Research Article

Effect of Roots of *Corallocarpus epigaeus* in Methanolic and Ethyl Acetate Extracts on Ccl_4-induced Hepatotoxicity in Rat

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ABSTRACT

To evaluate the hepatoprotective activity of different solvent extracts of *Corallocarpus epigaeus.* The root materials were shade dried and were extracted in a soxhlet apparatus successively with ethyl acetate and methanol. The solvent was removed by the process of distillation and the crude extract was dried under vacuum. The extract was preliminarily screened using thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) to know the types of compounds present in the extracts. TLC was developed in n hexane: ethyl acetate solvent systems of different polarities and then plates were visualized under Iodine vapour exposure, UV short wave 254nm and long wave 366 nm. Plates were sprayed with a solution of visualizing reagent 10% H_2SO_4 in methanol followed by heating in an oven at 110°C for up to five minutes. The methanolic extracts of *Corallocarpus epigaeus.* at different doses, Silymarin and Drug vehicle were administered *p.o* in sodium carboxy methyl cellulose suspension. The serum was used for the estimation of various biochemical parameters like SGOT, SGPT, ALKP, TBL, CHL, TPTN and ALB. The results clearly depicted that CCL_4 intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, where as decreased the levels of TPTN, ALB significantly when compared to control group. The crude extract from the selected plant produced significant hepatoprotective activity.

Keywords: CCl_4, Ethyl acetate, Hepatotoxicity, Methanol and *Corallocarpus epigaeus*.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, and energy provision and reproduce ion.[1] The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being. Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death.

In generally any hepatoprotective agent can act as antihepatotoxicity or hepatotropic agent but the vice versa is always not true. There are number of phytoconstituents from plants which have exhibited antihepatotoxic activity. Some of the reported constituents with pharmacologically/therapeutically proved claims may be enlisted as Silymarin, glycerryhizin, (+)- catechin, saikosaponins, curcumin, picroside 1 and 2 and gomisin etc. Acetylbergenin.[2] Kolaviron a, flaovanone was also reported for its hepatoprotective properties. [3]

Corallocarpus epigaeus: The herb belongs to the family Cucurbitaceae. The stems are glabrous, much branched, somewhat zigzag or angular at nodes. Leaves 3-5 lobed or angular, cordate, acute or rounded and obtuse sub entire sinuous to minutely, irregularly dentate, short hispid-to long hirsute-scabrid on both surfaces; petiole 1-1.5cm long, hispid to sub glabrous. Male flowers: peduncle 2-4 cm long, glabrous. Female flowers: peduncles 0.2-0.6 cm long, stout. Fruit 1.7-3 x 0.8 - 13cm including beak 0.6-0.8mm long, ovoid, circumcissile with line of dehiscence 2-3mm above base. Seeds few, 4-7 longer than broad. The herb is distributed both in Central Africa and Asia, growing in rich but well drained soil with some water and some sun. It was reported that Ecdysterone has been isolated from *C. epigaeus.*[3] Phytosterolins and a ketone were isolated from *C. epigaeus*.[4] It was reported that 3, 4 dimethoxy cinnamic acid and β-cyanin have been isolated from the species Trianthema.[5]

The potential use of higher plants as a source of new drugs is still poorly explored. In most cases, only pharmacological screening or preliminary studies have been carried out and out of estimated only 5000 species have been studied for their medicinal use.[6] As natural product research continues to be an important part of the drug discovery, the author developed interest in taking up

the phytochemical investigation of selected plant species to explore hepatoprotective.

Carbon tetrachloride (CCL_4)

The hepatotoxicity of CCL_4 is due to the metabolic formation of the highly reactive trichloroethylene free radical which attacks the polyunsaturated fatty acids of the membrane of the endoplasmic reticulum and initiates a chain reaction. It is enhanced by induction of hepatic microsomal enzyme system and vice by antioxidants which move up the free radicals. The first cells to be debagged are those in the centrilobular region where microsomal enzyme activity is the greatest. The initial damage produced is highly localized in the endoplasmic reticulum which results in loss of Cytochrome P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty, a characteristic of CCL_4 poisoning. If the damage is severe, it leads to disturbances in the water and electrolyte balance of hepatocytes leading to an abnormal increase in liver enzymes in plasma, there by impairing mitochondrial functions, followed by hepatocellular necrosis.[7]

MATERIALS AND METHOD

Plant Material

The roots of *C. epigaeus* were collected from Ananthagiri forest region, Visakhapatnam District, Andhra Pradesh, India in the months of March and May, 2006. The plant species were authenticated by Dr. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. The Voucher specimens (TDR-BG- 24-07-2006) were deposited in the institutional museum, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Extraction of the plant materials and sample preparation

The Root materials were shade dried and were extracted in a soxhlet apparatus successively with ethyl acetate and methanol. The solvent was removed by the process of distillation and the crude extract was dried under vacuum and stored in a dessicator prior to chromatographic separation. The extracts were subjected to hepatoprotective the extract producing significant activity was column chromatographed.

Preliminary extract screening

The extract was preliminarily screened using thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) to know the types of compounds present in the extracts. TLC was developed in n hexane: ethyl acetate solvent systems of different polarities and then plates were visualized under Iodine vapour exposure, UV short wave 254nm and long wave 366 nm. Plates were sprayed with a solution of visualizing reagent 10% H_2SO_4 in methanol followed by heating in an oven at 110°C for up to five minutes. The compounds develop various colours with this reagent.

Chromatographic techniques

The Column chromatography was done by standard procedure silica gel (400g, finer than 200#, ACME) was used as adsorbent. The column was eluted with n-hexane, ethyl acetate and finally with methanol. Thin-layer chromatography was simultaneously used to identify and further separate compounds from the fraction using the same solvent system. The developing reagent is 10% H_2SO_4 in methanol.

Experimental Animals

Wistar albino rats of either sex weighing between 150-200 g were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 + 20°C with an alternating 12h light-dark cycle and relative humidity of 50±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Reg. no. 516/01/A/CPCSEA). They were fed with standard laboratory diet (supplied by Ratan Brothers, India) and water *ad libitum* during the experiment.

Experimental Design

The rats were given doses orally with extracts at different dose as mentioned below. Group I normal rats treated with Drug vehicle (1% Sodium CMC) and served as normal control, Group II rats were treated with hepatogens and Group III rats were treated with the standard drug Silymarin at 25 mg/kg body weight. All the doses were administered orally according to the body weight of the animals.

The extracts were administered orally in the following order Group-IV Received methanolic extract of *C. epigaeus* 200 mg/kg Group-V Received methanolic extract of *C. epigaeus* 400 mg/kg Group-VI Received methanolic extract of *C. epigaeus* 800 mg/kg. Group-VII Received ethyl acetate extract of *C. epigaeus* 200 mg/kg Group-VIII Received ethyl acetate extract of *C. epigaeus* 400 mg/kg Group-IX Received ethyl acetate extract of *C. epigaeus* 800 mg/kg.

CCL_4 induced hepatotoxicity

Carbon tetrachloride (CCL_4): 50% v/v solution of carbon tetrachloride was prepared in liquid paraffin. The solution was administered at the dose of 1.25 ml/kg b. wt. I.p. Methanolic and Ethyl acetate extracts obtained from roots of *C. epigaeus in vivo* on preliminary basis, against CCL_4 induced toxicity by assessing them through biochemical parameters. Each set of experiment was divided into groups consisting of control, toxicant, standard, and test. Groups consisted of 5 rats each unless otherwise mentioned. The protocol followed for CCL_4 induced hepatotoxicity on preliminary basis[8] were given below.

The protocol for CCL_4 induced hepatotoxicity

Group	0h	24h	48h	72h
Control	Vehicle	Vehicle	Vehicle	With drawl of blood
CCl4	Vehicle	Vehicle+CCl_4	Vehicle	
Silymarin	Silymarin	Silymarin+CCl_4	Silymarin	
Test	Extract	Extract+CCl_4	extract	

Vehicle: 1% Sodium CMC,

Test: Extracts prepared in 1% Sodium CMC.

The rats of control group received three doses of 1% Sodium CMC (1 ml/kg p.o.) at 24 h intervals (0 h, 24 h and 48 h). The animals in CCL_4 treated group received vehicle at 0 h vehicle followed by followed by CCL_4 diluted in liquid paraffin (1:1 i.p.) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The test groups received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of CCL_4 and at 48 h the third dose of extracts. The positive control group received the first dose of silymarin (25 mg/kg) at 0 h, second dose of silymarin at 24 h followed by a dose of CCL_4 and at 48 h the third dose of silymarin. After 72 h blood was collected from all the groups, allowed to clot for the separation of serum. The serum was used for estimation of biochemical parameters. Serum Glutamic oxaloacetic transaminase (SGOT), serum Glutamic pyruvic transaminase (SGPT) were estimated by a UV – Kinetic method based on the reference method of international federation of clinical chemistry.[9] Alkaline phosphatase (ALKP) was estimated method by PNPP method,[10] while total bilirubin (TBL) by jendrassik and grof,[11] total cholesterol (CHL) by CHOD – PAP,[12] total protein (TPTN) by color complexation with copper ions in an alkali solution.[13] Albumin was estimated by bromo cresol green method (Webster, 1974).[14] All the estimations were carried out using standard kits on auto analyser of Merck make (300 TX, E. Merck-Micro Labs, Mumbai).

Statistical Analysis

The results are expressed as mean ± S.D from n=5 rats in each group. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS

Preliminary phytochemical analysis of the crude extracts was conducted according to the standard procedures and the results are tabulated;

Table 1: Percentage of the extractives obtained

S. no	Name of the plant part	Weight of the powdered material (kg)	% of Ethyl acetate soluble extractives	% of Methanol soluble extractives
1	*C. epigaeus* roots	2.0	8.80	5.20

Table 2: Preliminary phytochemical analysis of the crude extracts

Extract	$FeCl_3$	L.B reaction	Shinoda
C. epigaeus methanolic extract	+ve	+ve	+ve
C. epigaeus ethyl acetate extract	+ve	+ve	+ve

The methanolic and ethyl acetate extracts were concentrated at low temp (40-50°c) under reduced pressure. The ethyl acetate fractions of *C. epigaeus* were subjected to column chromatography, separately over silica gel column and the results are shown in Table. The fractions were monitored by using silica gel TLC and the fractions showing similar spots were mixed together.

STRUCTURAL ELUCIDATION OF COMPOUND CE-1

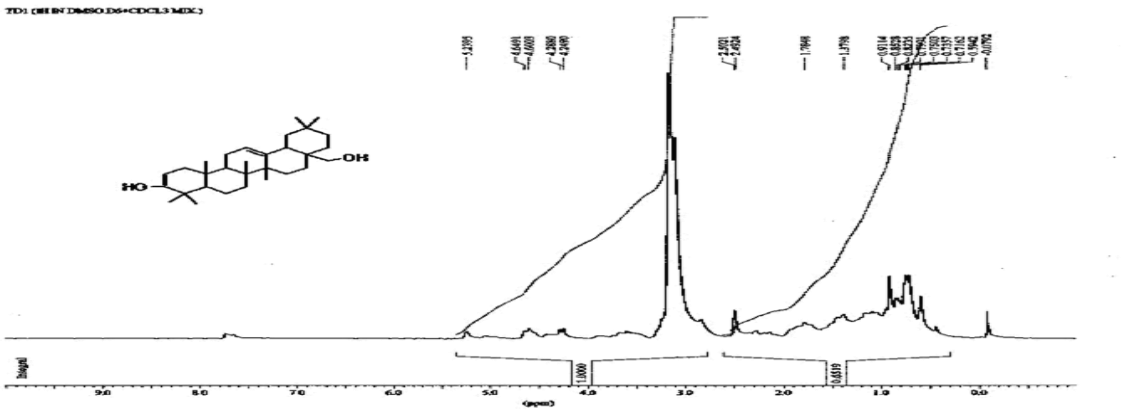
The compound was obtained as yellowish crystals from ethyl acetate and hexane with a molecular formula $C_{30}H_{50}O_2$.The absorption bands at 3400 cm^{-1} and at 1636 cm^{-1} in the IR spectrum indicates that the compound contains hydroxyl group and an unsaturation.

The group of peaks between δ 0.60 to 1.00 indicates that there are many methyl groups in the compound. The peak at δ 5.29 for one proton shows the presence of one unsaturated proton. The peaks at δ 4.24 and 4.62 are due to two protons under a hydroxyl group (CH_2OH). The peak at δ 3.54 is due to one more proton under a secondary hydroxyl group. The remaining signals in between δ 1.00 to 2.00 and absence of signals in the other regions of the spectrum indicates that the compound may be a triterpene with two hydroxyl groups and a double bond. The compound was identified as 8a-Hydroxymethyl-4, 4, 6a, 6b, 11, 11, 14b-heptamethyl-1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-eicosahydro-picen-3-ol. It was reported for the first time from *C. epigaeus.* (CE-1).

Table 3: Chromatography of the ethyl acetate fraction of *C. epigaeus* roots (CD):

Weight of extract taken	20 gm
Weight of silica gel (100-200 mesh) used for adsorption	40 gm
Column dimensions	60 mm × 210 cm
Weight of silica gel (100-200 mesh) used for packing the column	500 gm
Volume of each fraction collected	500 ml

Fraction No	Eluant composition	Weight of residue (g)	Compound isolated
1-7	Pure hexane	3.327	Oily yellow residue
8 -20	5% ethyl acetate in hexane	1.453	Waxy residue
21-40	8% ethyl acetate in hexane	0.490	CE-1
41-58	15% ethyl acetate in hexane	0.058	Yellowish residue
59-76	20% ethyl acetate in hexane	0.568	----
77-90	25% ethyl acetate in hexane	0.056	Mixture
91-102	30% ethyl acetate in hexane	1.103	crystalline residue
103-110	35% ethyl acetate in hexane	0.580	----
111-120	40% ethyl acetate in hexane	0.165	Mixture
121-138	50% ethyl acetate in hexane	0.019	Mixture
139-148	60% ethyl acetate in hexane	2.126	Dark reddish brownish residue
149-156	80% ethyl acetate in hexane	1.000	Dark brownish residue
157-170	100% ethyl acetate	1.089	Sticky matter
171-180	5% methanol in ethyl acetate	0.550	Sticky matter
181-195	10% methanol in ethyl acetate	0.257	Reddish yellow
196-208	20% methanol in ethyl acetate	1.361	Reddish residue
209-221	40% methanol in ethyl acetate	2.454	Greenish colouring matter
222-234	80%methanol in ethyl acetate	2.551	Dark red residue
235-260	100% methanol	1.983	intangible mass



1H-NMR Spectrum of CE-1

Table 4: Effect of Methanolic extracts of CE on CCl4 induced hepatotoxicity in rats:

Group	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	108.4±2.13	95.92±3.23	218.60±1.68	2.10±0.18	110.6±2.48	6.21±0.12	3.90±0.94
CCl_4	315.4±12.00	241.4±4.58	428.1±24.47	3.48±0.68	270.7±11.19	2.58±0.82	1.98±0.21
Silymarin	102.2±1.71*	104.4±0.8*	212.6±1.68*	1.38±11.19*	114.1±0.42*	6.98±0.17**	3.92±0.18**
CEM 200 mg/kg	265.6±2.82	232.6±2.99	360.1±3.20	2.80±0.25	255.1±3.21	3.94±1.06	2.99±0.31
CEM 400 mg/kg	138.4±2.01**	121.6±1.91*	241.7±1.98**	2.15±0.40*	149.7±2.01*	3.83±0.60**	3.92±0.41**
CEM 800 mg/kg	110.6±1.97*	110.5±2.55*	212.6±2.88*	1.85±0.80*	121.9±3.01*	4.80±0.18**	4.80±0.09**

Data expressed in mean ± s.e.m, n=5 *Significant reduction compared to hepatotoxic group (P<0.05) **Significant increase compared to hepatotoxic group (P<0.05)

Table 5: Effect of Methanolic extracts of CE on Percentage protection against CCl_4 induced hepatotoxicity in rats:

Group	SGOT	SGPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin	67.59	56.62	50.33	60.34	57.85	170.54	97.98
CEM 200 mg/kg	15.78	3.645	15.88	19.54	5.76	52.71	51.01
CEM 400 mg/kg	56.11	49.62	43.54	38.21	44.69	57.02	97.98
CEM 800 mg/kg	64.93	54.22	50.33	46.83	54.96	86.04	142.42

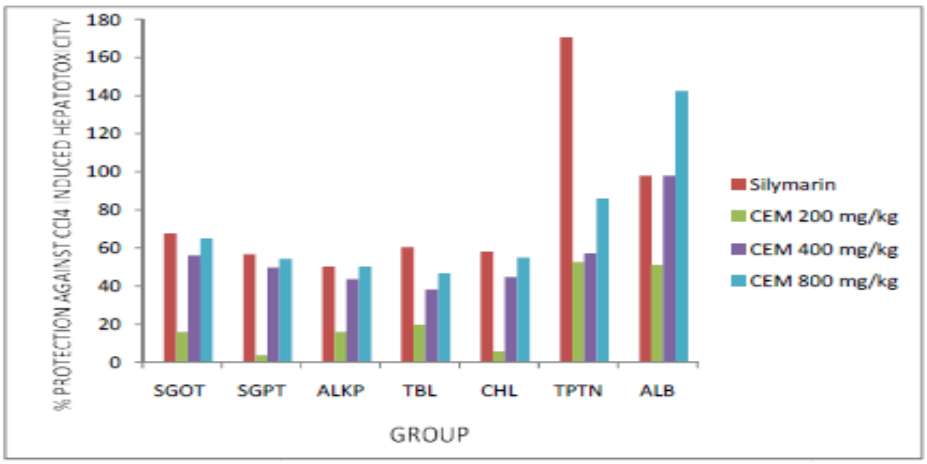


Figure 1: Effect of the crude extract of CEM 200, 400 and 800mg/kg along with silymarin 25 mg/ kg body wt. on percentage protection of various biochemical parameters against CCl_4 induced hepatotoxicity.

Results shows Silymarin the standard drug at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL with the values 102.2 ± 1.71, 104.4 ± 0.8, 212.6 ± 1.68, 1.38 ± 0.05, and 114.1± 0.42 respectively and increased the levels of TPTN and ALB 6.98 ± 0.17 and 3.92± 0.18 respectively. Methanolic extract of *C. epigaeus* at 400 mg/kg produced 138.4 ± 2.01, 121.6 ± 1.91, 241.7 ± 1.98, 2.15± 0.40, 149.7 ± 2.01, 3.83 ± 0.06 and 3.92 ± 0.41, where as methanolic extract of *C. epigaeus* at 800mg/kg produced 110.6 ± 1.97, 110.5 ± 2.55, 212.6 ± 2.88, 1.85 ± 0.80, 121.9 ± 3.01,4.80 ± 0.18 and 4.80 ± 0.09.

Table 6: Effect of Ethyl acetate extracts of CE on CCl4 induced hepatotoxicity in rats:

Group	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	108.4±2.13	95.92±3.23	218.60±1.68	2.10±0.18	110.6±2.48	6.21±0.12	3.90±0.94
CCl_4	315.4±12.00	241.4±4.58	428.1±24.47	3.48±0.68	270.7±11.19	2.58±0.82	1.98±0.21
Silymarin	102.2±1.71*	104.4±0.8*	212.6±1.68*	1.38±11.19*	114.1±0.42*	6.98±0.17**	3.92±0.18**
CEE 200 mg/kg	236.2±2.19	215.1±1.94	325.6±2.18	2.85±0.41	235.6±1.98	3.99±0.41	2.99±0.51
CEE 400 mg/kg	118.1±1.94*	110.4±1.90*	240.9±1.55*	1.99±1.92*	135.9±1.42*	4.89±0.12**	3.86±0.84**
CEE 800 mg/kg	102.4±2.01*	101.1±1.81*	190.2±1.67*	1.61±0.08*	108.9±1.54*	5.12±0.19**	5.29±0.78**

Data expressed in mean ± s.e.m, n=5 *Significant reduction compared to hepatotoxic group (P<0.05) **Significant increase compared to hepatotoxic group (P<0.05)

Table 7: Effect of Ethyl acetate extracts of CE on Percentage protection against CCl_4 induced hepatotoxicity in rats

Group	SGOT	SGPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin	67.59	56.62	50.33	60.34	57.85	170.54	97.98
CEE 200 mg/kg	25.11	10.89	23.94	18.10	12.96	54.65	51.01
CEE 400 mg/kg	62.55	54.26	43.72	42.81	49.79	89.53	94.94
CEE 800 mg/kg	67.53	58.11	55.57	53.73	59.77	98.45	167.17

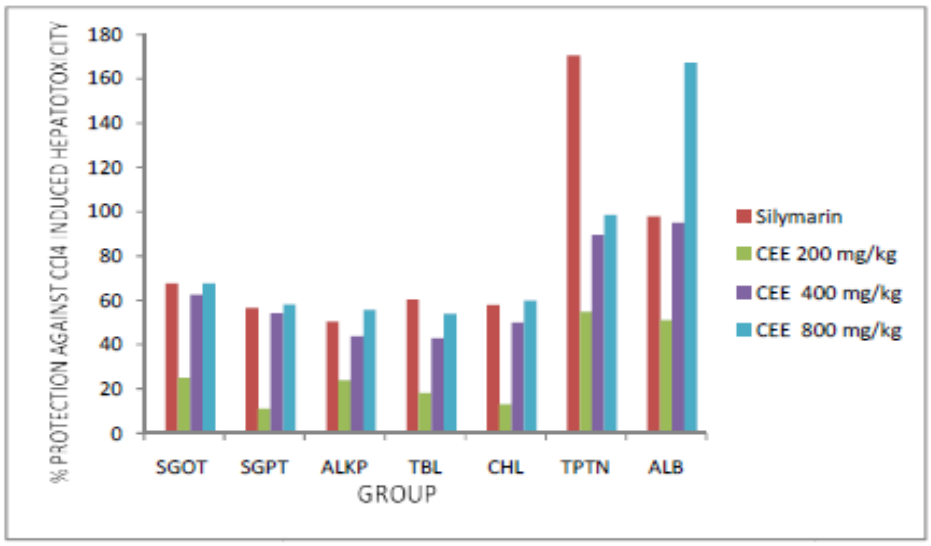


Figure 2: Effect of the crude extract of CEE 200, 400 and 800mg/kg along with Silymarin 25 mg/ kg body wt. on percentage protection of various biochemical parameters against CCl_4 induced hepatotoxicity.

Result shows Silymarin the standard drug at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL with the values 102.2 ± 1.71, 104.4 ± 0.8, 212.6 ± 1.68, 1.38 ± 0.05, and 114.1± 0.42 respectively and increased the levels of TPTN and ALB 6.98 ± 0.17 and 3.92± 0.18 respectively. Ethyl acetate extract of *C. epigaeus* at 400 mg/kg produced 118.1 ± 1.94, 110.4 ± 1.90, 240.9 ± 1.55, 1.99±1.92,135.9± 1.42, 4.89 ± 0.12, and 3.86 ± 0.84 and 3.68 ± 0.44,, where as ethyl acetate extract of *C. epigaeus* at 800m g/kg produced 102.4 ± 2.01, 101.1 ± 1.81, 190.2 ± 1.67, 1.61± 0.08, 108.9± 1.54, 5.12 ± 0.19 and 5.29 ± 0.78.

DISCUSSION

Liver is the most important organ that performs vital function of the body. Hepatotoxicity is becoming a leading cause of death world-wide and prevalence is increasing exponentially. There are many traditional as well as allopathic medicines available which impart hepatoprotection but the treatment of chronic liver

disease is still a challenge for health care professionals. For this purpose, rodents are routinely being used in the laboratory for induction of hepatotoxicity. Non-invasive methods that includes chemicals (CCL4, thioacetamide, aflatoxin B1, Acrylamide etc).[15] In present study we have observed that CCL_4 intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, where as decreased the levels of TPTN, ALB significantly when compared to control indicating acute hepatocellular damage and biliary obstruction leading to necrosis. The rats treated with the methanolic extracts of *C. epigaeus* and silymarin showed a significant ($P<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($P<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The rats treated with the ethyl acetate extracts of *C. epigaeus* and silymarin showed a significant ($P<0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($P<0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The ethyl acetate extract has produced better percent of inhibition when compared to the methanolic extracts.

CONCLUSION

The crude extract from the selected plant produced significant hepatoprotective activity and ethyl acetate extract has produced significant percent of inhibition when compared to the methanolic extracts. The extract must be studied further for dose dependency, toxicity studies and mechanism of action should be established.

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REFERENCES

1. Dwivedi Y, Rastogi R, Sharma SK and Dhawan BN. Picroliv afford protection against thioacetamide induced hepatic damage in rats. *Planta Medica*, 57, 1991, 25-28.
2. Lim HK, Kim HS and Choi HS. Effects of bergenin, the major constituent of Mallotus japonicas against D-galactosamine-induced hepatotoxicity in rats. Pharmacological Research, 42(5), 2000, 471-474.
3. Chintalwar GJ, Joshi NK and Chanda MS. Isolation of ecdysterone from Indian plants. *Phytochemistry*, 10(9), 1971, 2225-26.
4. Misra AN, Tiwari HP. Mass spectral studies of phytosterolins and a ketone from Trianthema pentandra. *Phytochemistry,* 12 (2), 1973, 393-395.
5. Singh BP, Singh RP and Jha OP., *Biological Bulletin of India*, 4, 1982, 157.
6. Mabberley DJ., *The plant-book: A Portable Dictionary of the Vascular Plants*, 2nd ed, Cambridge University Press; Cambridge, U.K, 1997.
7. Slater TF. Necrogemic action of carbon tetrachloride in the rat: A speculative mechanism based on activation. Nature, 209, 1966, 36.
8. Harsh M, Textbook of Pathology 5th ed. Jaypee Brothers Medical Publishers, New Delhi, 2005, pp. 610-613.
9. Bergmeyer HU, Horder MR and Rej R. IFCC methods for measurement of catalytic concentration of enzymes. Journal of Clinical Chemistry and Biochemistry, 23, 1985, 899-901.
10. Mac comb RB and Bowers GN. Study of optimum buffer conditions for measuring alkaline phosphate activity in human serum, Clinical Chemistry, 18, 1972, 97.
11. Chhaya G and Mishra SH. Antihepatotoxic activity of *p*-methoxy benzoic acid from *Capparis spinosa*. Journal of Ethnopharmacology, 66, 1999, 187-192.
12. Richmond W. Preparation and properties of a cholesterol oxidase from nocardia sp. And its application to the enzymatic assay of total cholesterol in serum. Clinical Chemistry, 19, 1973, 1350-1356.
13. Peters T. Propsals for standardization of total protein assays. Clinical chemistry 14, 1968, 1147-1159.
14. Webster D. The measurement of albumin in serum and plasma. Clinical Chimica Acta, 53, 1974, 109-115.
15. Iqubal A., Kashif M. Experimental hepatotoxicity inducing agents: A Review. International Journal of pharmacological research, 6(11), 2016, 325-335.

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