

Protective effect of *ervatamia coronaria* in CCl₄ Induced hepatic damage in mice

P.Philomina mary¹, P.Sampath kumar¹, S.Stalin^{2*}

¹Department of chemistry & Biosciences, Srinivasa Ramanujam centre, SASTRA University, Kumbakonam, India.

²School of chemical and biotechnology, SASTRA University, Thanjavur, India.

*Corres. Author: stalin@scbt.sastra.edu

Abstract: The present study is aimed to evaluate the hepatoprotective effects of leaves and flower extracts of *ervatamia coronaria* against CCl₄ induced hepatic damage. The results indicated that both flower and leaves extract of *ervatamia coronaria* exhibited significant effect against CCl₄ induced hepatic damage. We also observed the restoration of elevated levels liver marker enzymes includes SGOT, SGPT, ACP, ALP and GGT in a dose dependent manner compared to CCl₄ induced experimental groups. These findings confirm the hepatoprotective efficiency of flower and leaves extract fo *ervatamia coronaria* against CCl₄ induced hepatic damage.

Key words : *ervatamia coronaria* leaves and flowers, SGOT, SGPT, GGT, hepatoprotective activity.

Introduction

Ervatamia coronaria is a common weed found in moist grassy places distributed around the India (Terblarhe *et al.*, 1996). An ornamental shrub leading to 3 meters tall, native probably of northern India. The large shiny leaves are deep green and 6 or more inches in length and about 12 inches in width. Grape jasmines in spring but flowers appear sporadically all year. The waxy blossoms are white five pedaled pinwheels that are borne in small clusters on the stem tips [1]. Based on the authors knowledge, none of the reports indicated the pharmacological efficiency of *Ervatamia coronaria*. So, the present study is aimed to evaluate the hepatoprotective effects of *Ervatamia coronaria*. Numerous reports in the literature suggested that plant phytochemicals possess better hepatoprotective against CCl₄ induced hepatic damage. Bodakhe *et al.*

suggested that *Bauhinia variegata* bark extract exhibited significant hepatoprotective activity by maintaining serum liver marker enzyme SGOT and SGPT [2]. Another reports on ethanolic extract of *Momordica dioica Roxp.*, *Clerodendrum intermel*, *Andrographis paniculata Nees.*, *Antrodia camphorate*, *Armillariella tabescens*, *Cytisus scoparius* and *Pergularia daemia* restored the elevated levels of serum enzymatic levels of serum glutamate oxaloactate transaminase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphates (ASLP) and total bilirubin against CCl₄ induced hepatic damage [3,4,5,6,7,8]. The above mentioned hepatoprotective effects of ethanolic extracts of plant mainly attributed to their phytochemicals includes flavonoids, alkaloids, saponins and anthocyanins. In this juncture, we decided to evaluate the hepatoprotective effect *Ervatamia coronaria* against CCl₄ induced hepatic

damage and correlates with their phytochemical constituents.

Materials and Methods

Sample Collection

The fresh leaves and flowers of *Ervatamia coronaria* were collected from Mayiladuthurai during January 2008. The fresh leaves and flowers were washed with water, shade dried at room temperature for 7 days. The dried materials (leaves and flowers) were ground and coarsely powdered.

Ethanol Extraction

The powdered sample (leaves and flowers) and the solvent (ethanol and water) were taken in the Soxhlet apparatus in the ratio of 1:2. The collected extracts were concentrated by distillation and stored in the refrigerator [9].

Quantitative Phytochemical analysis

Dried leaves and flowers were weighed and immediately frozen in liquid nitrogen and finely ground after addition of glass powder using a mortar and pestle. The powder was mixed with HPLC-grade methanol using 2 ml of every 100mg of dried leaf and flower powder. After vigorous shaking and centrifugation for 15 min at 7000 rpm at 4°C, the supernatant was removed and evaporated under a nitrogen gas stream and the remaining substance is redissolved in HPLC- grade methanol at 100 $\mu\text{l g}^{-1}$ powder. The components of the leaf and flowers extracts were separated on a Hewlett Packard 1090 liquid chromatograph equipped with a C-18 column (Alltec Altima, 250 x 4.6mm) using the following solvents. A-ultra pure water containing 0.09% Trifluoroacetic acid; B-Acetonitrile (HPLC purified) containing 0.09% Trifluoroacetic acid. The column was eluted isocratically with solvent A for 5 min and a linear gradient of 0-100% Acetonitrile between 5 mins and 25 mins at ambient temperature with a flow rate of 1 ml min⁻¹. Eluting compounds were monitored between 250 nm and 450 nm using a diode array detector. The concentration of flavonoids, alkaloids, tannins, saponins, lignins, glycosides and phenols were calibrated against their respective standards [10].

Hepatoprotective Activity

Animals

Male albino mice (23-25gm) were used in the present study maintained with standard pellet diet, and water *ad libitum* for 8 weeks. Animals were maintained under constant conditions environmental (temperature 26 \pm 2°C) and grouped as follows and each consists of six animals.

Group 1 - Control received normal saline

Group II- CCl₄ treated (1mg/kg).

Group III- Orally treated with ethanolic leaf extract of *Ervatamia coronaria* (200mg/kg body weight).

Group IV- Orally treated with ethanolic flower extract of *Ervatamia coronaria* (200mg/kg body weight).

Group V- Pretreated with extract of *Ervatamia coronaria* leaf (200mg/kg body weight) and CCl₄ induced.

Group VI- Pretreated with extract of *Ervatamia coronaria* flower (200mg/kg body weight) and CCl₄ induced.

Group VII- Standard drug Silymarin was administered (25 mg/kg).

The mice were treated with 200mg/kg of Leaf and Flower extract of *Ervatamia coronaria* through oral administration. The development of Liver damage in the mice was done by the oral dose of 1mg/kg (1:1) of CCl₄ in olive oil. After the treatment, animals were fasted overnight and were sacrificed under mild chloroform anesthesia. Blood was collected by jugular vein puncture and the liver was quickly excised off, washed in saline, bottled and stored at 4°C and used for biochemical analysis includes total protein in serum, liver tissue protein, SGOT, SGPT, ACP, ALP, Gamma Glutamy1 transpeptidase using auto analyzer SECOMSM.

Statistical analysis

The results in the present investigation were analyzed by mean \pm standard deviation. Significance between the groups was estimated by students 't' test. * P<0.01 is considered as significant value in the treated groups when compared to control.

Table 1: Quantitative analysis of phytochemicals of *Ervatamia coronaria*

S. No.	Phytochemicals	Result	
		Leaf	Flower
1.	Total Alkaloids (mg/kg)	0.59*	0.89*
2.	Total Flavonoids (mg/kg)	0.75*	1.72*
3.	Tannins (mg/kg)	0.27*	0.18
4.	Lignin (mg/kg)	0.12	0.09
5.	Glycosides (mg/kg)	0.07	0.16
6.	serpentines (mg/kg)	0.03	0.19

Values are expressed as mean. * considered as significantly high amount.

Results

Phytochemical Analysis

Table-1 indicates the various phytochemicals of leaves and flowers of *Ervatamia coronaria* which were analysed quantitatively. The results show the amount of secondary metabolites in leaves and flowers of *Ervatamia coronaria* such as total Alkaloids, total Flavonoids, Tannins, Lignin, Glycosides and Serpentines, which possess various biochemical activities such as hepatoprotective activity, anticancer activity, antidiabetic activity, etc.

Hepatoprotective activity

Effect on Serum and Liver Tissue Protein level

Oral administration of CCl_4 causes a significant elevation in the total protein level in

serum and liver tissue to 55.08 ± 0.89 , 3.22 ± 0.46 respectively when compared to normal group (Table 2). After the oral administration of 200 mg/kg of *Ervatamia coronaria* leaf and flower extract, show no significant effect on liver tissue and serum protein level when compared to normal. The administration of *Ervatamia coronaria* leaf and flower extract in CCl_4 intoxicated mice causes significant decrease ($P < 0.01$) in protein level in serum and liver tissue when compared to the treated group. There is a significant reduction in the protein level observed in the silymarin treated group which is compared to the treated group ($P < 0.01$). This result indicates that ethanolic extract of *Ervatamia coronaria* leaves and flowers have good protein lowering effect on CCl_4 induced mice (Table 2).

Table 2: Effect of ethanolic extract of leaf and flower of *Ervatamia coronaria* on Liver Tissue and Serum Protein levels in CCl_4 induced mice.

S. No.	Groups	Liver tissue Protein mg / g	Serum protein mg / dl
1.	Control (Normal Saline)	2.74 ± 2.26	49.89 ± 1.70
2.	CCl_4 (1 mg / kg)	2.74 ± 2.26	55.08 ± 0.89
3.	<i>Ervatamia coronaria</i> leaf extract (200 mg/kg)	$2.67 \pm 0.56^*$	$48.75 \pm 0.68^*$
4.	<i>Ervatamia coronaria</i> flower Extract (200 mg / kg)	$2.42 \pm 0.38^*$	$48.35 \pm 0.33^*$
5.	200 mg / kg <i>Ervatamia coronaria</i> leaf extract and CCl_4 induced	$2.97 \pm 0.21^{**}$	$52.36 \pm 0.54^{**}$
6.	200 mg / kg <i>Ervatamia coronaria</i> Flower extract and CCl_4 induced	$2.66 \pm 0.24^{**}$	$53.01 \pm 0.28^{**}$
7.	CCl_4 and silymarin (25 mg / kg)	$2.75 \pm 0.27^{**}$	$50.89 \pm 1.71^{**}$

Values are expressed as mean \pm S.D (n = 6); * $P > 0.05$ Vs Control (Not significant) (Group III and Group IV) by Students 't' test. ** $P < 0.01$ Vs control (Group V, VI and VII) by students 't' test.

Effect of hepatic enzymes in serum

Oral administration of CCl_4 causes a significant elevation in the serum enzymes such as SGOT, SGPT, ACP, ALP, and GGT, are 20.67 ± 0.28 , 50.16 ± 0.10 , 90.11 ± 0.02 , 5.75 ± 0.23 , 80.28 ± 3.21 respectively. The treatment with 200 mg/kg of *Ervatamia coronaria* leaves and flowers extract in CCl_4 induced group significantly reduced ($P < 0.05$) the levels of hepatic marker enzymes in serum, when compared to the CCl_4 induced group. There was no significant difference in the hepatic marker enzymes in pretreated with leaves and flowers extract of *Ervatamia coronaria* when compare to normal group. There is a significant reduction in the serum hepatic marker enzymes noticed in the animals treated with silymarin alone in CCl_4 induced group. Thus this result shows that administration of *Ervatamia coronaria* leaf and flower extract lower the level of hepatic marker enzymes in CCl_4 induced mice which prevent hepatic damage (Table 3).

Effect on Hepatic Marker Enzymes in Liver Tissue

Oral administration of CCl_4 to hepatotoxic animal causes a significant increase in the level of SGOT, SGPT, ACP, and ALP, in the liver tissue to 18.66 ± 0.17 , 14.87 ± 0.12 , 19.87 ± 0.03 , 6.3 ± 0.22 . There was no significant difference ($P > 0.05$) marker enzymes in liver tissue to is observed by the administration of ethanolic extract of leaf and flowers of *Ervatamia coronaria* alone when compared to normal group. Administration of *Ervatamia coronaria* leaf and flower extract causes a significant reduction in the hepatic marker enzymes in the liver tissue in the CCl_4 induced hepatotoxic mice when compared to treated groups. Treatment with silymarin also significantly reduced the level of enzymes in liver tissue in CCl_4 treated mice, (Table 4). Thus the treatment with 200mg/kg flower and leaf extract reversed these changes towards the normal values.

Table 3: Effect of ethanolic extract of leaf and flower of *Ervatamia coronaria* on serum hepatic marker enzymes in CCl_4 induced mice

S. No.	Groups	SGOT (IU / L)	SGPT (IU / L)	ALP (KA)	ACP (KA)	GGT (UL / L)
1.	Control (Normal Saline)	13.37 ± 0.19	14.09 ± 0.27	11.20 ± 0.05	3.97 ± 0.19	48.24 ± 1.78
2.	CCl_4 (1 mg / kg)	20.67 ± 0.28	15.16 ± 0.10	19.11 ± 0.02	5.75 ± 0.23	80.28 ± 3.21
3.	<i>Ervatamia coronaria</i> leaf extract (200 mg/kg)	15.62 ± 0.26*	14.90 ± 0.60*	11.45 ± 0.20*	4.02 ± 0.27*	51.26 ± 2.45*
4.	<i>Ervatamia coronaria</i> flower Extract (200 mg / kg)	14.89 ± 0.32	13.93 ± 0.07*	11.02 ± 0.41*	4.15 ± 0.19	52.33 ± 2.80*
5.	200 mg / kg <i>Ervatamia coronaria</i> leaf extract and CCl_4 induced	17.20 ± 0.24	14.54 ± 0.10*	10.99 ± 0.46**	4.69 ± 0.23**	59.11 ± 1.20**
6.	200 mg / kg <i>Ervatamia coronaria</i> Flower extract and CCl_4 induced	16.90 ± 0.21	14.21 ± 0.21**	10.52 ± 0.65**	4.32 ± 0.34**	61.03 ± 0.75**
7.	CCl_4 and silymarin (25 mg / kg)	14.38 ± 0.20	15.10 ± 0.28**	12.21 ± 0.06	4.98 ± 0.20*	49.25 ± 2.79**

Values are expressed as mean \pm S.D (n = 6); * $P > 0.05$ Vs Control (Not significant) (Group III and Group IV) by Students 't' test. ** $P < 0.01$ Vs control (Group V, VI and VII) by students 't' test.

Table- 4 : Effect of ethanolic extract of leaf and flower of *Ervatamia coronaria* on serum hepatic marker enzymes in CC14 induced mice.

S. No.	Groups	SGOT (IU / L)	SGPT (IU / L)	ALP (KA Units)	ACP (KA Units)
1.	Control (Normal Saline)	11.08 ± 0.17	12.05 ± 0.21	11.8 ± 0.21	4.26 ± 0.20
2.	CC1 ₄ (1 mg / kg)	18.66 ± 0.17	14.87 ± 0.12	19.87 ± 0.03	6.3 ± 0.22
3.	<i>Ervatamia coronaria</i> leaf extract (200 mg/kg)	13.87 ± 0.13*	12.55 ± 0.31*	12.55 ± 0.4*	4.32 ± 0.18*
4.	<i>Ervatamia coronaria</i> flower Extract (200 mg / kg)	13.26 ± 0.09*	12.38 ± 0.21*	11.22 ± 0.05*	4.38 ± 0.13*
5.	200 mg / kg <i>Ervatamia</i> <i>coronaria</i> leaf extract and CC1 ₄ induced	16.40 ± 0.16**	12.24 ± 0.54**	10.73 ± 0.34**	5.02 ± 0.45**
6.	200 mg / kg <i>Ervatamia</i> <i>coronaria</i> Flower extract and CC1 ₄ induced	16.02 ± 0.69**	12.1 ± 80.6**	10.25 ± 0.10**	5.28 ± 0.31**
7.	CC1 ₄ and silymarin (25 mg / kg)	12.09 ± 0.18	13.06 ± 0.22	12.9 ± 0.22	4.27 ± 0.21

Values are expressed as mean ± S.D (n = 6); * P > 0.05 Vs Control (Not significant) (Group III and Group IV) by Students 't' test. ** P < 0.01 Vs control (Group V, VI and VII) by students 't' test.

Discussion

Scientific studies available on a good number of medicinal plants indicate that used to treat liver problems. The principle causes of CC1₄ induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzyme and generation of free radicals [11]. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with CC1₄. CC1₄ is an extensively studied liver toxicant and its metabolites such as trichloromethyl peroxy radical (CC1₄ O²) are known to be involved in the pathogenesis of liver damage [12]. The present study evaluates the hepatoprotective effect of leaves and flowers of *Ervatamia coronaria* in CC1₄ induced hepatotoxicity in mice.

Phytochemical screening

A number of investigations have shown that tannins and other polyphenolic compounds (e.g., coumarins), Flavonoids, triterpenoids, saponins and

a other plant secondary metabolites possess hypoglycemic, hypotensive, anti-inflammatory and other pharmacological properties in various experimental animal models [13]. Studies of flavonoids have produced the most compelling data for the antihepatotoxic activities of plant secondary metabolites in various type of liver damage and several flavonoids have been shown to inhibit liver damage while exhibiting antioxidant activities in various animal models [14]. Flavonoids have been reported to multiple biological effects including antibacterial, antiviral, antitoxic and anti-inflammatory activities. In the present study, the leaf and flower of *Ervatamia coronaria* possess significant amount of flavonoids, which may contribute to hepatoprotective activity against hepato damage caused by CC1₄ [15]. Saponins, Glycosidic surfactants produced by plant cells used to solubilize membrane proteins are also capable of decreasing tumor cell proliferation, inducing apoptosis or cell cycle arrest or inhibiting DNA synthesis in a variety of liver cells lines [16, 17]. Saponins have

anticarcinogenic properties. Immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cells and cholesterol lowering activity. The presence of saponins in the leaf and flower extract of *Ervatamia coronaria* may contribute to hepatoprotective activity against CCl_4 intoxication. Tannins are plant derived polyphenolic compounds which can be classified into two groups, hydrolysable commonly called tannic acid (TA). TA is widely found in food and plants. Recently it has been shown that TA exerts liver cancer chemopreventive activity in various animal models. For example, TA dietary intake in low doses can exert a strong dose dependent chemopreventive activity against spontaneous liver tumor development in male mice [18]. In our study, the leaf and flower or *Ervatamia coronaria* possess significant amount of Tannins which may prevent the hepatic damage caused by CCl_4 .

Effect on the level of serum and liver tissue protein

An obvious sign of hepatic injury leaking of cellular enzymes into the plasma due to the disturbance causes in the transport function of hepatocytes. When liver cell is damaged a variety of enzyme level in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage [19]. The liver is the largest organ in the vertebrate body and the site for intense metabolism. In living system, liver is considered to be highly sensitive to toxic agent. The liver is an important site for protein synthesis and it has the highest rate of synthesis in liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs. The rise in serum and liver tissue levels of protein has been attributed to the damage in structural integrity of liver [20].

The present study indicates that both the ethanolic extract of leaf and flower of *Ervatamia coronaria* exhibited significant hepatoprotective effect in normal and CCl_4 induced mice. Protein level in both serum and liver tissue markedly increase in the liver damage caused by CCl_4 . In mice, administrated with ethanolic extract of leaves and flowers of *Ervatamia coronaria* (200 mg / kg) significantly reverts the level to normal, when compared to silymarin, reference drug. Thus, the result infers a protective effect of *Ervatamia coronaria* leaf and flower extract on impaired function caused by CCl_4 . Support the present hypothesis, similarly the antihelatoxic effect of chloroform extract of leaves of *Polygala arvensis*.

Effect on hepatic marker enzymes in serum and liver

In the present investigation, it is observed that the animals treated with CCl_4 resulted in significant hepatic damage as shown by the elevated levels of liver tissue and serum enzymes. These changes in the marker levels will reflect the hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, with play a vital role in the conversion of conversion of amino acids to keto acids [21]. The pretreatment with leaf and flower extract of *Ervatamia coronaria* in CCl_4 induced liver damage, both at the dose 200 mg / kg, significantly attenuated the elevated liver of the serum markers. The normalization of serum markers by leaf and flower suggests that they are able to change the condition of the hepatocytes so as to protect the membrane integrity against CCl_4 induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Serum ALP on the other hand is related to hepatic cell damage. Increase in serum and level of ALP and ACP is due to increased synthesis in presence of increase biliary pressure. Elevated levels of ALP and ACP have been found out in group V, VI and VII suggests that is ethanolic extract of leaves and flowers of *Ervatamia coronaria* reduced hepatic damage, compared with silymarin as reference drug [22]. The present study investigates the hepatoprotective activity of ethanolic extract of leaves and flowers of *Ervatamia coronaria* on CCl_4 induced liver damage in mice.

Conclusion

The present study evaluated the phyto constituents of *Ervatamia coronaria* and hepatoprotective effects against CCl_4 induced hepatic damage. The result showed that the decreased levels of total protein, in serum and tissue as well as the hepatic marker enzymes such as SGOT, SGPT, ACP, ALP and GGT in serum to be noticed in *Ervatamia coronaria* treated groups. These findings confirm that leaves and flower of *Ervatamia coronaria* possess a similar hepatoprotective activity, which is due to the presence of its peculiar phyto constituents. This study will help for future investigators to identify a suitable agent for the treatment of liver disease.

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