HEPATOPROTECTIVE EFFECT OF *PLECTRANTHUS AMBOINICUS* (LOUR.) AGAINST CCL_4 INDUCED LIVER DAMAGE IN ALBINO RATS BY ITS ANTIOXIDANT PROPERTY

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Summary

Plectranthus amboinicus is a medicinally important plant, finds its use in traditional as well as in medicinal system to treat a range of ailments like dyspepsia, indigestion, diarrhoea etc., The aim of the present study was to investigate the effect of ethanolic extract of *Plectranthus amboinicus* (Lour.) (EEPA) against carbon tetra chloride induced lipid peroxidation in rats. Administration of CCL_4 for 7 days induces lipid peroxidation leading to liver damage. Hence the enzyme levels such AST and ALT which are good markers of liver condition have been elevated and also the serum bilirubin. Simultaneous administration of EEPA at the dose of 200 mg/kg reduced these elevated AST and ALT levels significantly ($p<0.001$) and serum bilirubin level was reduced significantly ($p<0.05$) substantiating that EEPA is inhibiting the CCl_4 induced liver damage in rats. This was further supported by the evidence of significantly ($p<0.001$) increased glutathione level indicating increased antioxidant enzyme level and significantly ($p<0.001$) decreased malondialdehyde level indicating the decreased oxidative stress. So, the present study supports the traditional claim of the *plectranthus amboinicus* for the hepatoprotective potential possibly through its antioxidant property.

Keywords: *Plectranthus amboinicus*; Hepatoprotective, Lipid peroxidation, Antioxidants.

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Introduction

Plectranthus is an important large genus containing about 300 species, among which *Plectranthus amboinicus* (Lour.) belonging to the family Lamiaceae , commonly called as Indian Borage is most frequently cited species in Tropical Africa, Asia and Australia [1]. It finds its use in traditional as well as in medicinal system to treat a range of ailments like dyspepsia, indigestion, diarrhoea and as carminative [2]. It is also used in the treatment of burns, wounds, sores, insect bite, skin ulceration caused Leishmanis brazilliensis [3], juice of leaves used to treat skin allergies [4], urinary diseases [5], to relieve kidney troubles and to treat vaginal discharges [2]. It is known to treat congestive heart failure [2], meningitis [6], epilepsy, and convulsions [7]. Literatures revealed that this plant is used in various Ayurvedic preparations as a liver tonic [8]. Even though this medicinal plant possessing various medicinal properties it is remained unexplored scientifically for many of its claimed activities. As there is paucity in the scientific evidence for the hepatoprotective activity and anti oxidant activity an effort has been made to evaluate the hepatoprotective activity and anti oxidant activity of *Plectranthus amboinicus* (Lour.)

Materials and Methods

The leaves of *Plectranthus amboinicus* (Lour.) were collected from the local areas of Kollam district, Kerala state in the month of November and were identified by Prof. Dr. D. Stephen, Dept. of Botany, American College, Madurai. A voucher specimen (PA-01) has been deposited at the departmental herbarium, K.M.College of Pharmacy, Madurai.

Preparation of the Extract

The Leaves were washed, shade dried, pulverized and successively extracted with ethanol by using soxhlet apparatus. The extract was phytochemically investigated [9].

Assay of Marker enzymes

Serum bilirubin was estimated by following method suggested by Wooton [10]. ALT (Alanine Transaminase) and AST (Aspartate Transaminase) were estimated by the method suggested by Reitman & Frankel [11].

Animals

The study was carried out after obtaining clearance from the Institutional Animal Ethics Committee (IAEC) and CPCSEA guidelines were adhered to throughout the study.

Albino rats of either sex weighing 125-150 g were acclimatized to our laboratory conditions. They were fed with standard commercial diet and water *ad libitum*.

Acute toxicity studies

Acute toxicity studies were carried out according to OECD guidelines [12] and the extract was found to be safe up to 2000 mg/kg.

Hepatoprotective activity [13]

The animals were divided into four groups viz. G1, G2, G3 and G4, each containing of a maximum of six animals. G1, which served as normal control, received normal saline (0.5ml) intraperitoneally (i.p).

G2 received CCL_4 0.5ml/kg, i.p once daily for 7 days [14]. G3 received received CCL_4 0.5ml/kg, i.p and silymarin 200mg/kg, p.o. simultaneously for 7 days. G4 received CCL_4 0.5ml/kg and 200 mg /kg of ethanolic extract of *Plectranthus amboinicus* (Lour) (EEPA), p.o. simultaneously for 7 days. At the end of the treatment, blood samples collected by retro orbital puncture and were kept at room temperature for about half an hour, the clot was dispersed with a glass rod and then centrifuged for 15-20 min at 3000 rpm to separate the serum. The serum was subjected to biochemical investigations viz., total bilirubin, SGOT and SGPT.

Estimation of peroxidation product and antioxidant enzyme

The level of peroxidation product viz. Malondialdehyde (MDA) was measured in blood [15] where the reaction depends on the formation of a coloured complex between malondialdehyde (MDA) and thiobarbituric acid (TBA) having an absorption maximum at 532 nm.

Similarly the level of glutathione (GSH) content in blood was measured [16] where 5-5^1-Dithiobis 2-nitro benzoic acid (DTNB) is reduced by glutathione, forming highly coloured yellow anion. The optical density of this yellow substance is measured at 412 nm.

Statistical analysis

Results were expressed as mean ± SEM and evaluated for statistical significance using ANOVA followed by Student-Newman-Keuls multiples comparison test. $p<0.05$ implied significance.

Results and Discussion

The phytochemical studies showed the presence of terpenes, flavonoids, saponins, phenols and tannins. The effect of EEPA on CCL_4 induced liver damage in albino rats with reference to biochemical changes in serum in shown table 1. There was a significant increase in the serum ALT , AST and bilirubin ($p<0.001$) in G2. G3 showed significant reduction in the levels of ALT, AST ($p<0.001$) and bilirubin ($p<0.01$). G4 significantly reduced the serum ALT ($p<0.001$), AST ($p<0.001$) and bilirubin ($p<0.05$). Decreased levels of MDA and increased level of GSH was observed significantly ($p<0.001$) with the EEPA treated group G4, which was shown in table 1.

Many medicinal plants are screened for the hepatoprotective activity because of the presence of active phytochemical constituents such as phenolics, flavonoids etc. Many Phenolic compounds have been reported to show antioxidative activity against CCL4 induced lipid peroxidation.[17]. Phenolics, the antioxidant principles and flavonoids are known to present in the plant [18]. Moreover this plant is used in Ayurvedic liver tonic preparations. This fostered us to evaluate the hepatoprotective activity and antioxidant activity of this plant. One of the mechanisms in this model is considered to be initiated by an enzymatic reaction of CCL_3 from CCL_4 in liver microsomal cytochrome P_{450} system and that in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation [19].

Table 1: Effect of EEPA on serum lipids and antioxidant enzymes

Group	AST (U/L)	ALT (U/L)	Total Bilirubin (mg/dl)	MDA nmol/ 100 ml)	GSH (mg/ 100 ml)
G1	44.6 ± 0.80	62.02 ± 1.13	0.33 ± 0.009	14.82 ± 2.12	62.44 ± 1.52
G2	131.11 ± 2.1[a]	140.6 ± 4.2[a]	1.12 ± 0.02[a]	160.42 ± 5.22[a]	35.23 ± 4.34[a]
G3	100.2 ± 5.9***	88.09 ± 2.04***	0.64 ± 0.18**	125.14 ± 1.22***	56.12± 3.42***
G4	113.0 ± 0.59***	121.1 ± 3.2***	0.816 ± 0.007*	110.82 ± 4.32***	58.12 ± 3.12***

ANOVA followed by Student Newman-Keuls multiple comparisons test.
a $p< 0.001$ when compared to G1
*$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ when compared to G2.
Group 1 (G1) – Normal control.
Group 2 (G2) – Rats treated with 0.5ml/k g of CCl_4.
Group 3 (G3) – Rats treated with 0.5ml/kg of CCl_4 and 200mg/kg of Sylimarin.
Group 4 (G4) – Rats treated with 0.5ml/100 g of CCl_4 and 200mg of EEPA.

The peroxidative products induce hypo function of the membrane and finally cytosolic enzyme aberrates to blood. Therefore lipid peroxidation of hepatocytes has been recognized to be a major factor in the liver injury model. Serum bilirubin, AST and ALT are the most sensitive tests employed in the diagnosis of hepatic diseases [20]. The significant increase in serum total bilirubin, AST and ALT in CCl_4 treated group are indicative of liver damage [21] which was reduced significantly by EEPA comparable with standard drug Silymarin showed the protective effect of *Plectranthus amboinicus* Lour. Moreover there was a well-marked increase in MDA in G2 following CCl_4 administration. Hepatic GSH depletion or even extra hepatic GSH depletion can provide a useful indication of the protective role of GSH against toxic foreign compounds [22] . In our study, GSH was found to be decreased in G2, which substantiates the increased lipid peroxidation and decrease in antioxidant enzymes following CCl_4 administration. Simultaneous administration of EEPA decreased MDA and increased glutathione levels, which upholds that, the free radical scavenging effect and antioxidant property of the plant may be the intended mechanism, which could be correlated with earlier studies [21] [23].

In conclusion the above findings are suggesting the hepatoprotective effect of *Plectranthus amboinicus* (Lour.) probably by its antioxidative property and rationalize its use as an ingredient in Ayurvedic formulations. Further extensive studies using some more models of experimental hepatic damage may help in establishing a definite rationale for its therapeutic use as a hepatoprotective drug.

Acknowledegments

The authors are thankful to the director and management of KM College of pharmacy, Uthangudi, Madurai and The Oxford College of pharmacy, Bangalore for the encouragement throughout the study.

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