



Hepatoprotective Activity of *Macrotyloma uniflorum* (Lam.) Verdc. Seeds Extracts Against Paracetamol Induced Hepatotoxicity in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MR and AJSJS developed the protocol and designed the experimental study. Authors AJSJS and AK involved in the collection of literature, preparation of extracts and screening of phytoconstituents present in the extracts. Authors MR, AJSJS and KB involved in the evaluation of hepatoprotective activity and biochemical analysis. Authors MR and AJSJS involved the histopathological studies, data interpretation and performed the statistical analysis. Author AJSJS wrote the first draft of manuscript. Authors MR and AK edited and completed the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Macrotyloma uniflorum* (Horse gram) seeds are used in Ayurvedic medicine for treating numerous health disorders including rheumatism, worm, conjunctivitis and piles. The seeds were boiled with water and used as a folklore medicine for treatment of jaundice in Andhra Pradesh, India.

Aim: The present study was aimed to find out the therapeutic potentials of *M. uniflorum* seeds

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extract against paracetamol- induced hepatotoxicity and also to scientifically prove its traditional claim.

Methodology: Hepatotoxicity was induced in wistar albino rats by the oral administration of paracetamol (400 mg/kg b.wt.) for 10 days. The ethanolic extract and water extract (400 mg/kg b.wt.) were orally administered to the respective group once daily for 10 days. Silymarin (20 mg/kg b.wt.) was used as standard. Biochemical parameters such as ASAT, ALAT, ALP, TP, albumin, globulin, total bilirubin and GGT were determined to assess the hepatoprotective effect.

Results: The oral administration of both ethanolic and water extracts significantly ($P < 0.001$) restored the biochemical parameters in the hepatotoxicated rats which were well compared with standard drug silymarin. The hepatoprotective activity of ethanolic extract provides therapeutically better efficacy which was further supported by histopathological examination. The phytochemical screening reveals the presence of phytoconstituents such as phenolic compounds, flavonoids and alkaloids which might be responsible for the hepatoprotective activity.

Conclusion: The scientific establishment of the *M. uniflorum* was traditionally claimed by folklore medicine was validated.

Keywords: *Macrotyloma uniflorum* seeds; ethanolic extract; water extract; hepatoprotective; paracetamol; silymarin.

1. INTRODUCTION

The liver is the second largest organ in the body and plays a major role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Liver has to perform different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver. Total bilirubin test measures the amount of bile pigment in the blood. If the bilirubin level in blood becomes elevated that leads to jaundice, one of the hepatic disorders which results from bilirubin deposition in the skin and mucous membrane and the patient may have a yellow colour to the skin and eyes [1].

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants which naturally biosynthesize and accumulate certain secondary metabolites. The medicinal plants have been used for treatment of illness and disease since the dawn of time. Researchers have found that people in different parts of the world tend to use the same or similar plants for treating the same illnesses [2]. Recently the World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care [3].

The edible seeds of *Macrotyloma uniflorum*, generally known as horse gram are a kind of highly nutritious bean and commonly used as numerous culinary purposes in many Indian

states. It is widely used in Ayurvedic medicine for treating numerous health disorders including rheumatism, worm, conjunctivitis and piles. They have astringent and diuretic properties and also used to control fever. The beans are beneficial for extracting phlegm. They are believed to be helpful for keeping the body warm during winter. The phenol content of the seeds helps to reduce excessive weight. They also help to lower cholesterol levels. Horse gram was boiled into water and given to treat jaundice in Andhra Pradesh, India [4]. Therefore, the present study was undertaken to investigate the hepatoprotective effect of *M. uniflorum* seeds extract against paracetamol (PCM) induced hepatotoxicity in wistar albino rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Macrotyloma uniflorum seeds were purchased from Renga Trading and Manufacturing (M) Sdn Bhd, Senai, Johor, Malaysia in the month of May 2016. The seeds were authenticated by Pharmacognosist, KPJ Healthcare University College, Nilai, Negeri Sembilan, Malaysia (Reference No: KPJUC/CRI/PA/2016 (01)). The seeds were dried in hot air oven at 60°C for three days. The dried seeds were pulverized into coarse powder [5,6].

2.2 Preparation of Extracts

Coarsely powdered seeds of *M. uniflorum* (500 gm) was separately taken in an aspirator bottle

and extracted with ethanol and water respectively by cold maceration technique for 6 days [7]. At the end of each extraction, the extracts were separately collected by filtration using whatmann filter paper. The filtered extract was evaporated and concentrated using rotatory vacuum evaporator under reduced pressure and kept in desiccator until further use [8].

2.3 Preliminary Phytochemical Screening

The qualitative chemical tests were performed for establishing the presence and absence of phytoconstituents such as alkaloids, carbohydrates and glycosides, proteins and amino acids, fatty acids, terpenoids, tannins and phenolic compounds, saponins, sterols, volatile oils, flavonoids, gums and mucilage in the extracts of *M. uniflorum* seeds using standard methods [9].

2.4 Pharmacological Screening

2.4.1 Animals

Healthy adult albino rats of wistar strain weighing 200 – 250 g were obtained from KPJUC vivarium, KPJ Healthcare University College, Nilai, Malaysia. The animals were kept in spacious, hygienic cages during the course of the experimental period by maintaining standard environmental conditions at an ambient temperature (25±2°C) with 12 h light-dark cycle. The animals were fed with standard pellets and water *ad libitum* [10]. The experimental protocol has been approved by KPJUC Animal & Ethical committee (Reference No. KPJUC/CRI/BPS/EC/2016/19).

2.4.2 Evaluation of hepatoprotective activity

Paracetamol (PCM) at dose of 400 mg/kg b.wt. *p.o.* was used to induce hepatotoxicity in the selected animals. Ethanolic and water extracts of *M. uniflorum* seeds (400 mg/kg b.wt. *p.o.*) were evaluated for their hepatoprotective activity in PCM induced hepatotoxic rats for 10 days. Silymarin (20 mg/kg b.wt. *p.o.*) was used as standard drug [11].

2.4.3 Experimental design

20 albino rats were taken and equally distributed into 5 groups. Group 1 served as solvent control and received 0.5 ml of normal saline. A single dose of PCM (400 mg/kg b.wt.) was orally administered into Group 2 which served as

hepatotoxic control [12]. Group 3 – 5 were orally administered a single dose of PCM (400 mg/kg b.wt. *p.o.*) thirty minutes after the first dose of ethanolic extract, aqueous extract of *M. uniflorum* seeds (400 mg/kg b.wt.) and standard drug, silymarin (20 mg/kg b.wt.) respectively for 10 days [13].

2.4.4 Biochemical analysis

On the 11th day, all the animals were mild anesthetized using ether and the blood samples of each animal were separately collected by orbital sinus puncture and transferred into Eppendroff's tubes (1 ml) containing 50 µl of anticoagulant (10% trisodium citrate). Then the plasma was separated by centrifuging at 6000 rpm for 15 min [14]. The serum was used to analyze serum biochemical parameters such as aspartate amino transaminase (ASAT), alanine amino transaminase (ALAT), alkaline phosphatase (ALP), total protein (TP), albumin, globulin, total bilirubin (TB) and gamma glutamyl transferase (GGT). The serum biochemical parameters were estimated using standard assay kits [15].

2.4.5 Statistical analysis

Data are expressed as Mean ± S.E.M. and subjected to one way ANOVA followed by Student's T Test. Values of P<0.01 and P<0.001 were considered statistically significant [16].

2.4.6 Histopathological studies

After collection of blood sample, all the animals were sacrificed by cervical dislocation and liver was removed and immersed in 10% buffered formalin solution. A portion of the liver samples were used for histopathological studies, as per the standard procedure. 10% neutral phosphate buffer formalin and the hydrated tissue sections were used for the histological examination. Liver pieces of 5 µm in thickness were fixed and were stained with hematoxylin and eosin. The sections were examined under light microscope [5,8].

3. RESULTS

3.1 Qualitative Phytochemical Screening

The presence of alkaloids, flavonoids, phenolic compounds and tannins and fixed oils and fats were observed in both ethanolic and water extract of *M. uniflorum* seeds (Table 1).

Table 1. Qualitative phytochemical screening of *Macrotyloma uniflorum* seeds extracts

Phytoconstituents	Ethanollic extract	Water extract
Alkaloids	+	+
Carbohydrates and glycosides	-	+
Proteins and amino acids	-	-
Sterols	+	-
Fixed oils and fats	+	+
Tannins-phenolic compounds	+	+
Triterpenoids	-	-
Saponins	-	+
Gum and Mucilage	-	-
Flavones and Flavanones	+	+

+ = Positive; - = Negative

3.2 Hepatoprotective Activity

A significant ($P < 0.001$) increase in serum level of ASAT, ALAT, ALP, globulin, total bilirubin and GGT and the significant ($P < 0.001$) decrease in the serum level of TP and albumin were noted in the animals intoxicated with PCM (400 mg/kg, b.wt. p.o.) when compared with control group (Table 2). Animals intoxicated with PCM were treated with ethanolic and water extract of *M. uniflorum* seeds (400 mg/kg, b.wt. p.o.) and silymarin (20 mg/kg, b.wt. p.o.) for 10 days. Among both extracts treated, the ethanolic extract of *M. uniflorum* treated group showed a significant ($P < 0.001$) restoration of all the biochemical parameters followed by water extract. The results were well comparable to the standard drug, silymarin (Table 2).

3.3 Histopathological Studies

Histopathological studies supported the hepatoprotective activity of both ethanolic and water extracts of *M. uniflorum* seeds. The histopathological photographs of the ethanolic and water extracts treated groups (Figs. 1c & 1d) were well comparable with silymarin treated group (Fig. 1e). The ethanolic extract of *M. uniflorum* treated group (Fig. 1c) showed mild degenerative changes of hepatocytes, less degree of perihepatitis in few hepatic lobules and centrilobular necrosis were observed when compared with normal group (Fig. 1a).

4. DISCUSSION

Paracetamol is a common antipyretic agent that is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic dose. PCM toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for PCM induced hepatotoxicity. Elevated levels of serum enzymes are the indicative of liver damage, cellular leakage and loss of functional integrity [17].

The administration of PCM to the animals result in a significant ($P < 0.001$) reduction in the levels of total protein and albumin and the significant ($P < 0.001$) increase in serum levels of ASAT, ALAT, ALP, globulin, total bilirubin and GGT. The PCM intoxicated animals treated with ethanolic extract, water extract (400 mg/kg) and silymarin (20 mg/kg), the toxic effect of PCM was observed partially. Comparing with PCM treated group, the ethanolic extract and silymarin groups exhibit a significant ($P < 0.001$) increase in total protein and albumin and also a significant ($P < 0.001$) decrease in the serum levels of ASAT, ALAT, ALP, globulin, total bilirubin and GGT (Table 2).

PCM used as tool to induced hepatotoxicity in experimental rats, which leads to covalent bonding of its toxic metabolite N-acetyl-p-benzoquinoneimine to sulfhydryl groups of proteins. An increase level ASAT, ALAT, ALP, globulin, total bilirubin and GGT are an indication of cellular leakage and loss of functional integrity of the hepatic cell membranes. Administration of the ethanolic extract of *M. uniflorum* seeds shows a significant ($P < 0.001$) hepatoprotective effect which was well compared with silymarin (Table 2) [13].

The animals in control group showed normal liver histological arrangements (Fig. 1a). The PCM intoxicated group of animals showed perihepatitis characterized by degeneration and coagulative necrosis of majority of the hepatic cells. There was a centrilobular necrosis and coagulative necrosis to central vein featured by Karyorrhesis, Karyolysis of the hepatocytes, along with infiltration of stray macrophages (Fig. 1b). The groups of animals treated with ethanolic extract, water extract (400 mg/kg) and silymarin (20 mg/kg) revealed mild to moderate degenerative changes of hepatocytes (Figs. 1c, 1d and 1e).

Table 2. Hepatoprotective effect of various extracts of *M. uniflorum* seeds and silymarin on the biochemical parameters in serum of PCM intoxicated rats

Treatment group (mg/kg)	ASAT (U/l)	ALAT (U/l)	ALP (U/l)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total bilirubin (mg/dl)	GGT (U/l)
Control	141.50 ± 5.44	82.72 ± 3.27	183.12 ± 6.26	8.22 ± 0.33	2.90 ± 0.08	4.82 ± 0.16	0.58 ± 0.03	9.45 ± 0.41
PCM (400)	258.58 ± 0.95***	137.30 ± 6.40***	259.72 ± 9.15**	5.80 ± 0.18***	1.25 ± 0.13***	6.20 ± 0.24***	1.61 ± 0.13***	14.72 ± 0.26***
Ethanol extract (400) + PCM (400)	169.58 ± 11.81***	94.72 ± 2.52***	189.32 ± 5.48***	7.38 ± 0.23***	1.94 ± 0.16***	5.70 ± 0.26**	0.62 ± 0.04***	10.30 ± 0.46***
Water extract (400) + PCM (400)	185.50 ± 8.61***	101.02 ± 0.64***	194.08 ± 5.68***	6.75 ± 0.23	1.39 ± 0.18	6.10 ± 0.25	0.78 ± 0.05**	10.88 ± 0.37***
Silymarin (20) + PCM (400)	147.25 ± 6.05***	83.50 ± 0.62***	185.58 ± 5.39***	7.95 ± 0.16***	1.80 ± 0.15**	5.98 ± 0.31**	0.79 ± 0.03***	10.85 ± 0.31***

1. *P<0.05, **P<0.01, ***P < 0.001 PCM VS Control; 2. *P<0.05, **P<0.01, ***P< 0.001 extract treated groups VS PCM; 3. Values are mean ± S.E.M., 4. n = 4

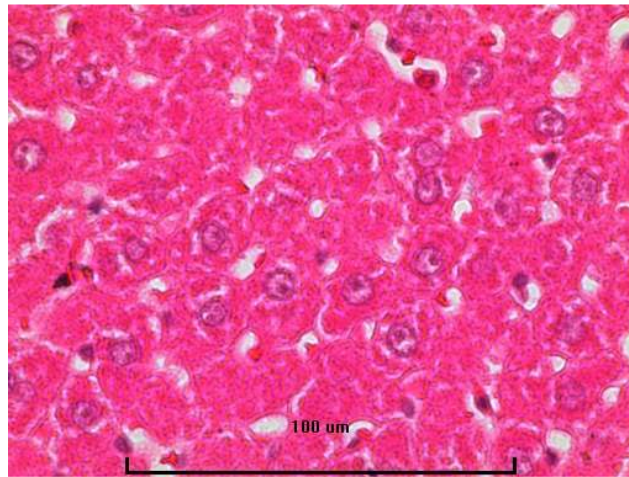


Fig. 1a. Control rat liver

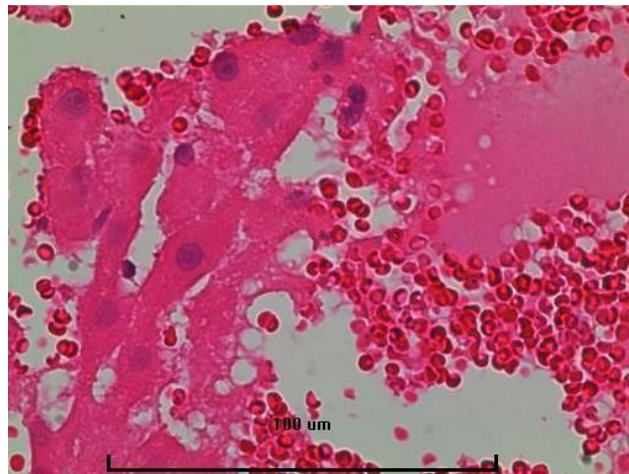


Fig. 1b. Paracetamol intoxicated rat liver

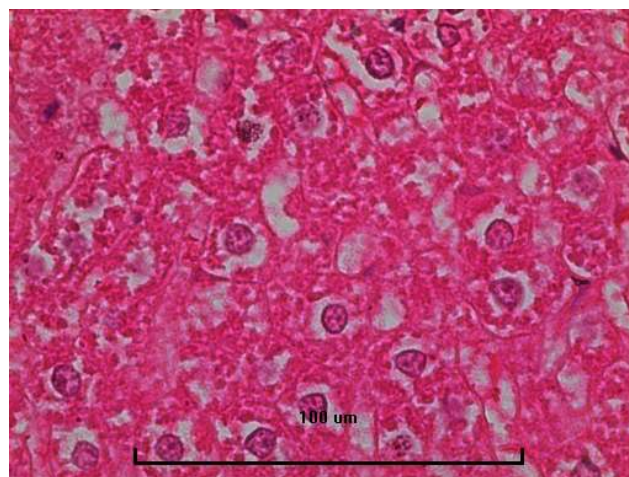


Fig. 1c. Ethanolic extract (400 mg/kg) treated group

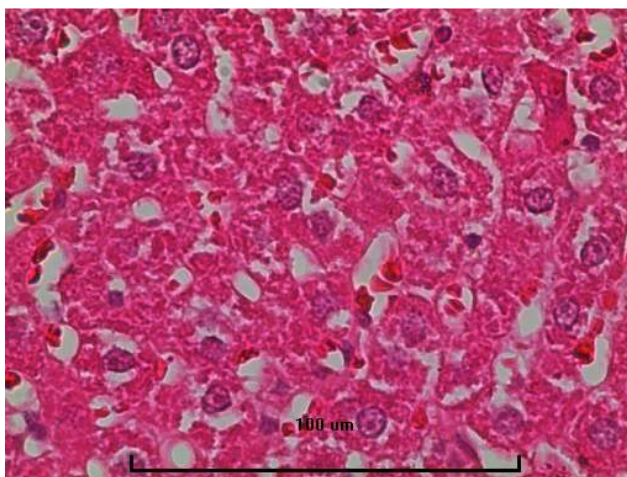


Fig. 1d. Water extract (400 mg/kg) treated group

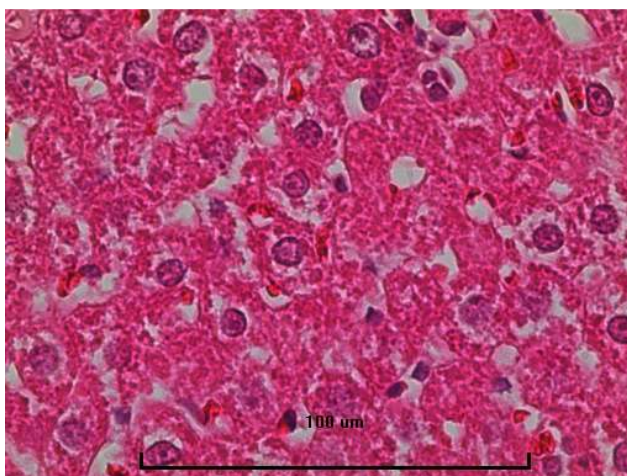


Fig. 1e. Silymarin 20 mg/kg treated rat liver

Fig. 1. Hepatoprotective effect of various extracts of *M. uniflorum* seeds against PCM intoxicated rat liver

The phytoconstituents such as flavonoids and phenolic compounds present in the ethanolic extract of *M. uniflorum* seeds could be responsible for the hepatoprotective activity. Number of medicinal plants has been reported with good hepatoprotective activity with potential antioxidative mechanism due to the presence of flavonoids and phenolic compounds [18,19]. The correlation between antioxidant activity and quantity of the flavonoids is still under discussion, a good linear relationship was observed in some published works for the hepatoprotective efficacy [20]. The protective role of this plant against PCM intoxicated hepatotoxicity might be through antioxidative effect of flavonoids in *M. uniflorum* seeds.

5. CONCLUSION

The present study scientifically proves the traditionally claim for the hepatoprotective activity of *M. uniflorum* seeds. The hepatoprotective activity of ethanolic extract of *M. uniflorum* seeds provides the therapeutic efficacy against PCM induced hepatotoxicity. The scientific establishment of *M. uniflorum* was traditionally claimed by folklore medicine was validated. Such a study in the field of indigenous medicine would put a firm foundation for standardization and scientific evaluations on the above therapeutically claim. Further investigations on the presence of phytochemicals and the exact mechanism of action are

necessary to explore the potent natural hepatoprotective agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" (Reference No. KPJUC/CRI/BPS/EC/2016/19).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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