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Research Article

HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACTS OF *CLITORIA TERNATEA* L. AND *CASSIA ANGUSTIFOLIA* VAHL LEAF AGAINST CCl_4 INDUCED LIVER TOXICITY IN RATS

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ABSTRACT
Hepatoprotective activity of ethanol extracts of leaf of *Clitoria ternatea* (EECT) and *Cassia angustifolia* (EECA) against carbon tetrachloride (CCl_4) in rats was evaluated. Administration of standard drug silymarin, EECT and EECA showed significant hepatoprotection against CCl_4 induced hepatotoxicity in rats. Hepatoprotective activity of EECT and EECA was due to the decreased levels of serum marker enzymes viz (AST, ALT, ALP, ACP and LDH) and increased total protein, total conjugated and unconjugated bilirubins. The phytoconstituents were identified from the ethanol extracts of *Clitoria ternatea* and *Cassia angustifolia* leaf.

KEY WORDS: Hepatoprotien, serum marker enzymes, CCl_4

INTRODUCTION

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic function and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals [1].In the absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief [2].Attempts are being made globally to get scientific evidence for these traditionally reported herbal drugs. While searching for hepatoprotective agents in natural products, highly encouraging results were obtained in our laboratory with *Clitoria ternatea* L and *Cassia angustifolia* Vahl.These two plants are extensively used by *Kanikkar* tribals of Agasthiarmalai Biosphere Reserve, Tamil Nadu to treat liver diseases. However, no systematic attempts have been made to establish the scientific basis of the beneficial effects of *Clitoria ternatea* and *Cassia angustifolia* leaf extracts. Hence, the aim of the present study was to investigate the hepatoprotective activity of ethanol extracts of *Clitoria ternatea* and *Cassia angustifolia* leaves on carbon tetrachloride induced liver toxicity in rats.

MATERIALS & METHODS

Plant Material

Clitoria ternatea L and *Cassia angustifolia* Vahl plants were freshly collected from the Karaiyar, Agasthiarmalai Biosphere Reserve, Tamil Nadu.The plants were identified and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin Tamil Nadu.

Preparation Of Plant Extracts For Phytochemical Screening And Hepatoprotective Activity

The leaves of *Clitoria ternatea* and *Cassia angustifolia* were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Clitoria ternatea* and *Cassia angustifolia* leaves were packed in a Soxhlet apparatus separately and extracted with ethanol. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[3,4,5].The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extracts were used for hepatoprotective activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum.*

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [6]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design

In the investigation, a total of 25 rats (20 rats CCl_4 hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

Group I: Rats received normal saline was served as a normal control.

Group II: Rats received 2.5 ml/kg body weight of CCl_4 alternate days for three times and served as hepatic toxicity induced control (Liver injury rats).

Group III: Liver injured rats received ethanol extract of *Clitoria ternatea* (EECT) leaf at the dose of 100 mg/ kg body weight daily orally by using IGC for 21 days .

Group IV: Liver injured rats received ethanol extract of *Cassia angustifolia* (EECA) leaf at the dose of 100 mg/kg body weight daily orally by using IGC for 21 days.

Group V: Liver injured rats received silymarin, a standard drug at the dose of 100 mg/kg body weight daily orally by using IGC for 21 days.

Biochemical Analysis

The animals were sacrificed at the end of the experimental period of 21 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum aspartate transaminase (AST), alanine transaminase (ALT) was measured spectrophotometrically by using the method of Reitman and Frankel [7].Serum alkaline phosphatase (ALP) and acid phosphatase (ACP) were measured by the method of King and Armstrong [8] and Lactate dehydrogenase (LDH) was determined by the method of Mercer [9]. Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw [10]. The unconjugated bilirubin concentration was calculated as the difference between total and conjugated bilirubin concentrations. Serum total protein was estimated by the method of Lowry *et al* [11].

Statistical Analysis

The data were analyzed using student's t-test statistical method. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

RESULTS

The ethanol extracts of leaf of *Clitoria ternatea* and *Cassia angustifolia* were subjected for phytochemical analysis showed the presence of alkaloid, anthraquinone, catechin, flavonoid, phenol, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotien.The ethanol extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

The effects of EECT and EECA on serum transaminase, alkaline phosphatase, acid phosphatase and lactate dehydrogenase levels in CCl_4 induced hepatotoxicity in rats are shown in Table.1. There was a significant increase in serum AST, ALT, ALP, ACP and LDH. Treatment with silymarin (100mg/kg), EECT (300mg/kg) and EECA (300mg/kg) prevented the elevation of serum marker enzymes AST, ALT, ALP, ACP, and LDA. The decreases in the level of AST, ALT, ALP, ACP and LDH were found to be greater in standard drug silymarin followed by EECT and EECA.

The effects of EECT and EECA on total, conjugated, unconjugated bilirubins and total protein levels in CCl_4 induced hepatotoxicity in rats are summarized in Table 2. A significant elevation of total, conjugated and unconjugated bilirubins and decreased level of total protein in the serum of CCl_4 intoxicated rats (Group II) when compared to normal control (Group I). The extracts of EECT and EECA reduced the levels of total, conjugated and unconjugated bilirubins and reversed the altered total protein.

DISCUSSION

CCl_4 produces an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures[12].The toxic metabolite, CCl_4 radical ,is produced and further reacts with oxygen to give trichloromethyl peroxy radical . Cytochrome P_{450} is the enzyme responsible for this conversion. This radical binds covalently to the macro molecule and causes peroxidative degradation of lipid membrane of the adipose tissue, which leads to leakage of serum marker enzymes. It is possible that hepatocellular damage occurs when the free radicals generation exceeds the cellular radicals scavenging capacity [13]. Assessments of liver toxicity was done by measuring the marker enzymes such as AST, ALT, ALP, ACP and LDH, which are originally present in high concentration in the cytoplasm. When there is hepatic injury these enzymes leak into blood stream inconformity with extent of hepatotoxicity. Leaf extracts of *Clitoria ternatea* (EECT) and *Cassia angustifolia* (EECA) at the dose of 300mg/kg significantly restored the elevated levels of serum marker enzymes. The normalization of serum markers by EECT and EECA suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl_4 induced leakages of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Increase in serum bilirubin levels may be found in hepatocellular damage, haemolytic jaundice or hepatitis.CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total bilirubin[14].The significant reduction of total, conjugated and unconjugated bilirubins levels in the serum when administrated with the EECT and EECA extracts, which indicates that the conjugating function of the liver was improved. The reduction of the bilirubins level by the extracts suggest that, the extracts may activate the constitutive androstane receptor (CAR) which is a key regulator in bilirubin clearance in the liver[15].The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of EECT and EECA significantly reversed these changes may be increasing protein synthesis. This indicates the hepatoprotective activity of ethanol extracts of *Clitoria ternatea* (EECT) and *Cassia angustifolia* (EECA) leaves against damage by CCl_4.

In earlier reports, plants are found to have hepatoprotective activity due to presence of antioxidants, flavonoid, terpenoid, tannin and steroid nature[16,17,18,12]. EECT and EECA shows the presence of terpenoid, flavonoid, tannin and steroid which may act as antioxidant principle with EECT and EECA.The results of the present study demonstrate that, ethanol extract of *Clitoria ternatea* and *Cassia angustifolia* leaves have potent hepatoprotective activity against CCl_4 induced hepatotoxicity in rats. Further investigations are ongoing in our laboratory to determine the exact phytochemicals responsible for the hepatoprotective properties of these two plant extracts.

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Table 1: Effect of ethanol extracts of *Clitoria ternatea* and *Cassia angustifolia* leaf on AST, ALT, ALP, ACP and LDH in the serum of CCl_4 intoxicated male albino rats

S. No.	Treatment Group	Hepatic Marker Enzyme				
		AST(U/L)	ALT(U/L)	ALP (U/L)	ACP (U/L)	LDH(U/L)
1	Group – I	24.58 ± 0.8	19.39 ± 0.7	29.37 ± 0.73	34.42 ± 0.46	54.35±1.2
2	Group – II	69.56 ± 0.5*	58.05 ± 1.5*	54.65 ± 0.93**	65.41 ± 0.83*	98.57±3.2
3	Group – III	26.25 ± 0.7	19.31 ± 0.7	29.53 ± 0.75	36.91 ± 0.38	40.36±1.1
4	Group – IV	39.52 ± 0.9*	24.16 ± 0.7	41.58 ± 0.57*	39.50 ± 0.43*	52.19±1.9
5	Group – V	24.54 ± 0.2	17.12 ± 0.3*	26.63 ± 0.68	38.54 ± 0.41	43.41±1.6

Each Value is SEM ± 5 individual observations * $p<0.05$, ** $p < 0.01$ Compared with control hepatic toxicity induced control and drug treated.

Table 2: Effect of *Clitoria ternatea* and *Cassia angustifolia* leaf on concentration of total protein, bilirubin, conjugated bilirubin and unconjugated bilirubin in the serum of CCl_4 intoxicated male albino rats

SNO	Treatment group	Parameters			
		Total Bilirubin (µmol/L)	Conjugated (µmol/L)	Unconjugated (µmol/L)	T.Protein (mg/Dl)
1	Group – I	1.23 ± 0.04	0.26 ± 0.05	0.87± 0.04	7.9 ± 0.7
2	Group – II	5.21 ± 0.46*	0.92 ± 0.06*	3.79 ± 0.11*	5.8 ± 0.5*
3	Group – III	1.66 ± 0.05*	0.35 ± 0.02	1.76 ± 0.05	6.4 ± 0.3
4	Group – IV	2.98 ± 0.04	0.44 ± 0.01	1.49 ± 0.05	6.9 ± 0.4
5	Group – V	1.26 ± 0.03*	0.18 ± 0.05*	0.77 ± 0.03	6.7 ± 0.5

Each Value is SEM ± 5 individual observations * $p < 0.05$ Compared with control hepatic toxicity induced control and drug treated.

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