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## Hepatoprotective effect of ethanolic leaf extract of *Calycópterus floribunda* Lam on cadmium induced hepatotoxicity in rats

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### ABSTRACT

Ethanolic leaf extract of *Calycópterus floribunda* was investigated for hepatoprotective activity against cadmium induced liver damage. Various biochemical parameters, serum glutamate oxalo acetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase and total protein were determined to assess the effect of the leaf extract on the cadmium induced hepatic damage. The animals treated with cadmium recorded elevated concentration indicating severe hepatic damage by cadmium, whereas the blood samples from the animals treated with 200 mg/kg (b.w) and 400 mg/kg (b.w) of ethanolic leaf extract of *Calycópterus floribunda* showed significant reduction in the serum markers indicating the effect of the leaf extract in restoring the normal functional ability of the hepatocytes. Silymarin (100 mg/kg, p.o.) was given as reference drug. The present study concluded that the ethanolic leaf extract of *Calycópterus floribunda* showed significant hepatoprotection against cadmium-induced hepatocellular injury.

**Keywords:** *Calycópterus floribunda*, ethanolic leaf extract, hepatoprotective activity, Cadmium liver damage

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## INTRODUCTION

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common ailment resulting into serious debilities ranging from severe metabolic disorders to even mortality [1]. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [2-4]. *Calycopteris floribunda* (Family: Combretaceae) is a species found in Malabar and other parts of South India and is synonymous with *Combretum extensum* Roxb [5-7]. The leaf of the plant *Calycopteris floribunda* reported to contain tannins, flavanol, Octacesanol, sitosterol, 3'-O-methylcalycopterin, 4-O-methylcalycopterin, ellagic acid, gossoypol, quercetin, proanthocyanidin, calycopterin, calycopterin methyl ester and oxymethyl calycopterin [8]. The leaf, stem and root of this plant are being used in the ayurvedic formulation *Marma Gutika* [9]. The leaves of *Calycopteris floribunda* was reported to have laxative and anthelmintic activity [5]. Antibacterial and antifungal activity was reported for petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Calycopteris floribunda* for ten human pathogenic bacteria and five phytopathogenic fungi [10]. Jia-Jia Liu [11] reported the antimicrobial activity of the volatile oil isolated from the leaves and barks of *Calycopteris floribunda*. Toxicity studies of *Calycopteris floribunda* reported in calf rabbit and rats [12]. Hunse-Ara Ali isolated a flavanol, Pachypodol and reported the *in vitro* anti cancer activity [13]. *Calycopteris floribunda* has been reported to possess significant antioxidant activity *in vitro* studies. However, it is not known whether it is equally effective *in vivo*. Further, significant hepatoprotective activity of the methanolic stem extract of *Calycopteris floribunda* against  $\text{CCl}_4$  induced toxicity in rats was reported. There is no report on the hepatoprotective effect of *Calycopteris floribunda* leaves against cadmium induced toxicity in rats. Hence the present study was undertaken to validate the traditional claims and to study the *in vivo* antioxidant and hepatoprotective activity of ethanolic leaf extract of *Calycopteris floribunda*.

## MATERIALS AND METHODS

### Chemicals

Cadmium was procured from S.D. Fine Chemicals Ltd. (India), Silymarin was obtained as gift sample from Ranbaxy (Devas, India), standard kit of SGPT, SGOT and ALP was obtained from Jain Scientific Industries, Moradabad, India. All other reagents used were of analytical grade.

### Plant materials

The leaves of *Calycopteris floribunda* was collected from Malapuram district, Kerala. The plant was authenticated at Botanical survey of India, Coimbatore. A voucher specimen was deposited at Department of Pharmacognosy, KMCH College of Pharmacy, and Coimbatore for future reference.

## Preparation of plant extract

The fresh dried powdered leaves of *Calycopteris floribunda* was extracted with 70% ethanol in a Soxhlet apparatus at 68°C for 72 h. The extract obtained was filtered on Whatman No 1 filter paper and concentrated using rotary vacuum evaporator (Eyele, Japan) at 40° - 45°C (4 % w/w).

## Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure described by Kokate [14] and Harborne [15] (Table 1).

**Table 1. Preliminary phytochemical screening of ethanolic leaf extract of *Calycopteris floribunda***

Type of constituents	Ethanolic leaf extract
Triterpenes	+
Steroids	+
Carbohydrates	-
Tannins	+
Flavonoids	+
Alkaloids	-
Glycosides	-
Saponins	-

+ indicates present and – indicates absent

## TLC

TLC for ethanolic leaf extract of *Calycopteris floribunda* was performed on precoated silica gel GF<sub>254</sub> (Merck) using the mobile phase n-Hexane: Ethylacetate (7:3) and detected under UV light and Anisaldehyde-Sulphuric acid reagent.

## Experimental animals

Swiss male mice (20 - 25 g) and albino adult Wistar male rats (150 - 200 g) were obtained from the animal house of KMCH College of Pharmacy Tamilnadu, India. The study protocol was approved by the institutional animal ethics committee, Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi, India. The animals were housed in plastic cages (47×34×18 cm) in an air-conditioned environment with 10/cage and 6/cage for mice and rats, respectively.

## Acute toxicity study [16]

Acute oral toxicity was determined according to the guidelines of organization for Economic Co-operation and Development (Fixed dose method-OECD Guideline 420). Eight groups of six male mice each were used for the study. The control group received 0.5 ml of 0.5% w/v sodium carboxymethylcellulose orally. The other groups received 3000 mg/kg of

ethanol extract in 0.5% sodium carboxymethylcellulose orally immediately after dosing, the animals were observed continuously for the first 4 h for behavior, occasionally up to 6 h, and then kept for up to 14 days post-treatment in order to observe for any toxic symptoms and mortality.

### Hepatoprotective activity [17]

Five groups of male Wistar albino rats of six each were used for the study. Animals of group I served as normal control and received normal saline 5 ml/kg (b.w) daily for 14 days. Group II animals constituted the hepatotoxic group, and treated similarly as group I. Group IV group V received ethanolic leaf extract (200 and 400 mg/kg/day respectively) suspended in 0.5% sodium carboxymethylcellulose for 14 days and group III were fed with standard drug silymarin 100 mg/kg; p.o daily for 14 days. On the seventh day, cadmium suspension was given by oral route, in a dose of 2 g/kg (b.w) to all rats except the rats in group I. At the end of the experimental period, the rats were fasted overnight and sacrificed by ether anesthesia. Blood and liver samples were collected for biochemical and histological studies.

### Biochemical studies

The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT [18] SGPT, ALP, serum bilirubin [19] and total protein [20] (Table 2).

### Statistical analysis

For determination of significant inter-group differences, each parameter was analyzed separately and one way analysis of variance (ANOVA) was carried out. Dunnet's test was used for individual comparisons. The  $p < 0.05$  or less considered statistically significant.

## RESULTS

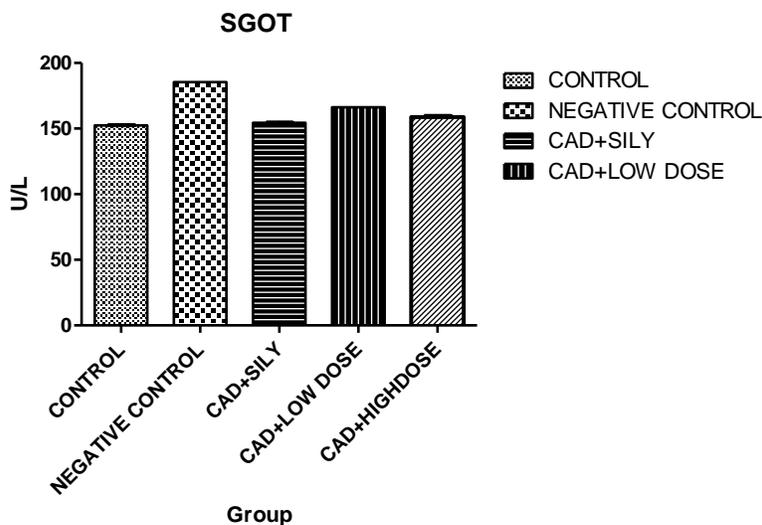
The preliminary phytochemical investigations of the ethanolic leaf extract of *Calycopteris floribunda* confirmed the presence of triterpenes, steroids, tannins and flavonoids. The ethanolic leaf extract of *Calycopteris floribunda* not produced any mortality of mice up to the dose of 3000 mg/kg (b.w) and hence a dose of 200 and 400 mg/kg were selected for the evaluation. The effect of ethanolic leaf extract of *Calycopteris floribunda* on serum transaminase, alkaline phosphatase, bilirubin and total protein levels in cadmium-induced liver damage in rats are summarized in Table 2. Administration of cadmium (5 mg/kg; b.w), as cadmium chloride (0.5 ml) for 4 weeks intoxication resulted a significant ( $P < 0.05$ ) elevation of hepatospecific serum markers SGOT, SGPT, ALP and total protein in cadmium-treated group, in comparison with the normal control group. On administration of ethanolic leaf extract of

*Calycotris floribunda* (Group IV & V) and Silymarin at the dose of 100 mg/kg (Group III) the level of these enzymes were found retrieving towards normalcy.

**Table 2 Estimation of serum biochemical parameters**

Parameters	Groups (n=6)				
	Control	Cadmium treated (5 mg/kg) (Negative control)	Cadmium(5 mg/kg) + Silymarin (100 mg/kg)	Cadmium(5 mg/kg) +Ethanolic leaf extract of <i>Calycotris floribunda</i>	
				200 mg/kg	400 mg/kg
SGOT (U/L)	152.65 ± 1.436	185.45 ± 2.644	154.62 ± 2.844***	166.24 ± 1.455***	159 ± 1.654***
SGPT(U/L)	59.75 ± 1.391	81.62 ± 2.725	60.76 ± 1.684***	78.75 ± 1.307***	70.45 ± 1.725***
ALP(U/L)	142.25 ± 1.668	162.75 ± 1.941	149.65 ± 1.256***	159.44 ± 1.702***	152.35 ± 1.684***
Bilirubin (mg/dl)	152.65 ± 1.436	80.48 ± 0.667	62.13 ± 0.996***	75.13 ± 2.066***	65.92 ± 2.088***
Total protein (g/dl)	2.074 ± 1.248	1.08 ± 1.538	2.072±1.384***	2.03 ± 1.237***	0.05 ± 1.028***

Values are expressed as the mean ± S.E.M., (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's \* P<0.05, \*\* P<0.01, and \*\*\* P<0.001 between treatment Groups and cadmium treated group.



**Fig1. Estimation of SGOT**

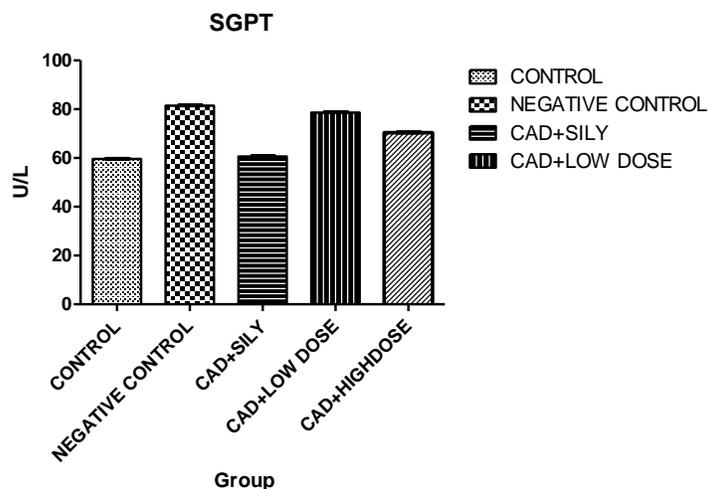


Fig 2. Estimation of SGPT

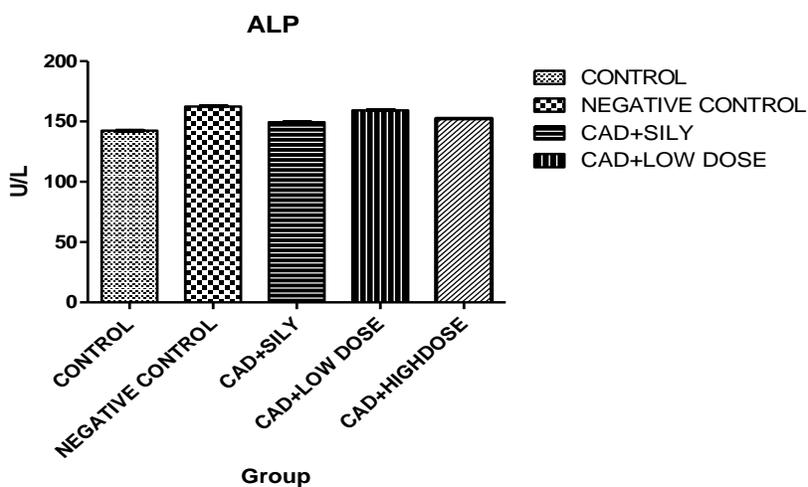


Fig 3. Estimation of ALP:

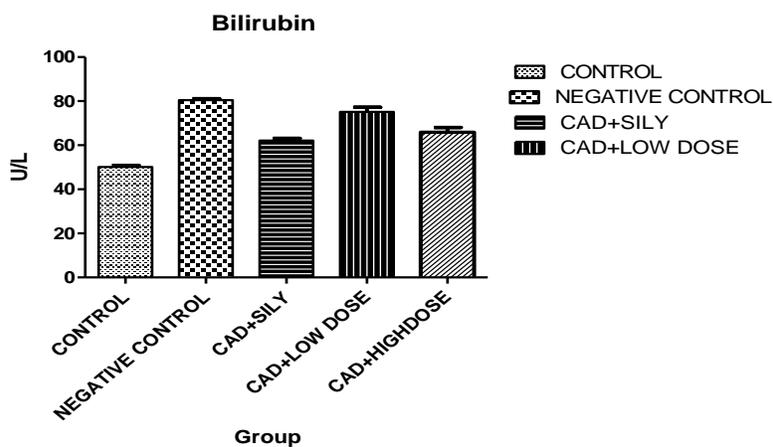


Fig 4. Estimation of Bilirubin

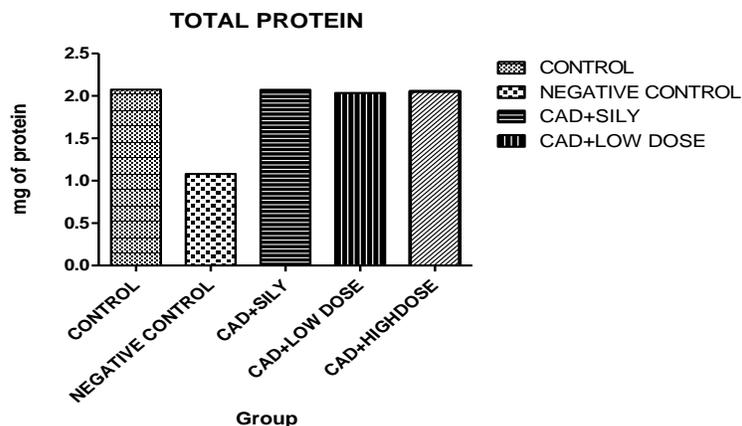


Fig 5. Estimation of total protein

### DISCUSSION

Chronic intake of cadmium in contaminated food or air produces organ dysfunction as a result of cell death, resulting in pulmonary, hepatic and renal tubular diseases [21]. The liver is the most important target organ when considering Cd-induced toxicity because Cd primarily accumulates in the liver [22]. Cadmium is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [23,24]. Cadmium induces oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles [25]. However, the hepatotoxicity of cadmium has been attributed to the formation of toxic metabolites when a part of cadmium is activated by hepatic cytochrome P-450 [26] to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI) [27]. The glutathione protects hepatocytes by combining with the reactive metabolite of cadmium thus preventing their covalent binding to liver proteins [28]. The study of different enzyme activities such as SGOT, SGPT, ALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage [29].

In the present investigation, the animals treated with cadmium at a dose of 5 mg/kg resulted in significant hepatic damage as shown by the elevated levels of serum markers. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids [30]. The pretreatment with ethanolic leaf extract of *Calycopteris floribunda*, both at the dose of 200 mg/kg and 400 mg/kg, significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by ethanolic leaf extract of *Calycopteris floribunda* suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against cadmium 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 and bilirubin levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence

of increasing biliary pressure [31]. The histopathological observations in cadmium-treated rats showed severe necrosis, with disappearance of nuclei. This could be due to the formation of highly reactive radicals because of oxidative threat caused by cadmium. Based on the above results, it could be concluded that ethanolic leaf extract of *Calycopteris floribunda* exerts dose dependent hepatoprotection against cadmium-induced toxicity.

### CONCLUSIONS

The ethanolic leaf extract of *Calycopteris floribunda* exhibited significant hepatoprotective activity. Thus the scientific validation for the traditional claims for hepatoprotective use of plants has been justified. Further studies are needed to explore the possible mechanism of claimed actions of these plants.

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