

Hepatoprotective activity of *Plectranthus amboinicus* against paracetamol induced hepatotoxicity in rats

Smita Shenoy^{1*}, Hemanth Kumar², Thashma³, Veena Nayak¹, Krishnananda Prabhu⁴, Priya Pai⁴, Indu Warriar⁴, Somayaji⁵, Venu Madhav⁵, KL Bairy¹, Anoop Kishore⁶

¹Department of Pharmacology, Kasturba Medical College, Manipal, Manipal University, Karnataka, India

²Department of Pharmacology, Al- Ameen Medical College, Bijapur, Karnataka, India

³Department of Pharmacology, Kasturba Medical College International Centre, Manipal, Manipal University, Karnataka, India

⁴Department of Biochemistry, Kasturba Medical College, Manipal, Manipal University, Karnataka, India

⁵Department of Anatomy, Manipal Melaka Medical College, Manipal, Manipal University, Karnataka, India

⁶Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal, Manipal University, Karnataka, India

ABSTRACT

Background: *Plectranthus amboinicus*, belonging to the genus *Plectranthus*, is widely used for medicinal purposes. Its leaves have been used traditionally as a hepatoprotective.

Objectives: The present study was aimed at evaluating the hepatoprotective activity of ethanolic extract of *Plectranthus amboinicus* against paracetamol induced hepatotoxicity in rats.

Materials and methods: Six groups of wistar rats were used in the study. The two control groups received gum acacia and paracetamol orally respectively. The three test groups were treated orally with paracetamol followed by 300, 600 and 900 mg/kg of ethanolic extract of *Plectranthus amboinicus* respectively. The sixth group (standard hepatoprotective) received paracetamol followed by N-acetylcysteine 100 mg/kg orally. The hepatoprotective activity was evaluated by estimating serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, tissue malondialdehyde levels and by histopathological analysis of the liver tissue. Results were analysed by one-way ANOVA followed by Dunnett's multiple comparison test.

Results: *Plectranthus amboinicus* in doses of 600 mg/kg and 900 mg/kg significantly ($p < 0.05$) altered paracetamol induced changes in the serum and tissue enzyme levels to near normal values. It also improved the liver histopathology profile.

Conclusion: The results of the study indicate that the ethanolic extract of *Plectranthus amboinicus*, possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Key words: *Plectranthus amboinicus*, hepatoprotective, paracetamol, N-acetylcysteine

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INTRODUCTION

Plectranthus amboinicus, belonging to the genus *Plectranthus*, has been used traditionally for medicinal purposes. These include skin ulcerations, scorpion bite, skin allergy, wounds, diarrhea and fever.^[1] The plant has been shown to have antioxidant,^[2] antiinflammatory,^[3] anticlastogenic,^[4] antiepileptic,^[5] antimicrobial,^[6] urolithiatic,^[7] wound healing^[8] and mast cell stabilization properties.^[9] The leaves of the plant have been used traditionally as a hepatoprotective.^[10] Studies in rats have demonstrated its hepatoprotective effect against

carbon tetrachloride induced hepatotoxicity.^[11]

Paracetamol (acetaminophen) is a commonly used antipyretic and analgesic agent. Hepatotoxicity is an acute adverse effect of paracetamol overdosage which could be fatal.

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* Correspondence : smtshenoy@gmail.com

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The hepatotoxicity is due to its toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI) which is normally detoxified by glutathione. In paracetamol overdose, excess of NAPQI is formed which binds to proteins and other macromolecules resulting in hepatic necrosis. The standard therapy for paracetamol overdose is N-acetylcysteine. It restores hepatic glutathione which detoxifies NAPQI.^[12] Though N-acetylcysteine is used for the treatment of paracetamol toxicity, some cases do not respond.^[13] Since *Plectranthus amboinicus* has been demonstrated to have antioxidant action and protective effect against carbon tetrachloride induced liver damage, the present study was conducted to evaluate its hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by Institutional Animal Ethics Committee, Manipal. Healthy, adult albino wistar rats of either sex weighing 180-200 g were selected. Each rat was housed singly in a polypropylene cage (U.N.Shah manufacturers, Mumbai, India) and provided with standard rat feed (Amrut lab animal feed, Pranav Agro industries Ltd., Sangli, Maharashtra) and water *ad libitum*. The rats were maintained under standard conditions in animal house approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The temperature maintained was $(23 \pm 2) ^\circ\text{C}$, humidity $50 \pm 5\%$, light and dark cycle of 10-12 hours.

Chemicals

Paracetamol 500mg tablet (Nirmal Prime, Mumbai), N-acetylcysteine 100mg tablet (Hitech Medicals, Mangalore), 2% gum acacia (Nice Chemicals, Kochi), ketamine (Neon laboratories, Mumbai) were used. *Plectranthus amboinicus* was procured locally and authenticated by Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal. Serum enzymes and hepatic MDA

were estimated using kits form Aspen Laboratories Pvt. Ltd., Delhi.

Preparation of the ethanolic extract of *Plectranthus amboinicus*^[14]

The dried leaves of *Plectranthus amboinicus*, weighing 500 g, were soaked in absolute alcohol measuring about 3 litres in a round flask for about 24 hours. The process of extraction was done by reflux condensation at $60^\circ - 80^\circ\text{C}$ for three hours. After cooling, the alcohol was drained through a muslin cloth into a conical flask following which 3 litres of fresh absolute alcohol was put into the flask containing the plant materials. The procedure of reflux condensation was repeated twice. The extract obtained was concentrated by distillation till a syrupy consistency was obtained. Finally, the extract from three batches was put in a china dish and evaporated to dryness on a water bath.

Acute toxicity studies^[15]

Healthy wistar rats of either sex were chosen and divided into four groups ($n = 6$ in each group). They were fasted overnight. They were administered ethanolic extract of *Plectranthus amboinicus* orally in single increasing doses of 100 mg/kg, 300 mg/kg, 1000 mg/kg and 3000 mg/kg body weight of the rat respectively. They were observed continuously for 2h, then occasionally for 4h and finally for overnight mortality. The dose upto 3000 mg/kg was well tolerated without producing any alteration in gross behaviour, signs of toxicity and mortality. The dose selected for the study was 10% of the maximum tolerated dose, that is, 300 mg/kg orally (p.o.). Two other doses (in increasing doses of 300) were selected - 600 mg/kg and 900 mg/kg for administration to the rats.

Study design

Six groups of rats, each with six rats ($n = 6$), were used for the study. The experimental groups were as follows:

Group - I (control), received 2% gum acacia dissolved in distilled water at 1, 24 and 48 h p.o.

Group - II (hepatotoxic drug control) were administered paracetamol (3 g/kg)^[16] as single dose on day one and received distilled water at 1, 24 and 48 h p.o. following paracetamol administration.

Group - III (test group) received paracetamol (3 g/kg) as a single dose on day one followed by ethanolic extract of *Plectranthus amboinicus* (300 mg/kg) at 1, 24 and 48 h p.o. following paracetamol administration.

Group - IV (test group) received paracetamol (3 g/kg) as a single dose on day one followed by ethanolic extract of *Plectranthus amboinicus* (600 mg/kg) at 1, 24 and 48 h p.o. following paracetamol administration.

Group - V (test group) received paracetamol (3 g/kg) as a single dose on day one followed by ethanolic extract of *Plectranthus amboinicus* (900 mg/kg) at 1, 24 and 48 h p.o. following paracetamol administration.

Group - VI (standard hepatoprotective) received paracetamol (3 g/kg) as a single dose on day one followed by N-acetylcysteine (100mg/kg) at 1, 24 and 48 h p.o. following paracetamol administration.

Assessment of hepatic damage

Biochemical estimation

At the end of 72 h, blood was collected by retro-orbital puncture and serum was separated for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) by using kits. The rats were sacrificed using excess ketamine. The liver was excised immediately and perfused with cold normal saline. The perfused liver was suspended in 10% w/v ice cold 0.1M phosphate buffer, cut into small pieces, and the required quantity was weighed and homogenized using Teflon homogenizer. The homogenate was used for the estimation of malondialdehyde (MDA).^[17]

Histopathological studies

The liver tissue was collected, fixed in 10% formalin and stained with hematoxylin and eosin for photomicroscopic observations.

Statistical analysis

The data was represented as mean \pm SEM. Results were analysed by one-way ANOVA followed by Dunnett's multiple comparison test using SPSS software. The minimum level of significance was set at $p < 0.05$.

RESULTS

Biochemical parameters

There was a significant ($p < 0.001$) increase in the serum levels of ALT, AST, ALP and tissue MDA levels in rats treated with paracetamol as compared to control indicating paracetamol induced hepatotoxicity. Treatment of rats in Group III with ethanolic extract of *P. amboinicus* 300 mg/kg did not alter the enzyme levels as compared to paracetamol treated rats. Rats that were treated with paracetamol along with either N-acetylcysteine or *P. amboinicus* 600 mg/kg and 900 mg/kg showed a significant ($p < 0.05$) reduction in the serum levels of ALT, AST, ALP and tissue MDA levels as compared to paracetamol treated group. The reduction in serum and tissue enzyme levels by N-acetylcysteine was significant ($p < 0.05$) as compared to those treated with *P. amboinicus* 600 mg/kg. There was no significant difference in enzyme levels between N-acetylcysteine treated rats and those treated with 900 mg/kg of *P. amboinicus* (Table 1).

Histopathological examination

The normal control group and N-acetylcysteine treated groups showed normal hepatic parenchyma and stroma. Hepatocyte cords, sinusoids and stroma were histologically normal (Figure-1a and Figure -1f respectively).

The histopathological examination of paracetamol treated group showed necrosis of hepatic cells with generalized congestion; binuclearity of hepatocytes and moderate increase in hepatic macrophages. Portal tract showed infiltration (mostly plasma cells and eosinophils), marked periportal congestion associated with necrotic debris; some parts revealed hepatocyte necrosis with bridging fibrosis and minimal cholestasis (Figure-1b).

The section of hepatic tissue of rats treated with paracetamol and ethanolic extract of *Plectranthus amboinicus* 300 mg/kg (group III) revealed periportal congestion and infiltration; focal necrosis was also seen with binuclearity of hepatocytes. Some cells were distorted. (Figure - 1c).

The section of liver tissue from rats treated with paracetamol and ethanolic extract of *Plectranthus amboinicus* 600 mg/kg showed slight peripheral congestion; hepatocytes were almost normal with only occasional hepatocytes being binucleated. Sinusoids and portal tract appeared normal (Figure-1d).

The section of liver of rats treated with paracetamol and ethanolic extract of *P. amboinicus* 900 mg/kg showed normal hepatocytes with sinusoids and portal tract showing recovery. There was no apparent sign of necrosis, fibrosis or periportal infiltration (Figure-1e).

DISCUSSION

Paracetamol is normally metabolised mainly by glucuronidation and sulfation. Consumption of large amount of paracetamol results in saturation of these pathways and formation of a toxic metabolite N-acetyl-p

-benzoquinoneimine (NAPQI). The large amount of NAPQI formed cannot be completely detoxified by the liver resulting in hepatic and renal damage. N-acetylcysteine is administered to patients with paracetamol overdose. It detoxifies NAPQI.^[12] Some patients still develop severe hepatic damage inspite of acetylcysteine therapy. In this study, ethanolic extract of *Plectranthus amboinicus* was given to rats following paracetamol administration.

Estimation of levels of serum ALT, AST and ALP is used to assess hepatic function. Loss of integrity of membranes of hepatocytes and cell damage results in release of enzymes AST and ALT into circulation.^[16] The elevated level of these enzymes in the paracetamol treated rats indicated drug induced hepatotoxicity whereas their decreased levels in rats treated with higher doses of *P. amboinicus* denotes hepatoprotective effect of the plant. Cholestasis and increased biliary pressure result in an increase in serum ALP levels.^[18] The decrease of serum enzyme levels in *P. amboinicus* treated groups (600 mg/kg and 900 mg/kg) could be due to an improvement in structure and function of hepatocytes.

Table 1: Effect of ethanolic extract of *Plectranthus amboinicus* on paracetamol induced hepatotoxicity in rats.

Group/Drug, dose	Serum AST (IU/L)	Serum ALT (IU/L)	Serum ALP (IU/L)	Hepatic MDA (nmol/mg protein)
I/ Gum acacia, 2 ml	92.88 ± 1.21	47.02 ± 1.89	107.47± 2.93	66.13±1.51
II/ Paracetamol (3g/kg)	284.72 ± 2.63 [*]	200.42 ± 2.48 [*]	392.27± 9.70 [*]	246.52±2.66 [*]
III/ Paracetamol (3 g/kg) + <i>P. amboinicus</i> (300 mg/kg)	283.77 ± 1.51	165.90 ± 8.74	386.80 ± 3.96	191.00±2.31
IV/ Paracetamol (3 g/kg) + <i>P. amboinicus</i> (600 mg/kg)	172.45 ± 2.87 ^{†,‡}	110.38 ± 2.53 [†]	187.92 ± 2.28 ^{†,‡}	150.31±2.98 [†]
V/ Paracetamol (3 g/kg) + <i>P. amboinicus</i> (900 mg/kg)	107.84 ± 2.94 ^{†,‡,§}	95.05 ± 4.60 ^{†,‡}	122.80 ± 5.99 ^{†,‡}	97.15±1.78 ^{†,‡,§}
VI/ Paracetamol (3 g/kg) + N-acetylcysteine(100mg/kg)	100.38 ± 4.86 ^{†,§}	51.39 ± 3.49 ^{†,§}	113.67 ± 1.70 ^{†,§}	87.25±1.50 ^{†,§}

Values expressed as mean ± SEM.

^{*}P < 0.001 vs. group I

[†]P < 0.05 vs. group II

n = 6 in each group.

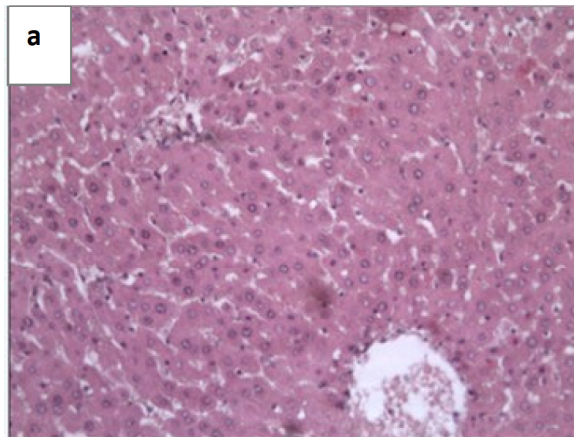
[‡]P < 0.05 vs. group III

[§]P < 0.05 vs. group IV

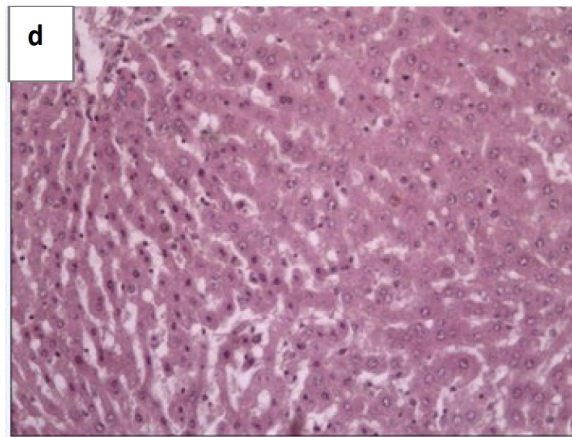
Figure 1: Effect of ethanolic extract of *Plectranthus amboinicus* on paracetamol induced hepatotoxicity in rats.

Histological figures of liver sections of rats treated with

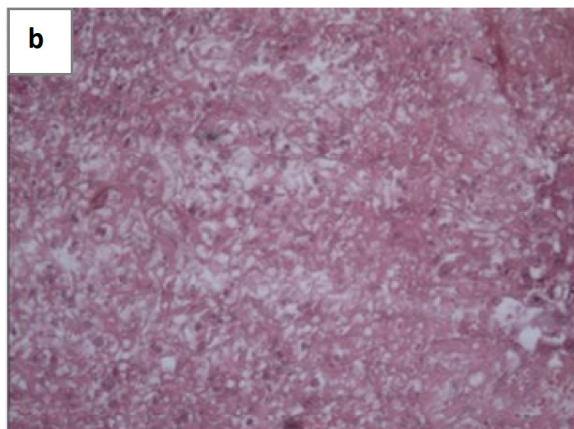
a) gum acacia shows normal hepatic architecture.



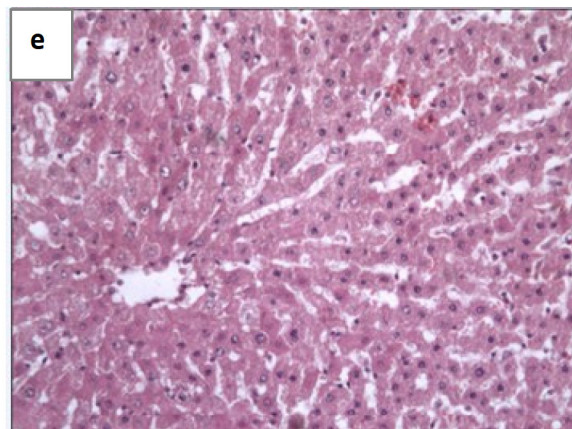
d) paracetamol and *Plectranthus amboinicus* 600 mg/kg shows near normal hepatocytes, sinusoids and portal tract.



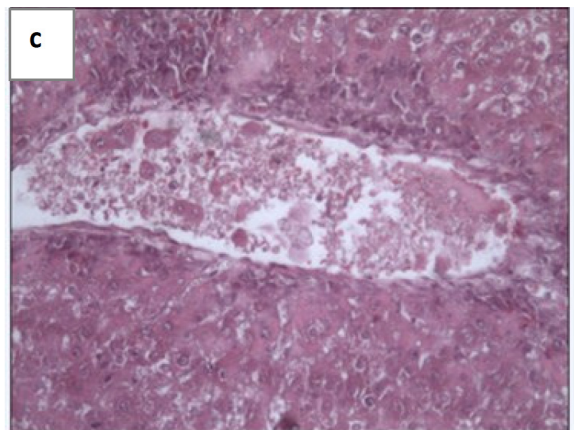
b) paracetamol exhibits hepatic necrosis, congestion and portal tract infiltration.



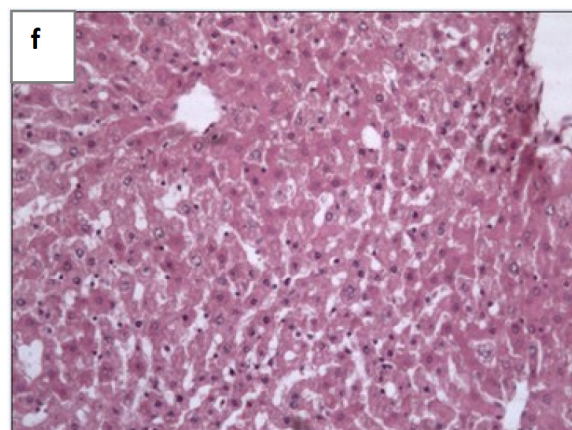
e) paracetamol and *Plectranthus amboinicus* 900 mg/kg reveals normal hepatocytes and portal tract.



c) paracetamol and *Plectranthus amboinicus* 300 mg/kg shows hepatic necrosis, periportal infiltration and congestion.



f) paracetamol and N-acetylcysteine shows normal hepatic parenchyma and stroma.



Malondialdehyde is a lipid peroxidation product.^[19] An increase in lipid peroxidation and depletion of antioxidants may have resulted in elevated hepatic MDA levels in the paracetamol treated group.

P. amboinicus has been demonstrated to have a membrane stabilising effect.^[3] This may contribute to the structural integrity of the hepatocyte membrane. *P. amboinicus* has polyphenols like flavonoids and tannins which scavenge free radicals resulting in an antioxidant effect.^[2, 20] The antioxidant action of *P. amboinicus* may have resulted in a decrease in lipid peroxidation product (MDA) in the liver. Thus, these phytoconstituents may have exerted a hepatoprotective effect. N-acetylcysteine restores glutathione levels in the liver which resulted in its hepatoprotective effect. Thus, a decrease in serum and tissue enzymes indicates recovery of hepatocytes and restoration of function. The hepatoprotective effect of high dose (900 mg/kg) of *P. amboinicus* was comparable to N-acetylcysteine.

Paracetamol produced cell necrosis and

generalised congestion. Treatment with higher doses of *P. amboinicus* (600 mg/kg and 900 mg/kg) produced near normal to normal hepatic architecture indicating hepatoprotective effect of the plant extract.

In this study, different doses of plant extract were used and were administered after exposure to paracetamol which is often the clinical scenario. The limitation of the study is that blood samples could be collected at earlier time intervals to know the onset of hepatoprotective effect.

To conclude, in this study, higher doses of *Plectranthus amboinicus* decreased both serum and tissue enzymes and restored hepatic structure which were altered following paracetamol administration. The present study thus demonstrated its hepatoprotective effect against paracetamol induced hepatotoxicity in rats. Further studies are required to elucidate the mechanism of its hepatoprotective action.

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Not reported.

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