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Hepatoprotective activity of *Musa paradisiaca* on experimental animal models

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ABSTRACT

Objective: To investigate the hepatoprotective activity of stem of *Musa paradisiaca* (*M. paradisiaca*) in CCl_4 and paracetamol induced hepatotoxicity models in rats. **Methods:** Hepatoprotective activity of alcoholic and aqueous extracts of stem of *M. paradisiaca* was demonstrated by using two experimentally induced hepatotoxicity models. **Results:** Administration of hepatotoxins (CCl_4 and paracetamol) showed significant biochemical and histological deteriorations in the liver of experimental animals. Pretreatment with alcoholic extract (500 mg/kg), more significantly and to a lesser extent the alcoholic extract (250 mg/kg) and aqueous extract (500 mg/kg), reduced the elevated levels of the serum enzymes like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin levels and alcoholic and aqueous extracts reversed the hepatic damage towards the normal, which further evidenced the hepatoprotective activity of stem of *M. paradisiaca*. **Conclusions:** The alcoholic extract at doses of 250 and 500 mg/kg, p.o. and aqueous extract at a dose of 500 mg/kg, p.o. of stem of *M. paradisiaca* have significant effect on the liver of CCl_4 and paracetamol induced hepatotoxicity animal models.

1. Introduction

Liver is one of the largest organs in the human body and carries out various functions like carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins[1]. Liver disease is still a worldwide health problem. Jaundice and hepatitis are two major hepatic disorders that account for high death rate[2]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects[3].

Musa paradisiaca (*M. paradisiaca*) (Musaceae) is commonly known as banana (English). It is cultivated in tropical and semi-tropical countries. *M. paradisiaca* root is used as tonic for congestion of the liver and to prevent scurvy, anaemia, veneral disease. The leaves are used in inflammation of eye, healing wounds and ulcers. The flowers check excessive bleeding during menstruation and are used in the case of diabetes. The fruits are used in diarrhea, indigestion and flatulence. The stems are used for ulcer, jaundice, nervous disorder, hysteria, diarrhea, dysentery, antidote for opium poisoning, asthma, hair loss, treatment of piles[4]. Literature reviews indicated that the hepatoprotective activity of stem of *M. paradisiaca* has not been scientifically evaluated so far. An active and safe drug is needed for the treatment of jaundice. In view of this, the present study was aimed at evaluating the hepatoprotective activity of stem of *M. paradisiaca* against CCl_4 and paracetamol induced hepatotoxicity in albino rats.

2. Materials and methods

2.1. Plant

Stem of *M. paradisiaca* was collected from Kunigal, Tumkur district, Karnataka and authenticated by Sreenath KP, taxonomist, Department of Botany, Bangalore University, Jnana Bharathi Campus, Bengaluru. The material was dried under shade, powdered mechanically and stored in air tight container.

2.2. Preparation of plant extracts

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300 g powder of stem of *M. paradisiaca* was subjected for soxhlation. It was refluxed with ethanol for 18 h in batches of 100 g each. The extracts were pooled together and concentrated by using rotary evaporator. The yield was 9.08% w/w. 300 g powder of stem of *M. paradisiaca* was macerated for 3 days with distilled water containing chloroform (2.5 mL/1 000 mL) and the residue was removed by filtration and the filtrate was concentrated by using rotary evaporator to obtain aqueous extract. The yield was 11.45% w/w.

2.3. Preliminary phytochemical investigation

Preliminary phytochemical investigation of alcoholic and aqueous extracts of stem of *M. paradisiaca* was carried out by using standard procedure given by Kokate[5] and Khandelwal[6].

2.4. Drugs and chemicals

Silymarin was obtained from Micro Labs, Bengaluru and all biochemical kits were purchased from Anjan Distributors, Bengaluru.

2.5. Experimental animals

Wistar albino rats of (150-200 g) were purchased from Bioneeds, Nelamangala, Tumkur and were acclimatized for 7 days under standard husbandry conditions, *i.e.*, room temperature of (26±10) ℃, relative humidity of (45%-55%) and light and dark cycle of 12 h:12 h in the animal house of PES College of Pharmacy, Bengaluru. All the experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) of PES College of Pharmacy, Bengaluru, and conducted according to the Committee for the Purpose of the Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

2.6. Acute oral toxicity studies

Alcoholic and aqueous extracts of stem of *M. paradisiaca* were studied for the acute oral toxicity according to the guidelines set by Organization for Economic Co-operation and Development (OECD) guidelines number 423[7]. Healthy Wistar rats weighing (150-180 g) were used for the study. Three doses of 500, 1 000 and 2 000 mg/kg, p.o. of the alcohol and aqueous extacts were given to 6 groups containing 5 animals in each group. The treated groups were monitored for mortality and general behaviour for 14 days. The extracts were devoid of any toxicity in rats when given in dose up to 2 000 mg/kg by oral route. Hence, for further study (250-500) mg/kg doses of extracts were selected.

2.7. Hepatoprotective activity

Hepatoprotective effect of alcoholic and aqueous extracts of stem of *M. paradisiaca* was demonstrated by using two experimentally induced hepatotoxicity models.

2.7.1. Carbon tetrachloride (CCl_4) induced hepatotoxicity in rats

The effect of the extracts was studied in CCl_4 induced Wistar rats of either sex weighing (150-180 g) for 7 days. They were divided into 7 groups containing 6 animals each. Rats in group 1 (normal control) received normal saline (1 mL/kg b.w., p.o.); group 2 (toxic group) received CCl_4 (1 mL/kg b.w., s.c.); group 3 (standard group) received standard drug silymarin (100 mg/kg b.w., p.o.) and CCl_4 (1 mL/kg bw, s.c.); groups 4 and 5 (test groups), received a dose of (250 and 500 mg/kg b.w., p.o.) of alcoholic extract and CCl_4 (1 mL/kg b.w., s.c.), respectively; groups 6 and 7 (test groups), received a dose of (250 and 500 mg/kg b.w., p.o.) of aqueous extract and CCl_4 (1 mL/kg b.w., s.c.), respectively. Animals were sacrificed and blood was collected directly through retro-orbital plexus. Serum was separated after coagulating at 37 ℃ for 30 min and centrifuged at 1 200-1 500 rpm for 15-20 min. The serum was used for the estimation of biochemical parameters, namely, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin (total and direct) by using autoanalyser[8] and the liver tissues collected were subjected to histopathology[9].

2.7.2. Paracetamol induced hepatotoxicity in rats

The effect of the extracts was studied in paracetamol induced Wistar rats of either sex weighing (150-180 g) for 5 days. Animals were divided into 7 groups of 6 animals each. Rats in group 1 (normal control) received normal saline (1 mL/kg b.w., p.o.) for 5 days; group 2 (toxic group) received saline (1 mL/kg b.w., p.o.) for 5 days and paracetamol (2 g/kg b.w., p.o.) on the 2nd and 3rd day; group 3 (standard group) received silymarin (100 mg/kg b.w., p.o.) for 5 days and paracetamol (2 g/kg b.w., p.o.) on the 2nd and 3rd day, 30 min after sylimarin administeration; groups 4 and 5 (test groups), received alcoholic extract (250 and 500 mg/kg b.w., p.o.) for 5 days and were administered paracetamol (2 g/kg b.w., p.o.) on the 2nd and 3rd day, 30 min after alcoholic extract administeration; groups 6 and 7 (test groups), received aqueous extract (250 and 500 mg/kg b.w., p.o.) for 5 days and were administered paracetamol (2 g/kg b.w., p.o.) on the 2nd and 3rd day, 30 min after aqueous extract administration. Animals were sacrificed and blood was collected directly through retro-orbital plexus. Serum was separated after coagulating at 37 ℃ for 30 min and centrifuged at 1 200-1 500 rpm for 15-20 min. The serum was used for the estimation of biochemical parameters, namely, SGPT, SGOT, ALP and bilirubin (total and direct) by using autoanalyser[10] and the liver tissues collected were subjected to histopathology[9].

2.7.3. Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes[9].

2.8. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results were expressed as mean±SEM from six rats in each group. P values < 0.05 were considered significant.

3. Results

3.1. Preliminary phytochemical investigation

Preliminary phytochemical screening revealed the presence of alkaloids, phytosterols, phenolic compounds, proteins and amino acids, flavonoids, tannins and carbohydrates in alcoholic extract and aqueous extract showed the presence of alkaloids, carbohydrates, flavonoids, phenolic compound, tannins, proteins and amino acids.

3.2. Effect of alcoholic and aqueous extracts of stem of M. paradisiaca against CCl_4-induced hepatotoxicity in rats

In CCl_4 induced hepatotoxicity animal model pretreatment with silymarin (100 mg/kg, p.o.) and alcoholic extract of stem of *M. paradisiaca* at a dose of 500 mg/kg reduced SGOT, SGPT, ALP, direct bilirubin, total bilirubin significantly (P<0.001), as compared with CCl_4 intoxicated group and at 250 mg/kg reduced SGOT, SGPT, direct bilirubin, total bilirubin significantly (P<0.01), as compared with CCl_4 intoxicated group. Aqueous extract of stem of *M. paradisiaca* at 500 mg/kg reduced SGPT, direct bilirubin, significantly (P<0.01), as compared with CCl_4 intoxicated group; total bilirubin significantly (P<0.001), as compared with CCl_4 intoxicated group; SGOT, ALP significantly (P<0.05), as compared with CCl_4 intoxicated group. However, aqueous extract of 250 mg/kg dose has not decreased the level of the serum enzymes significantly (Table 1).

3.3. Effect of alcoholic and aqueous extracts of stem of M. paradisiaca against paracetamol-induced hepatotoxicity in rats

In paracetamol induced hepatotoxicity animal model, pretreatment with sylimarin (100 mg/kg, p.o.) and alcoholic extract of stem of *M. paradisiaca* at a dose of 500 mg/kg reduced SGOT, SGPT, ALP, direct bilirubin, total bilirubin significantly (P<0.001), as compared with paracetamol intoxicated group and at 250 mg/kg reduced SGOT, SGPT significantly (P<0.01), as compared with paracetamol intoxicated group; ALP significantly (P<0.01), as compared with paracetamol intoxicated group. Aqueous extract of stem of *M. paradisiaca* at 500 mg/kg reduced total bilirubin significantly (P<0.001), as compared with paracetamol intoxicated group; SGPT significantly (P<0.01), as compared with paracetamol intoxicated group; SGOT, direct bilirubin significantly (P<0.05), as compared with paracetamol intoxicated group. However, aqueous extract of 250 mg/kg dose has not decreased the level of the serum enzymes significantly (Table 2).

3.4. Histopathology

In histopathological study of CCl_4 induced hepatotoxicity model liver section of normal liver showed central vein and cords of hepatocytes; CCl_4 intoxicated group rat liver section showed hepatocellular degeneration with fatty changes; Sylimarin treated group rat liver section showed normal central vein with mild hepatocytic changes. Alcoholic extract (250 mg/kg) treated group rat liver section showed mild hepatocellular degeneration; alcoholic extract (500 mg/kg) treated group rat liver section showed mild perilobular hepatocellular fatty changes degeneration. Aqueous extract (250 mg/kg) treated group rat liver section showed high hepatocellular degeneration; aqueous extract (500 mg/kg) treated group rat liver section showed dilated central vein with perilobular hepatocellular changes (Figure 1).

In histopathological study of paracetamol induced hepatotoxicity model liver section of normal liver showed central vein and cords of hepatocytes; paracetamol intoxicated group rat liver section showed hepatocellular

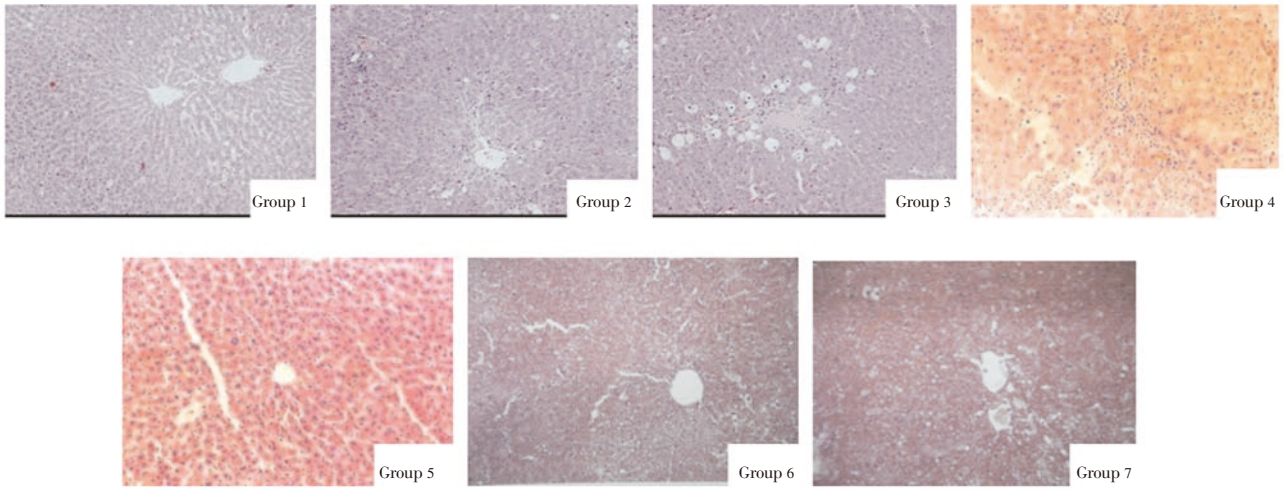

Figure 1. Effect of alcoholic and aqueous extracts of stem of *M. paradisiaca* on CCl_4 induced hepatotoxicity in rats (histopathology).

Table 1
Effect of alcoholic and aqueous extracts of stem of *M. paradisiaca* on CCl_4 induced hepatotoxicity in rats (Mean±SEM) (n=6).

Groups	Serum biochemical parameters				
	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)
Group 1	32.080±1.412	26.080±1.412	31.15±1.375	0.182±0.004	0.182±0.004
Group 2	219.600±24.810	100.400±5.627	82.48±4.866	1.828±0.157	1.758±0.06
Group 3	43.620±6.201***	34.920±1.605***	37.920±1.605***	0.274±0.007***	0.274±0.007***
Group 4	149.300±26.630*	73.460±14.310*	71.050±4.705	1.467±0.245*	1.485±0.068*
Group 5	78.640±5.669***	58.680±2.419***	57.290±1.855***	0.769±0.157***	0.686±0.123***
Group 6	180.900±14.290	96.390±2.705	80.830±2.792	1.667±0.176	1.668±0.066
Group 7	140.900±9.357*	71.130±4.036*	65.580±6.008*	1.018±0.075**	0.933±0.096***

*P<0.05 when compared with disease control (CCl_4); **P<0.01 when compared with disease control (CCl_4); ***P<0.001 when compared with disease control (CCl_4).

Table 2
Effect of alcoholic and aqueous extracts of stem of *M. paradisiaca* on paracetamol induced hepatotoxicity in rats (Mean±SEM) (n=6).

Groups	Serum biochemical parameters				
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)
Group 1	26.080±1.412	32.080±1.412	31.150±1.375	0.182±0.004	0.182±0.004
Group 1	100.200±7.343	220.400±25.250	82.480±2.817	1.828±0.290	1.693±0.071
Group 3	34.750±1.418***	42.610±6.663***	38.590±1.733***	0.233±0.026***	0.345±0.022***
Group 4	73.460±14.310*	147.600±25.270*	62.210±5.300**	1.617±0.321	1.470±0.050
Group 5	58.680±2.419***	78.480±6.822***	58.690±4.137***	0.455±0.178***	0.635±0.116***
Group 6	96.390±2.705	179.200±14.440	78.390±3.912	1.800±0.236	1.615±0.101
Group 7	71.130±4.036*	138.800±9.861**	69.190±4.450	0.966±0.2011*	0.851±0.166***

*P<0.05 when compared with disease control (paracetamol); **P<0.01 when compared with disease control (paracetamol); ***P<0.001 when compared with disease control (paracetamol).

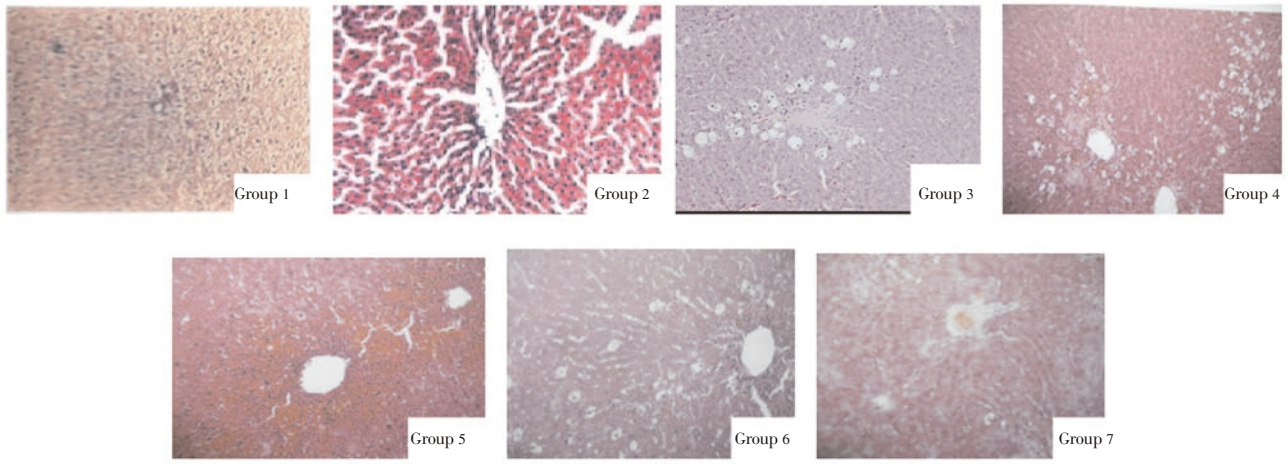

Figure 2. Effect of alcoholic and aqueous extracts of stem of *M. paradisiaca* on paracetamol-induced hepatotoxicity in rats (histopathology).

degeneration with fatty changes. Sylimarin treated group rat liver section showed normal central vein with mild hepatocytic changes. Alcoholic extract (250 mg/kg) treated group rat liver section showed less fatty changes vacuolated; alcoholic extract (500 mg/kg) treated group rat liver section showed normal central vein. Aqueous extract (250 mg/kg) treated group rat liver section showed dilated central vein with less hepatic changes; aqueous extract (500 mg/kg) treated group rat liver section showed mild hepatocytes with fatty changes (Figure 2).

4. Discussion

Liver plays an important role in the metabolism of drug and nutrients. Because of its central role in drug metabolism, it is the most vulerable tissue for drug toxicity. According to the reports published by USFDA, more than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20%-40% of all instances of hepatic failure[11].

The hepatoprotective effects of both alcoholic and aqueous extracts of the stem of *M. paradisiaca* were studied in rats by using CCl_4 induced hepatotoxicity and paracetamol induced hepatotoxicity models at the doses of 250 and 500 mg/kg bw. Liver damage was assessed by biochemical studies (SGOT, SGPT, ALP, total and direct bilirubin) and histopathological examinations.

In CCl_4-induced hepatotoxicity model, upon administration of CCl_4 to animals, it undergoes enzymatic activation, majorly by CYP2E1, into the trichloromethyl free radical (CCl_3) within the membrane of the endoplasmic reticulum. This is followed by chloromethylation, saturation, peroxidation

and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids[12]. These processes are known as lipid peroxidation, leading to functional and structural disruption of hepatocytes[13]. During hepatic damage, cellular enzymes like SGPT, SGOT, ALP, bilirubin (direct and total) will leak into the serum resulting in elevation of their serum concentrations. Hepatology of the damaged liver showed histological changes, such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed[14].

Pretreatment with sylimarin (100 mg/kg, p.o.), alcoholic extract (250 mg/kg and 500 mg/kg) and aqueous extract (500 mg/kg) of stem of *M. paradisiaca* for 7 days offered significant protection against the CCl_4-induced hepatic damage. Both alcoholic and aqueous extracts of stem of *M. paradisiaca* prevented the histological changes caused by CCl_4, which further confirmed its hepatoprotective activity against CCl_4-induced hepatic damage. The possible mechanism of action may be associated with scavenging of free radicals responsible for CCl_4 toxicity.

Paracetamol induced hepatotoxicity is one of the well known and commonly used animal model for studying the hepatoprotective property of the plant. Administration of paracetamol at a dose of (1-3 g/kg/day), p.o. results in hepatic damage. The toxic metabolic N-acetyl-p-benzoquineimine is an oxidative product of paracetamol formed by the action of cytochrome P-450 and it reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinine to undergo covalent bonding with cellular sulphydryl groups of protein and leads to cell death. Histopathology of the liver shows necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis, eosinophilic cytoplasm and large excessive hepatic lesions[15].

Pretreatment with sylimarin (100 mg/kg, p.o.), alcoholic extract (250 mg/kg and 500 mg/kg) and aqueous extract (500 mg/kg) of stem of *M. paradisiaca* for 7 days has significantly reduced the elevated serum enzyme level. Both alcoholic and aqueous extracts of stem of *M. paradisiaca* reduced the histological changes caused by paracetamol, which further confirmed its hepatoprotective activity against paracetamol induced hepatic damage. The possible mechanism of action may be associated with the antioxidant property of stem of *M. Paradisiaca*.

These results showed that alcoholic extract (250 mg/kg and 500 mg/kg) and aqueous extract (500 mg/kg) of stem of *M. paradisiaca* possessed significant protection against experimentally induced hepatotoxic models. The possible mechanism behind the hepatoprotective property of stem of *M. paradisiaca* may be associated with the antioxidant property of the phytoconstituents of stem of *M. paradisiaca*.

Conflict of interest statement

We declare that we have no conflict of interest.

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