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ABSTRACTS


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ORIGINAL ARTICLE

Antidiabetic and antihyperlipidemic effects of the stem of *Musa sapientum* Linn. in streptozotocin-induced diabetic rats

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Introduction

In India, the use of herbal drugs based on Ayurveda is common.¹ *Musa sapientum* syn. *Musa paradisiaca* Linn. (*kela* in Hindi, *banana* in English) is a herbaceous plant of the Musaceae family. Different parts of the plant are used in the treatment of a wide array of human diseases. The fruit of the banana aids in

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Abstract

Background: *Musa sapientum* Linn. is a herbaceous plant of the Musaceae family. It has been used in India for the treatment of gastric ulcer, hypertension, diarrhea, dysentery, and diabetes. The antidiabetic effect of the fruit, root, and flower has been demonstrated. The aim of the present study was to assess the antidiabetic and antihyperlipidemic effects of the stem of *M. sapientum* Linn.

Methods: Diabetes was induced in rats by streptozotocin injection (45 mg/kg, i.p.). Diabetic rats were treated for 2 weeks with different doses of lyophilized stem juice of *M. sapientum* Linn. (25, 50, and 100 mg/kg) to select the most effective dose. The effects of 4 weeks treatment with this dose (50 mg/kg) on fasting and postprandial plasma glucose (FPG, PPG) levels, body weight, lipid profile, HbA1c, insulin, liver enzymes (i.e. glucokinase, glucose-6-phosphatase and 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase) and muscle and liver glycogen were evaluated.

Results: The most effective dose of lyophilized stem juice of *M. sapientum* Linn. was 50 mg/kg. Four weeks treatment with this dose resulted in significant decreases in FPG and PPG (*P* < 0.05). Serum insulin increased (*P* < 0.05) whereas HbA1c decreased (*P* < 0.05). Diabetes-induced changes to the lipid profile, muscle and liver glycogen, and enzyme activity (i.e. glucokinase, glucose-6-phosphatase, and HMG-CoA reductase) were restored near to normal levels (*P* < 0.05).

Conclusion: Diabetic rats responded favorably to treatment with lyophilized stem juice of *M. sapientum* Linn., which exhibits antidiabetic and antihyperlipidemic effects.

Keywords: diabetes, *Musa sapientum*, stem juice, streptozotocin.

Significant findings of the study: Lyophilized stem juice of *M. sapientum* Linn. has significant antidiabetic and antihyperlipidemic effects.

What this study adds: The stem of *M. sapientum* Linn. has the potential to be used in the treatment of diabetes.


combating diarrhea and dysentery, and promotes healing of intestinal lesions in ulcerative colitis. A water extract of the leaves is used to treat hypertension, whereas stem extracts have been shown to have antivenom, analgesic, and hepatoprotective activities and are also used to dissolve urinary stones. Various parts of the *Musa sapientum* plant have been investigated for antidiabetic effects, including the flowers/inflorescence, unripe fruits, and fruit peel. Various biologically important compounds found in the banana plant include tannins, pectins, dopamine, serotonin, noradrenaline, sitosterol, and stigmastosterol. Recently, the hypoglycemic effect of the pseudostem has been reported. The trunk, or the pseudostem, of the banana plant is not a true stem, but rather a cluster of cylindrical aggregations of leaf stalk bases. The true stem begins as an underground corm, which grows upwards, pushing its way through the center of the pseudostem, 10–15 months after the banana plant has been planted. This true stem of the banana is edible and is used in India in the preparation of curries and chutneys. In the present study, we analyzed the antidiabetic potential of this part of the banana plant. Because hyperglycemia and hyperlipidemia coexist in diabetes, the aim of the present study was to investigate the antidiabetic and antihyperlipidemic effects of the stem of *M. sapientum* in a rat model of diabetes.

**Methods**

**Animals**

Male Wistar rats, weighing 150–200 g, were housed in an air-conditioned room (25 ± 3°C) with relative humidity of 42 ± 3%. Standard light and dark cycles were maintained throughout the experimental period. Animals were fed with a standard laboratory diet and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee, UCMS (IAEC) of Animal Research (IAEC-AR) at University College of Medical Sciences and Guru Teg Bahadur (GTB) Hospital (UCMS/IAEC/16–17/30 December 2009). Experiments were performed as per the guidelines of the committee.

**Induction of diabetes**

Rat were made diabetic by a single intraperitoneal injection of streptozotocin (45 mg/kg), in citrate buffer, pH 4.0. Fasting plasma glucose (FPG) levels were estimated at 3-day intervals until the blood glucose levels stabilized. Rats with FPG ≥126 mg/dL and postprandial plasma glucose (PPG) ≥200 mg/dL were considered diabetic and were used in the present study.

**Plant material**

The stem of *M. sapientum* Linn. was collected from the local area and authenticated by National Institute of Science Communication and Information Resources, New Delhi (voucher no. NISCAIR/RHMD/Consult/2007–08/895/79).

**Preparation of stem juice of *M. sapientum***

Freshly cut stems (500 g) of *M. sapientum* Linn. were used to make the juice. The upright concentric layers of leaf sheaths forming the pseudostem were peeled off to reveal the central pale white stem. This was cut into tiny pieces that were then crushed mechanically using a hand grinder and filtered through a sterile muslin cloth to yield the stem juice, which was lyophilized and stored at 2–8°C (yield 2%). The lyophilized juice was dissolved in water prior to the treatment of rats.

**Phytochemical studies**

Stem juice was analyzed qualitatively for terpenoids, phenolic compounds, saponins, and pectins using methods described in detail elsewhere.

**Experimental design**

Two types of experiments were conducted to investigate the antidiabetic and antihyperlipidemic effects of the stem of *M. sapientum* Linn., as described below.

**Experiment I**

To select the most effective dose of stem juice, rats were divided into six groups of six animals each and treated for 2 weeks via orogastric intubation once daily. Group I was the healthy control (non-diabetic) group and was administered saline (0.5 mL/kg); Group II was the diabetic control group administered saline (0.5 mL/kg); Groups III, IV, and V were diabetic rats administered 25, 50, and 100 mg/kg stem juice, respectively; and Group VI consisted of diabetic rats treated with 0.6 mg/kg glibenclamide. Fasting blood samples were collected before (Day 0) and after (Day 15) treatment to estimate plasma glucose levels.
**Experiment II**

Rats were divided into five groups of six rats each and treated for 4 weeks. Group A was the healthy control (non-diabetic) group administered saline (0.5 mL/kg); Group B was the healthy control (non-diabetic) group administered stem juice (50 mg/kg); Group C was the diabetic control group administered saline (0.5 mL/kg); Group D consisted of diabetic rats administered stem juice (50 mg/kg); and Group E consisted of diabetic rats treated with 0.6 mg/kg glibenclamide.

**Collection of blood samples**

Blood samples were collected from the retro-orbital eye plexus of overnight-fasted rats. Blood samples were collected in vials containing oxalate fluoride mixture for blood glucose determination, EDTA vials for determination of HbA1c, and in plain vials for determination of the serum lipid profile and insulin. After collection of fasting blood samples, rats were given an oral glucose load of 2 g/kg using feeding tube and blood samples were collected after 2 h to measure PPG. Both FPG and PPG were determined before the start of treatment and then again after 4 weeks treatment.

**Collection of tissue samples**

After blood sampling, overnight-fasted rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and killed by cervical dislocation so that liver tissue and skeletal muscles could be collected. Samples were washed with cold saline and stored at −80°C until analysis.

**Biochemical parameters**

Plasma glucose levels were estimated via the glucose oxidase/peroxidase method using a commercially available kit (Accurex Biomedical, Mumbai, India). Total cholesterol (TC), high-density lipoprotein–cholesterol (HDL-C), and triacylglycerol were estimated in fasting serum samples using commercially available kits (Accurex Biomedical). To determine levels of low-density lipoprotein (LDL) + very low-density lipoprotein (VLDL)-cholesterol, HDL-C values were subtracted from those of TC. An ion-exchange resin method was used to estimate HbA1c levels in whole blood using a commercially available kit (Transasia, Daman, India). Serum insulin levels were determined by ELISA (DRG Diagnostics, Marburg, Germany). Glycogen was isolated from tissues according to the method of Good et al. Homogenized tissues were boiled with 50% KOH in a boiling water bath until a homogeneous solution was obtained. Glycogen was precipitated by the addition of 95% ethanol and the supernatant was discarded. The precipitated glycogen was dissolved in water and estimated according to the method of Caroll et al. using anthrone reagent. Glucokinase was determined according to the method of Porter et al. by measuring the glucose-6-phosphate formed following the reduction of NADP⁺ in presence of glucose-6-phosphate dehydrogenase. Glucose-6-phosphatase was estimated according to the method of Harper by measuring the amount of inorganic phosphate formed following incubation of the liver homogenate with glucose-6-phosphate. The inorganic phosphate formed was determined according to the method of Fiske and Subbarow. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was estimated according to the method of Rao and Ramakrishnan. Liver homogenate HMG-CoA and mevalonate were estimated colorimetrically and the ratio of HMG-Co A : mevalonate was taken as an index of enzyme activity, with a decreased ratio indicating increased activity and vice versa.

**Statistical analysis**

Data are expressed as the mean ± SEM. Statistical analyses were performed using repeated-measures analysis of variance (ANOVA) followed by Tukey’s test. Two-tailed significance was set at 5%.

**Results**

In the present study, diabetic rats were treated with the stem juice of *M. sapientum* and the effects treatment on glycemic control and improvements in the lipid profile were assessed.

**Determination of maximum effective dose**

Table 1 lists FPG levels in normal and diabetic rats after treatment for 15 days with different doses of stem juice. Diabetic control rats had significantly higher FPG levels than the healthy control rats, implying that the diabetic animal model was efficient. There was a significant decrease in FPG levels in diabetic rats treated with either stem juice or glibenclamide compared with the untreated diabetic controls. When the dose of the stem juice was increased from 25 to 50 mg/kg (i.e. Groups III and IV, respectively), there was a greater decrease in FPG in Group IV compared with Group III (22% vs. 14%, respectively). However, when the dose was increased to 100 mg/kg (Group V), no further effects on FPG were observed. Thus, a dose of...
50 mg/kg stem juice was selected for use in further studies.

**Effect on glycemic control**

Table 2 lists the effects of 4 weeks treatment with 50 mg/kg stem juice on FPG, PPG, and body weight. Both FPG and PPG levels increased significantly in untreated diabetic rats compared with healthy control rats. There was a significant decrease in both parameters in the stem juice- and glibenclamide-treated diabetic groups compared with the untreated diabetic group. However, stem juice treatment of healthy rats had no significant effect on either FPG or PPG. It is of note that the treatment did not produce hypoglycemia in healthy rats.

Healthy rats, with and without treatment, exhibited an increase in body weight during the treatment period. In contrast, untreated diabetic rats exhibited a 21% decrease in body weight over the same period. Treatment of diabetic rats with either the stem juice or glibenclamide resulted in a significant body weight gain compared with the diabetic control group ($P < 0.001$).

Figure 1 shows the effect of stem juice treatment on serum insulin levels in the different groups. Serum insulin levels in the diabetic control rats were significantly lower than in the healthy control. Treatment of diabetic rats with stem juice or glibenclamide significantly increased serum insulin levels ($P < 0.05$). There was no significant difference in serum insulin levels

Table 2  Effects of 4 weeks treatment with stem juice of *Musa sapientum* Linn. (50 mg/kg per day) on body weight and fasting and postprandial plasma glucose levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose levels (mmol/L)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Group A</td>
<td>3.8 ± 0.2</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>Group B</td>
<td>3.9 ± 0.4</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Group C</td>
<td>7.9 ± 0.3</td>
<td>13.7 ± 0.8</td>
</tr>
<tr>
<td>Group D</td>
<td>8.1 ± 0.5</td>
<td>13.4 ± 1.1</td>
</tr>
<tr>
<td>Group E</td>
<td>8.2 ± 0.4</td>
<td>13.6 ± 0.6</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM of six animals in each group. *$P < 0.05$, **$P < 0.001$ compared with Group C.

Postprandial glucose levels were determined 2 h after a 2 g/kg glucose load.

Group A, healthy control (non-diabetic) group administered saline (0.5 mL/kg); Group B, healthy control (non-diabetic) group administered stem juice (50 mg/kg); Group C, diabetic control group administered saline (0.5 mL/kg); Group D, diabetic rats administered stem juice (60 mg/kg); Group E, diabetic rats treated with 0.6 mg/kg glibenclamide.

Figure 1  Effects of 4 weeks treatment with stem juice of *Musa sapientum* Linn. (50 mg/kg per day) on serum insulin in the different experimental groups. Group A, healthy control (non-diabetic) group administered saline (0.5 mL/kg); Group B, healthy control (non-diabetic) group administered stem juice (50 mg/kg); Group C, diabetic control group administered saline (0.5 mL/kg); Group D, diabetic rats administered stem juice (50 mg/kg); Group E, diabetic rats treated with 0.6 mg/kg glibenclamide. Data are the mean ± SEM ($n = 6$ rats in each group). *$P < 0.05$ compared with Group C.
Antidiabetic effect of *Musa sapientum* Linn.

Figure 2 Effects of 4 weeks treatment with stem juice of *Musa sapientum* Linn. (50 mg/kg per day) on HbA1c in the different experimental groups. Group A, healthy control (non-diabetic) group administered saline (0.5 mL/kg); Group B, healthy control (non-diabetic) group administered stem juice (50 mg/kg); Group C, diabetic control group administered saline (0.5 mL/kg); Group D, diabetic rats administered stem juice (50 mg/kg); Group E, diabetic rats treated with 0.6 mg/kg glibenclamide. Data are the mean ± SEM (n = 6 rats in each group). *P < 0.05 compared with Group C.

Effects on muscle and liver glycogen

Figure 3 shows glycogen levels in the muscle and liver of all five groups. The glycogen content of the muscle and liver was lower in the diabetic control group compared with that in the untreated and treated healthy controls. Following the treatment of diabetic rats with stem juice or glibenclamide, the liver and muscle glycogen content increased significantly.

Effects on liver enzymes

Table 3 lists the effects of 4 weeks treatment with stem juice on various liver enzymes. The activity of glucokinase, which is involved in glycolysis, decreased, whereas that of glucose-6 phosphatase (involved in gluconeogenesis) and HMG-CoA reductase (involved in cholesterol biosynthesis) increased in diabetic control rats compared with the normal untreated or treated groups. However, following treatment of diabetic rats with stem juice or glibenclamide, glucokinase activity increased significantly, whereas that of glucose-6 phosphatase (*P < 0.05*) and HMG-CoA reductase decreased (*P < 0.001*).

Effects on serum lipid parameters

Figure 4 shows serum TC, HDL-C and LDL + VLDL–cholesterol levels in the five groups before and after 4 weeks treatment. In diabetic control rats, TC and LDL + VLDL-C levels were significantly higher, and HDL-C levels were lower, than in the healthy control

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between the healthy control and stem juice-treated healthy rats.

Effects of stem juice treatment on HbA1c are shown in Fig. 2. In the diabetic control group, HbA1c was significantly increased compared with levels in the healthy control or stem juice-treated healthy control rats. Treatment of diabetic rats with either stem juice or glibenclamide restored HbA1c to near to normal levels.

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Table 3 Effects of 4 weeks treatment with stem juice of *Musa sapientum* Linn. (50 mg/kg per day) on liver enzymes

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucokinase (U/mg protein)</th>
<th>Glucose-6-phosphatase (U/mg protein)</th>
<th>HMG-CoA reductase (HMG-CoA/mevalonate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0.45 ± 0.01</td>
<td>0.13 ± 0.03</td>
<td>1.71 ± 0.11</td>
</tr>
<tr>
<td>Group B</td>
<td>0.51 ± 0.03</td>
<td>0.14 ± 0.04</td>
<td>1.82 ± 0.21</td>
</tr>
<tr>
<td>Group C</td>
<td>0.20 ± 0.01</td>
<td>0.46 ± 0.04</td>
<td>0.83 ± 0.12</td>
</tr>
<tr>
<td>Group D</td>
<td>0.36 ± 0.02*</td>
<td>0.21 ± 0.11*</td>
<td>1.28 ± 0.20**</td>
</tr>
<tr>
<td>Group E</td>
<td>0.34 ± 0.02*</td>
<td>0.22 ± 0.12*</td>
<td>1.34 ± 0.22**</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM of six animals in each group. *P < 0.05, **P < 0.001 compared with Group C.

Group A, healthy control (non-diabetic) group administered saline (0.5 mL/kg); Group B, healthy control (non-diabetic) group administered stem juice (50 mg/kg); Group C, diabetic control group administered saline (0.5 mL/kg); Group D, diabetic rats administered stem juice (50 mg/kg); Group E, diabetic rats treated with 0.6 mg/kg glibenclamide; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase.
and an increase in HDL-C at the end of 4 weeks in diabetic rats treated with stem juice or glibenclamide.

Serum triacylglycerol levels in the five groups before (Week 0) and after 4 weeks treatment are shown in Fig. 5. Triacylglycerol levels were significantly higher at Week 0 in diabetic control rats compared with the healthy control or stem juice-treated healthy control groups and had increased further by the end of Week 4. In diabetic rats treated with either stem juice or glibenclamide, significant decreases were seen in triacylglycerol levels compared with the diabetic control group by the end of Week 4 (\(P < 0.05\)).

**Phytochemicals in stem juice**

Preliminary phytochemical screening of the stem juice of *Musa sapientum* Linn. revealed the presence of phenolic compounds, terpenoids, saponins, and pectins (Table 4).

**Discussion**

The present study has shown a beneficial effect of the stem juice of *Musa sapientum* in a diabetic model, suggesting that it causes insulin secretion, with consequent changes in hepatic and muscle glycogen, liver enzymes, and serum lipid levels. We have performed these studies in moderately diabetic rats and used glibenclamide as a reference drug.\(^\text{33}\) The results of the present study indicate that the juice of the central part of stem of the *Musa sapientum* Linn. has a significant hypoglycemic effect (\(P < 0.05\)), with the most effective dose being 50 mg/kg. The efficiency of this dose, based on FPG and PPG levels, was comparable to that of glibenclamide. Diabetic rats treated with this dose of stem juice for 4 weeks exhibited a significant decrease in FPG and PPG levels. The significant finding of the present study is that stem juice treatment does not induce hypoglycemia in normal rats, which shows that it does not interfere with the normal regulatory mechanisms of glucose homeostasis.

In the present study, we found decreased levels of glycogen in the muscle and liver of diabetic rats. In this respect, our results are in agreement with those reported
in previous studies.\textsuperscript{34,35} After stem juice treatment, there was a significant recovery in hepatic and muscle glycogen, which may be related to the effect of the stem juice on increasing the release of the insulin. Insulin stimulates the synthesis of the glucokinase enzyme and increases its activity.\textsuperscript{36} We found increased glucokinase activity in diabetic rats treated with the stem juice. The increased glucokinase activity may have contributed to the reduction in blood glucose levels by enhancing the flux of glucose into the glycolytic pathway. Glucose-6-phosphate formed by the action of glucokinase stimulates dephosphorylation and activation of glyco-
gen synthase.\textsuperscript{36} Increased glucokinase activity may have resulted in an increase in the glycogen content of the liver and muscle. Stem juice treatment also decreased gluconeogenesis in diabetic rats, as evidenced by decreased levels of glucose-6-phosphatase.

A reliable index of long-term glycemic control is HbA1c.\textsuperscript{37} In the present study, diabetic rats exhibited increased HbA1c levels. However, significant decreases in HbA1c were seen in treated diabetic rats, indicating that stem juice treatment was effective in controlling blood glucose levels.

Hyperglycemia and dyslipidemia coexist in diabetes mellitus. A drug that also corrects lipid abnormalities will be beneficial for the treatment of diabetes. We observed increased TC, LDL + VLDL-C, and triacylglycerol levels in untreated diabetic rats and this finding is consistent with previous reports.\textsuperscript{33,38} Following treatment with either stem juice or glibenclamide, significant decreases were observed in TC, LDL + VLDL-C and triacylglycerol in diabetic rats, whereas HDL-C levels increased significantly. Moreover, we observed decreased HMG-CoA reductase activity in the liver after treatment. This enzyme catalyses the first step in cholesterol biosynthesis. The decrease in cholesterol may be due to decreased synthesis of cholesterol in treated diabetic rats. The hypolipidemic effect of stem juice may be related to its effect in increasing the release of insulin. This lipid-lowering effect makes the stem juice very useful for the treatment of diabetes.

Pectins isolated from the inflorescence stalk and terpenoids obtained from the fruit pulp of \textit{M. sapientum} Linn. have been shown to have significant hypoglycemic effects in alloxan-diabetic rats.\textsuperscript{14,16} Phenolic compounds from rice bran have also been shown to have hypoglycemic effects.\textsuperscript{39} Phytochemical analysis of the stem juice in the present study indicated the presence of pectins, terpenoids, and phenolic compounds. All three compounds have been shown to have hypoglycemic actions in previous studies.\textsuperscript{14,16,39} Thus, the presence of phenolic compounds, pectins, and terpenoids in the stem juice of \textit{M. sapientum} Linn. may contribute to its significant hypoglycemic effects in diabetic rats.

**Conclusion**

The present study has demonstrated that the stem juice of \textit{M. sapientum} Linn. has significant antidiabetic and antihyperlipidemic effects. The antidiabetic and antihyperlipidemic effects of the stem juice may be related to its insulinogenic effect.

**Acknowledgment**

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**Disclosure**

The authors declare that they have no competing financial interests.

**References**


Hepatoprotective effect of stem of *Musa sapientum* Linn in rats intoxicated with carbon tetrachloride

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ABSTRACT

Methods. The study was designed to evaluate the hepatoprotective activity of aqueous extract of central stem of *Musa sapientum* (AqMS) against carbon tetrachloride induced hepatotoxicity in rats. Animals were divided into six groups. Group I served as normal control. Group II, III, IV, V & VI were administered CCl₄ mixed with olive oil 1:1 (1.5 mL/kg) I.P., twice a week for 5 weeks. Group II was maintained as CCl₄ intoxicated control. Group III, IV and V received AqMS at a dose of 25, 50 and 100 mg/kg. Group VI received silymarin 100 mg/kg for 5 weeks orally once daily. Marker enzymes of hepatic functions estimated in serum were AST, ALT and ALP. Antioxidant parameters estimated were MDA and GSH in blood and liver and SOD in blood, after fifth week, animals were sacrificed, livers dissected out and evaluated for histomorphological changes. Results. There was significant rise in AST, ALT and ALP in CCl₄ intoxicated control group II. Treatment with AqMS prevented rise in levels of these enzymes. There was significant rise in MDA and fall in GSH in blood and liver in group II, indicating increased lipid peroxidation and oxidative stress upon CCl₄ administration. Treatment with AqMS prevented rise in MDA & increased GSH in treated group. SOD levels were decreased in group II while groups treated with AqMS showed significant rise (p < 0.05). Maximum hepatoprotective effect was observed with 50 mg/kg dose. Hepatoprotective effect observed with this dose was comparable to standard hepatoprotective drug silymarin. The results of pathological study also support the results of biochemical findings. Conclusion. the results of the present study indicate that stem of *Musa sapientum* possess hepatoprotective effect and probably it is due to it’s antioxidant property.


INTRODUCTION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver diseases such as cirrhosis, fatty liver and chronic hepatitis are important world health issues. Conventional and synthetic drugs used in liver disease are inadequate and sometimes have serious side effects.¹

The use of herbal remedies for the treatment of liver diseases has long history starting with Ayurvedic treatment and extending to the Chinese, European and other systems of traditional medicine. A large number of plants and formulations have been claimed to have hepatoprotective effect. Some plants which have shown genuine utility in liver disorders are *Silybium marimum*,² *Picrorhiza kurroa*,³ *Andrographis paniculata*, *Phyllunthus niruri* and *Eclipta alba*.⁴ *Musa sapientum* Linn is an herbaceous plant of Museace family, it has great medicinal value. Fruits and roots have antidiabetic activity.⁵,⁶ Rhizome and stem aqueous extract is used to dissolve urinary stones.⁷ Aqueous extract of flower is useful in dysentery and diarrhea.⁸ Aqueous extract of stem have antivenom⁹ and analgesic activity.¹⁰ Various biologically important compounds reported from banana plant are tannins, pectins, dopamine, serotonin, noradrenaline, sitostetrol, stigmasitosterol, etc.¹¹-¹³ However to best of our knowledge this plant has not been explored for its hepatoprotective effect.

We have investigated hepatoprotective effect of aqueous extract of central part of stem of *Musa*
sapientum Linn (AqMS). This central part of stem is true stem of banana plant. What appears like stem of this plant is pseudo stem formed by upright concentric layers of leaf sheaths. The true stem begins as an underground corm which grows upwards pushing its way through the centre of pseudo stem eventually producing inflorescence which later bear fruits. This central part of stem is edible part of plant. We have found aqueous extract of this part of plant to have significant hypoglycemic effect (unpublished data). Hepatic damage was induced in rats by giving them carbon tetrachloride (CCl4) intraperitonealy. Ability of AqMS to ameliorate CCl4 induced heptatotoxicity and its effects on important antioxidant parameters were studied.

MATERIAL AND METHODS

Chemicals

Technical grade carbon tetrachloride (purity 99.4%) was obtained from Merck, India and silymarin was obtained from Sigma Chemicals, USA. Stem of Musa sapientum was collected from local area and was authenticated by National Institute of Science Communication and Information Resources, New Delhi (Voucher no.NISCAIR/RHMD/Consult/2007-08/895/79). All other reagents used were of analytical grade and obtained either from Sisco Research Laboratories or Qualigens Fine Chemicals, Mumbai, India.

Animals

Male Wistar rats (aged 10-12 weeks, weight range 150-200 g) were obtained from central animal house facility of the institute. The animals were kept under standard conditions (temp. 22 ± 2 °C, 80% humidity with 12 h light/dark cycle). Food and water were accessible ad libitum. Body weights and food consumption were recorded weekly. Records were maintained to comply with the conditions as desired by Institutional Ethical Committee-Animal Research, (IEC-AR) University College Medical Sciences, Delhi and the experiments were carried out as per the guidelines given by IEC-AR.

Induction of hepatotoxicity

Hepatotoxicity was induced in rats by giving intraperitoneal injection of CCl4 in olive oil (1:1, 1.5 mL/kg) twice a week for 5 weeks.14

Preparation of aqueous extract of central part of stem (AqMS)

Fresh stems of Musa sapientum Linn were used. The upright concentric layers of leaf sheaths forming the pseudo stem were peeled off to reveal the central pale white stem. This central pale white stem was cut into pieces. One hundred gram stem was crushed with 20 mL water in a mixer followed by filtration through a sterile muslin cloth to get aqueous extract. Whole process was carried out at room temperature. Extract was lyophilized and stored in refrigerator (yield 2%).

Phytochemical studies

Phytochemical analysis of the lyophilized aqueous extract was done for terpenoids, phenolic compounds, saponins and pectins according to the methods described.15 Total phenolic contents were analyzed using Folin Ciocalcateu reagent16 and results are expressed as gallic acid equivalent.

Experimental design

Animals were randomly divided into six groups (six rats per group). Group I served as normal control. Groups II, III, IV, V & VI were treated with CCl4 mixed with olive oil in ratio of 1:1 at a dose of 1.5 mL/kg intraperitonealy twice a week for five weeks. Group II animals were maintained as CCl4 intoxicated control without any drug treatment. Group III, IV & V were administered AqMS at a dose of 25, 50 and 100 mg/kg respectively and group VI received silymarin (100 mg/kg in 5% gum acacia) orally once daily for five week17 in addition to CCl4 twice a week as mentioned above.

Biochemical parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were estimated in serum using commercially available kits of Transasia Pvt. Ltd. Mumbai. Malondialdehyde (MDA) was estimated in serum and liver.18 Reduced glutathione (GSH) was estimated in whole blood and liver,19 activity of superoxide dismutase (SOD) was assayed in erythrocytes.20

Assessment of liver damage

After five weeks overnight fasted rats were anaesthetized with pentobarbital (50 mg/kg i.p.)21 and sa-
crificed by cervical dislocation. Blood was collected from heart by the heart puncture technique in EDTA vial for estimating SOD and GSH and plain vial for estimating MDA, ALT, AST & ALP. Liver tissue was taken out washed with cold saline and stored at -20 °C for estimating the antioxidant parameters.

**Histopathological studies**

Part of liver samples was submitted in 10% buffered formaldehyde for histopathological examinations. Sections were cut on glass slides (4-6 µm thick) and stained with hematoxylin and eosin (H & E) for routine histomorphology. The extent of liver damage was assessed by a histopathologist under light Olympus microscope.

**Statistical analysis**

The results are expressed as means ± S.D. The difference between experimental groups compared by one way ANOVA followed by Tukey’s test at 5% significance level.

**RESULTS**

**Effect on serum enzymes**

Treatment with CCl₄ at a dose of 1.5 mL/kg twice a week for five weeks increased levels of AST, ALT and ALP significantly in group II (Table 1). Group III, IV and V which received treatment with AqMS orally once daily in addition to CCl₄ twice a week showed significant fall in levels of these parameters. Maximum effect was obtained with a dose of 50 mg/kg which produced 85, 80 and 51% fall in levels of AST, ALT & ALP respectively (p < 0.05). Further increase in dose had no additional benefit. Fall in AST, ALT and ALP with 50 mg/kg dose was comparable to fall in these parameter by standard hepatoprotective drug i.e. silymarin.

**Markers of oxidative damage**

Treatment of rats with CCl₄ increased lipid peroxidation as evident by significant rise in the levels of MDA in blood in group II (Table 2). Simultaneous

### Table 1. Effect of five weeks treatment with aqueous extract of central stem of Musa sapientum (AqMS) on hepatic enzymes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment groups</th>
<th>Aspartate aminotransferase (IU/L)</th>
<th>Alanine aminotransferase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal-control</td>
<td>37.8 ± 3.8</td>
<td>30 ± 5.4</td>
<td>71 ± 15.3</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄ treated (1.5 mL/kg)</td>
<td>716 ± 22†</td>
<td>454 ± 27.1†</td>
<td>247 ± 43.5†</td>
</tr>
<tr>
<td>III</td>
<td>CCl₄ + AqMS(25 mg/kg)</td>
<td>600 ± 18*</td>
<td>350 ± 23*</td>
<td>240 ± 38</td>
</tr>
<tr>
<td>IV</td>
<td>CCl₄ + AqMS(50 mg/kg)</td>
<td>120 ± 12*</td>
<td>87.5 ± 22.4*</td>
<td>122 ± 23*</td>
</tr>
<tr>
<td>V</td>
<td>CCl₄ + AqMS(100 mg/kg)</td>
<td>125 ± 10*</td>
<td>92 ± 18*</td>
<td>129 ± 16*</td>
</tr>
<tr>
<td>VI</td>
<td>CCl₄ + silymarin(100 mg/kg)</td>
<td>178 ± 20*</td>
<td>89 ± 14*</td>
<td>122 ± 32.1*</td>
</tr>
</tbody>
</table>

The results are the mean ± SD for six rats in each group. Significantly different from normal control: †P < 0.05; Significantly different from CCl₄ treated: *P < 0.05.

### Table 2. Effect of five weeks treatment with aqueous extract of central stem of Musa sapientum (AqMS) on antioxidant parameters in blood.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment group</th>
<th>Malondialdehyde (nmol/mL)</th>
<th>Reduced glutathione (mg/g Hb)</th>
<th>Superoxide dismutase (u/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal-control</td>
<td>2.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>2448 ± 269</td>
</tr>
<tr>
<td>II</td>
<td>CCl₄-treated</td>
<td>6.1 ± 1.3†</td>
<td>0.8 ± 0.1†</td>
<td>1492 ± 223†</td>
</tr>
<tr>
<td>III</td>
<td>CCl₄ + AqMS(25 mg/kg)</td>
<td>5.2 ± 0.8</td>
<td>0.9 ± 0.2</td>
<td>1550 ± 198</td>
</tr>
<tr>
<td>IV</td>
<td>CCl₄ + AqMS(50 mg/kg)</td>
<td>5.2 ± 1.2*</td>
<td>1.4 ± 0.4*</td>
<td>1931 ± 208*</td>
</tr>
<tr>
<td>V</td>
<td>CCl₄ + AqMS(100 mg/kg)</td>
<td>3.2 ± 1.1*</td>
<td>1.38 ± 0.3*</td>
<td>1840 ± 188*</td>
</tr>
<tr>
<td>VI</td>
<td>CCl₄ + silymarin(100 mg/kg)</td>
<td>3.1 ± 0.80*</td>
<td>1.5 ± 0.3*</td>
<td>1966 ± 236*</td>
</tr>
</tbody>
</table>

The results are the mean ± SD for six rats in each group. Significantly different from normal control: †P < 0.05; Significantly different from CCl₄ treated: *P < 0.05.
treatment with AqMS decreased MDA in blood by 15% in group III, 47.5% in group IV & 46% in group IV. Amount of reduced glutathione decreased in blood of group II rats by 47%. Group III, IV and V rats which received AqMS treatment as mentioned above had 12.5, 75 and 72% rise in GSH, respectively. Antioxidant enzyme SOD was decreased in erythrocytes of group II rats while treatment prevented fall in activity of SOD in all AqMS treated groups with maximum rise in group IV. Findings with respect to GSH, MDA and SOD in group IV which received AqMS at 50 mg/kg were comparable with group treated with standard hepatoprotective drug silymarin.

Effect of treatment with AqMS on liver MDA & GSH is shown in table 3. In liver there was 230% rise in MDA in group II. While group III, IV and V showed fall in levels of MDA. Maximum fall was obtained in group IV. GSH was decreased in group II but group III, IV and V which received treatment showed 7, 150, 123% rise in levels of GSH (P < 0.05), respectively. Results obtained in groups IV which received 50 mg/kg dose were comparable with that of hepatoprotective drug silymarin.

### Table 3. Effect of five weeks treatment with aqueous extract of central stem of Musa sapientum (AqMS) on antioxidant parameters in liver.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Malondialdehyde (nmol/mg protein)</th>
<th>Reduced Glutathione (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal-control</td>
<td>1.3 ± 0.3</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>II</td>
<td>CCl4-treated</td>
<td>4.3 ± 0.6†</td>
<td>2.6 ± 0.9†</td>
</tr>
<tr>
<td>III</td>
<td>CCl4 + AqMS (25 mg/kg)</td>
<td>4 ± 0.4</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>IV</td>
<td>CCl4 + AqMS (50 mg/kg)</td>
<td>2.2 ± 0.2*</td>
<td>6.5 ± 1.2*</td>
</tr>
<tr>
<td>V</td>
<td>CCl4 + AqMS (100 mg/kg)</td>
<td>2.4 ± 0.4*</td>
<td>5.8 ± 1.8*</td>
</tr>
<tr>
<td>VI</td>
<td>CCl4 + silymarin (100mg/kg)</td>
<td>2.5 ± 0.5*</td>
<td>8.5 ± 1.3*</td>
</tr>
</tbody>
</table>

The results are the Mean ± SD for six rats in each group. Significantly different from normal control: †P < 0.05; Significantly different from CCl4 treated: * P < 0.05.

**Figure 1.** Photomicrograph of rat liver sections H & E X 40; (a) Normal. (b) CCl4 treated (1.5 mL/kg) showing severe macro vascular steatosis, centrilobular hepatocellular necrosis and periportal inflammation. (c) CCl4 + Aqueous extract of Musa sapientum(50mg/kg) (d) CCl4 + silymarin (100 mg/kg); Both group c & d shows only mild periportal inflammation, reduced amount of steatosis without any significant hepatocyte necrosis.
Phytochemicals in AqMS

Phytochemical analysis showed presence of phenolic compounds, terpenoids, saponins and pectins in AqMS. Total phenolic content was found to be 50 mg/gm of lyophilized extract.

Histopathological studies

Histopathology of liver of group I, II, IV & V was done. Group IV in AqMS treated group was selected as it showed maximum hepatoprotective effect. The histology of liver in group I showed a normal cell morphology with hexagonal lobular architecture (Figure 1A). While the group II showed marked hepatocellular damage in the form of severe macrovesicular steatosis, centrilobular hepatocellular necrosis and periportal inflammation (Figure 1B). These findings seems to be ameliorated by treatment with AqMS in group IV and silymarin in group VI. There was only mild periportal inflammation and reduced amount of steatosis without any significant hepatocyte necrosis (Figure 1C and 1D).

DISCUSSION

Liver is the principle site for CCl₄ induced effect to manifest themselves.22 Plasma membrane, endoplasmic reticulum, mitochondria, and Golgi apparatus are the main subcellular structures affected by CCl₄ exposure.23 Damage to plasma membrane of hepatocytes results in release of enzymes in circulation. In the CCl₄ treated group ALT, AST, ALP levels increased dramatically compared to normal control group indicating severe hepatocellular damage.

The ability of a hepatoprotective drug to reduce injurious effect or preserve the normal hepatic physiological function is the index of its hepatoprotective effect. Treatment with AqMS lowered levels of these enzymes. Similar effects have been obtained with other plants with hepatoprotective activity.21,24,25

CCl₄ metabolism begins with formation of trichloro methyl free radical CCl₃• through the action of Cytochrome P450 oxygenase system. The major cytochrome P450 isozyme involved is CYP2E1, however at higher concentration CYP3A contributes significantly in this activation. CCl₃• radical is converted trichloromethyl peroxy radical CCl₃COO• in the presence of oxygen.

CCl₃COO• is more active then CCl₃• and both react with various biologically important substances like proteins, nucleic acids and lipids, altering their functions. CCl₃• and CCl₃COO• both can abstract a hydrogen from polyunsaturated fatty acids and initiate the process of lipid peroxidation which can affect in two ways:

1. By compromising membrane function, and
2. By covalent binding of reactive intermediate leading to the liver cell necrosis.26, 27

CCl₄ also affects hepatocellular levels of Ca²⁺ and continued exposure leads to increased cytosolic Ca²⁺. Uncontrolled levels of Ca²⁺ in cells destroy cytoskeletal structures and activate number of catabolic enzymes whose actions result in cell death via apoptosis or necrosis.28, 29 One of the causes for cell damage due to CCl₄ is loss of Ca²⁺ homeostasis.

CCl₄ treatment increased lipid peroxidation as evident by rise in MDA, a marker of lipid peroxidation in group II. Others have also reported similar rise in MDA on CCl₄ intoxication.30,31 Treatment with AqMS and sylimarin decreased levels of MDA. Sylimarin is known to decrease MDA due to its free radical scavenging activity.32 A positive correlation has been reported in phenolic contents and antioxidant activity.33 Saponins have also been reported to confer hepatoprotective and antioxidant properties.34

Phytochemical studies show the presence of saponins and phenolic compounds in aqueous extract of Musa sapientum. Phenolic content of AqMS was found to be 50 mg/gm. Therefore decrease in MDA may be due to antioxidant activity of the saponin and phenolic compounds present in AqMS. Reduced glutathione (GSH) is a non enzymatic antioxidant, widely distributed in liver cells. It’s main functions is mainly concerned with the removal of free radicals species such as H₂O₂, superoxide radicals and maintenance of membrane protein thiols.35 AqMS treatment prevented depletion of GSH in liver and blood.

CCl₄ has been shown to induce the formation of free radicals which may decrease the activity of SOD as has been observed in CCl₄ treated animals, however increase in SOD activity in AqMS treated group indicates that this treatment prevents formation of free radicals.

The results of histopathological study also support the results of biochemical parameters and are the in-situ evidence of hepatoprotective effect of AqMS. There was marked hepatocellular damage in CCl₄ alone treated group. Simultaneous treatment of AqMS with CCl₄ exhibited significantly less damage to the hepatic cells compared to rats treated with CCl₄ alone. The reduction in cellular damage seen in AqMS treated group was morphologically similar to silymarin treated group.
CONCLUSION

It may be concluded from the present study that AqMS possess hepatoprotective activity against model hepatotoxicant CCl₄ and maximum beneficial effect was observed at a dose of 50 mg/kg. The hepatoprotective action is probably related to its potential antioxidant activity. Further investigations are required to characterize the active hepatoprotective principle.

ABBREVIATIONS

- AqMS: Aqueous extract of Musa sapientum.
- i.p: Interaperitoneal.
- AST: Aspartate aminotransferase.
- ALT: Alanine aminotransferase.
- ALP: Alkaline phosphatase.
- MDA: Malonaldehyde.
- SOD: Superoxide dismutase.
- H&E: Hematoxylin & eosin.
- S.D.: Standard deviation.
- %: Percent.
- ANOVA: Analysis of variance.
- IU: International unit.
- nmol: Nano mole.
- mg: Milligram.
- g: Gram.
- Hb: Hemoglobin.
- mL: Milliliter.
- ICMR: Indian council of medical research.

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REFERENCES