peel of banana fruit 'Dwarf Cavendish' (*Musa acuminata* var. *cavendish*) was studied by gas chromatography-mass spectrometry. Fatty acids, sterols, and steryl esters are the major families of lipophilic components present in banana. Fatty acids are more abundant in the banana pulp (29-90 per cent of the total amount of lipophilic extract), with linoleic, linolenic, and oleic acids as the major compounds of this family. In banana peel, sterols represent about 49-71 per cent of the lipophilic extract with two triterpenic ketones (31-norcyclolaudenone and cycloeucalenone) as the major components (Oliveira *et al.*, 2008).

Englyst *et al.* (1992) analysed the starch content of raw banana and reported that it contains 82 per cent starch of which 50-66 per cent is amylase resistant starch as evidenced from *in vitro* experiments. It was also noted that the amount of banana starch not hydrolyzed and absorbed from human small intestine depends on the state of ripeness.

Feldman and Lee (2009), employed radio-enzymatic analysis and thin layer chromatography for the estimation of serotonin content and estimated that ripe fruits of plantain and banana contain 30.3 ± 7.5 and 15.0 ± 2.4 µg serotonin per gram of fresh fruit respectively. Higher content of serotonin was observed in the fruit peel and seed containing areas of both plantain and banana.

2.2.2 Gastro protective effect

Lewis *et al.* (1999) reported that dried unripe banana (*Musa sapientum*) powder, a natural flavonoid leucocyanidin extracted by solvent extraction from unripe banana and purified synthetic leucocyanidin showed a significant protective effect on gastric mucosa of rats against aspirin-induced erosions.

Goel *et al.* (2001) studied the role of gastric anti-oxidant and anti-ulcerogenic activity of methanol extract of plantain banana (*Musa sapientum var. paradisiaca).* Administration of the extract (50 mg/kg), twice daily for 5 days showed significant anti-ulcer effect and antioxidant activity in gastric mucosal homogenates, where it decreased the ulcer index, lipid peroxidation and increased SOD activity.

Pannangpetch *et al.* (2001) reported that separate oral administration of ethanol extracts of Palo and Hom varieties of bananas at 0.5 and 1 g/kg respectively, significantly reduced the development of indomethacin-induced gastric ulcers. Oral administration of Hom banana extract at doses of 0.5 and 1 g/kg showed a tendency to accelerate healing of acetic acid induced chronic ulcers whereas, Palo banana extracts at the same doses did not show any appreciable effect.

Methanol extract of mature green fruits of *Musa sapientum var. paradisiaca* (100 mg/kg) showed better ulcer protective effect when administered for 6 days in streptozotocin (70 mg/kg) induced non-insulin dependent diabetes mellitus rats subjected to cold-restraint stress induced gastric ulcers. The effect was compared with standard ulcer protective drug, sucralfate (500 mg/kg) and standard anti-diabetic drug, glibenclamide (0.6 mg/kg). The standard drugs and the extract showed an increase in mucosal glycoprotein level, but cell proliferation was enhanced in case of extract alone. The ulcer protective effect of *Musa* extract could be due to its effect on mucosal defensive factors like increase in mucin secretion, life span of mucosal cells, synthesis of mucosal glycoprotein, cell proliferation, antioxidant. systems and decrease in the generation of free radicals (Kumar *et al.*, 2006).

2.2.3 Antilithiatic property

Kailash and Varalakshmi (1992) studied the influence of stem extract of banana on glycolic acid oxidase and lactate dehydrogenase enzymes, calcium, phosphorus, oxalate and glycolic acid in liver tissues of sodium glycolate-induced hyperoxaluric rats. They reported that activities of glycolic acid oxidase and LDH significantly lowered in the extract-treated rats compared to that of the glycolate-fed rats.

Prasad *et al.* (1993) tested the fresh juice of *Musa* (Paradisiaca Linn. Cultivar, Puttubale) stem for its antilithiatic activity. Zinc discs were implanted in the urinary bladder of albino rats to induce urolithiasis. The stones formed were mainly of magnesium ammonium phosphate with traces of calcium oxalate. *Musa* stem juice (3 ml/rat/day orally) was found to be effective in reducing the formation of stones and also in dissolving the pre-formed stones.

Aqueous banana stem extract significantly reduced urinary oxalate, glycolic and glyoxylic acid and phosphorus excretion in the hyperoxaluric rats. The extract appeared to have no effect on urinary calcium excretion (Poonguzhali and Chegu, 1994).

2.2.4 Antimicrobial and antioxidant activity

Pari and Umamaheshwari (2000) studied the effect of *Musa sapientum* flowers on lipid peroxidation in alloxan diabetic rats. They reported that oral administration of the chloroform extract of the flowers at 0.15, 0.20 and 0.25 g/kg doses for 30 days resulted in a significant reduction in TBARS and an increase in

GSH, glutathione peroxidase, SOD and catalase in liver and kidney tissues clearly showing the antioxidant property of the extract.

Krishnan and Vijayalakshmi (2005) assessed the level of alterations in lipids and lipid peroxidation in rats fed with flavonoid rich fraction of banana (*Musa paradisiaca*) fruit from high background radiation area. Flavonoid rich fraction of *Palayamkodan* and *Rasakadali* varieties grown in Veli (control area) showed significant hypolipidaemic and antioxidant activities while lowered level of activities were observed in rats administered with flavonoid rich fraction of banana grown in high background radiation area, particularly in Karunagappally area.

Antimicrobial and antioxidant activities of chloroform, ethyl acetate and aqueous extracts of green peel of banana (*Musa* AAA, Cavendish) fruits were evaluated *in vitro*. The ethyl acetate and aqueous extracts displayed high antimicrobial and antioxidant activity, respectively. Malic acid and 12-hydroxystrearic acid isolated from ethyl acetate fraction were the most active components against all Gram-negative and positive bacterial species tested (Mokbel and Hashinaga, 2005).

Perez-Perez *et al.* (2006) studied the *in vitro* antioxidant effect of immature green banana fruits *viz.*, interspecies crossed varieties of *Musa acuminate* and *Musa balbisiana*, named Harton plane (*Musa* AAB, subgroup plane), Cavendish banana, (*Musa* AAA, subgroup banana), and Manzano banana (*Musa* AAB, subgroup plane). Addition of phosphate buffered saline (PBS) extracts of fruits from all these varieties in rat brain homogenate decreased the concentration of lipid hydroperoxides and malondialdehyde in a manner comparable to melatonin and vitamin E.

Matsuda *et al.* (2006) reported that feeding mature green banana fruit pulp

(2 g/kg) mixed with skimmed milk powder to calves for 5 days significantly increased the number of T- lymphocytes, $CD3^+$, $CD3^+CD45R^-$ and $CD3^+TcR^+$ cells.

Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of aqueous extracts of fruits of banana were assessed. FRAP, TRAP and TEAC values for banana were 2.28 mM Fe^{2+} /kg fresh weight[3], 1.05 and 0.64 mM Trolox/kg fresh weight respectively (Pellegrini *et al.*, 2009).

2.2.5 Hypoglycaemic and hypolipidaemic activity

Gomathy *et al.* (1989) reported that fresh juice from stem of *Musa sapientum* (20 mg/100 g) when administered for a period of 45 days produced significant decrease in the levels of cholesterol and triglycerides in serum and liver of rats fed with both cholesterol free diet and high fat cholesterol diet. There was decreased cholesterogenesis in the liver as evident from decreased activity of hydroxymethylglutaryl coenzyme A reductase. There was significant increase in hepatic bile acid content, fecal excretion of neutral sterols and bile acids.

Usha *et al.* (1989) evaluated the effect of feeding isolated dietary fiber from banana (*Musa paradisiaca*) on the metabolism of carbohydrates in liver. They found that fiber fed rats showed significantly lower levels of fasting blood glucose and higher concentration of liver glycogen. Moreover, the activities of glucose-1-phosphate uridyl transferase, glycogen synthase, Glucose-6-phosphatase, Glucose-6-phosphate dehydrogenase were significantly higher while phosphoglucomutase, hexokinase and pyruvate kinase showed lower activity. Fructose 1-6 bisphosphatase activity was not affected.

Juice of inflorescence stalk of plantain (*Musa sapientum,* 20 mg/100 g) showed significant hypoglycaemic effect in alloxan diabetic rats. There was increase in the concentration of hepatic glycogen and increased glycogenesis as evident from the increased activity of glycogen synthetase. Glycogenolysis and gluconeogenesis were lower as evident from the decreased activity of glycogen phosphorylase and gluconeogenic enzymes (Gomathy *et al.*, 1990).

Horigome *et al.* (1992) assessed the cholesterol-lowering effect of pulp of banana (*Musa* sapientum *L. var. Cavendishii*) fruit in rats fed on a cholesterol-containing diet. Freeze-dried banana pulp showed a marked cholesterol-lowering effect when incorporated into the diet both at the levels of 300 and 500 g/kg feed, while the banana pulp dried in a hot-air current did not show any effect. They observed that both soluble and insoluble fibers fractionated from banana pulp had a cholesterol-lowering effect, with the exception of cellulose and suggested that the browning reaction undergone during hot-air drying might be related to the disappearance of the hypo-cholesterolaemic effect of banana pulp.

Pari and Umamaheshwari (2000) evaluated the hypoglycaemic effect of chloroform extract of the *Musa sapientum* flowers in alloxan diabetic rats. Oral administration of the extract (0.15, 0.20 and 0.25 g/kg) for 30 days resulted in a significant reduction in blood glucose, HbA1c and an increase in total haemoglobin. It was also noted that the extract prevented a decrease in body weight. The effect was highly significant in rats treated with the extract at 0.25 g/kg.

Ojewole and Adewunmi (2003) reported that methanol extract of mature, green fruits of *Musa paradisiaca* (100 to 800 mg/kg) induced significant, dose-dependent reduction in blood glucose concentration of both normal and diabetic mice which was comparable to that of chlorpropamide (250 mg/kg) treated mice. They

also suggested that hypoglycaemic effect could be due, at least in part, to stimulation of insulin production and subsequent glucose utilization.

Dhanabal *et al.* (2005) observed that oral administration of the ethanol extract of flowers of *Musa sapientum* (200 mg/kg) showed significant blood glucose lowering and free radical scavenging effects in alloxan diabetic rats. They opined that the antidiabetic activity might be attributed to the presence of flavonoids, alkaloids, steroids and glycosides present in the extract.

Materials and Methods

Plate 1. Mature green fruits of *Musa* AAA (Chenkadali)

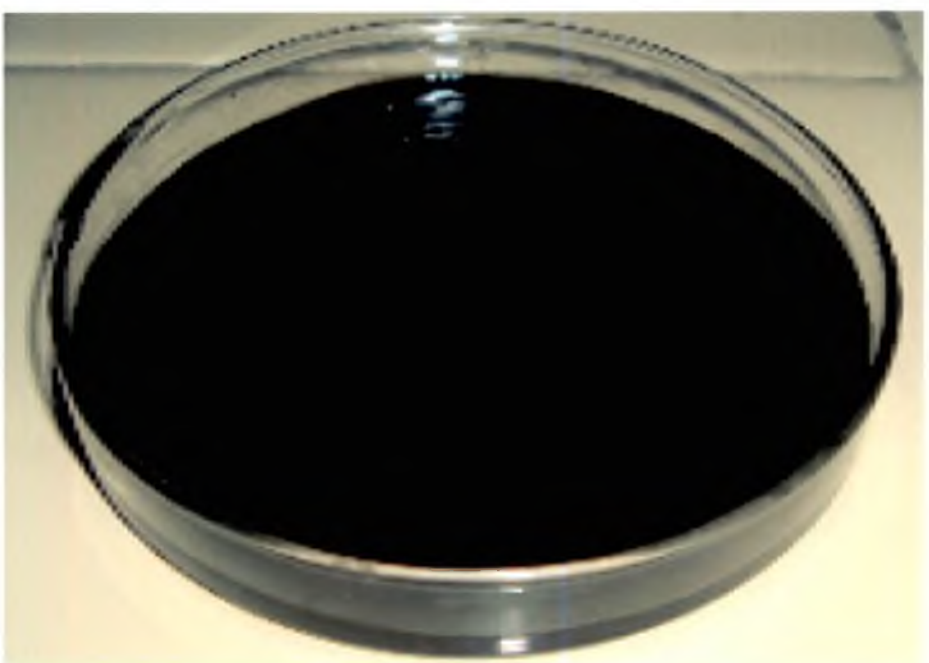

Plate 2. Dry extract of mature green fruits of *Musa* AAA (Chenkadali)

3. MATERIALS AND METHODS

3.1 COLLECTION OF PLANT MATERIAL

Fresh mature green fruits of *Musa* AAA (Chenkadali) were purchased from Banana Research Station, Kannara, Kerala Agricultural University, Thrissur. The fruits were identified and authenticated by Dr. Rema Menon,Professor, Banana Research station, Kannara, Kerala Agricultural University, Thrissur (Plate 1). The fruits were cut into small pieces, dried under shade, coarsely powdered in a pulverizer and used for the preparation of alcohol extract.

3.2 PREPARATION OF ETHANOL EXTRACT

Alcohol extract of *Musa* fruits was prepared using ethyl alcohol in a soxhlet apparatus. The extract was concentrated in a rotary vacuum evaporator under reduced pressure and at 50°C. The concentrated extract was then dried by keeping at room temperature. On an average, dried fruits of *Musa* AAA yielded 12 % (w/w) of dry extract (Plate 2). The crude extract thus prepared was kept in the refrigerator at 4°C till the time of administration. The extract was dissolved in aqueous solution of gum acacia (5 %) to obtain a final concentration of 50 mg of dry extract per ml.

3.3 CHEMICALS

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) was procured from Sigma-Aldrich India,

Bangalore. Trichloroacetic acid (TCA) was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai. All the other chemicals were procured from Merck India Ltd, Mumbai.

3.4 PHYTOCHEMICAL SCREENING

The ethanol extract of *Musa* AAA (Chenkadali) fruits were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harbone (1991).

3.4.1 Tests for detection of steroids

3.4.1.1 Salkowski Test

Mixed 5 mg of the extract with 3 ml of chloroform and then shaken with 3 ml of concentrated sulphuric acid. Development of red colour indicates the presence of steroids.

3.4.1.2 Leiberman Burchardt Test

Mixed 5 mg of the extract with 3 ml of chloroform. Then 5 drops of acetic anhydride and 1 ml of concentrated sulphuric acid was added to it through the sides. Development of a reddish ring at the junction of two layers indicates the presence of steroids.

3.4.2 Tests for detection of alkaloids

Mixed 0.5 g of the extract with 5 ml ammonia solution and then extracted with equal volume of chloroform. To this, equal quantity of 0.1N hydrochloric acid was added. The acid layer obtained was used for the following tests.

3.4.2.1 Mayer's Test

To 1 ml of acid layer obtained, three drops of Mayer's reagent ((1.358 g of Mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of potassium iodide in 10 ml of water; then made up the volume to 100 ml with distilled water) were added. Development of a creamy white precipitate indicates the presence of alkaloids.

3.4.2.2 Hager's Test

To 1 ml of acid layer, three drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were added and mixed. Development of yellow precipitate indicates the presence of alkaloids.

3.4.2.3 Dragendroff's Test

Two drops of Dragendroff's reagent was mixed with 1 ml of acid layer. Development of a reddish brown precipitate indicates the presence of alkaloids.

Dragendroff's reagent:

Stock solution (1): 0.6 g of bismuth subnitrate was dissolved in 2 ml concentrated HCl and 10 ml of water was added.

Stock solution (2): 6 g of potassium iodide was dissolved in 10 ml of water.

Both stock solutions were mixed together and then mixed with 7 ml of concentrated HCl and 15 ml of water. Sufficient amount of distilled water was added to make up the volume to 400 ml.

3.4.3 Tests for detection of Tannins

3.4.3.1 Ferric chloride test

To 2 mg of the extract, 3 ml of 1 % ferric chloride solution was added and mixed. Development of blue, green or brownish colour indicates the presence of tannins.

3.4.4 Tests for detection of Flavonoids

3.4.4.1Ferric chloride Test

To 2 ml of alcoholic solution of the extract, (0.5 g extract in 10 ml methanol), three drops of neutral ferric chloride solution was added and mixed. Development of green colour indicates the presence of flavonoids.

3.4.4.2 Lead acetate Test

To 2 ml of alcoholic solution of the extract, (0.5 g extract in 10 ml methanol), three drops of 10% lead acetate was added and mixed. Development of a yellow precipitate indicates the presence of flavonoids.

3.4.5 Tests for detection of Glycosides

3.4.5.1 Benedict's Test

To about 1 ml of the extract (0.5 g of extract in one ml water), 5 ml of Benedict's reagent was added. The mixture was boiled for 2 minutes. Development of brown to red colour indicates the presence of glycosides.

3.4.5.2 Sodium hydroxide Test

Mixed 5 mg of the extract with 1 ml water and added 5 drops of 10 % sodium hydroxide solution. Development of a yellow colour indicates the presence of glycosides.

3.4.6 Tests for the presence of Phenolic Compounds

5 mg of the extract was mixed with 1 ml of water and 5 drops of 10 % ferric chloride solution was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

3.4.7 Tests for detection of Diterpenes

5 mg of the extract was mixed with 3 ml of 5 % copper acetate solution. Development of green colour indicates the presence of diterpenes.

3.4.8 Tests for detection of Triterpenes

3.4.8.1 Salkowski's Test

Mixed 5 mg of the extract with 3ml of chloroform and then shaken with 3 ml concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicates the presence of triterpenes.

3.4.8.2 Leiberman Burchardt Test

Three drops of acetic acid and 1 ml of concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (3 mg of extract in 3 ml chloroform). Development of deep red ring at the junction of two layers indicates the presence of triterpenes.

3.4.9 Tests for detection of Saponins

3.4.9.1 Foam Test

5 mg of the extract was shaken with 3 ml of water. Development of the foam that persists for ten minutes indicates the presence of saponins.

3.5 EXPERIMENTAL ANIMALS

The study was conducted in 48 adult female Sprague Dawley rats weighing 150-200 g procured from Small Animal Breeding Station, College of Veterinary &

Animal Sciences, Mannuthy. All the rats were housed in a well ventilated experimental animal room under 12:12 hour LD cycle at 22 to 28°C with free access to standard rat diet and drinking water. An acclimatization period of one week was allowed before the commencement of the experiment. The experiment was conducted for a period of 30 days.

3.6 INDUCTION OF EXPERIMENTAL DIABETES

A total number of 48 rats were selected for the study. Out of the 48 rats, 32 were fasted over night and made diabetic by subcutaneous (s/c) injection of alloxan @ 130 mg/kg body weight (b.w). Animals were monitored for glucose levels on the 3rd day and again on 7th day to confirm maintenance of high blood glucose level. Animals showing blood glucose level of 200 mg/dl or above were only selected for the study. All the animals injected with alloxan showed a blood glucose level above 200 mg/dl.

3.7 ADMINISTRATION OF EXTRACT AND REFERENCE DRUG

From 8th day after alloxan injection (day 0), rats were administered orally (using orogastric tube) with ethanol extract of *Musa* fruits (Chenkadali) and the standard antidiabetic drug, glibenclamide (Daonil®), continuously for a period of 30 days as detailed below:

3.8 EXPERIMENTAL DESIGN

Sixteen normal rats were divided into two groups, G1 and G3 and 32 alloxan diabetic rats were divided into four groups, G2, G4, G5 and G6, of 8 animals in each group. From 8th day after alloxan injection, rats were administered orally (using

orogastric tube) with ethanol extract of *Musa* fruits (Chenkadali) and the standard antidiabetic drug, glibenclamide, continuously for a period of 30 days as detailed below:

roup	Treatment
1	Normal control administered with 5 % solution of gum acacia @ 0.6 ml/100 g b.w.
2	Diabetic control given s/c injection of alloxan @ 130 mg/kg b.w
3	Normal animals administered with ethanol extract of fruits of Chenkadali @ 500 mg/ kg b.w.
4	Diabetic animals administered with ethanol extract of fruits of Chenkadali @ 500 mg/ kg b.w.
5	Diabetic animals administered with ethanol extract of fruits of Chenkadali @ 1000 mg/kg b.w.
6	Diabetic animals administered with glibenclamide @ 0.5 mg/ kg b.w.

Weight of the animals was recorded on days -7, 0, 15 and 30. In order to adjust the dose of the extract and glibenclamide, the animals were also weighed at weekly intervals. All animals were euthanized on day 30 (30 days after treatment).

3.9. COLLECTION OF BIOLOGICAL MATERIALS

3.9.1 Collection of blood and separation of serum

Blood samples were collected from all animals with and without anticoagulants on day –7 (before alloxan injection), day 0 (7 days after alloxan

administration), day 15 (15 days after treatment) and day 30 (30 days after treatment) from the retro orbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes. Blood samples were also collected without anticoagulant on day 21 for estimation of fructosamine. The tubes containing samples for serum separation were kept undisturbed for one hour at room temperature. Then transferred to 4°C and kept for 30 minutes for clot retraction, after which the tubes were thawed and centrifuged at 3000 rpm for 10 minutes at 15°C to separate the serum.

3.9.2 Collection of internal organs

The animals were euthanized by cervical dislocation, dissected upon and collected the liver and pancreas. The organs were washed in ice-cold saline to remove blood clots and kept in chilled saline. Weight of liver was recorded.

Liver and pancreas tissue homogenates were prepared (detailed under sections 3.10.7 and 3.10.8) for the estimation of lipid peroxides and reduced glutathione. Tissue samples of liver and pancreas were preserved in 10 % formalin for histopathological examination.

3.10 BIOCHEMICAL PARAMETERS ANALYSED

Haemoglobin, serum glucose, serum triacylglycerols, serum cholesterol and serum ALT activity were determined using commercially available kits. Except haemoglobin and glucose, the values of all other parameters were directly measured from semi-automatic blood analyzer, Microlab-200 (M/s E. Merck India, Ltd. Mumbai).

3.10.1 Haemoglobin

Haemoglobin was estimated photometrically by cyanmethemoglobin method using commercial kit (Beacon Diagnostics Pvt Ltd, Navsari, India).

Principle:

In alkaline medium, haemoglobin and its derivatives are oxidized in the presence of potassium ferricyanide and get converted to methemoglobin. It reacts with potassium cyanide to form purple red coloured cyanmethemoglobin complex. The intensity of colour is measured in a spectrophotometer (Spectronic 1001) at 546 nm.

Reagent 1: Drabkin's solution

Contains:

Sodium bicarbonate	1g
Potassium cyanide	50 mg
Potassium ferricyanide	200 mg
Distilled water	1000 ml

Reagent 2 Cyanmethemoglobin standard

Procedure:

	Blank	Test
Drabkin's solution	5.0 ml	5.0 ml
Blood	--------	20 µl

Mixed the tubes well and allowed to stand at room temperature for 5 minutes. The absorbance of the test was measured against the blank at 546 nm or green filter. The absorbance of Cyanmethemoglobin standard was taken directly without adding Drabkin's solution, against blank at 546 nm or green filter.

Calculation:

$$\text{Haemoglobin in g \%} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 15.06 \text{ (concentration of standard)}$$

3.10.2 Glucose

Glucose was estimated photometrically by GOD-POD method using commercial kit (Merckotest® Glucose).

Principle:

Glucose is oxidized by glucose oxidase to gluconic acid and H_2O_2 is liberated. The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase. Intensity of colour generated is directly proportional to glucose concentration.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4- aminoantipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\,H_2O$$

Reagent 1: Phosphate buffer pH 7.0	100 mmol/l
Phenol	5 mmol/l
4-aminoantipyrine	0.5 mmol/l
Glucose oxidase	≥ 15 KU/l
Peroxidase	≥ 1 KU/l
Reagent 2: Glucose Standard	100 mg/dl

Procedure:

	Blank	Standard	Sample
Sample	---	---	10 µl
Standard	---	10 µl	---
Distilled water	10 µl	----	---
Reagent 1	1000 µl	1000 µl	1000 µl
Mixed and incubated for approximately 15 minutes at 37°C. The absorbance was read against the reagent blank at 546 nm with in 30 minutes.			

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (mg/dl)}$$

3.10.3 Triacylglycerol

Concentration of serum triacylglycerol was estimated using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of triacylglycerol involves enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

$$\text{Triacylglycerol} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4\,H_2O$$

Reagents:

Reagent 1:	Good's buffer, pH 7.2	50 mmol/L
	4-chlorophenol	4 mmol/L
	ATP	2 mmol/L
	Mg^{2+}	15 mmol/L
	Glycerokinase	≥0.4 kU/L
	Peroxidase	≥2 kU/L
	Lipoprotein lipase	≥2 kU/L
	4-aminoantipyrine	0.5 mmol/L
	Glycerol-3-phosphate oxidase (GPO)	≥1.5 kU/lL
Standard:	Triacylglycerol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl
Mixed, incubated for 5 minutes at 37°C. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Triacylglycerol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}$$

3.10.4 Cholesterol

Concentration of serum cholesterol was estimated by Cholesterol oxidase phenol amino antipyrine (CHOD-PAP) method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase.

Reagents:

Reagent 1:	PIPE's buffer, pH 7.5	99 mmol/L
	Salicylic alcohol	3.96 mmol/L
	Peroxidase	≥1000 U/L
	4-aminoantipyrine	0.5 mmol/L
	Cholesterol oxidase	≥100 U/L
	Cholesterol esterase	≥100 U/L

Standard:

Cholesterol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl

Mixed, incubated for 5 minutes at 37°C. Absorbance was read against the blank at 546 nm within 60 minutes.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.10.5 Alanine amino transferase (ALT) activity

Serum ALT activity was determined using commercially available kit, Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of serum ALT activity is based on reference method of International Federation of Clinical Chemistry (IFCC).

$$\text{L-Alanine} + \text{2-Oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^{+} \xleftrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^{+}$$

The rate of NADH consumption is measured photometrically and is directly proportional to the ALT concentration in the sample.

Reagents:

Reagent 1:	TRIS, pH 7.5	100 mmol/L
	L-Alanine	500 mmol/L
	Lactate dehydrogenase (LDH)	≥ 1.2 kU/L
Reagent 2:	2-Oxoglutarate	15 mmol/L
	NADH	0.18 mmol/L

Procedure:

Reagent 1 and Reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

Serum	100 µl
Working reagent	1000 µl

Mixed, after 1 minute, read the decrease in absorbance (ΔA) every minute for three minutes at 340 nm.

Calculation:

ALT activity (U/L) = ΔA × 1746

3.10.6 Fructosamine

The level of fructosamine in serum was estimated using the method described by Kumar *et al.* (1988).

Principle:

The differential reduction of free hexose and hexose bound non-enzymatically with 2.0 mg and 20 mg of sodium borohydride followed by phenol sulphuric acid reaction is estimated colorimetrically at 480 nm. Sodium borohydride at low concentrations completely reduces glucose. But glycated proteins require higher concentration of sodium borohydride for reduction. The difference in colour intensities measured between the two systems gives a measure of glycated protein concentration reduced by sodium borohydride.

Reagents

Sodium borohydride (freshly prepared 10 % solution, containing 0.1 ml of ammonium hydroxide /ml)

Phenol (80 %)

Concentrated sulphuric acid

Glucose standard (200 µg / 20 µl)

Distilled water

Procedure

Five test tubes were taken and marked Sample A1, Sample A2, Sample blank, Standard and Standard blank. Same sample of serum was taken in tubes A1 and A2 and proceeded as follows:

	Sample A1	Sample A2	Sample blank	Standard	Standard blank
Serum	20µl	20µl	20µl	---	---
Glucose standard	---	---	---	20µl	---
Sodium borohydride	20µl	200µl	200µl	---	---
Distilled water	180 µl	---	---	200µl	220µl
Incubated for 15 minutes at room temperature and added the following					
Distilled water	780 µl	780 µl	780 µl	780 µl	780 µl
Phenol	50 µl	50 µl	---	50 µl	50 µl
Concentrated sulphuric acid	3000 µl	3000 µl	3000 µl	3000 µl	3000 µl

Mixed each tube well and incubated for 30minutes at room temperature. Absorbance of A1 and A2 was read against the sample blank at 480 nm and that of standard was read against the standard blank.

Calculation:

$$\text{Fructosamine (mM/l)} = \frac{O.D_{A1} - O.D_{A2}}{O.D \text{ of standard}} \times \frac{200}{20} \times \frac{1000}{180}$$

3.10.7 Estimation of lipid peroxides in tissues

Level of lipid peroxides in tissue homogenate was determined by the method of Ohkawa *et al.* (1979).

Principle:

Thiobarbituric acid reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. 1,1,3,3 tetra methoxy propane was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

Reagents:

8.1 % SDS

20 % acetic acid solution, pH adjusted to 3.5 with NaOH

0.8 % aqueous solution of TBA

1.15 % KCl

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and pancreas were prepared in a ratio of 1 g of wet tissue to 9 ml of 1.15 % KCl solution (10 % w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of lipid peroxides.

To 100 µl of the supernatant, added 200 µl of 8.1 % SDS, 1.5 ml 20 % acetic acid solution (pH 3.5) and 1.5 ml of 0.8 % aqueous solution of TBA. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95°C for 60 minutes. After cooling under tap water, 1 ml of distilled water and 5 ml of n-butanol were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, absorbance of the organic layer was taken at 532 nm.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 nM to 5 nM of TMP in deionised double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nM of malondialdehyde/g of wet tissue.

3.10.8 Estimation of reduced glutathione in tissues

Level of reduced glutathione in tissue homogenate was estimated by the method of Moron *et al.* (1979).

Principle:

Reduced glutathione is measured by its reaction with DTNB to give a yellow coloured complex with an absorption maximum at 412 nm.

Reagents:

0.2 M phosphate buffer, pH 8

25 % TCA

5 % TCA

0.6 mM DTNB

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and pancreas were prepared in a ratio of 1 g of wet tissue to 9 ml of 0.2 M phosphate buffer, pH 8 (10 % w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

Added 125 µl of 25 % TCA to 500 µl of supernatant/serum for the precipitation of proteins. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 575 µl of 5 % TCA. Centrifuged the tubes for 5 minutes and 300 µl of resulting supernatant was taken for the estimation of reduced glutathione. The volume of aliquot was made up to 1 ml with 0.2 M phosphate buffer (pH, 8). Added 2 ml of freshly prepared 0.6 mM DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. The level of reduced glutathione was measured from the standard curve.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 1 µg to 10 µg of reduced glutathione dissolved in 5 % TCA. The volume of standard solution was made up to 1 ml with 0.2 M phosphate buffer (pH, 8). Added 2 ml of freshly prepared 0.6 mM DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between optical density and concentration of the

standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly from the calibration curve and expressed as µg/g of wet tissue.

3.10.9 Estimation of liver glycogen

Liver glycogen was estimated by Anthrone method (Narasimhan, 1971).

Principle:

The sulphuric acid medium of anthrone reagent causes dehydration of sugar to a furfural derivative, which presumably condenses with anthrone to form a blue colored compound, which was measured in a spectrophotometer at 620 nm.

Reagents:

A. KOH solution (30 %): Dissolved 300 g of reagent grade potassium hydroxide pellets in distilled water in a beaker, cooled and transferred quantitatively into one litre volumetric flask and diluted to one litre with distilled water.

B. Sulphuric acid (95 %): Mixed 950ml of concentrated sulphuric acid with 50 ml of distilled water and cooled.

C. Anthrone reagent (0.2 %): The reagent was prepared by dissolving 0.2 g of anthrone in 100 ml of 95 % sulphuric acid. The reagent was prepared fresh whenever required.

D. Standard glucose solution (20 µg/ml): The stock standard was prepared by dissolving one gram of highest purity anhydrous glucose in saturated benzoic acid solution and diluted to 100 ml with the same. The working standard was prepared by diluting one ml of stock standard to 500 ml with distilled water.

Procedure:

Approximately 0.5 g of liver tissue was taken in a test tube containing 3 ml of 30 % KOH solution. The tissue was digested by heating the tube for 20 minutes in a boiling water bath. The sample was then cooled and quantitatively transferred into a 50 ml volumetric flask and diluted up to the mark with distilled water. After thorough mixing, 5 ml of the solution was pipetted into a second 50 ml volumetric flask and diluted up top the mark with distilled water. 5 ml of this was taken as the unknown sample.

Sample:	5 ml of digesta prepared at the end of second dilution of 50ml.
Standard:	5 ml of glucose working standard
Blank:	5 ml of distilled water

The sample, standard and blank were kept in a cold water bath and added 10 ml of anthrone reagent to each of the three tubes from a fast flowing burette. Mixed the reactants by swirling the test tubes. After cooling, covered the mouth of test tubes with glass stoppers and heated for 10 minutes in boiling water bath. Then immediately cooled by placing them in cold water bath. The optical densities were taken against the blank at 620 nm in a spectrophotometer.

Calculation:

$$\text{Liver glycogen (g percent)} = \frac{A_u}{A_s} \times \frac{1}{1.11} \times \frac{500}{5} \times \frac{100}{\text{weight of tissue in g}} \times \frac{100}{1000000}$$

A_u = O.D of unknown sample

A_s = O.D of standard

Concentration of standard in μg =100

$$\text{Correction factor for conversion of glucose to glycogen} = \frac{1}{1.11}$$

$$\text{Dilution factor} = \frac{500}{5}$$

$$\text{Factor for expressing value in \%} = \frac{100}{1000000}$$

3.11 HISTOPATHOLOGICAL EXAMINATION OF LIVER AND PANCREAS

Representative samples of liver and pancreas obtained from the dissected animals were fixed in 10 % formalin. They were processed and paraffin embedded as described by Sheehan and Hrapchak, (1980). The sections were stained with haematoxyline and eosin as per the technique followed by Bancroft and Cook, (1984). The sections were examined in detail under light microscope.

3.12 STATISTICAL ANALYSIS OF THE DATA

Data obtained were analyzed by the following statistical tools (Snedecor and Cochran, 1994) to determine the level of significance. The value of $P<0.05$ was considered statistically significant.

1. Compared by analysis of variance (ANOVA) followed by Duncan's multiple range test
2. Weight gain for different groups was analyzed by analysis of co-variance (ANCOVA)
3. Period wise comparison of means was done by paired t-test

Results

4. RESULTS

4.1 PHYTOCHEMICAL SCREENING

Ethanol extract of mature green fruits of *Musa* AAA (Chenkadali) was screened for the presence of the following active principles:

Active principles	Tests	Observations	Inference
Steroids	Salkowski test	Solution turned red in colour	Presence of steroids
	Lieberman Burchardt test	Reddish ring at the interface	
Alkaloids	Mayer's test	No precipitate	Absence of alkaloids
	Hager's test	No precipitate	
	Dragendroff's test	No precipitate	
Tannins	Ferric chloride test	No colour change	Absence of tannins
Flavonoids	Ferric chloride test	Solution turned green in colour	Presence of flavonoids
	Lead acetate test	Yellow precipitate	
Glycosides	Benedict's test	Solution turned brown in colour	Presence of glycosides
	Sodium hydroxide	Solution turned yellow in colour	

Phenolic compounds	Ferric chloride test	Solution turned dark blue in colour	Presence of phenolic compounds
Diterpenes	Copper acetate test	Solution turned green in colour	Presence of diterpenes
Triterpenes	Salkowski test	Lower layer turned yellow	Presence of triterpenes
	Lieberman Burchardt test	Deep red ring at the interface	
Saponins	Foam test	Foam disappeared before ten minutes.	Absence of saponins

4.2 EFFECT OF ETHANOL EXTRACT OF FRUITS OF *Musa* AAA (Chenkadali) IN DIABETIC AND NORMAL RATS

4.2.1 Body weight

Body weight of different groups is presented in Table 1 and Fig.1. Significant ($P < 0.05$) variation in the body weight was not observed in the alloxan administered groups on day 0 when compared with normal rats. Though the diabetic control exhibited a decreased body weight on day 15, it became significant ($P < 0.05$) only on

Table 1. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on body weight (g) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	169.17 ± 4.55[a]	180.15 ± 5.77[a,b]	244.49 ± 3.16[a]	284.08 ± 4.23[a]
G2	153.33 ± 1.054[a]	178.26 ± 1.54[b]	219.66 ± 2.47[a]	241.78 ± 3.01[b]
G3	155.00 ± 2.58[a]	182.27 ± 2.11[a,b, c]	227.80 ± 9.80[a]	266.54 ± 8.06[a]
G4	169.17 ± 3.52[a]	185.99 ± 5.83[a,c]	243.65 ± 5.69[a]	271.58 ± 8.85[a]
G5	170.00 ± 3.69[a]	182.58 ± 4.94[a,b, c]	240.22 ± 3.52[a]	280.21 ± 6.15[a]
G6	171.67 ± 5.58[a]	188.26 ± 4.90[c]	243.36 ± 3.27[a]	278.30 ± 4.36[a]

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

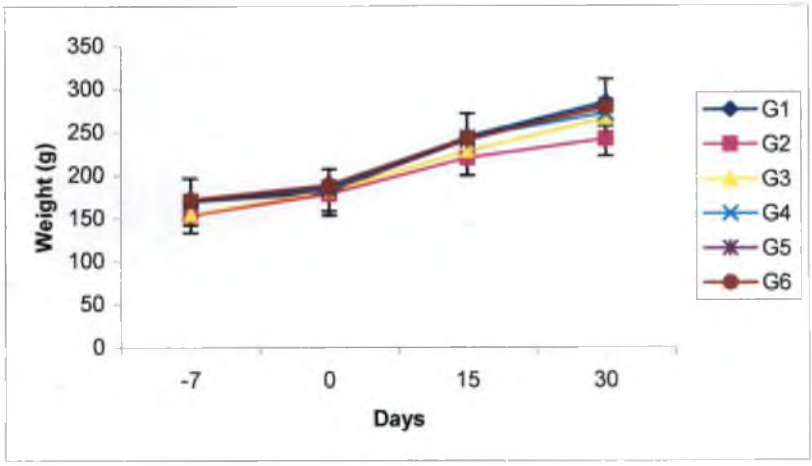

Fig 1. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on body weight (g) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 –Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

day 30 when compared with normal control and treated groups. Among the treated groups, there was no significant difference in body weight between the extract treated groups, G4 (271.58 ± 8.85 g) and G5 (280.21 ± 6.15 g) and the group treated with the reference drug, glibenclamide, G6 (278.30 ± 4.36 g). Body weight of normal animals administered with the extract also did not show any significant variation from the normal control group and the treated groups at any stage of analysis.

4.2.2 Weight of liver

Liver weight of different groups is presented in Table 2 and Fig. 2. Significant decrease (1.09 ± 0.06 mM/L) was observed in the liver weight of G2 (4.04 ± 0.11g) in comparison with the normal control, G1 (6.12 ± 0.08 g). All the treated groups, G4 (6.08 ± 0.13 g), G5 (6.04 ± 0.20 g) and G6 (5.93 ± 0.10 g) showed a significant ($P < 0.05$) increase in liver weight compared with the diabetic control and were homogenous with the normal control. Liver weight of normal animals treated with *Musa* fruit extract (5.97 ± 0.09 g) did not differ significantly from that of normal control after 30 days of study.

4.2.3 Haemoglobin

Haemoglobin concentration of different groups is presented in Table 3 and Fig. 3. All the groups administered with alloxan showed a significant ($P < 0.05$) reduction in the level of haemoglobin on 0^{th} day when compared with the normal control. Administration of *Musa* fruit extract for 15 days significantly increased haemoglobin concentration of G4 (12.70 ± 0.14 g %) and G5 (12.46 ± 0.10 g %) when compared to the diabetic control (9.91 ± 0.13 g %). Treatment with glibenclamide for 15 days also significantly ($P < 0.05$) increased haemoglobin

Table 2. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on liver weight (g) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	31st day
G1	6.12 ± 0.08^{a}
G2	4.04 ± 0.11^{b}
G3	5.97 ± 0.09^{a}
G4	6.08 ± 0.13^{a}
G5	6.04 ± 0.20^{a}
G6	5.93 ± 0.10^{a}

Values not bearing a common superscript letter (a and b) in a column differ significantly ($P<0.05$).

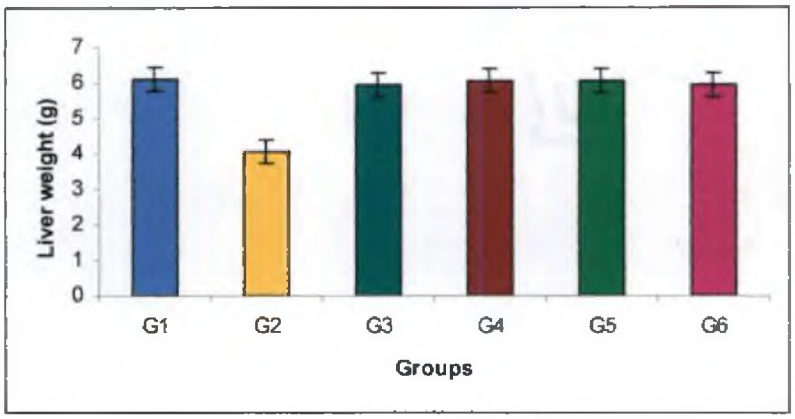

Fig. 2. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on liver weight (g) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

Table 3. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on haemoglobin level (g %) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	13.35 ± 0.45^{a}	13.30 ± 0.32^{a}	13.87 ± 0.20^{a}	14.01 ± 0.20^{a}
G2	12.75 ± 0.21^{a}	10.72 ± 0.23^{b}	9.91 ± 0.13^{b}	8.44 ± 0.13^{b}*
G3	13.41 ± 0.52^{a}	13.33 ± 0.22^{a}	13.81 ± 0.30^{a}	13.95 ± 0.21^{a}
G4	13.07 ± 0.12^{a}	10.77 ± 0.19^{b}	12.70 ± 0.14^{c}	13.38 ± 0.11^{a}*
G5	13.12 ± 0.22^{a}	10.61 ± 0.11^{b}	12.46 ± 0.10^{c}	12.84 ± 0.46^{c}*
G6	13.29 ± 0.33^{a}	10.88 ± 0.17^{b}	10.91 ± 0.17^{d}	11.52 ± 0.43^{d}

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$). Means bearing * indicates significant ($P<0.05$) difference between day 0 and day 30.

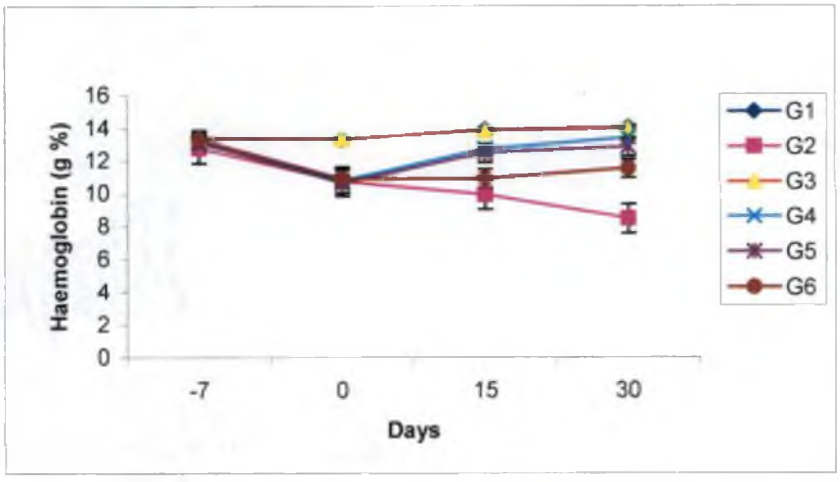

Fig. 3. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on haemoglobin concentration (g %) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 –Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

concentration (10.91 ± 0.17 g %) when compared with the diabetic control, while the values were significantly ($P < 0.05$) lower than that of the extract treated groups. Significant ($P < 0.05$) increase was observed in the level of haemoglobin after 30 days of administration of both doses of the extract when compared with diabetic control. The value obtained in G4 (13.38 ± 0.11 g %) did not show any significant difference from that of normal control (14.01 ± 0.20 g %). Though, there was significant ($P < 0.05$) increase in haemoglobin level in the group treated with glibenclamide (11.52 ± 0.43 g %), the level was significantly ($P < 0.05$) lower than that of normal control. Comparison between the haemoglobin concentration of 0^{th} and 30^{th} days showed significant ($P < 0.05$) increase in the extract treated groups, while the group treated with glibenclamide showed an increase, but not significant. Normal animals treated with *Musa* fruit extract @ 500 mg/kg dose showed a value similar to that of normal control at all stages of analysis.

4.2.4 Glucose

Serum glucose concentration of different groups is presented in Table 4 and Fig. 4. Compared with the normal control (G1), all the groups administered with alloxan showed a significant ($P < 0.05$) increase in the level of glucose on 0^{th} day. Administration of *Musa* fruit extract for 15 days significantly ($P < 0.05$) decreased glucose level of G4 (204.49 ± 2.05 mg/dl) and G5 (198.38 ± 26.71 mg/dl) as compared with diabetic control (384.71 ± 13.65 mg/dl). Treatment with glibenclamide for 15 days also significantly ($P < 0.05$) decreased glucose level (135.09 ± 2.24 mg/dl), when compared with the diabetic control and the values were significantly ($P < 0.05$) lower than that of extract treated groups. Administration of the extract for thirty days at both doses significantly ($P < 0.05$) decreased the level of blood glucose G4 (122.47 ± 1.54 mg/dl) and G5 (133.90 ± 2.24 mg/dl) when

Table 4. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum glucose (mg/dl) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	88.80 ± 4.58^{a}	89.76 ± 3.15^{a}	91.23 ± 3.80^{a}	88.43 ± 2.54^{a}
G2	88.48 ± 3.84^{a}	353.90 ± 14.90^{b}	384.71 ± 13.65^{b}	397.06 ± 2.48^{b}*
G3	88.89 ± 3.58^{a}	90.52 ± 3.77^{a}	89.38 ± 3.56^{a}	91.63 ± 3.53^{a}
G4	87.51 ± 3.85^{a}	353.64 ± 8.69^{b}	204.49 ± 2.05^{c}*	122.47 ± 1.54^{c}*
G5	87.66 ± 3.72^{a}	354.10 ± 5.81^{b}	198.38 ± 26.71^{c}	133.90 ± 2.24^{c}*
G6	88.47 ± 5.49^{a}	354.22 ± 13.56^{b}	135.09 ± 2.24^{d}	131.84 ± 1.76^{c}*

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly (P<0.05). Means bearing * indicates significant (P<0.05) difference between day 0 and day 30.

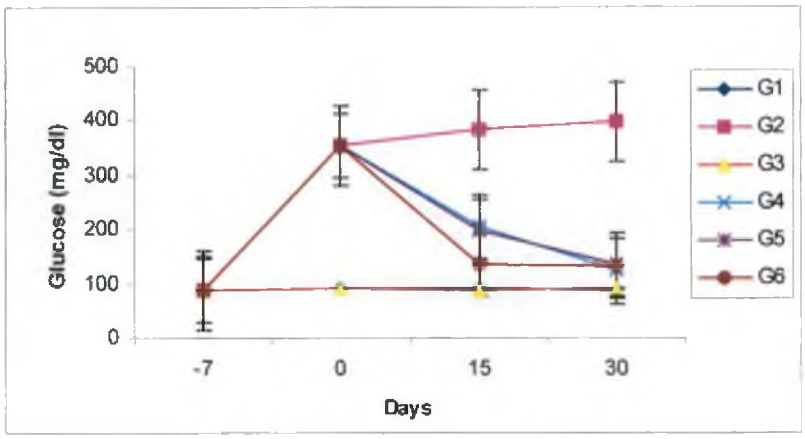

Fig. 4. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum glucose (mg/dl) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

compared with diabetic control (397.06 ± 12.48 mg/dl) and the levels were similar to that of the group treated with glibenclamide (131.84 ± 1.76 mg/dl). Comparison between the effects of two doses of the extract, the dose of 500 mg/kg showed a better reduction in the glucose level, though not significant. Normal animals treated with *Musa* fruit extract did not show any significant variation from that of normal control at any stage of analysis.

4.2.5 Fructosamine

Fructosamine level of different groups is presented in Table 5 and Fig. 5. Significant ($P < 0.05$) increase in fructosamine level was observed in all alloxan-administered groups on day 0 when compared with the normal control. Administration of *Musa* fruit extract for 21 days produced significant ($P < 0.05$) decrease in fructosamine level of G4 (1.40 ± 0.04 mM/L) and G5 (1.33 ± 0.13 mM/L) when compared with diabetic control (2.09 ± 0.04 mM/L), but the levels were significantly ($P < 0.05$) higher than that of normal control (1.09 ± 0.06 mM/L). Glibenclamide treated group viz. G6 (1.10 ± 0.05 mM/L) also showed significantly ($P < 0.05$) lower level of fructosamine when compared with diabetic control and it was homogenous with normal control. Normal animals treated with *Musa* extract (1.05 ± 0.07 mM/L) showed no significant difference from the values shown by normal control (1.09 ± 0.06 mM/L).

4.2.6 Liver glycogen

Liver glycogen level of different groups is presented in Table 6 and Fig. 6. Level of liver glycogen decreased significantly ($P < 0.05$) in diabetic control, G2 (1.24 ± 0.05 g %) when compared with the normal control, G1 (2.20 ± 0.08 g %). G2 values were almost half of the normal control. Significant ($P < 0.05$) increase in liver

Table 5. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on fructosamine level (mM/L) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days		
	-7th day	0th day	21st day
G1	1.13 ± 0.10[a]	1.17 ± 0.11[a]	1.09 ± 0.06[a]
G2	1.13 ± 0.54[a]	1.64 ± 0.04[b]	2.09 ± 0.04[b*]
G3	1.22 ± 0.05[a]	1.26 ± 0.05[a]	1.05 ± 0.07[a]
G4	1.15 ± 0.09[a]	1.84 ± 0.06[b]	1.40 ± 0.04[c*]
G5	1.05 ± 0.13[a]	1.72 ± 0.05[b]	1.33 ± 0.13[c*]
G6	1.03 ± 0.09[a]	1.77 ± 0.05[b]	1.10 ± 0.05[a*]

Values not bearing a common superscript letter (a, b, and c) in a column differ significantly (P<0.05). Means bearing * indicates significant (P<0.05) difference between day 0 and day 21.

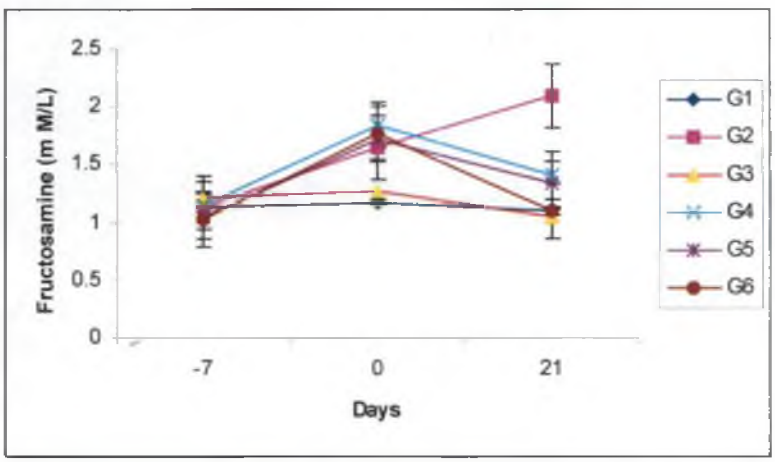

Fig. 5. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on fructosamine level (mM/L) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

Table 6. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of liver glycogen (g %) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Liver glycogen
G1	$2.20 \pm 0.08^{a,c}$
G2	1.24 ± 0.05^{b}
G3	2.27 ± 0.05^{a}
G4	2.27 ± 0.10^{a}
G5	$2.20 \pm 0.23^{a,c}$
G6	1.79 ± 0.06^{c}

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

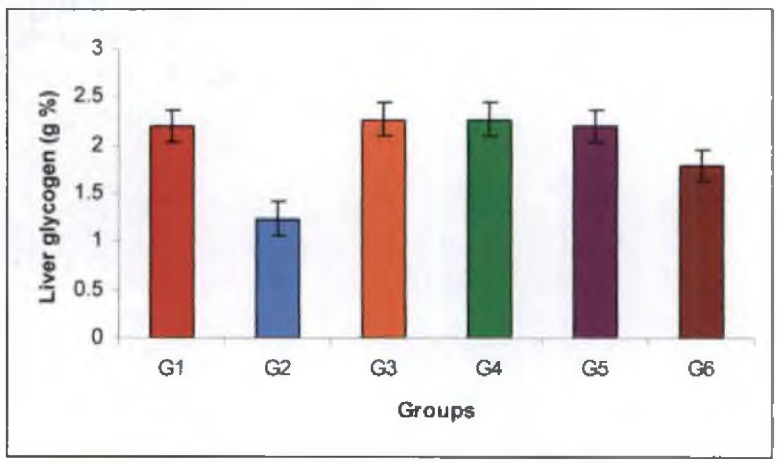

Fig. 6. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of liver glycogen of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

values were almost half of the normal control. Significant ($P < 0.05$) increase in liver glycogen was observed in G4 (2.27 ± 0.10 g %) and G5 (2.20 ± 0.23 g %) as compared with the diabetic control. Group treated with the standard antidiabetic drug, G6 also showed a significant ($P < 0.05$) increase in liver glycogen (1.79 ± 6.88 g %) compared to the diabetic control. Although there was no significant difference between the treated diabetic groups with that of normal control, the extract treated groups showed higher glycogen level and was significant in G4 than glibenclamide treated animals. Liver glycogen level in normal animals treated with the extract (2.27 ± 0.05 g %) did not differ significantly from that of the normal control (2.20 ± 0.08 g %).

4.2.7 Triacylglycerol

Level of triacylglycerol of different groups is presented in Table 7 and Fig. 7. A significant increase ($P < 0.05$) was observed in the level of triacylglycerols on day 0 of all alloxan administered groups when compared with the normal control. Administration of the extract for 15 days significantly ($P < 0.05$) decreased triacylglycerol level of G4 (71.00 ± 5.33 mg/dl) and G5 (79.17 ± 2.96 mg/dl) when compared with diabetic control (223.33 ± 2.03 mg/dl) and a similar effect was observed in the group treated with glibenclamide (79.83 ± 2.87 mg/dl). There was no significant difference in the level of triacylglycerol between G4 and normal control while that of G5 and G6 were significantly ($P < 0.05$) higher when compared with normal control. The values obtained in the treated groups were less than half on 15^{th} day when compared with that on 0^{th} day. After 30 days treatment, the triacyl glycerol level in the treated groups (G4, G5 and G6) became homogeneous with that of normal control. Triacylglycerol level of normal animals treated with *Musa* fruit extract was homogeneous with that of normal control at all stages of analysis.

Table 7. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum triacylglycerol (mg/dl) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	57.50 ± 4.01^{a}	58.83 ± 3.00^{a}	57.17 ± 3.12^{a}	$59.17 \pm 2.56^{a,c}$
G2	57.83 ± 2.00^{a}	165.17 ± 12.64^{b}	223.33 ± 2.03^{b}	234.83 ± 10.81^{b}*
G3	57.67 ± 3.05^{a}	58.17 ± 1.66^{a}	56.67 ± 1.41^{a}	58.50 ± 2.63^{a}
G4	58.33 ± 2.78^{a}	165.00 ± 7.87^{b}	$71.00 \pm 5.33^{a,c}$	75.50 ± 3.99^{c}*
G5	58.00 ± 2.56^{a}	164.67 ± 7.37^{b}	79.17 ± 2.96^{c}	75.50 ± 3.89^{c}*
G6	58.17 ± 3.28^{a}	165.17 ± 11.18^{b}	79.83 ± 2.87^{c}	$72.83 \pm 2.73^{a,c}$*

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$). Means bearing * indicates significant ($P<0.05$) difference between day 0 and day 30.

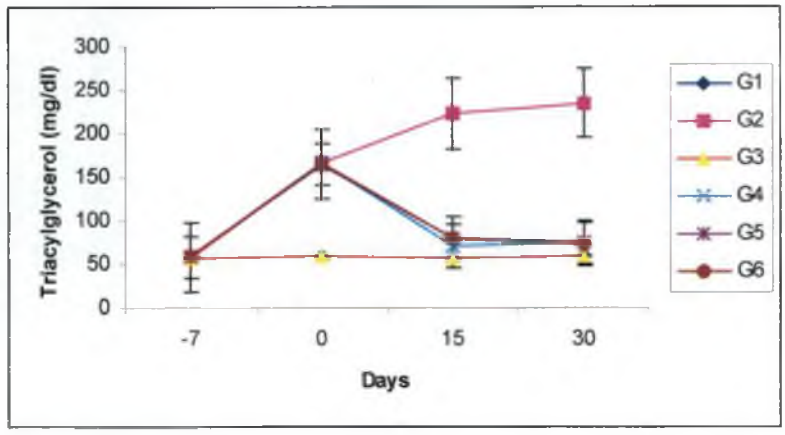

Fig. 7. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum triacylglycerol (mg/dl) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

4.2.8 Cholesterol

Serum cholesterol level of different groups is presented in Table 8 and Fig. 8. Significant increase was observed in the level of serum cholesterol of all alloxan-administered groups on day 0. Treatment with both doses of the extract and glibenclamide for 15 days produced significant ($P < 0.05$) decrease in the level of cholesterol as compared with diabetic control (80.50 ± 2.60 mg/dl) but the levels were significantly ($P < 0.05$) higher than that of the normal control (57.00 ± 1.57 mg/dl). Administration of the extract for 30 days @ 500 mg/kg significantly ($P < 0.05$) decreased the level of cholesterol in G4 (56.67 ± 1.59 mg/dl) as compared with diabetic control (91.83 ± 2.32 mg/dl) and was homogenous to that of the normal control (59.67 ± 1.36 mg/dl). The group administered with the extract at 1000 mg/kg for 30 days though showed a significantly ($P < 0.05$) lower level (68.00 ± 2.43 mg/dl) than the diabetic control, the level was significantly ($P < 0.05$) higher than that of the normal control.

Comparing the levels of cholesterol between day 0 and day 30, in G5, the extract failed to produce a significant decrease but prevented the increase in serum cholesterol level as compared to that of diabetic control. Analysis of serum cholesterol level of normal animals treated with *Musa* fruit extract did not show any significant variation from that of normal control at any stage of analysis.

Table 8. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum cholesterol (mg/dl) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	57.33 ± 3.68^{a}	56.83 ± 2.12^{a}	57.00 ± 1.57^{a}	59.67 ± 1.36^{a}
G2	57.50 ± 2.35^{a}	69.17 ± 2.52^{b}	80.50 ± 2.60^{b}	91.83 ± 2.32^{b}*
G3	56.67 ± 1.69^{a}	57.17 ± 1.78^{a}	56.83 ± 2.60^{a}	58.00 ± 1.77^{a}
G4	57.33 ± 1.93^{a}	68.33 ± 3.52^{b}	66.00 ± 2.49^{c}	56.67 ± 1.59^{a}
G5	56.50 ± 2.60^{a}	67.33 ± 1.15^{b}	70.33 ± 1.96^{c}	68.00 ± 2.43^{c}
G6	56.83 ± 2.36^{a}	67.50 ± 0.64^{b}	64.00 ± 1.48^{c}	50.83 ± 2.17^{d}*

Values not bearing a common superscript letter (a, b, and c) in a column differ significantly ($P<0.05$). Means bearing * indicates significant ($P<0.05$) difference between day 0 and day 30.

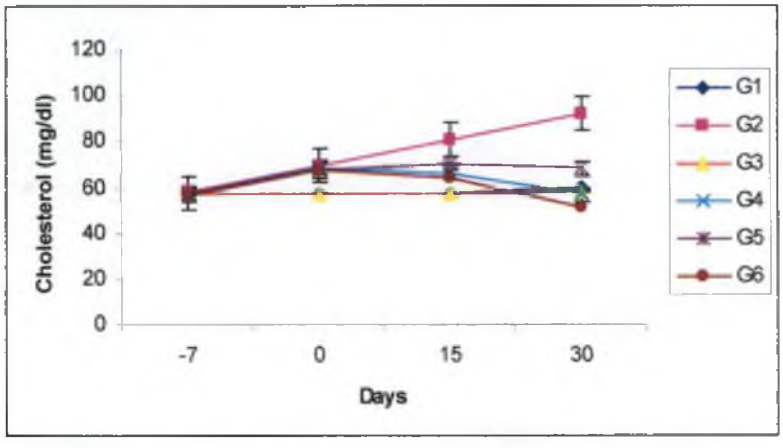

Fig. 8. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on serum cholesterol (mg/dl) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

4.2.9 Alanine amino transferase activity

Level of ALT in different groups is presented in Table 9 and Fig. 9. Alloxan administration caused a significant ($P < 0.05$) increase in ALT level when compared with the normal control. Administration of *Musa* fruit extract for 15 days produced a significant ($P < 0.05$) decrease in ALT level in G4 (71.17 ± 3.00 IU/L) when compared with diabetic control (87.17 ± 1.40 IU/L) while the level of ALT in G5 (78.17 ± 1.56 IU/L) and G6 (81.17 ± 2.12 IU/L) did not differ significantly from that of diabetic control. The level observed in G4 was homogeneous to that of normal control. Serum ALT level further decreased in G4 (65.50 ± 3.03 IU/L) following the extract administration for another 15 days and a similar effect was observed in the glibenclamide treated group, G6 (64.33 ± 2.99 IU/L) after 30 days administration of the drug as compared with diabetic control (103.67 ± 0.14 IU/L). The values observed in G4 and G6 were homogeneous to that of normal control (62.33± 0.38 IU/L). Following 30 days administration of the extract, the level of ALT in G5 (78.50 ± 4.42 IU/L) significantly ($P < 0.05$) decreased from that of diabetic control but was significantly ($P < 0.05$) higher than that of the normal control. Moreover, no significant variation was observed from that on day 0. From the results, it appears that in G5, the extract could prevent a rise in the level of ALT as compared to diabetic control. Normal animals treated with *Musa* fruit extract did not show any significant variation from that of normal control at any stage of analysis.

4.2.10 Lipid peroxides in liver and pancreas

Level of lipid peroxides in liver and pancreas of different groups are presented in Table 10 and Fig. 10. Significant ($P < 0.05$) increase in the liver lipid peroxides was observed in G2 (511.43 ± 15.38 nM/g) when compared with the normal control, G1 (299.38 ± 27.16 nM/g). Though G4 (346.25 ± 22.5 nM/g) and G5 (400 ± 23.78 nM/g) showed a significant ($P < 0.05$) decrease in liver lipid

Table 9. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on ALT level (IU/L) of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Days			
	-7	0	15	30
G1	60.67 ± 2.04^{a}	58.67 ± 1.54^{a}	65.17 ± 6.07^{a}	62.33 ± 0.38^{a}
G2	60.00 ± 1.79^{a}	81.67 ± 1.33^{b}	87.17 ± 1.4^{b}	$103.67 \pm 0.14^{b*}$
G3	60.33 ± 2.00^{a}	59.17 ± 1.68^{a}	65.83 ± 1.78^{a}	61.5 ± 0.99^{a}
G4	59.50 ± 1.61^{a}	82.17 ± 1.69^{b}	$71.17 \pm 3.00^{a,c}$	$65.5 \pm 3.03^{a*}$
G5	60.83 ± 1.89^{a}	82.5 ± 3.40^{b}	$78.17 \pm 1.56^{b,c}$	78.5 ± 4.42^{c}
G6	61.33 ± 1.65^{a}	83.25 ± 2.26^{b}	81.17 ± 2.12^{b}	$64.33 \pm 2.99^{a*}$

Values not bearing a common superscript letter (a, b, and c) in a column differ significantly ($P<0.05$). Means bearing * indicates significant ($P<0.05$) difference between day 0 and day 30.

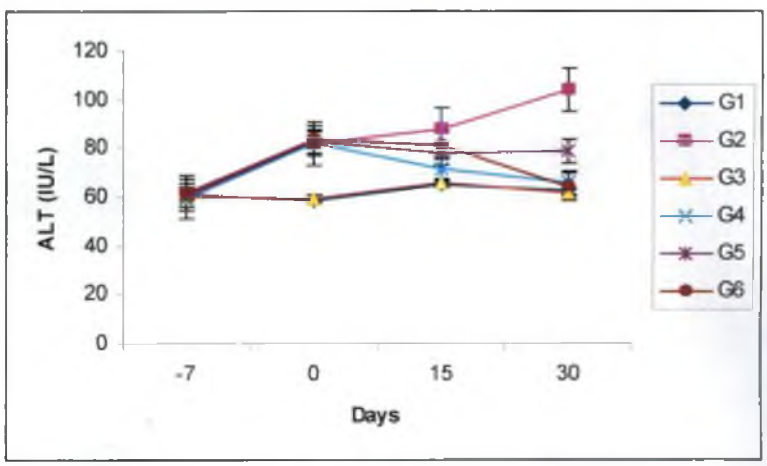

Fig. 9. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on ALT level (IU/L) of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

Table 10. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of lipid peroxides (nM/g) in the liver and pancreas of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Liver	Pancreas
G1	299.38 ± 27.16[a]	357.5 ± 21.20[a]
G2	511.43 ± 15.38[b]	450 ± 17.37[b]
G3	327.22 ± 13.50[a]	393.33 ± 6.67[a]
G4	346.25 ± 22.50[a,c]	348.33 ± 11.01[a]
G5	400 ± 23.78[c]	395 ± 13.10[a]
G6	325.84 ± 11.65[a]	379.17 ± 16.85[a]

Values not bearing a common superscript letter (a and b) in a column differ significantly ($P<0.05$).

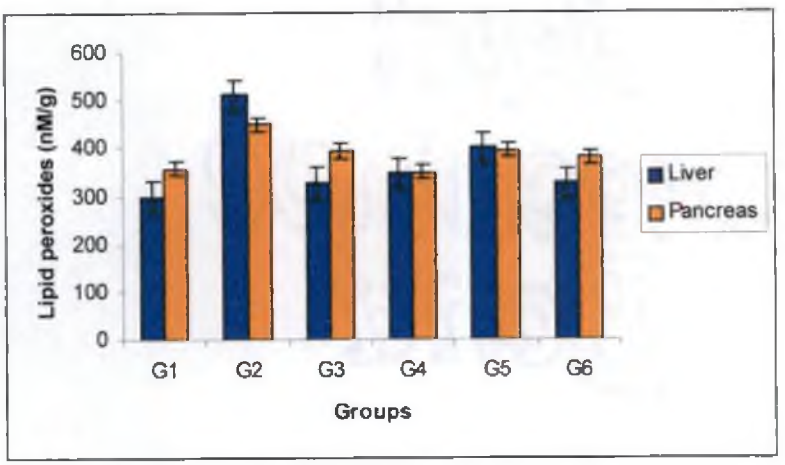

Fig. 10. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of lipid peroxides (nM/g) in the liver and pancreas of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

peroxides compared to the diabetic control, the level in G4 did not differ signicantly from that in normal control and glibenclamide treated group, G6 (325.84 ± 11.65 nM/g). Liver lipid peroxides of normal animals treated with *Musa* fruit extract (G3 - 327.22 ± 13.5 nM/g) did not differ significantly from that of normal control.

Diabetic control group exhibited a significantly ($P < 0.05$) higher level of lipid peroxides in pancreas (450 ± 17.37 nM/g) when compared with the normal control (357.5 ± 21.2 nM/g). Both the extract and glibenclamide treated groups showed a significantly ($P < 0.05$) lower level of lipid peroxides in pancreas when compared with diabetic control and the levels did not differ significantly from that of normal control. The level of lipid peroxides in pancreas of normal animals treated with the extract (393.33 ± 6.67 nM/g) was similar to that of normal control.

4.2.11 Reduced glutathione in liver and pancreas

Level of GSH in liver and pancreas of different groups are presented in Table 11 and Fig.11. A significant ($P < 0.05$) decrease was observed in the level of liver GSH of diabetic control, G2 (424.29 ± 25.71 µg/g) when compared with the normal control, G1 (795 ± 52.20 µg/g). Both the extract and glibenclamide treated groups, G4 (586.25 ± 14.63 µg/g), G5 (571.82 ± 45.38 µg/g) and G6 (588.33 ± 19.73 µg/g) showed a significant ($P < 0.05$) increase in liver GSH compared with the diabetic control. However, none of the treated diabetic groups showed values homogenous to that of normal control. The level of liver GSH showed by normal animals treated with the extract (672.22 ± 12.22 µg/g) did not reveal any significant difference from that of the normal control.

Table 11. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of GSH ((µg/g) in the liver and pancreas of normal and diabetic rats (Mean ± SE, n = 6)

Groups	Liver	Pancreas
G1	795 ± 52.20[a]	712.5 ± 54.24[a]
G2	424.29 ± 25.71[b]	464.29 ± 4.94[b]
G3	672.22 ± 12.22[a]	783.33 ± 25.55[a]
G4	586.25 ± 14.63[c]	771.25 ± 43.44[a]
G5	571.82 ± 45.38[c]	682.72 ± 47.62[a]
G6	588.33 ± 19.73[c]	655 ± 23.20[a]

Values not bearing a common superscript letter (a, b and c) in a column differ significantly (P<0.05).

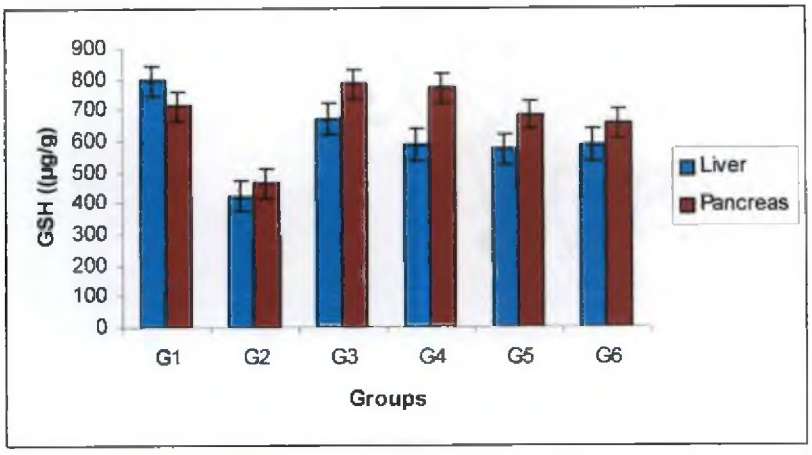

Fig. 11. Effect of ethanol extract of *Musa* AAA (Chenkadali) fruits on the level of GSH (µg/g) in the liver and pancreas of normal and diabetic rats

(G1– Normal control rats, G2 – Diabetic control rats, G3 – Normal rats administered with the extract @ 500 mg/kg, G4 –Diabetic rats administered with the extract @ 500 mg/kg, G5 – Diabetic rats administered with the extract @ 1000 mg/kg, G6 – Diabetic rats administered with glibenclamide @ 0.5 mg/kg)

Reduced glutathione level decreased significantly ($P < 0.05$) in the pancreas of diabetic control group (464.29 ± 4.94 µg/g) in comparison with normal control (712.5 ± 54.24 µg/g). Treatment with the extract at both doses as well as glibenclamide significantly ($P < 0.05$) increased the level of GSH in G4 (771.25 ± 43.44 µg/g), G5 (682.72 ± 47.62 µg/g) and G6 (655 ± 23.20 µg/g) when compared with the diabetic control. Level of GSH in all the treated diabetic groups did not vary significantly from that of normal control. Normal animals treated with the extract showed the highest level of GSH (783.33 ± 25.55 µg/g) but it was not significantly different from that of normal control (712.5 ± 54.24 µg/g). Though not significantly different, diabetic group treated with the extract at 500 mg/kg also showed a higher GSH content compared with the normal control.

4.3 Histopathology of liver and pancreas

Representative samples of liver and pancreas of all groups were subjected to histopathological examination. Microscopic examination of the pancreas (Fig. 12) and liver (Fig. 13) of control group (G1) revealed normal histological architecture.

In the diabetic control group (G2), histopathological changes could be seen in both the pancreas (Fig. 14) and liver (Fig. 15). Pancreas showed scattered necrosis of islet cells, reduction in the cell numbers and cellular debri in the zone. There was loss of compactness of the structure and the necrosed cells appeared shrunken with pyknotic nuclei. The acinar cells remained intact with congestion of inter-acinar capillaries. Zymogen granules in the acinar cells appeared prominent. Multifocal diffuse haemorrhage and necrosis, focal coagulation of hepatocytes and central venous congestion were the lesions observed in the liver.

In pancreas of normal rats treated with the extract (G3), well developed zones of islet were observed in many of the acini (Fig. 16). Islet cells were more compact and evenly distributed. The nucleus and the cytoplasmic details of the cells were well preserved. Acinar cells with zymogen granules remained in normal architecture. In the liver (Fig. 17), radiating cells around central vein with vesicular nuclei were observed. Sinusoids in the liver remained normal.

In diabetic rats treated with the extract at 500 (G4) and 1000 mg/kg (G5) doses, both islet and acinar zones remained active (Fig. 18 and Fig. 20). The islet cells were intact, well preserved and some of the cells appeared regenerating with hyperchromatic nuclei and abundant cytoplasm. Liver showed focal haemorrhage and congestion (Fig. 19 and Fig. 21). Binucleated hepatocytes and hepatocytes with distended nuclei and condensed chromatin with moderately basophilic cytoplasm appeared which indicated regeneration.

In glibenclamide treated group (G6), islet cell zones in most of the acini appeared normal except a few in which necrosed and hyalinised cells could be seen (Fig. 22). In such zones, regenerating islet cells appearing hyperchromatic were seen around the necrosed ones. Both the islet zones and cellularity were more in a few of the acini. In case of liver, most of the lobules remained normal (Fig. 23). In certain lobes, the hepatocytes in the midzonal regions appeared regenerating. The cells were prominent, moderately crowded and contained large nuclei with condensed chromatin and pale to basophilic cytoplasm. Amidst this or away from this, necrosed hepatocytes could be seen.

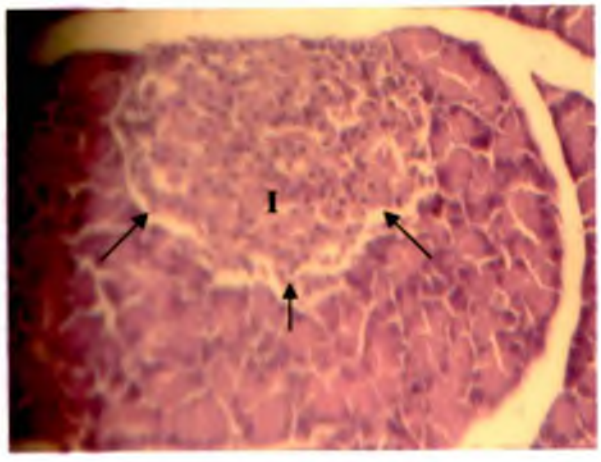

Fig. 12

Normal control – Pancreas
(H&E × 100)
I – Intact islet cells

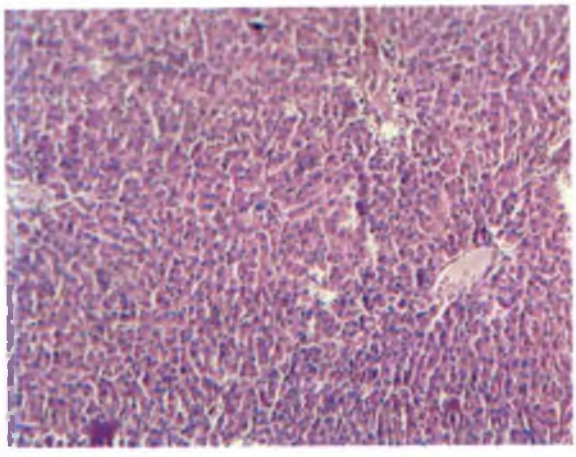

Fig. 13

Normal control – Liver
(H&E × 100)

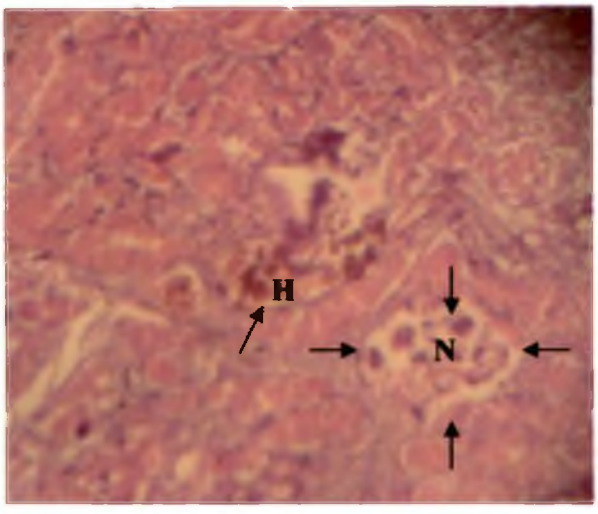

Fig.14.

Diabetic control - Pancreas
(H&E × 100)
N– Necrosed islet cells
H - Haemorrhage

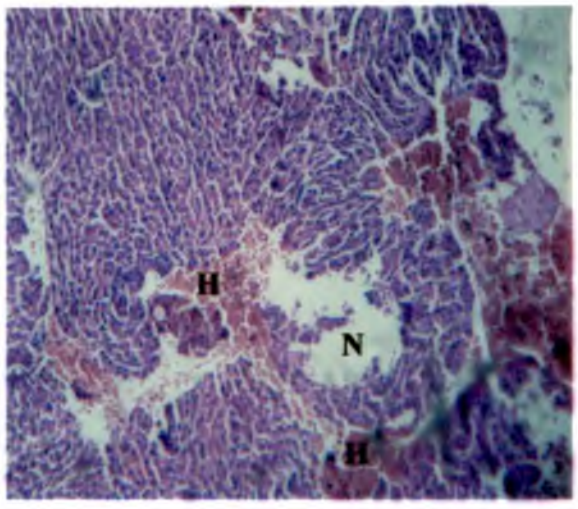

Fig.15

Diabetic control -Liver
(H&E × 100)
H- Haemorrhage
N – Necrosed zone

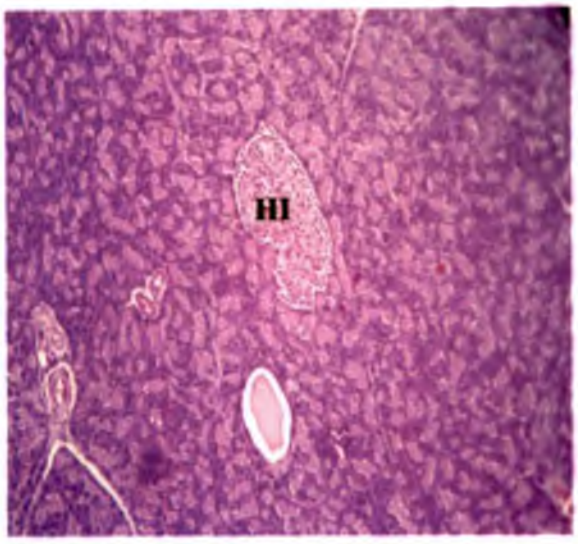

Fig.16

Normal rats given extract
Pancreas (H&E × 100)
HI-Highly compact islet cells

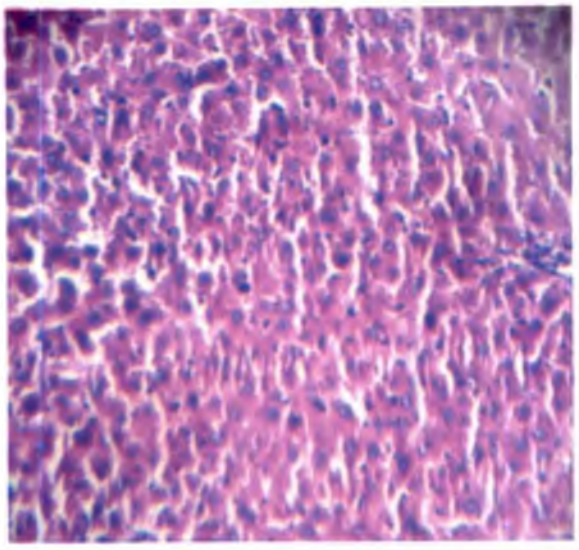

Fig. 17

Normal rats given extract
Liver (H&E × 100)
Normal hepatocytes

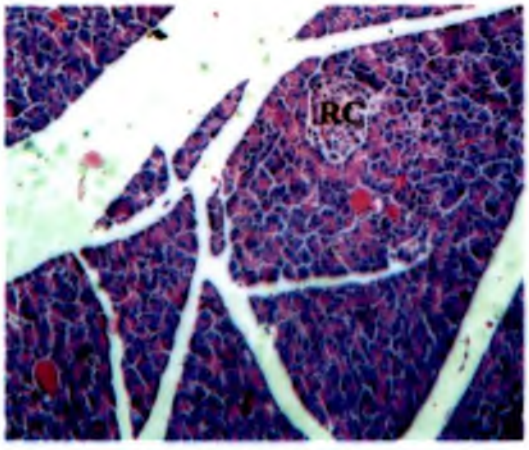

Fig.18

Treated with the extract (500 mg/kg)
Pancreas (H&E × 100)
RC - Regenerating cells with hyperchromatic nucleus

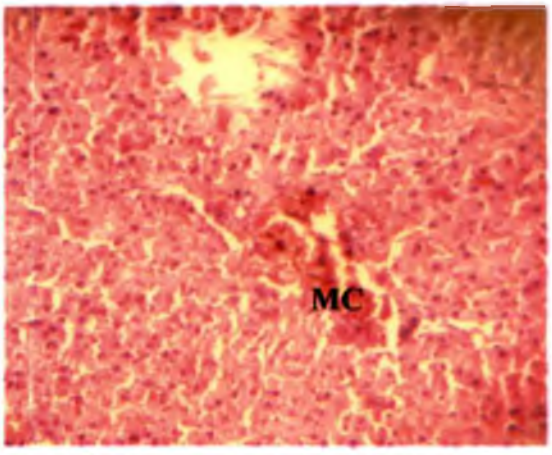

Fig.19

Treated with the extract (500 mg/kg)
Liver (H&E × 100)
MC- Mild congestion

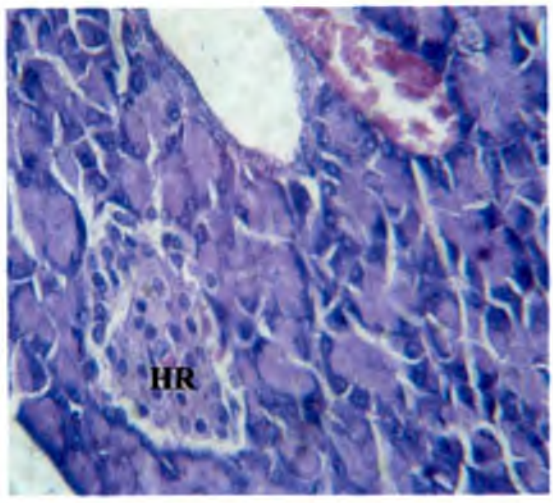

Fig. 20
Treated with the extract (1000 mg/kg)
Pancreas ((H&E × 400)
HR- Large hyperchromatic cells indicating regeneration

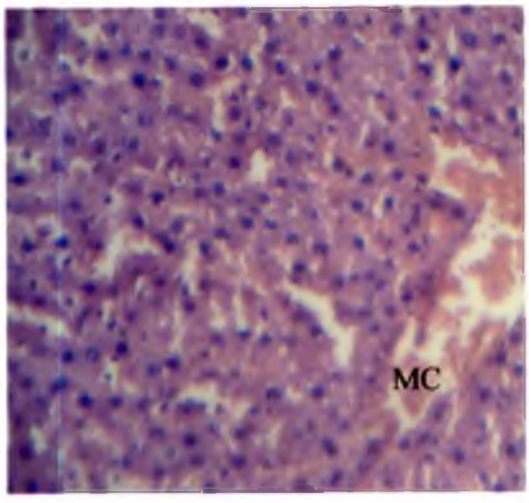

Fig. 21
Treated with the extract (1000 mg/kg)
Liver (H&E × 100)
L – Normal hepatocytes
MC – Mild congestion

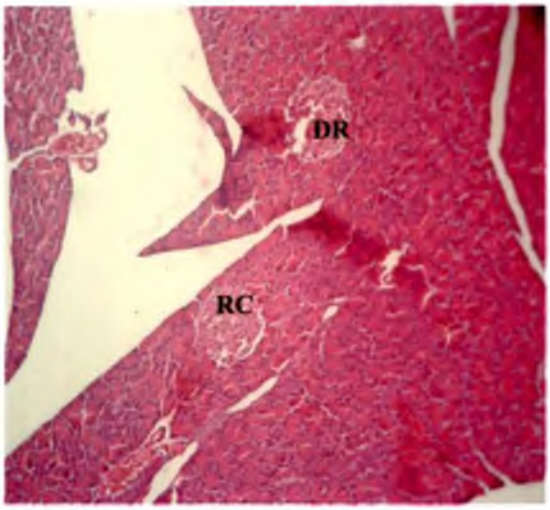

Fig. 22

Treated with glibenclamide (500 µg/kg)
Pancreas (H&E × 100)
DR - Damaged cells undergoing regeneration
RC- Regenerated cells

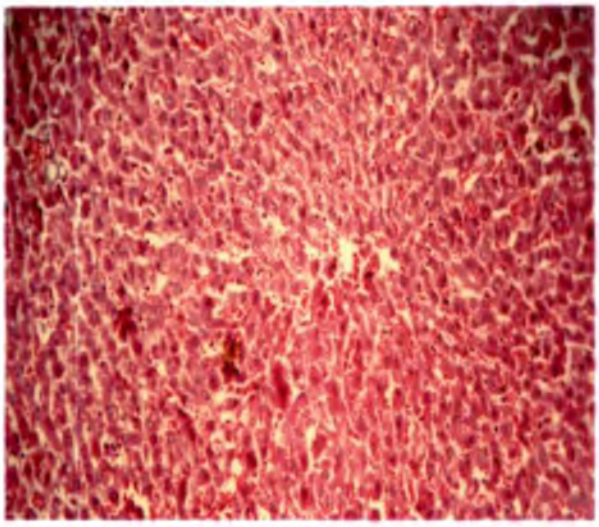

Fig. 23

Treated with glibenclamide (500 µg/kg)
Liver (H&E × 100)
L – Apparently normal hepatocytes

Discussion

5. DISCUSSION

The present study was undertaken to assess the therapeutic effect of ethanol extract of the fruits of *Musa* AAA (Chenkadali) in diabetes mellitus (alloxan induced diabetic rats). Banana plant (*Musa* sp.) is reported to have many medicinal effects and is used to treat anaemia, gastric ulcers, high blood pressure, constipation, oxidative stress induced neuro-degenerative diseases, depression and urolithiasis (Jain *et al.*, 2007). Inflorescence, stem, flowers and roots have been used traditionally for the treatment of diabetes mellitus (Jain, 1968). Oral antihyperglycaemic drugs and insulin injections are routinely used for the treatment of diabetes mellitus. Antihyperglycaemic drugs cause side effects such as weight gain, fluid retention, peripheral oedema, central nervous system disorders, skin reactions, aplastic/hemolytic anaemia, flatulence, bloating and nausea (Ryysy, 2001). Insulin and other medications are expensive and not affordable, especially for poor patients. In this context, the study is relevant in exploring the antidiabetic potential of fruits of *Musa* AAA (Chenkadali), which are cheap and easily available.

Phytochemical analysis

Phytochemical analysis of the extract revealed the presence of detectable levels of steroids, flavonoids, glycosides, phenolic compounds, diterpenes and triterpenes. Alkaloids, tannins and saponins were not detected in the extract. Many of the above mentioned compounds were detected in various other plant extracts. Dhanabal *et al.* (2005) demonstrated the presence of flavonoids and glycoside principles in the ethanol extract of flowers of *Musa sapientum*. Raghavan and Krishnakumari (2006) observed the presence of tannins, saponins and flavonoids in the extract of bark of *Terminalia arjuna*. Presence of alkaloids, glycosides and saponins were detected in alcohol extract of *Celosia argentea* seeds (Vetrichelvan *et*

al., 2002). Phytochemical analysis of aqueous extract of leaves of *Cocculus hirsutus* showed presence of alkaloids, phenolic compounds, flavonoids and glycosides (Badole *et al.*, 2008). Ethanol extract of *Cassia kleini* leaves contained terpenoids, coumarins and saponins (Babu *et al.*, 2003).

The hypoglycaemic, hypolipidaemic and antioxidant properties of the extract could be attributed to the presence of these principles. Flavanoids, phenolic compounds (Badole *et al.*, 2008), terpenoids, coumarins (Babu *et al.*, 2003) and diterpenoids (Latha and Pari, 2004) have already been reported to have antidiabetic effect.

Induction of diabetes mellitus

Diabetes mellitus could be induced experimentally using diabetogens such as, alloxan or streptozotocin (STZ). Alloxan is a chemical diabetogen and the product of its reduction, dialuric acid, establishes a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive 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 β cells (Szkudelski, 2001).

Streptozotocin, is a broad-spectrum antibiotic from *Streptomyces achromogenes*, enters the β cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation that leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a

result of the streptozotocin action, β cells undergo destruction by necrosis (Szkudelski, 2001).

In the present study, alloxan was used to induce diabetes mellitus in rats. Pancreatic beta cell cytotoxicity of alloxan was also reported by earlier workers. The chemical induces the production of H_2O_2, O_2^- and HO^- that first damage and later bring about the death of beta cells (Heikkila *et al.*1976; Soto *et al.* 1994). Winterbourn and Munday (1989) suggested the formation of ROS, during the redox cycle of alloxan, which was responsible for the destruction of beta cells of islets of Langerhans leading to diabetogenic effect. Alloxan administration produced elevated level of lipid peroxides, hydroperoxides and conjugated dienes which was a clear indication of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage (Sabu and Kuttan, 2004). Free radical generation and elevated level of lipid peroxides indicated the damage of other organs such as liver, kidney and the haemopoietic system though, at a lesser extent in comparison to β cells of pancreas (Sabu *et al.*, 2002).

5.1 PHYSIOLOGICAL PARAMETERS

5.1.1 Body weight

There was no substantial reduction in the body weight of alloxan-administered rats in comparison with the normal rats on day 0 (7 days after alloxan administration). Moreover, diabetic control rats also did not show any significant reduction in the body weight by day 15, but it became significant by day 30 of experiment when compared to the normal control. Absence of significant reduction in the body weight of diabetic control rats during the first three weeks following alloxan administration might be due to the utilization of other body reserves, such as fat, for energy purpose.

which might not have reflected in a substantial reduction in the body weight. Significant reduction in the body weight observed by day 30 might be due to increased catabolism of proteins, that led to muscle wasting, which is in agreement with the reports of Vetrichelvan *et al.* (2002). Moreover, absolute or relative deficiency of insulin in alloxan-induced diabetic rats led to inhibition of anabolic processes such as, glycogenesis, lipogenesis and protein synthesis. This also agrees with the findings of Ahmed *et al.* (2005), who observed a significant reduction in body weight of alloxan diabetic rats in a period of 21 days. Badole *et al.* (2008) reported a decrease in the body weight in a period of 28 days by the intravenous administration of alloxan. Ene *et al.* (2007) observed a 10 % decrease in body weight of diabetic control rats in 10 weeks time by intravenous injection of alloxan. Streptozotocin diabetic rats also showed a significant reduction in the body weight in a period of 15 days (Babu *et al.*, 2003).

Diabetic rats treated with the extract at both doses and the reference drug, glibenclamide, did not show any significant variation in body weight as compared with normal control. This might be due to its ability to increase the utilization of glucose for body's energy needs. This is in accordance with the findings of earlier workers on diabetic rats treated with hypoglycaemic plant extracts. According to the studies of Vetrichelvan *et al.* (2002), alcoholic extract of seeds of *Celosia argentea* prevented weight loss in extract treated diabetic rats when administered for 15 days. *Trigonella foenum graecum* seed powder caused a significant and consistent weight gain in alloxan diabetic rats when incorporated at the level of 5 % in the diet for 21 days (Raju *et al.*, 2001). Similar effect was observed when aqueous extract of leaves of *Cocculus hirsutus* was administered in alloxan diabetic mice for 28 days (Badole *et al.*, 2008). Another study by Aguilar *et al.* (2005) showed that administration of dichloromethane extract of *Ibervillea sonorae* root for 41 days significantly improved body weight of alloxan diabetic rats. Oral administration of aqueous suspension of

Cuminum cyminum for 6 weeks to alloxan diabetic rats prevented reduction in body weight when compared to diabetic control rats (Dhandapani *et al.*, 2002). Pari and Latha (2002) noted that administration of aqueous extract of flowers of *Cassia auriculata* in alloxan diabetic rats brought the body weight back to that of normal control rats, which was similar to that observed in glibenclamide treated group. Babu *et al.* (2003) observed that oral administration of ethanol extract of *Cassia kleini* leaves for 15 days significantly prevented reduction in body weight of streptozotocin diabetic rats.

The body weight of normal rats administered with *Musa* fruit extract did not show any significant difference from that of normal control at all stages of analysis. Similar observation was reported by Veeramani *et al.* (2008) when ethanol extract of leaves of *Cardiospermum halicacabum* was administered to normal rats. Oral administration of powdered leaves of *Eclipta alba* to normal rats for 60 days also did not produce significant variation in body weight compared to normal control rats (Ananthi *et al.*, 2003).

5.1.2 Weight of liver

Significant decrease in liver weight was observed in diabetic control rats. which was in accordance with the reports of Raju *et al.* (2001). El-Soud *et al.* (2007) observed a significant reduction in liver weight in streptozotocin diabetic rats. Insufficient insulin level might have, enhanced catabolic processes including glycogenolysis, caused a reduction in the liver weight.

Treatment with *Musa* extract, at 500 and 1000 mg/kg b.w, was effective in increasing the liver weight and the weight of liver in these groups was similar to that observed in normal control and glibenclamide treated group. Leite *et al.* (2007)

reported that aqueous extract of aerial parts of *Parkinsonia aculeata* caused a significant rise in the liver weight of alloxan diabetic rats when administered orally for 16 days. *Trigonella foenum graecum* seeds incorporated at the level of 5 % in the diet for 21 days significantly increased liver weight in alloxan diabetic rats (Raju *et al.*, 2001).

Restoration of liver glycogen might account for the increase in liver weight in treated diabetic animals (Hakkim *et al.*, 2007). Glibenclamide stimulates the production of insulin leading to glycogenesis and inhibits glycogenolysis in liver that might have contributed to the maintenance of liver weight (Akhtar, 2007).

Administration of the extract to normal rats did not produce any significant difference in the liver weight. This was in accordance with the findings of Afaf *et al.* (2008). They reported that oral administration of aqueous extract of *Aloe vera* leaves for 6 days did not produce any change in the liver weight of normoglycaemic rats. This shows that the extract might not have a stimulatory effect on glycogen synthesis in normoglycaemic rats.

5.2 BIOCHEMICAL PARAMETERS

5.2.1 Haemoglobin

Haemoglobin level significantly decreased in alloxan-administered rats. Earlier workers reported similar observations (Pari and Latha, 2002, Ananthi *et al.*, 2003, Kaleem *et al.*, 2006, Chauhan *et al.*, 2007, Rajagopal and Sasikala, 2008). Similar observation was also reported in streptozotocin diabetic rats (Gupta *et al.*, 2005, Veeramani *et al.* 2008).

Lowering of haemoglobin level may be due to the increased formation of glycated haemoglobin (HbA1c) along with depressed synthesis of haemoglobin. In uncontrolled or poorly controlled diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin, increases the level of HbA1c (Pari and Satheesh, 2004). A study by Inomata *et al.* (1997) suggested that structural renal abnormality, possibly interstitial fibrosis and factors such as, glycation of erythropoetin, modification of erythropoetin receptor by glycation, hyperglycemia induced damage of erythroid precursor cells or abnormal erythrocyte half-life due to increased osmotic stress might play a role in the etiology of anaemia in diabetes mellitus. In addition, hyperglycaemia induces the exposure of phosphatidylserine on the red cell surface, a signal in aged RBC, lead to red cell recognition and removal by the reticuloendothelial system from circulation (Thomas *et al.*, 2005).

Administration of the extract at both the doses increased the level of haemoglobin, which is in agreement with the findings of many other workers, where various plant extracts were used to treat diabetic rats. Comparison between the extract and glibenclamide revealed that the extract was better in increasing haemoglobin level and the level was similar to that of normal control.

Pari and Latha (2002) noted that administration of aqueous extract of flowers of *Cassia auriculata* in alloxan diabetic rats brought the haemoglobin level back to that observed in normal control rats. The level was also comparable with that of the glibenclamide treated group. Oral administration of aqueous suspension of *Cuminum cyminum* for 6 weeks to alloxan diabetic rats resulted in significant increase in the level of haemoglobin (Dhandapani *et al.*, 2002). Pari and Venkateswaran (2002) noted that oral administration of aqueous extract of *Scoparia dulcis* leaves for 45 days resulted in a significant increase in haemoglobin levels of alloxan diabetic rats. Rajagopal and Sasikala (2008) reported that administration of ethanol extract of

flowers of *Nymphea stellata* to alloxan diabetic rats significantly increased the level of haemoglobin. Oral administration of aqueous and methanol extract of *Picrorhiza kurrooa* for 15 days resulted in significant increase in the level of haemoglobin in alloxan diabetic rats (Chauhan *et al.*, 2007). Streptozotocin diabetic rats when treated with both aqueous and ethanol extracts of *Cyanodon dactylon* plant and glibenclamide separately for 45 days significantly increased haemoglobin to almost normal level (Mahesh and Brahatheeswaran, 2007). Kaleem *et al.* (2006) reported that administration of aqueous leaf extract of *Annona squamosa* to streptozotocin diabetic rats significantly increased the level of haemoglobin.

Haemoglobin level in normal rats administered with the extract did not show any significant variation from that of normal control. Similar observations were made by earlier workers on diabetic rats treated with other plant extracts. Veeramani *et al.* (2008) reported that ethanol extract of leaves of *Cardiospermum halicacabum* when administered orally for 45 days produced no change in haemoglobin level of normal rats. Administration of *Eclipta alba* leaf suspension for 60 days in normal rats did not cause any significant variation in haemoglobin level (Ananthi *et al.*, 2003). Studies of Mahesh and Brahatheeswaran (2007) also showed that oral administration of aqueous and ethanol extracts of *Cyanodon dactylon* plant for 45 days was not effective in bringing about any significant variation in haemoglobin levels of normal rats.

Increased haemoglobin level in the extract treated groups might be due to reduced haemoglobin glycation subsequent to lowered blood glucose level. The extract might also have a stimulatory effect on the erythropoetic cells or might have protected the erythropoetin or erythropoetin receptor from modification by glycation since the extract exhibited an antihyperglycaemic effect. Better effect showed by the extract in comparison to glibenclamide might be due to the presence of minerals and

other nutritional factors in the extract that stimulate the production of red blood cells and thereby elevating the level of haemoglobin in addition to its hypoglycaemic effect. The findings of Nadkarni (1976) support this observation. He reported the use of banana fruits in the treatment of anaemia due to its high content of iron, which might have a stimulatory effect on the production of haemoglobin.

5.2.2 Glucose

Significant increase was observed in the level of blood glucose after alloxan administration. Similar observations have also been reported by earlier workers (Raghavan and Krishnakumari, 2006, Yousef *et al.*, 2006, Ebong *et al.*, 2008, Gokce *et al.*, 2008). Alloxan induces the formation of ROS with simultaneous increase in cytosolic calcium concentration resulting in rapid destruction of β cells of pancreas (Szkudelski, 2001), which in turn reduces the level of insulin, decrease tissue glucose utilization, augments hepatic glycogenolysis, gluconeogenesis and finally ends up in hyperglycemia (Raju, 2001).

Administration of the extract for 30 days in diabetic rats at both the doses caused significant reduction in blood glucose level, while it did not reach to that of normal control. Comparison between the effects of the extract and glibenclamide showed that the hypoglycaemic effect of the extract after 30 days treatment was almost similar to 15 days treatment with the drug, glibenclamide. From the results it appears that the action of the extract is slow and steady while glibenclamide exhibited a rapid hypoglycaemic action. Moreover, treatment with 500 mg/kg dose of the extract decreased the glucose level than that of glibenclamide treated group.

Pari and Latha (2002) compared the effects of glibenclamide and aqueous extract of flowers of *Cassia auriculata* in alloxan diabetic rats and found that both

showed similar blood glucose lowering effect when administered orally for a period of 30 days. Oral administration of ethanol extract of aerial parts of *Bacopa monnieri* for 10 days produced significant lowering of blood glucose level in alloxan diabetic rats and was comparable with glibenclamide treatment (Ghosh *et al*, 2008). They opined that the insulin-like action of the extract might have caused an increase in peripheral glucose utilization and thereby decreased blood glucose concentration. Kumar *et al.* (2008) reported that separate administration of ethanol and petroleum ether extract of leaves of *Phyllanthus reticulatus* produced a significant reduction in glucose level in alloxan diabetic rats. Oral administration of ethanol extract of whole plant of *Posidonia oceanica* for 15 days at 50, 150, and 250 mg/kg also resulted in dose-dependent decrease in blood glucose level (Gokce *et al.*, 2008). Independent administration of ethanol extracts of leaves of *Coccinia cordifolia* and *Catharanthus roseus* in single doses reduced blood glucose by 14 days in alloxan diabetic rats. The possible mechanisms explained were by increasing glycogenesis, inhibiting gluconeogenesis in the liver or inhibiting the absorption of glucose from the intestine (Akhthar *et al.*, 2007).

Normal rats administered with the extract did not show any reduction in the blood glucose level. This shows that the extract do not exert any hypoglycaemic effect by increasing the utilization of glucose in normoglycaemic animals whereas, it effectively prevented the rise in blood glucose level and at the same time increased the utilization of glucose in hyperglycaemic animals. This was in accordance with the findings of Viana *et al.* (2004). They observed that aqueous extract of leaves of *Cissus sicyoides* did not produce any significant change in the blood glucose level of normal rats whereas it effectively lowered the elevated blood glucose level in alloxan diabetic rats. In another study conducted by Vetrichelvan *et al.* (2002) showed that treatment with alcoholic extract of *Celosia argentea* seeds for a period of 15 days produced a significant decrease in the blood glucose levels of diabetic rats, but not in

the normal rats. They opined that it might be due to increased tissue uptake of blood glucose and its utilization.

Hypoglycaemic action of the extract, only in hyperglycaemic subjects is similar to that of biguanid group of hypoglycaemic drugs. These drugs do not cause hypoglycaemia in normal subjects even when taken in excessive dose. It acts by inhibiting hepatic glucose production and increase the uptake of glucose by muscle tissues (Afaf *et al.* 2008).

Phytochemical analysis of the extract revealed the presence of flavonoids, terpenoids, phenolic compounds, glycosides and steroids. Flavonoids, sterols, triterpenoids and phenolics are known to be bioactive antidiabetic principles (Bever, B.O., 1986, Ivora *et al.*, 1989, Rhemann, Z.K.A., 1989, Rao *et al.*, 1997).

In vitro studies demonstrated that some flavonoids modify the insulin-secreting capacity of islet cells. Genistein, an isoflavone increased glucose-stimulated insulin secretion in the beta-pancreatic cell line MIN6 (mouse-derived) (Ohno *et al.*, 1993) as well as in cultured islet cells from mice (Jonas *et al.*, 1995) and rats (Sorenson *et al.*, 1994). Flavonoids were reported to reduce β cell apoptosis, a common feature of diabetes mellitus (Pinent, 2008) and prevent cytokine induced pancreatic cell death (Matsuda *et al.*, 2005). They modulate cell proliferation in osteoblasts (Harmon and Harp, 2001) and fibroblasts (Notoya *et al.*, 2004) and hence might have an effect on proliferation of β cells in addition to increasing the level of pancreatic antioxidant enzymes (Pinent, 2008). In alloxan-induced diabetes, flavonoids like epicatechin have also shown to act by β cell regeneration (Chakravarthy *et al.*, 1980). It has been demonstrated that triterpenoids have an insulin-like activity *in-vitro* and *in-vivo* (Sakurai *et al.*, 2002). *In vitro* studies conducted in rat hepatoma cells showed that phenolic compounds induced the mRNA

expression of glycolytic enzymes and inhibited the expression of enzymes associated with gluconeogenesis (Valentova *et al.*, 2007). Therefore, it is suggested that the extract might be potentiating insulin secretion from remaining β-cells of pancreas or decreasing β-cell death and increasing its proliferation/ regeneration or it might be due to insulin-like activity of the compounds present in the extract. It is also suggested that increasing the expression of mRNA of glycolytic enzymes or inhibiting the expression of mRNA associated with gluconeogenesis and glycogenolysis could be responsible for fighting hyperglycaemia. It may also be due to retardation of intestinal glucose absorption (Hakkim *et al.*, 2007).

The observations made in the study show that treatment with the extract for 30 days could significantly decrease the level of blood glucose in diabetic rats, but the level was significantly higher than normal control. Therefore, it is suggested that administration of the extract for a prolonged period (more than 30 days) might be required to bring back the glucose level in the diabetic rats to that of normal control.

Glibenclamide comes under sulphonyl urea class of antidiabetic drugs, which inhibits ATP-sensitive K+ channels causing depolarization of the cells and insulin secretion. Lowering of blood glucose is brought about by the enhanced production of insulin (Akhtar, 2007).

5.2.3 Fructosamine

Glucose molecules are joined to the free amino group of serum proteins by a nonenzymatic process to form stable ketoamines known as fructosamine. The amount of fructosamine in serum increases in diabetes mellitus owing to the abnormally high concentration of glucose in blood. The concentration of fructosamine in serum thus reflects the degree of glycaemic control attained by the

diabetic patient and is useful in monitoring the effectiveness of therapy over a period of 2-3 weeks, in a manner analogous to the determination of glycated hemoglobin (Armbruster, 1987). In the present study, fructosamine levels were estimated on day –7, 0 and 21 since, serum protein turn over takes place in every 2-3 weeks.

Alloxan administration resulted in an increase in fructosamine level. Zhang and Cui (2007) and Hannan *et al.* (2006) also observed high level of fructosamine in alloxan diabetic rats. A study conducted by Pavana *et al.* (2008) showed an elevated level of hexosamine in the serum of streptozotocin diabetic rats. Ananthi *et al.* (2003) observed that intraperitoneal administration of alloxan resulted in an increase in glycated haemoglobin. Gupta *et al.* (2005) reported that streptozotocin diabetic rats showed significantly higher levels of glycated haemoglobin compared to that of normal control.

Treatment with the extract at both doses significantly decreased the level of fructosamine as compared to the diabetic control, but the levels were significantly higher than the normal control. However, glibenclamide treated group showed a level similar to that of normal control. This observation correlates with the blood glucose concentration of extract and glibenclamide treated groups. The extract treated groups showed a gradual decrease in the blood glucose concentration while glibenclamide treated group showed a rapid reduction, which is reflected correspondingly in the level of fructosamine of the treated groups. Zhang and Cui (2007) noted that oral administration of *Tremella aurantialba* broth significantly reduced the levels of fructosamine and glycosylated haemoglobin in alloxan diabetic rats when administered orally for a period of 4 weeks. The soluble dietary fibre fraction of *Trigonella foenum graecum* when administered orally twice daily for 28 days produced significant lowering of serum fructosamine level in streptozotocin diabetic rats (Hannan *et al.*, 2003). In another study conducted by Hannan *et al.*

(2006), administration of hot water extract of husk of *Plantago ovata* for a period of 28 days significantly reduced fructosamine level in streptozotocin diabetic rats. Pavana *et al.* (2008) assessed the role of ethanol extract of seeds of *Tephrosea purpurea* on hexosamine concentration in streptozotocin diabetic rats. They observed that oral administration of the extract for 45 days significantly decreased the elevated hexosamine level and brought back to that of normal control. Ghosh *et al.* (2008) reported that ethanol extract of aerial parts of *Bacopa monnierei* prevented significant elevation of glycosylated hemoglobin in alloxan diabetic rats when administered orally for 10 days.

Normal animals administered with the extract did not show any variation in fructosamine level. In a study conducted by Veeramani *et al.* (2008), administration of ethanol extract of *Cardiospermum halicacabum* to normal rats did not show any significant change in the level of glycated haemoglobin. In normal rats, non-enzymatic glycation of serum proteins occur only to a limited extent based on the concentration of blood glucose. It appears that the extract has no action on decreasing the level of glycated proteins in normoglycaemic animals.

5.2.4 Liver glycogen

Liver plays an important role in buffering the postprandial hyperglycemia and is involved in synthesis of glycogen. Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen (Hornbrook, 1970).

Alloxan administration produced a significant decrease in liver glycogen level, which is in agreement with earlier reports (Daisy *et al.*, 2007, Fernandes *et al.*, 2007, Dewanjee *et al.*, 2008, Gokce *et al.*, 2008). Reduction in liver glycogen level might be due to alloxan induced islet cell damage and insufficient production of

insulin. Administration of streptozotocin too significantly decreased the level of liver glycogen (Babu *et al.*, 2003, Mahesh and Brahatheeswaran, 2007).

Diabetic rats treated with both doses of the extract showed significant increase in liver glycogen and the values were similar to that of normal control rats. In comparison with glibenclamide treated group, a significant increase in the level of liver glycogen was noticed in the rats treated with 500 mg/kg dose. Though, not significant, the glycogen level was also higher in rats treated with 1000 mg/kg extract when compared with glibenclamide treated group. Previous studies conducted using other plant extracts also showed similar results. Gokce *et al.* (2008) reported that oral administration of ethanol extract of leaves of *Posidonea oceanica* for 15 days produced dose-dependent increase in liver glycogen in alloxan diabetic rats. Oral administration of methanol extract of matured fruits of *Diospyros peregrina* for 14 days, showed a significant increase in liver glycogen content of alloxan diabetic rats which was comparable to glibenclamide treated rats (Dewanjee *et al*, 2008). Administration of ethanol extract of Bitter melon *(Momordica charantia)* fruits produced 100 % increase in liver glycogen compared with the diabetic control (Fernandes *et al.*, 2007). Aqueous and ethanol extracts of aerial parts of Bermuda grass (*Cyanodon dactylon*), when administered separately for 45 days showed a significant increase in liver glycogen of streptozotocin diabetic rats (Mahesh and Brahatheeswaran, 2007).

Presence of various compounds in the extract such as, flavonoids, phenolics, phytosterols and terpenoids might have contributed to increase the level of liver glycogen. Flavonoids increase glucose stimulated insulin secretion from islet cells, which enhances glycogenesis by the reactivation of the glycogen synthase system (Pinent, 2008). Phenolics, phytosterols and flavonoids are antioxidant compounds, that might have decreased the oxidative stress either by scavenging the free radicals

or by increasing the level of antioxidant enzymes (Pinent, 2008). Reduced oxidative stress in islet cells might have favoured production of sufficient insulin to maintain normal carbohydrate metabolism. Insulin-like activity of triterpenoids also prevents glycogenolysis and enhances glycogenesis, thereby increase the level of liver glycogen. Action of glibenclamide is to stimulate the production of insulin and thereby increase the level of liver glycogen (Akhtar, 2007).

Administration of the extract to normal rats did not show any significant variation in liver glycogen level from that of normal control rats which agrees with the findings of Mahesh and Brahatheeswaran (2007) who observed that administration of aqueous and ethanol extracts of *Cyanodon dactylon* for 45 days failed to cause any variation in liver glycogen level of normal rats.

5.2.5 Triacylglycerol and Cholesterol

Administration of alloxan produced hyperlipidemia in rats with a significant increase in the levels of serum triacylglycerol and serum 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 (Gupta *et al.*, 2005, Akhthar *et al.*, 2007, Tenpe and Yeole, 2009). Similar observations were reported in streptozotocin diabetic rats too (Ignacimuthu and Amalraj, 1998, Bhandari *et al.*, 2002, Fernandes *et al.*, 2007).

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 resulting in insufficient production of insulin resulting in diabetes mellitus. Insufficient insulin stimulates hormone sensitive lipase and causes increased mobilization of free fatty acids from the peripheral fat depots leading to abnormally

high concentration of serum lipids. The marked hyperlipidaemia in the diabetic state might, therefore, be regarded as a consequence of the uninhibited actions of lipolytic enzymes on the fat depots (Al-Shamaony *et al.*, 1994). Other hormones such as glucagon and catecholamines, known to increase during diabetes, compound the effect by stimulating lipolysis. High concentration of free fatty acid increases β-oxidation of fatty acids, producing more acetyl CoA leading to more cholesterol formation during diabetes mellitus (El-Soud *et al.*, 2007).

Treatment with the extract at both the doses as well as glibenclamide for a period of 15 days significantly decreased the triacylglycerol level. The level observed in rats treated with 500mg/kg extract was similar to that of normal control. In the other two treated groups, the triacylglycerol level was significantly higher than that of normal control. Treatment for 30 days with both doses of the extract and glibenclamide caused a significant reduction in the level of triacylglycerol and the levels were homogeneous to that of normal control. In the case of serum cholesterol, treatment for 30 days with 500 mg/kg extract and glibenclamide were found to be effective for a significant reduction. Both the extract and drug showed similar effect in reducing the level of triacylglycerol and cholesterol and reached a level similar to that of normal control by 30 days. However, treatment with 1000 mg/kg prevented significant increase in the level of cholesterol as compared to that of days 0, 15 and diabetic control.

These findings are in accordance with the reports of earlier studies using various plant extracts in diabetic rats. Akhthar *et al.* (2007) reported a significant reduction in serum triglycerides and cholesterol on 14^{th} day after treating alloxan diabetic rats independently with a single dose of the ethanol extracts of leaves of *Coccinia cordifolia* and *Catharanthus roseus*. They opined that the reduction could have resulted from the presence of flavonoids and carotene present in the extracts.

Dhandapani *et al.* (2002) demonstrated that oral feeding of an aqueous suspension of *Cuminum cyminum* treatment resulted in a significant reduction in cholesterol and triglycerides of alloxan diabetic rats. Bopanna *et al.* (1997) noted that aqueous suspension of neem kernel powder reduced serum cholesterol and triglycerides in alloxan diabetic rats. Administration of aqueous extract of whole plant of *Scoparia dulcis* for 6 weeks (Pari and Latha, 2006) and ethanol extract of *Cassia kleini* leaves for 2 weeks (Babu *et al.*2003) showed hypolipidaemic effect in a similar manner to glibenclamide in streptozotocin diabetic rats.

Normal rats administered with the extract did not show any hypolipidaemic effect, which is contrary to the findings of Krishnan and Vijayalakshmi (2005). They reported that Flavanoid Rich Fraction extracted from the fruits of two varieties of *Musa paradisiaca* (*Palayamkodan* and *Rasakadali*) produced significant lowering of triglycerides and cholesterol when fed to normal rats. Studies conducted by many others using plant extracts in normal rats agree with the present findings. Kumarappan *et al.* (2007) reported that polyphenolic root extract of *Ichnocarpus frutescens* did not lower hyperlipidaemic indicators such as, total cholesterol and triglycerides in normal rats. Mahesh and Brahatheeswaran (2007) showed that oral administration of aqueous and ethanolic extracts of whole plant of *Cyanodon dactylon* for 45 days to normal rats did not produce any significant reduction in the levels of triglycerides and cholesterol compared to normal control rats. Horigome *et al.* (1992) studied the protective effect of freeze-dried banana pulp in rats, when incorporated into a high fat (lard-50 g/kg, cholesterol-5 g/kg) diet at 300 and 500 g/kg, observed that the pulp at both doses prevented a rise in serum cholesterol level.

The hypolipidaemic effect observed in the present study could be attributed to the presence of flavonoids, terpenoids and phenolic compounds present in the extract (Hakkim *et al.*, 2007). The reduction of serum lipids by *Musa* fruit extract might also

be due to inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract. Since terpenoids are reported to have insulin-like activity (Sakurai *et al.*, 2002), rate of lipogenesis might be normalized by the extract in a way similar to the effect of insulin on lipid metabolism. Or, it could be due to the achievement of normoglycemia by the action of flavonoids (Pinent, 2008) where there was no further degradation of already accumulated lipid for otherwise glucose starved cells.

Hypolipidaemic action of glibenclamide is through the production of insulin. Insulin inhibits lipoprotein lipase and thereby prevents lipolysis. So the elevated level of triglycerides and cholesterol are reverted back to their normal levels.

5.2.6 Alanine amino transferase

The activity of Alanine aminotransferase in the serum is a useful indicator of liver damage since, it is located at a high level in the cytoplasm of hepatocytes and is released into circulation after hepatic damage.

Alloxan diabetic rats showed a significantly increased serum ALT activity which is in agreement with earlier findings (Aguilar *et al.*, 2005, Gokce *et al.*, 2008, Selvan *et al.*, 2008). In diabetes mellitus, ROS, several immunomodulatory factors and chronic inflammatory state might contribute to insulin resistance and liver injury. Nuclear Factor-κB (NF-κB)-dependent inflammatory mediators produced in hepatocytes, such as Tumor Necrosis Factor-α (TNF-α), are most likely to act in a paracrine manner to down regulate insulin sensitivity in the liver and to favour liver injury (El-Soud *et al.*, 2007).

Diabetic rats treated with the extract at 500 mg/kg for 15 days significantly decreased serum ALT activity and was homogeneous with normal control while no significant reduction was observed with 1000 mg/kg and glibenclamide. Thirty days administration of the extract at 500 mg/kg and glibenclamide showed the ALT level similar to that of normal control while the rats treated with 1000 mg/kg, the activity of ALT did not show any variation from that of 15 days treatment. Thus, a dose independent trend was noted in the effectiveness of the extract. Normal animals administered with the extract did not show any change in the serum ALT level.

Earlier studies using various plant extracts in diabetic animals support our findings. Aguilar *et al.* (2005) observed that oral administration of dichloromethane extract from the root of Wereki (*Ibervillea sonorae)* for a period of 41 days reduced the level of serum ALT in alloxan diabetic mice. Methanol extract of aerial parts of Artanema (*Artanema sesamoides*) when administered for a period of 14 days produced significant reduction in the levels of ALT in streptozotocin diabetic rats (Selvan *et al.*, 2008). Kaleem *et al.* (2008) reported that aqueous extract of leaves of *Annona squamosa* when administered orally for 30 days significantly reduced the elevated levels of ALT observed in streptozotocin diabetic rats. Hakkim *et al.* (2007) demonstrated that oral administration of aqueous extract of flowers of *Cassia auriculata* for 30 days significantly decreased the ALT level in alloxan diabetic rats, and the effect was comparable with that of glibenclamide.

Our observations suggest that the various compounds such as flavonoids, phenolic compounds, terpenoids and phytosterols present in the extract might have protected the hepatic tissue from oxidative injury associated with diabetes mellitus, by fighting hyperglycaemia or by scavenging the free radicals.

5.2.7 Lipid peroxidation

Oxidation of lipid molecules of biological membrane causes membrane damage resulting in the development of several physiological and pathological disorders (Chaturvedi, and Sigale 2007). Malondialdehyde, a secondary product of lipid peroxidation, is known to cause cross-linkage of membrane components containing amino groups that makes the membrane fragile. Lipid peroxidation causes serious damage to cell membranes thereby leakage of intracellular enzymes resulting in loss of cell function and death of cells. Estimation of thiobarbituric acid reactive substances are diagnostic indices of lipid peroxidation and tissue injury due to oxidative stress (Blaha, 2004).

A significant increase in the level of lipid peroxides was observed in the tissues of pancreas and liver by the administration of alloxan, which is in agreement with the findings of earlier workers (Latha and Pari, 2004, Kaleem *et al.*, 2006, El-Soud *et al.*, 2007). Significant increase in the level of lipid peroxides in liver (Sabu and Kuttan, 2004, Ghosh *et al.* 2008) and pancreas (Chauhan *et al.*, 2008) of alloxan diabetic rats were also reported by others. Increased levels of TBARS in liver and pancreas of streptozotocin diabetic rats were also reported (Bhandari *et al.*, 2002).

Hyperglycaemia as well as the redox cycle products of alloxan might be attributed to increased formation of free radicals in diabetes mellitus. It has been suggested that increased blood glucose concentration, which upon autoxidation generate free radicals and attenuate antioxidant mechanisms, creating a state of oxidative stress and endothelial injury (El-Soud *et al.*, 2007). In addition, glucose is known to induce lipid peroxidation through activation of lipoxygenase enzymes (Rajeswari *et al.*, 1991). Diabetogenic agent, alloxan also generates free radicals during its redox cycle (Szkudelski, 2001).

Treatment with *Musa* fruit extract at 500 mg/kg dose proved to be effective in decreasing the lipid peroxidation in the tissues of liver and pancreas of alloxan diabetic rats, which was comparable to that of glibenclamide treated rats and normal control. However, on comparison with diabetic control, though there was a significant reduction in the level of lipid peroxides in liver and pancreas of rats treated with 1000 mg/kg of the extract, the level in liver was significantly higher and no significant difference was observed in pancreas when compared to glibenclamide treated group. Between the two doses of the extract, 500 mg/kg dose was found to be more effective in reducing the level of lipid peroxides in both tissues.

Studies using various plant extracts in alloxan diabetic rats showed similar findings. Ghosh *et al.* (2008) reported that oral administration of ethanol extract of aerial parts of *Bacopa monnieri* for 10 days significantly decreased the levels of TBARS in liver tissue of alloxan diabetic rats. They reported that the effect was comparable with glibenclamide. A study conducted by Latha and Pari (2004) showed that aqueous extract of *Scoparia dulcis* plant was comparable to glibenclamide in reducing the elevated TBARS and hydro-peroxides in streptozotocin diabetic rats when administered orally for 6 weeks. Administration of chloroform extract of *Musa sapientum* flowers for 30 days significantly reduced the level of TBARS in alloxan diabetic rats (Pari and Umamaheshwari, 2000). Sabu and Kuttan (2004) reported that methanol extract of leaves of *Aegle marmelos* when administered for a period of 12 days significantly lowered the level of lipid peroxides in the liver of alloxan diabetic rats. Perez-Perez *et al.* (2006) reported the *in vitro* antioxidant effect of phosphate buffered saline (PBS) extract of fruits of interspecies crossed varieties of *Musa acuminate* and *Musa balbisiana* on rat brain homogenates. They observed that extracts decreased the concentration of lipid hydroperoxides and malondialdehyde in brain tissue. Oral administration of aqueous and methanolic extracts of *Picrorhiza*

kurrooa for 15 days resulted in significant decrease in the level of lipid peroxides in pancreas of alloxan diabetic rats (Chauhan *et al.*, 2008).

Hypoglycaemic activity of the extract could contribute to the lowering of free radical formation and lipid peroxides in tissues. Phytochemical analysis of the extract showed the presence of flavonoids, diterpenoids and phenolic compounds, which are antioxidants that scavenge the free radicals, generated during diabetes mellitus. Many *in vitro* studies have demonstrated the antioxidant potential of phenolics as direct aqueous phase radical scavengers (Rice-evans *et al.*, 1995). Free radicals are compounds that have unpaired electrons and are deleterious to the cells. Being good electron donors, phenolic compounds show the reducing power and prevent the formation of lipid peroxides (Pinent, 2008).

5.2.8 Reduced glutathione

Reduced glutathione is an important antioxidant, functions as free radical scavenger. It is a potent-free radical scavenger within the islet of β-cell and is an important factor against the progressive destruction of the β-cell. Decreased glutathione levels in diabetes mellitus and the generation of ROS have been considered to be an indicator of increased oxidative stress.

Administration of alloxan greatly reduced the level of GSH in the liver and pancreas, which indicates the oxidative stress in these tissues. The observations are in accordance with the earlier findings (Sabu and Kuttan, 2004, Kaleem *et al.*, 2006, Ghosh *et al.*, 2008). Significant decline in hepatic GSH in alloxan diabetic rats (Gokce *et al.*, 2008) and mice (El-Shenawy and Abdel-Nabi, 2006) as well as pancreatic GSH in streptozotocin diabetic rats (Yazdanparast *et al.*, 2007) were also reported.

Treatment with *Musa* fruit extract at both doses proved to be effective in replenishing the level of GSH in tissues of liver and pancreas. The effect of the extract was comparable to that of glibenclamide in both tissues. However, GSH content of pancreas in both the extract treated groups were higher than that of glibenclamide treated rats though, not significant. Among the two doses administered, 500 mg/kg increased the level of GSH in pancreas and the level was higher than that of normal control though, there was no significant difference. Many other workers have reported similar observations in their studies with various other plant extracts (Latha and Pari, 2004, Kaleem *et al.*, 2006, Gokce *et al.*, 2008). Gokce *et al.* (2008) observed that oral administration of ethanol extract of leaves of *Posidonea oceanica* produced dose-dependent increase in GSH level of liver tissue of alloxan diabetic rats. Administration of ethanol extract of *Bacopa monnieri* increased the content of GSH in liver of alloxan diabetic rats, which was comparable to that of glibenclamide treated group (Ghosh *et al.*, 2008). Administration of aqueous extract of *Achillea santolina* plant for 30 days significantly increased the level of reduced glutathione in streptozotocin diabetic rats (Yazdanparast *et al.*, 2007).

The antioxidant activity of *Musa* AAA (Chenkadali) might be attributed to the presence of flavonoids and phenolic compounds present in the extract. It has been suggested that dietary polyphenolics play an important role in protecting the body against chronic diseases such as, cancer, cardiovascular diseases (Mojzisova and Kuchta, 2001; Karaca *et al.* 2006) and diabetes mellitus (Knekt *et al.* 2009). Flavonoids can exert their antioxidant activity by various mechanisms, such as, scavenging free radicals, chelating metal ions, or inhibiting enzymatic systems responsible for free radical generation (Blaha *et al.* 2004).

Although, no significant difference was observed in the level of GSH in normal rats administered with the extract, it was higher than that of normal control. These observations agree with the reports of Latha and Pari (2004) who have shown that oral administration of aqueous extract of *Scoparia dulcis* plant for 6 weeks did not produce any significant change in liver GSH of normal rats.

5.3 HISTOPATHOLOGICAL EXAMINATION OF PANCREAS AND LIVER

In the present study, representative samples from tissues of pancreas and liver of all groups of animals were subjected to histopathological examination. Administration of alloxan induced histological changes in the tissues of pancreas and liver.

In the diabetic control (G2) rats, lesions in the pancreas consisted of scattered necrosis of islet cells, reduction in the cell number. There was presence of cellular debri in the islet zone. Hyalinisation and congestion of acinar capillaries were observed. Lesions in liver sections include multifocal diffused haemorrhage, central venous congestion, diffused areas of necrosis and focal coagulation. These observations are in agreement with the findings of Sharma *et al.* (2007). They observed that alloxan administration produced severe necrotic changes in islet cells with disappearance of nuclei. Relative reduction in size and number of islets was observed. Ahmed *et al.* (2005) reported extensive damage to the islets of langerhans and reduced dimensions of islets in alloxan diabetic rats. Similar findings were reported in the histopathological examination of pancreas (Noor *et al.*, 2008) and liver (El–soud *et al.*, 2007, Noor *et al.*, 2008) of streptozotocin diabetic rats. They reported that the hepatocytes of diabetic control rats showed shrunken nuclei, periportal necrosis, granular cytoplasm, congested portal vessels as well as areas of inflammatory cell infiltration.

Alloxan administration induces the production of superoxide radicals that undergoes dismutation to form hydrogen peroxide. Along with the formation of highly reactive free radicals, cytosolic calcium concentration increases massively resulting in rapid destruction of β cells (Szkudelski, 2001). Alloxan produces time- and concentration-dependent degenerative lesions of the pancreatic ß-cells. In diabetes mellitus, ROS, several immunomodulatory factors and chronic inflammatory state might contribute to insulin resistance and liver injury (El- Soud *et al.*, 2007).

Treatment with *Musa* fruit extract at both the doses showed a tendency to normalize the histological architecture of pancreas and liver. Cells undergoing regeneration with hyperchromatic nucleus were seen in the pancreas of both these groups. Cells remained active. The hepatocytes remained apparently normal except for focal congestion. Pancreas and liver of glibenclamide treated group also showed similar appearance on histological examination. These findings correlate with the biochemical parameters of the treated groups.

Earlier workers have also reported regeneration of islet cells following treatment with various plant extracts as well as glibenclamide. Oral administration of aqueous extract of leaves of *Elephantopus scaber* when administered for 12 weeks resulted in regeneration of islet cells in alloxan diabetic rats (Daisy *et al.* 2007). Partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia were observed in alloxan diabetic rats after oral administration of aqueous and cold extracts of leaves of *Terminalia catappa* for a period of 21 days. Similar results were observed by administration of glibenclamide also (Ahmed *et al.*, 2005). Jothivel *et al.* (2007) observed that alloxan diabetic rats treated with methanol leaf extract of *Costus pictus* for a period of 2 weeks exhibited normal histological architecture of liver and pancreas. Treatment with ethanol extract of leaves of *Aloe*

vera for 21 days resulted in the regeneration and enlargement of islet cells in pancreas and partial reduction in degenerative changes of liver of streptozotocin diabetic rats (Noor *et al.*, 2008).

The number of functionally intact β-cells in the islets of Langerhans is of decisive importance in the development, course and outcome of diabetes mellitus. Total β-cell mass reflects the balance between the renewal and loss of these cells. It was suggested that regeneration of islet β cells destroyed by alloxan may be the primary cause of recovery from diabetes mellitus in alloxan injected guinea pigs (Gonay *et al.*, 1986). In alloxan-induced diabetes, the flavonoids, epicatechin (Chakravarthy *et al.*, 1980) and *Vinca rosea* extract (Ghosh *et al.*, 2001) have also shown to act by β cell regeneration. Photomicrographical data in our studies confirm regeneration of islet cells of pancreas by the extract, as a plausible mechanism of its antidiabetic activity. This effect may be due to the presence of flavonoids in the extract, which was reported to cause regeneration of islet cells (Chakravarthy *et al.*, 1980). Moreover, the phenolics and flavonoids in the extract might have improved the antioxidant status of pancreas and liver by scavenging the excess free radicals formed during diabetes mellitus or inhibiting enzymatic systems responsible for free radical generation (Blaha *et al.*, 2004, Knekt *et al.*, 2009).

Administration of the extract to normal rats did not produce any variation in the histology of both tissues except for an increased compactness of islet cells of pancreas. Liver tissue resembled that of normal control rats. Noor *et al.* (2008) also reported that pancreas and liver of normal rats administered with ethanol extract of fresh leaves of *Aloe vera* for 21 days showed no difference from normal histological architecture.

Considering the histopathology findings in the present study, the mechanism involved in the antidiabetic effect of the extract appears to be both pancreatic, helping in regeneration of damaged pancreas and also extra-pancreatic.

5.4 CONCLUSION

The present study revealed that the ethanol extract of *Musa* AAA (Chenkadali) fruits possesses significant hypoglycaemic, hypolipidemic and antioxidant properties. Presence of flavonoids, terpenoids, phytosterols and phenolic compounds in the extract might account for these activities. The extract acted as an antihyperglycaemic agent and helped to maintain normal metabolic homeostasis by restoring the functions of pancreas and liver.

Observations made in this study suggested that both the doses (500 mg/kg and 1000 mg/kg) were effective in reducing the metabolic derangements and oxidative stress associated with diabetes mellitus. On comparison between the two doses, 500 mg/kg was found to be more effective, especially to increase the level of haemoglobin, reduce blood glucose, serum cholesterol, serum ALT and to alleviate the oxidative stress as evidenced by increased GSH content and decreased lipid peroxides in tissues of liver and pancreas. The effect of the extract (500 mg/kg) on parameters *viz.* body weight, triacylglycerols, ALT, liver lipid peroxides and liver reduced glutathione were comparable to the effect of glibenclamide, whereas haemoglobin, blood glucose and liver glycogen showed better effect than glibenclamide. GSH and lipid peroxides in pancreas also showed better effect than glibenclamide.

Musa AAA (Chenkadali) is a widely cultivated, easily and cheaply available variety of plantain. It has been calculated that one kg green fruits of this variety

consisted of 9-10 mature fresh green fruits, which showed that each fruit had a weight of 100-110 g. Dried weight of the fruits was about ¼ of wet weight and the yield of ethanol extract was about 12 per cent. Thus the estimated dry weight of each fruit is 25-27.5 g, which could yield 3-3.3 g of the extract. Conversion of rat dose to human dose showed that the dosage of ethanol extract is about 50 mg/kg body weight and an adult human being having a body weight of approximately 60 kg requires 3000 mg of the extract which is present in ~25 g of the dried fruit powder or in one fresh green fruit.

Since, the hypoglycaemic effect of the fruit extract is evident only in hyperglycaemic individuals and not in normoglycaemic individuals, about 25 g of dried fruit powder of Chenkadali could be recommended to hyperglycaemic individuals to prevent further complications of diabetes mellitus. Thus, the study suggested that mature green fruits of Chenkadali could be effectively used to treat hyperglycaemia and hyperlipidaemia associated with diabetes mellitus.

Summary

6. SUMMARY

The present study was undertaken to assess the effect of ethanol extract of mature green fruits of *Musa* AAA (Chenkadali) in alloxan-induced diabetes mellitus in rats. Phytochemical analysis of the extract revealed the presence of flavonoids, terpenoids, phenolic compounds, steroids and glycosides. The effect of the extract was compared with a reference antidiabetic drug, glibenclamide.

Experiments were carried out in 48 adult female Sprague-dawley rats, which were randomly divided into six groups. Group, G1 served as a normal control and G2 as diabetic control. G3 consisted of normal animals administered with the fruit extract @ 500 mg/kg body weight. G2, G4, G5 and G6 were made diabetic by s.c injection of alloxan @ 130 mg/kg body weight. Diabetic animals showing a serum glucose level above 200 mg/dl were selected for the study and the level was confirmed on 7^{th} day after alloxan administration. G4 and G5 were treated with the fruit extract at the dose of 500 and 1000 mg/kg respectively and G6 was treated with glibenclamide @ 0.5 mg/kg body weight.

Treatment was started on day 0 (8^{th} day after alloxan administration) after confirmation of diabetes. Weight of all the animals was recorded and blood samples were collected on days –7 (day of alloxan administration), 0, 15 and 30 of experiment. For fructosamine estimation, blood samples were also collected on day 21. All the animals were euthanized on day 31 of experiment. From the euthanized animals, liver was separated and weighed.

Biochemical parameters viz. haemoglobin, serum glucose, fructosamine, liver glycogen, triacylglycerol and total cholesterol were estimated. Oxidative stress in tissues of liver and pancreas were assessed by estimating the levels of lipid peroxides.

reduced glutathione and serum ALT activity. Representative samples of tissues of liver and pancreas were subjected to histopathological examination.

Body weight decreased significantly in diabetic control rats by day 30 when compared with all the other groups. All the treated diabetic groups showed a body weight near to that of normal control. Similar results were observed in liver weight also. Weight of liver decreased significantly in diabetic control group whereas in all the other groups it was similar to that of normal control.

Alloxan administered groups exhibited a significantly lowered haemoglobin level when compared with normal control rats. Administration of both doses of the extract and glibenclamide brought about a significant increase in the level of haemoglobin when compared with diabetic control. The level of haemoglobin in the extract treated group (500 mg/kg) did not show any significant difference from that of normal control. Though, there was a significant increase in haemoglobin level in the group treated with glibenclamide, it was significantly lower than that of normal control.

Compared with the normal control, all the groups administered with alloxan showed a significant increase in the level of serum glucose and fructosamine while the level of liver glycogen was significantly decreased. Treatment with the extract for thirty days, at both doses, significantly decreased the level of blood glucose and fructosamine when compared with diabetic control and the levels were similar to that of the group treated with glibenclamide. In case of liver glycogen, extract treatment restored the liver glycogen level and it was similar to that of normal control. Though, treatment with glibenclamide also brought about a significant increase in liver glycogen, it did not reach the normal level.

Administration of alloxan also caused an increase in the serum triacylglycerol, and cholesterol level. Treatment with both the doses of the extract and glibenclamide for 30 days significantly decreased the level of triacylglycerol and the level was similar to that observed in normal control. Administration of lower dose of the extract (500 mg/kg) and glibenclamide showed similar effect in decreasing the level of cholesterol in diabetic rats while 1000 mg/kg prevented an increase in the level of cholesterol when compared with diabetic control.

Alloxan administration resulted in significant oxidative injury as evidenced by increased levels of lipid peroxides in liver and pancreas, decreased levels of GSH in both tissues and increased activity of ALT. Treatment with both the doses exerted a significant antioxidant effect and the 500 mg/kg dose was found to be more effective. Significant decrease in ALT, tissue lipid peroxides and increase in GSH were observed in the treated groups. Treatment with glibenclamide also significantly decreased the level of ALT and tissue lipid peroxides to the normal level. Though glibenclamide treatment resulted in increased levels of pancreas GSH that approached normal level, liver GSH was significantly lower than that of normal control, but higher than diabetic control.

In the diabetic control rats, lesions in the pancreas consisted of scattered necrosis of islet cells, reduction in the cell number, hyalinisation and congestion of acinar capillaries. Lesions in liver sections include multifocal diffused haemorrhage, central venous congestion, diffused areas of necrosis and focal coagulation. Treatment with the extract at both the doses and glibenclamide showed a tendency to normalize the histological architecture of liver and pancreas.

Based on the findings of the present study on the antidiabetic effect of *Musa* AAA (Chenkadali) extract in rats, it could be summed up as follows:

1. The extract possesses significant antidiabetic, hypolipidemic, hepatoprotective and antioxidant effect at both the doses. The extract did not have any hypoglycemic effect in normal rats. These findings correlate with the histopathological observations.

2. 500 mg/kg was found to be more effective, especially to increase the level of haemoglobin, reduce blood glucose, serum cholesterol, serum ALT and to alleviate the oxidative stress as evidenced by increasing GSH content and decreasing lipid peroxides in tissues of liver and pancreas.

3. The effect of the extract (500 mg/kg) on parameters viz. body weight, triacylglycerol, ALT, liver lipid peroxides and liver reduced glutathione were comparable to the effect of glibenclamide whereas haemoglobin, blood glucose, liver glycogen and GSH and lipid peroxides in pancreas showed better effect than glibenclamide.

References

REFERENCES

Afaf, I., Abuelgasim., Maha, K.M., Osman and Elmahdi, B. 2008. Effects of *Aloe vera* (Elsabar) ethanolic extract on blood glucose level in wistar albino rats. *J. Appl. Sci. Res.*12: 1841-1845

Aguilar, F.J.A., Bermejo, F.C., Galicia, E.H., Angeles, C.R and Ramos, R.R. 2005. Acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. *J. Ethnopharmacol.* 3: 447-452

Ahmed, S.M., Swamy, V.B.M., Dhanapal, P.G.R and Chandrasekhara, V.M. 2005. Antidiabetic activity of *Terminalia catappa* Linn. leaf extracts in alloxan-induced diabetic rats. *Iranian journal of pharmacology & therapeutics.* 4: 36-39

Akhtar, A., Rashid, M., Wahed, M.I.I., Islam, M.R., Shaheen, S.M., Islam, M.A., Amran, M.S and Ahmed, M. 2007. Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats. *Res J Med and Med Sci.* 2: 29-34

Al-Shamaony, L., Al-khazraji S. M and Twaij, H. A. A.1994. Hypoglycaemic effect of *Artemisia herba alba.* II: Effect of a valuable extract on some blood parameters in diabetic animals. *J Ethnopharmacol.* 43 : 163 –171

Ananthi, J., Prakasam, A and Pugalendi, K.V. 2003. Antihyperglycemic activity of *Eclipta alba* leaf on alloxan-induced diabetic rats. *Yale J Biol Med.* 76: 99-102.

Armbruster, D.A.1987. Fructosamine: Structure, analysis and clinical usefulness. *Clin. chem.* 33: 2153-2156

Babu, V., Gangadevi, T., Subramoniam, A. 2003. Antidiabetic activity of *Cassia kleini* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J Pharmacol.* 35: 290-296

Badole, S., Patel, N., Bodhankar,S., Jain, B and Bhardwaj, S. 2008. Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan induced diabetic mice. *Indian J Pharmacol.* 38 : 49-53

Bancroft, J.D. and Cook, H.C. 1984. *Manual of Histological Techniques and their Diagnostic Application.* Second edition. Churchill Livingstone, Edinburgh. 457p.

*Bever, B.O. 1986. Medicinal plants in tropical West Africa, Cambridge University Press, London. pp.245-267.

Bhandari, U., Kanojia, R and Pillai, K.K. 2002. Effect of ethanolic extract of *Embelia ribes* on dyslipidemia in diabetic rats. *Int. Jnl. Experimental Diab. Res.* 3: 159–162

Blaha, L., Kopp, R., Simkova, K and Mares, J. 2004. Oxidative stress biomarkers are modulated in silver carp (*Hypophthalmichthys molitrix* Val.) exposed to

microcystin-producing cyanobacterial water bloom. *ACTA VET. BRNO.* 73: 477–482

Bopanna, K.N., Kannan, J., Gadgil, S., Balaraman, R and Rathod, S.P. 1997. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. *Indian J Pharmacol.* 29 : 162-167

Chakravarthy, B.K, Gupta, S, Gambir, S.S and Gode, K.D. 1980. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. *Ind J Pharmacol.* 12:123-127

Chaturvedi, P and Segale, M. 2007. Effects of different types of water decoctions of fruit of *Melia azedarach* on glucose induced hyperglycemia, liver transaminases, lipid peroxidation and reduced glutathione in normal albino rats. *Scientific Research and Essay.* 9: 384-389

Chauhan, N.S., Dixit, V.K. 2007. Antihyperglycemic activity of the ethanolic extract of *Curculigo orchioides Gaertn. Phcog Mag.* 12: 237-240

Clark, Z. 2003. Diabetes mellitus in a 6 month old Charolais heifer calf. *Can Vet J.* 44: 921–922

Daisy, P., Rayan, N.A and Rajathi, D. 2007. Hypoglycaemic and other related effects of *Elephantopus scaber* extracts on alloxan induced diabetic rats. *J.Biol.Sci.* 7: 433-437

*Dewanjee, S., Bose, S.K., Sahu, R and Mandal, S.C. 2008. Antidiabetic effect of matured fruits of *Diospyros peregrina* in alloxan-induced diabetic rats. *Indian J Pharmacol.* 33 : 130-137

Dhanabal, S.P., Sureshkumar, M., Ramanathan, M and Suresh, B. 2005. Hypoglycemic effect of ethanolic extract of *Musa sapientum* on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. *J Herb Pharmacother.*5: 7-19

Dhandapani, S., Subramanian, V.R., Rajagopal, S and Namasivayam, N. 2002. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol. Res..*46: 251-255

Ebong, P.E., Atangwho, I.J., Eyong, E.U and Egbung, G.E. 2008. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf), *Am. J. Biochem. & Biotech.* 4: 239-244.

Edwin, E., Sheeja, E., Dhanabal, S.P and Suresh, B. 2008. Antihyperglycemic activity of *Passiflora molissima* Bailey. *Indian J Pharm Sci.* 69: 570-571

El-Shenawy, N.S and Abdel-Nabi, I.M. 2006. Hypoglycemic effect of *Cleome droseri folia* ethanolic leaf extract in experimental diabetes, and on non-enzymatic antioxidant, glycogen, thyroid hormone and insulin levels. *Diabetol Croat.*35: 15-22

El-Soud, N.H.A., Khalil, M.Y., Hussein, J.S., Oraby, F.S.H and Farrag, A.R.H. 2007. Antidiabetic effects of fenugreek alkaloid extract in streptozotocin induced hyperglycaemic rats. *J. Appl. Sci. Res.* 3: 1073-1083

Ene, A.C., Nwankwo, E.A and Samdi, L.M. 2007. Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi L.*) oil on their body weight. *Res. J. Medicine & Med. Sci.* 2: 48-52

Englberger, L., Darton- Hill,I., Coyne,T., Fitzgerald,M.H. and Marks,G.C. 2003. Carotenoid rich bananas: A potential food source for alleviating vitamin A deficiency. *Food Nutr Bull.* 24: 303-318

*Englyst, H.N., Kingman, S.M and Cummings, J.H. 2009. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr.* 46: 33–50.

*Feldman, J.M and Lee, E.M. 2009. Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. *Am J Clin Nutr.*42: 639-643

Fernandes, N.P.C., Lagishetty, C.V., Panda, V.S and Naik, S.R. 2007. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract . *BMC Complement Altern Med.* 7: 29-35.

Ghosh, T., Maity,T.K., Sengupta,P., Dash,D.K and Bose, A .2008. Antidiabetic and *in vivo* antioxidant activity of ethanolic extract of *Bacopa monnieri* Linn.aerial parts: A possible mechanism of action. *Iranian journal of pharmaceutical research.*7: 61-68

Goel, R. K., Sairam, K and Rao, C. V. 2001. Role of gastric antioxidant and anti-*Helicobactor pylori* activities in antiulcerogenic activity of plantain banana (*Musa sapientum* var. *paradisiaca*). *Indian J Exp Biol.* 39: 719-722.

Gokce, G and Haznedaroglu, M.Z. 2008. Evaluation of antidiabetic, antioxidant and vasoprotective effects of *Posidonia oceanica* extract. *J Ethnopharmacol.* 115: 122–130

Gomathy, R., Vijayalekshmi, N.R and Kurup, P.A. 1990. Hypoglycemic action of the pectin present in the juice of the inflorescence stalk of plantain (*Musa sapientum*)—Mechanism of action. *J. Biosci.* 15: 297-303

Gomathy, R., Vijayalekshmi, N.R and Kurup, P.A.1989. Hypolipidemic principle of the inflorescence stalk of plantain (*Musa sapientum*). *J. Biosci.* 14 : 301-309.

*Gorray, K. C., Baskin, D., Brodsky, J., Fujimoto, W.Y. 1986. Responses of pancreatic β cells to alloxan and streptozotocin in the guinea pig. *Pancreas.* 1: 130-138

Gupta, R.K., Kesari, A.N., Watal, G., Murthy, P.S., Chandra, R., Maithal, K and Tandon.V. 2005. Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animals. *Curr Sci.* 8 :1244-1254

Hakkim, F.L., Girija, S., Senthilkumar, R and Jalaludeen, M.D. 2007. Effect of aqueous and ethanol extracts of *Cassia auriculata* L. flowers on diabetes using alloxan induced diabetic rats. *Int J Diabetes & Metabolism* . 15: 100-106

Hannan, J.M.A., Ali, L., Khaleque, J., Akhter, M., Flatt, P.R and Abdel-Wahab, Y.H.A. 2006. Aqueous extracts of husks of *Plantago ovata* reduce hyperglycaemia in type 1 and type 2 diabetes by inhibition of intestinal glucose absorption. *Br J Nutr.* 131-137

Hannan, J.M.A., Rokeya, B., Faruque, O., Nahar, N,Mosihuzzaman, M, Khan, A.K.A and Ali, L. 2003. Effect of soluble dietary fibre fraction of *Trigonella foenum graecum* on glycemic, insulinemic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *J Ethnopharmacol.* 88 : 73-77

Harbone, J.B.1991. Phytochemical methods – Guide to modern techniques of plant analysis . Second edition, Chapman and Hall, India, p 653

Harmon, A and Harp, J. 2001. Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. *Am J Physiol Cell Physiol.* 4: 807–813

Heikkila, R.E., Winston, B., Cohen, G and Barden, H.1976. Alloxan induced diabetes, evidence for hydroxyl radicals as a cytotoxic intermediate. *Biochem Pharmacol.*25: 1085-1092

Horigome, T., Sakaguchi,E and Kishimoto, C. 1992. Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing diet. *Br J Nutr.* 68: 231-244

*Hornbrook. K, R.1970: Synthesis of liver glycogen in starved alloxan diabetic rats. *Diabetes.*19: 916–923

Ignacimuthu, S and Amalraj, T. 1998. Effect of leaf extract of *Zizyphus jujuba* on diabetic rats. *Indian J Pharmacol.* 30: 107-108

*Inomata, S., Itoh, M., Imai, H., Sato, T. 1997. Serum levels of erythropoetin as a novel marker reflecting the severity of diabetic nephropathy. *Nephron.* 75 : 426-430

*Ivora, M.D., Paya, M., Villar, A. 1989. A review of natural products and plants as potent antidiabetic drugs. *J.Ethnopharmacol.* 27: 243-275

Jain, D. L., Baheti, A.M., Parakh, S.R., Ingale, S.P and Ingale, P.L. 2007. Study of antacid and diuretic activity of ash and extracts of *Musa sapientum* L. fruit peel. *Phcog Mag.* 10: 116-119

*Jain, S.R. 1968.Hypoglycaemic principle in *Musa sapientum* (L.) and its location. *Planta Medica.* 16:44-47

Jonas, J., Plant, T., Gilon, P., Detimary, P., Nenquin, M and Henquin,J. 1995. Multiple effects and stimulation of insulin secretion by the tyrosine kinase inhibitor genistein in normal mouse islets. *Br J Pharmacol* .4:872-88

Jothivel, N., Ponnusamy, S.P., Appachi, M., Singaravel, S.,Rasilingam, D and Thangavel, S. 2007. Antidiabetic activity of methanol leaf extract of *Costus pictus* in alloxan induced diabetic rats. *Journal of health.* 53: 655-663

Kailash, L and Varalakshmi. P. 1992. Effect of banana stem juice on biochemical changes in liver of normal and hyperoxaluric rats. *Indian J Exp Biol.* 30: 440-442

Kaleem, M., Asif, M., Ahmed, Q. U and Bano, B. 2006. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. *Singapore Med.* 8: 670- 678

Karaca, T., Cemek, M and Kanter, M. 2006. Lipid peroxidation and antioxidant levels, and alpha naphthyl acetate esterase activity of peripheral blood lymphocytes in Mallard, Muscovy and Pekin ducks. *ACTA VET. BRNO.* 75: 33–38

Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovara, M., Reunanen, A and Hakulinen, T. 2009. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr.* 76: 560–568

Krishnan, K and Vijayalakshmi, N.R. 2005. Alterations in lipids & lipid peroxidation in rats fed with flavonoids rich fraction of banana (*Musa paradisiaca*) from high background radiation area. *Indian J Med Res.* 122: 540-546

Kumar, A., Rao, P and Pattabiraman. 1988. A colorimetric method for the estimation of serum glycated proteins based on differential reduction of free and bound glucose by sodium borohydride. *Biochem Med Metab Biol.*39 : 296

Kumar, M.M., Joshi, M.C., Prabha, T., Dorababu, M and Goel, R.K. 2006. Effect of plantain banana on gastric ulceration in NIDDM rats: role of gastric mucosal glycoproteins, cell proliferation, antioxidants and free radicals. *Indian J Exp Biol.* 44:292-299

Kumar, S., Kumar, D., Deshmukh, R.R., Lokhande, P.D., More, S.N and Rangari, V.D. 2008. Antidiabetic potential of *Phyllanthus reticulatus* in alloxan-induced diabetic mice. *Fitoterapia.* 79: 21–23

Kumarappan, C.T., Rao, T.N and Mandal, S.C. 2007. Polyphenolic extract of *Ichnocarpus frutescens* modifies hyperlipidemia status in diabetic rats. *J Cell Mol Biol.* 6: 175-187

Latha, M and Pari, L. 2004. Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes. *Braz J Med Biol Res.* 37: 577-586

Leite, A.C.R., Araujo, T.G., Carvalho, B.M., Silva, N.H., Lima, V.L.M and Maia, M.B.S. 2007. *Parkinsonia aculeata* aqueous extract fraction: Biochemical studies in alloxan-induced diabetic rats. *J Ethnopharmacol.* 3 : 547-552

Lewis, A.D., Fields, W.N and Shaw, G.P. 1999. A natural flavonoid present in unripe *var.* plantain banana pulp (*Musa sapientum L. paradisiaca*) protects the gastric mucosa from aspirin-induced erosions, *J Ethnopharmacol.* 65:283-288

Mahesh, N and Brahatheeswaran, D. 2007. Anti hyperglycemic activities of aqueous and ethanolic extracts of *Cyanodon dactylon* (Linn) in streptozotocin diabetic rats. *Asian J.Biochem.*2: 66-72

Mai, T.T and Chuyen, N.V. 2007. Anti hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus (Roxb.) Merr and Perry. Biosci.Biotechnol.Biochem.* 71 : 69-76

Matsuda, K., Ohtsuka, H., Ichijho, T and Kawamura, S. 2006. Effect of dietary administration of bananas on immunocytes in F1 hybrid calves. *J Vet Med Sci.* 68:75-77

Matsuda, T., Ferreri, K., Todorov, I., Kuroda, Y., Smith, C., Kandeel, F and Mullen Y. 2005. Silymarin protects pancreatic beta cells against cytokine-mediated

toxicity: implication of c-Jun NH2-terminal kinase and janus kinase/signal transducer and activator of transcription pathways. *Endocrinology.* 1: 175–185

Medina,F., Gamez, M.J., Jimenez,I., Jimenez,J., Osuna,J.I and Zarzuelo, A. 1994. Hypoglycemic activity of "juniper berries". *Planta Med.* 60:197-200

Mojzisova, G and Kuchta, M. 2001. Dietary flavonoids and risk of coronary heart disease. *Physiol. Res.* 50: 529-535

Mokbel, M.S and Hashinaga, F. 2005. Antibacterial and antioxidant activities of banana (*Musa* AAA.cv.Cavendish) fruits peel. *Am.J.Biochem &Biotechnol.* 1: 125-131

Moron, M.S., Depierre, J.W. and Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem. Biophys. Acta.* 582: 67-78

Mostofa, M., Choudhury, M.E., Hossain, M.A., Islam, M.Z., Islam, M.S and Sumon. M.H 2007. Antidiabetic effects of *Catharanthus roseus, Azadirachta indica, Allium sativum* and glimepride in experimentally diabetic induced rats. *Bangl. J. Vet. Med.* 5: 99–102

Nadkarni, K.M. 1976. *The Indian Materia Medica.* Third edition. Popular Prakashan, Bombay. 1319p.

Narasimhan, T.R.1971. A comparative study of the chemical composition and some of the enzymes in the liver of ducks and chicken. MSc thesis, Calicut University, Calicut, p 111

Noor, A., Gunasekaran, S., Manickam, A.S and Vijayalakshmi, M.A. 2008. Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin induced diabetic rats. *Curr Sci.* 94:1070-1076

Notoya, M., Tsukamoto, Y., Nishimura, H.,Woo, J., Nagai, K., Lee, I and Hagiwara, H. 2004. Quercetin, a flavonoid, inhibits the proliferation, differentiation, and mineralization of osteoblasts in vitro. *Eur J Pharmacol.* 3:89–96

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351-358

Ohno, T., Kato, N., Ishi, C., Shimizu, M., Ito, Y., Tomono, S and Kawazu, S.1993. Genistein augments cyclic adenosine 3'5'-monophosphate (cAMP) accumulation and insulin release in MIN6 cells. *Endocr Res.* 4:273–85

Ojewole, J.A.and Adewunmi, C.O. 2003. Hypoglycemic effect of methanolic extract of *Musa paradisiaca (Musaceae)* green fruits in normal and diabetic mice, *Methods Find Exp Clin Pharmacol*, 25(6): 453

Okokon, J.E., Antia, B.S and Ita, B.N. 2007. Antidiabetic effects of *Homalium letestui* (Flacourtiaceae) in streptozotocin induced diabetic rats.*Res.J.Med.Plant.*1: 134-138

*Oliveira, L., Carmen, S., Freire., Armando, J.D., Silvestre and Cordeiro, N. 2008. Lipophilic extracts from banana fruit residues: A source of valuable phytosterols. *J Agric Food Chem.* 20: 9520-9524.

Pannangpetch, P., Vuttivirojana, A., Kularbkaew, C., Tesana, S., Kongyingyoes, B and Kukongviriyapan, V. 2001. The antiulcerative effect of Thai *Musa* species in rats. *Phytother Res.*15: 407-410

Pari, L and Latha, M. 2002. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Med J.* 43: 617-621

Pari, L and Satheesh, M.A. 2004. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *J Ethnopharmacol.* 91: 109-113

Pari, L and Umamaheshwari, J. 2000. Antihyperglycaemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. *Phytother Res.* 14: 136-138

Pari, L and Venkateswaran, S. 2002. Hypoglycaemic activity of *Scoparia dulcis* L. extract in alloxan induced hyperglycaemic rats. *Phytother. Res.* 16: 662-664

Pavana, P., Sethupathy, S and Manoharan. S. 2007. Antihyperglycemic and antil-lipid peroxidative effects of *Tephrosia purpurea* seed extract in streptozotocin induced diabetic rats. *Indian J Clin Biochem.* 22 : 77-83

Pellegrini,N., Serafini,M., Colombi, B., Rio, D.D., Salvatore, S., Bianchi, M and Brighenti, F. 2009. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different invitro assays. *J Nutr.*25: 2812-2819

Pérez-Pérez, E.M., Malaver, A.J.R., Padilla, N., Ramírez, G.M and Dávila, J. 2006. Antioxidant capacity of crude extracts from clones of banana and plane species. *J Med Food.* 9: 517–523

Pinent, M., Castell, A., Baiges, I., Montagut, G., Arola, L and Ardevol, A.2008. Bioactivity of flavonoids on insulin-secreting cells. *Comprehensive reviews in food science and food safety.*7: 2299-308

Ponnachan, P.T.C., Paulose, C.S., Panikkar, K.R.1991. Effect of *Aegle marmelose* leaf extract on alloxan induced diabetic rats. *Amala Research Bulletin* . 11: 47-51

Poonguzhali, P.K and Chegu, H.1994. The influence of banana stem extract on urinary risk factors for stones in normal and hyperoxaluric rats. *Br J Urol.* 74:23-25

Prasad, K. V., Bharathi, K and Srinivasan, K.K. 1993. Evaluation of *Musa* (*Paradisiaca* Linn. cultivar)-"Puttubale" stem juice for antilithiatic activity in albino rats. *Indian J Physiol Pharmacol.* 7: 337-341

Raghavan, B and Krishnakumari, S. 2006. Antidiabetic effect of *Terminalia arjuna* bark extract in alloxan induced diabetic rats. *Indian J Clin Biochem.* 21:123-128

Rajagopal, K and Sasikala, K. 2008. Antidiabetic activity of hydro-ethanolic extracts of *Nymphaea Stellata* flowers in normal and alloxan induced diabetic rats. *Afr. J. Pharm. Pharmacol.* 2: 173- 178

Rajeswari, P., Natarajan, R., Nadler, J. L., Kumar, D., and Kalra, V. K. 1991. Glucose induces lipid peroxidation and inactivation of membrane-associated ion-transport enzymes in human erythrocytes in vivo and in vitro. *J Cell Physiol. 119*: 100–109.

Raju, J., Gupta, D., Rao, A.R., Yadava, P.K and Baquer, N.Z. 2001.*Trigonella foenum graecum* (fenugreek seed) powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. *Mol Cell Biochem* .224: 45-51

Rand, J.S., Fleeman, L.M., Farrow, H.A., Appleton, D.J and Lederer, R.2009. Canine and feline diabetes mellitus: nature or nurture?. *J Nutr.* 5: 2072-2080

Rao, B.K., Giri, R., Kesavulu, M.M.and Apparao, C. H. 1997. Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika*, 1: 33- 35

Rao, N.K and Nammi, S. 2006. Antidiabetic and renoprotective effects of the chloroform extract of *Terminalia chebula* Retz. seeds in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine*. 6:33-39

Rao.N.K. 2006. Anti-hyperglycemic and renal protective activities of *Andrographis paniculata* roots chloroform extract. *Indian J Pharmacol.* 5:47-50

Rhemann, Z.K.A. 1989. Medicinal plants with hypoglycemic activity. *J Ethno pharmacol.* 26:1-55.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M and Pridham, J.B. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Rad. Res.* 4: 375-383

Roglic, G. 2005. The burden of mortality attributable to diabetes. *Diabetes Care.* 28:2130–2135

Ryysy, L. 2001. Insulin treatment in Type 2 diabetes. Academic dissertation. Department of Medicine, Division of Diabetes, University of Helsinki, Helsinki, Finland. p. 42-51

Sabu, M.C and Kuttan, R. 2004. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. *Indian J Physiol Pharmacol.* 48: 81-88

Sabu, M.C, Smitha, K and Kuttan, R. 2002. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol.* 83: 109-116

Safiyeh, S., Fathallah, F.B., Vahid, N., Hossine, N and Habib, S.S. 2007. Antidiabetic effect of *Equisetum arvense* L. (*Equisetaceae*) in streptozotocin induced diabetes in male rats. *Pak.J.Biol.Sci* . 10 : 1661-1666

Sakurai, T., Nishimura, T., Otake, N., Xinsheng, Y., Abe, K., Zeida, M., Nagasawa, H and Sakuda, S.2002. Assamicin I and II, novel triterpenoid saponins with insulin – like activity from *Aesculus assamica* griff. *Bioorg. Med.Chem.Lett.*12: 807-810

Saravanan, G., Pari, L and Venkateswaran, S. 2002. Effect of cogent db, a herbal drug on plasma insulin and hepatic enzymes of glucose metabolism in experimental diabetes. *Diabetes Obes Metab.* 6:394-398

*Saravanan, G., Pari, L., Venkateswaran, S. 2002. Effect of Cogent db, a herbal drug on plasma insulin and hepatic enzymes ooof glucose metabolism in experimental diabetes. *Diabetes Obes Metab.* 4: 394-398

Saravanan, R and Pari, L. 2005. Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. *BMC Complementary and Alternative Medicine.*14: 22-30

Selvan, V.T., Manikandan,L., Senthilkumar,G.P., Suresh,R., Kakoti,B.B., Gomathi,P., Kumar,D.A., Saha,p., Gupta,M and Mazumdar,U.K. 2008.Antidiabetic and antioxidant effect of methanol extracgt of *Artanema sesamoides* in streptozotocin induced diabetic rats. *International journal of applied research in natural products*. 1: 25-33

Sharma, S., Chaturvedi, M., Edwin, E., Shukla, S and Sagrawat, H. 2009. Evaluation of the phytochemicals and antidiabetic activity of *Ficus bengalensis*. *Int J Diab Dev C tries*. 27: 56-59

Sheehan, D.C. and Hrapchak, B.B. 1980. *Theory and Practice of Histotechnology*. Second edition. C.V. Monsby Co., St. Louis, Toronto, London. 481p

Snedecor, G.W and Cochran, W.G. 1994. *Statistical Methods*. Eighth edition. Oxford and IBH Publishing Company, Calcutta. 564 p.

Someya, S., Yumiko, Y and Kazuyoshi, O. 2002. Antioxidant compounds from bananas (*Musa Cavendish*). *Food Chem*. 3: 351-354

Sorenson, R., Brelje, T and Roth, C.1994. Effect of tyrosine kinase inhibitors on islets of Langerhans: evidence for tyrosine kinases in the regulation of insulin secretion. *Endocrinology*. 4:1975-1978

Soto, C, Muriel, P, Reyes,J.L. 1994. Pancreatic lipid peroxidation in alloxan-induced diabetes mellitus. *Arch Med Res*. 25: 377-380

Sridhar, S.B., Sheetal, U.D., Pai, M.R.S.M and Shastri , M.S. 2005. Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in streptozotocin-diabetic rats. *Braz J Med Biol Res.* 38: 463-468

Szkudelski,T. 2001. The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. *Physiol. Res.* 50: 536-546

Tedong, L, Dimo, T, Dzeufiet, P.D.D., Asongalem, A.E., Sokeng, D.S., Callard, P., Flejou, J.F and Kamtchouing. P. 2006. Antihyperglycemic and renal protective activities of A*nacardium occidentale* (Anacardiaceae) leaves in streptozotocin induced diabetic rats. *Afr. J. Trad. CAM.* 3: 23 – 35

Thomas.M., Salamandris, C., Issac, R.M and jerums, G. 2005. Anaemia in diabetes: An emerging complication of microvascular disease. *Current Diabetes Reviews.* 1: 107-126

Thomson, M., Al-Amin, Z.M., Al-Qattan, K.K., Shaban, L.H and Ali,M. 2007. Anti-diabetic and hypolipidaemic properties of garlic (*Allium sativum*) in streptozotocin-induced diabetic rats. *Int J Diabetes & Metabolism.* 15: 108-115

Usha, Vijayammal, P. L and Kurup, P.A. 1989. Effect of dietary fiber from banana (*Musa paradisiaca*) on metabolism of carbohydrates in rats fed cholesterol free diet. *Indian J Exp Biol.* 27: 445-451

Valentova, K., Truong, N.T., Moncion, A., Waziers, I and Ulrichova, J. 2007. Induction of glucokinse mRNA by dietary phenolic compounds in rat liver cells *in vitro. J.Agric.Food Chem.* 55: 7726-7731

Vanderslice, J.T and Higgs, D.J. 1991. Vitamin C content of foods: sample variability. *Am J Clin Nutr.* 2: 1323-1327

Veeramani, C, Pushpavalli, G and Pugalendi, K.V. 2008. Antihyperglycaemic effect of *Cardiospermum halicacabum* Linn. leaf extract on STZ induced diabetic rats. *J. Appl. Biomed.* 6: 19–26

Vetrichelvan, T., Jegadeesan, M and Devi, B.A.U. 2002. Antidiabetic activity of alcoholic extract of *Celosia argentea* Linn. Seeds in rats.*Biol.Pharm.Bull.* 4: 526-528

Viana, G.S.B., Medeiros, A.C.C., Lacerda, A.M.R., Leal, A.K., Vale, T.G and Matos, F.J.A. 2004. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. *BMC Pharmacology*. 4: 1471-1479

Wall, M.M. 2006. Ascorbic acid, vitamin A and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *J Food Comp Anal.*19: 434-445

Winterbourn, C.C, Munday, R. 1989. Glutathione-mediated redox cycling of alloxan. Mechanisms of superoxide dismutase inhibition and of metal-catalyzed OH formation. *Biochem Pharmacol* . 38: 271-277

Yazdanparast, R., Ardestani, A and Jamshi, S. 2007. Experimental diabetes treated with *Achillea santolina*: Effect on pancreatic oxidative parameters. *J Ethnopharmacol.* 112: 13-18

Yousef, M.I., Haroun, M., Mohamed, H, El-Masry and Ateia, R. 2006. Biochemical and immunological study on the effects of barley and its components as hypoglycemic agents in diabetic rats. *Am. J. Biochem. & Biotech.* 2: 1-8

Zhang, Z and Cui, F.2007. Non-enzymatic glycosylation reaction contributes to a rise of blood glucose in alloxan-induced diabetic rats. *Int J Diabetes & Metabolism.* 15: 52-59

* Originals not consulted

HYPOGLYCAEMIC EFFECT OF FRUITS OF *Musa* AAA (Chenkadali) ON DIABETIC RATS

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ABSTRACT

The present study was conducted to evaluate the hypoglycemic and hypolipidemic effects of ethanol extract of mature green fruits of *Musa* AAA (Chenkadali) in alloxan diabetic rats. The effects were also compared with a reference antidiabetic drug, glibenclamide.

Adult female Sprague-dawley rats were used for the experiment. Rats were randomly divided into six groups viz. G1 – normal control, G2 – diabetic control, G3 – normal rats administered with the extract (500 mg/kg), G4 and G5 – diabetic rats administered with the extract (500 and 1000 mg/kg respectively), G6 – diabetic rats administered with glibenclamide (0.5 mg/kg).

G2, G4, G5 and G6 were made diabetic by sub-cutaneous injection of alloxan @ 130 mg/kg body weight. Diabetes mellitus was confirmed on the 8th day of alloxan administration (day 0). Blood samples were collected on day –7, 0, 15 and day 30. Blood samples were also collected on day 21 for the estimation of fructosamine. Body weight was recorded at weekly intervals. Animals were euthanized on 31st day of the experiment.

Biochemical parameters viz. haemoglobin, serum glucose, triacylglycerol, total cholesterol, fructosamine and ALT activity were estimated. Oxidative damage to liver and pancreas were assessed by estimation of lipid peroxides and reduced glutathione levels in both tissues. Liver glycogen was estimated and weight of liver was noted. Representative samples of liver and pancreas tissues were subjected to histopathological examination.

Alloxan administration caused a significant increase in the level of serum glucose, fructosamine, triacylglycerol and cholesterol while a significant decrease

was noted in the body weight, weight of liver, haemoglobin and level of liver glycogen. Body weight of diabetic control rats was significantly lesser than all the other groups by 30th day of experiment. Increased level of lipid peroxides, decreased level of GSH and increased activity of serum ALT indicated oxidative injury to the tissues of liver and pancreas. In the diabetic control rats, lesions in the pancreas consisted of scattered necrosis of islet cells, reduction in the cell number, hyalinisation and congestion of acinar capillaries while multifocal diffused haemorrhage, central venous congestion, diffused areas of necrosis and focal coagulation were observed in the liver.

Both the doses (500 mg/kg and 1000 mg/kg) of the extract were effective in reducing the metabolic derangements and oxidative stress associated with diabetes mellitus. On comparison between the two doses, 500 mg/kg was found to be more effective, especially to increase the level of haemoglobin, reduce blood glucose, serum cholesterol, serum ALT and to alleviate the oxidative stress as evidenced by increasing GSH content and decreasing lipid peroxides in tissues of liver and pancreas. Histopathological examination of pancreas and liver of treated groups showed almost normalized histological architecture.

The effect of the extract (500 mg/kg) on parameters viz. body weight, triglycerides, ALT, liver lipid peroxides and liver reduced glutathione were comparable to that of glibenclamide whereas, haemoglobin, blood glucose, liver glycogen and GSH and lipid peroxides in pancreas showed better effect than glibenclamide.