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Effect of Wedelolactone on CCl4 Induced Liver Damage

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ABSTRACT

The hepatoprotective activity of ethanolic extract and wedelolactone isolated from EcliptaalbaL. (Family: Asteraceae) was studied against CCl_4 induced, acute hepatotoxicity in rats. Hepatoprotective activity was studied by estimating biochemical analysis of blood viz. serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), protein and bilirubin. The treatment with wedelolactone showed a dose-dependent reduction of CCl_4 induced toxicity, indicating the compound could preserve the normal functional status of the liver.

Key word : Hepatoprotective activity, Ecliptaalba, Hepatotoxicity

Introduction

Ecliptaalbais said to be the best drug widely used in India and other countries for the treatment of liver cirrhosis, infective (viral) hepatitis, liver enlargement, jaundice and other ailments of the liver and gall bladder. It is used in most of herbal formulations of liver diseases as it possesses various active principles responsible for treatment of such disorders.Ecliptaalba(L.) Hassk., commonly known as False Daisy and Bhringraj, is a small much branched annual herb, as "White Bhringraja" when in flower and as "Black Bhringraja" when in fruit stage of life cycle. Various parts ofEcliptaalbaand its chemical constituents are used as anticancer, analgesic, antioxidant, hypoglycemic, antimyotoxic, antihaemorrhagic, antihepatotoxic, antiviral, spasmogenic, hypotensive, antibacterial, ovicidal, promoter for blackening and growth of hair.

Materials and Methods

Collection of plant material:

Plants of E. alba were collected locally from botanical garden and surroundings of MaharshiDayanand University, Rohtak. The plant was duly authenticated and voucher specimens were duly deposited in the herbarium section of Department of Biosciences, MaharshiDayanand University, Rohtak (Haryana) India.

Plant extraction and fractionation:

The three months old 950 gm lyophilized leaves were Soxhlet extracted with methanol for 36h. The solvent was removed and the residues were suspended in water separately and heated on steam bath below 80°C for 30 min. After filtration, the aqueous phase was partitioned with ethyl acetate. The organic phase was dried, filtered and the solvents were evaporated to yield 6.8 gm light brown powder. The powder was subjected to fractionation by column chromatography on silica gel, eluted with the solvent of increased polarity i.e. Non-polar - polar - highly polar. The coumestans are polar compounds so the solvent combination found suitable for their elution was Chloroform + Methanol (70 + 30). They were eluted simultaneously in 37 to 48 fractions. The pooled sample was then subjected to TLC, the solvent system (Toluene : Acetone : Formic acid :: 11 : 6 : 1 v/v) showed two spots with Rfvalues 0.39 and 0.28 which matched with the Rfvalues of reference wedelolactone and demethylwedelolactone respectively (Courtesy M/s Natural Remedies, Bangalore, India).

Treatment of animals:

The Wistar rats of either sex (125-150gms) were fed on pelleted diet and water of ad libitum. They were housed under standard laboratory conditions (23±2°C), 60-70% relative humidity and 12hrs light/dark cycle. The experiments were carried out as per NIH guidelines and approved by “Institute Ethical Committee”.

Chemicals:

Chemicals for the estimation of various biochemical parameters were of analytical grade.

Hepatotoxin used to induce liver damage:

Carbon tetrachloride (CCl_4) diluted with olive oil (1:1) was administered orally with the aid of feeding tube. Animals were given 1.5ml/kg, p.o. in case of multiple dose treatment (biochemical studies).The control group animals were given equal volume of olive oil. Carbon tetrachloride, a well knownhepatotoxin is known to induce oxidative stress and liver injury by formation of trichloro methyl (CCl_3) radicals. When liver cell plasma membrane is damaged due to chronic exposure to carbon tetrachloride, a variety of enzymes normally located in the cytosol are released into the blood stream so determinations of the altered levels of enzymes like AST, ALT and alkaline phosphatase are considered as useful markers of liver function (Mitraet al., 1998).

Preparation of suspension of extracted sample:

The suspension of methanol extract of plant leaves, wedelolactone and demethylwedelolactone were prepared by using olive oil as vehicle. These suspensions were used for the treatment of animals against the CCl_4 induced toxicity.

Grouping and treatment schedule of animals:

Seven groups of animals were randomly made with eight animals in each group. The treatment schedule and experimental protocol for biochemical studies is as per Table 1.

Table 1: Biochemical studies -treatment schedule

S. No	Name of Group	#Treatment	Dose	Duration	Sample drawn for biochemical analysis
1	Normal	Vehicle (olive oil)	1.5ml/kg, p.o.	Once	After 24 hrs
2	Toxic Control*	CCl_4	1.5ml/kg, p.o.	Once	After 24 hrs and then after one week
3	Plant leaves	CCl_4	1.5ml/kg, p.o.	Once	After one week
		Plant leaves methanol extracts	250mg/kg, p.o.	One week multiple dose	
4	Wedelolactone	CCl_4	1.5ml/kg, p.o.	Once	After one week
		Wedelolactone	50mg/kg, p.o.	One week multiple dose	
5	Wedelolactone	CCl_4	1.5ml/kg, p.o.	Once	After one week
		Wedelolactone	100mg/kg, p.o.	One week multiple dose	
6	Demethylwedelolactone	CCl_4	1.5ml/kg, p.o.	Once	After one week
		Demethylwedelolactone	100mg/kg	One week multiple dose	
7	Standard control	CCl_4	1.5ml/kg, p.o.	Once	After one week
		Silymarin	70mg/kg, p.o.	One week multiple dose	
8	Self recovery	-	-	-	-

#The animals were starved overnight before the start of the experiment.

**Self-recovery group: Animals of second group were observed for one week before collecting blood samples.*

Biochemical studies:

The blood samples from each animal were collected separately in sterilized dry centrifuge tubes by carotid bleeding and allowed to coagulate for 30minutes at room temperature. The clear serum was separated by centrifugation at 2500rpm for 10 minutes and assessment of liver damage was done by biochemical investigations viz. serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, total protein and albumin.

Results

The ALT, AST, alkaline phosphatase and bilirubin mean values from normal samples taken before the start of the experiment were found elevated on CCl_4 toxicity

Table 2: Effect of various extracts on biochemical parameters studied.

Group No	Group	ALT (units^)	AST (units^^)	Serum Alkaline Phosphatase (units#)	Total Bilirubin (mg/dl)	Serum Total Protein (g/dl)	Serum Albumin (g/dl)
1	Normal	35.2±2.2	87.6±1.2	18.0±1.6	0.74±0.01	7.78±0.02	3.43±0.26
2	CCl_4 (control)	176.8±4.0	444.7±3.8	39.2±1.3	3.01±0.06	4.50±0.02	2.23±0.07
3	Plant leaves	121.4±2.1^{a}	226.0±1.6^{b}	32.4±3.2	2.54±0.03	4.78±0.03^{c}	2.36±0.04
4	Wedelolactone (50mg/kg)	95.8±2.4^{a}	118.4±2.0^{b}	24.4±1.1^{a}	1.62±0.02^{c}	5.86±0.03	2.98±0.10^{a}
5	Wedelolactone (100mg/kg)	58.8±1.3^{a}	93.0±1.2^{b}	19.8±1.6^{a}	1.42±0.02^{a}	6.81±0.11^{f}	3.23±0.08^{b}
6	Demethylwedelolactone	99.4±1.1^{d}	124.4±1.1^{a}	24.6±1.1^{a}	1.61±0.03	5.22±0.05^{d}	2.87±0.05
7	Silymarin	83.2±2.4^{a}	98.6±1.1^{a}	21.2±1.5	1.48±0.02	6.73±0.03^{c}	3.15±0.03
8	Self recovered	140.2±1.3	252.6±1.1	34.0±1.6	2.62±0.03	4.67±0.03	2.30 ±0.06

Student t-test was performed. Each value represents the meanS.E. (n = 8)

^Each unit is µmol pyruvate/min/l

^^Each unit is µmol oxaloacetate/min/l

#Each unit is µmol p-nitrophenol/min/mg of protein.

Significant difference from carbon tetrachloride group (Group 2), ap<0.10; bp<0.05; cp<0.02; dp<0.01 and ep<0.001

The elevated levels of ALT, AST, ALKP and total bilirubin whereas the decrease in level of total protein and albumin were observed significantly which indicates acute hepatocellular damage and biliary obstructions. On various treatments, different groups exhibited different recovery percentages on the basis of ALT, AST, ALKP and total bilirubin, with maximum in case of wedelolactone (100mg/kg) i.e. 66.74%, 79.08%, 49.48% and 52.82% respectively.

Discussion

Carbon tetrachloride has been widely used to induce experimental hepatic damage. It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration. In this study, a significant increase in the levels of bilirubin with significant increase in the activities of AST, ALT and ALP but, significant decrease in the levels of protein was observed. The elevation of enzyme activities and altered levels of bilirubin and protein are due to increased production of free radicals, which initiate lipid peroxidation leads to cellular damage. In the present study, wedelolactone administration possesses significant effect on CCl_4 induced hepatotoxicity. Decrease in the levels of serum bilirubin, the activities of AST, ALT and ALP with significant increase in protein after treatment withwedelolactone indicated the effectiveness of the compound against CCl_4 induced hepatotoxicity.

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Table 3: The percentage change in serum enzyme markers studied with reference to CCl_4 damage

Group No.	Group	ALT	AST	ALKP	Total Bilirubin
1	*E. alba* plant leaves	31.3	49.17	17.34	15.61
2	Wedelolactone (50mg/kg)	45.81	73.38	37.75	46.18
3	Wedelolactone (100mg/kg)	66.74	79.08	49.48	52.82
4	Demethylwedelolactone	43.78	72.20	37.24	46.51
5	Silymarin	52.95	77.82	45.91	50.83

The hepatoprotective effect has also been reported earlier with wedelolactone (Wagner et al., 1986). Similarly, the all fractions of E. albaat a concentration of 10mg/kg exhibited protection against CCl_4 hepatotoxicity (Singh et al.,1998). Another report showed recovery in paracetamol-intoxicated rats at a concentration of 100mg/kg of wedelolactone (Sagaret al., 2006). Reduction in alkaline phosphatase levels with concurrent depletion in bilirubin suggested the stability of the biliary function. Activity of alkaline phosphatase was brought down (49.25%) by wedelolactone (100mg/kg) which is better than earlier reports of 36% in E. albatreated rats (Saxenaet.al.,1993).The present dose dependent studies revealed that wedelolactone at 100mg/kg, reduced ALT, AST, alkaline phosphatase and bilirubin by 66.74%, 79.08%, 49.48% and 52.82% respectively as compared to treatment at a dose of 50mg/kg reducing 45.81%, 73.38%, 37.75% and 46.18% respectively. Similarly, in a study it has been observed that a reduction of ALT levels occurred in a dose dependent manner up to 18.4% for 100mg/100g/day and 31.18% for 250mg/100g/day in mice receiving E. albaextract and paracetamol than those given paracetamol alone. The degree of hepatic cell damage was of lesser magnitude in treated groups (Tabassumet al., 2004). Another study revealed that the fraction Eall containing both wedelolactone and demethylwedelolactone as major components with some minor constituents like apigenin, luteolin, 4-hydroxy benzoic acid and protocateuic acid showed dose dependent activity in preventing CCl_4 induced liver injury. The wedelolactone and demethylwedelolactone as major components exhibited maximum hepatoprotective activity. The protection of the hepatocytes was based on its ability to improve the functional status of hepatic drug metabolizing enzymes (Singh et al., 2001). An ethanolicextract of E. alba significantly counteracted carbon tetrachloride induced inhibition of the hepatic microsomal drug metabolising enzyme amidopyrine N-demethylase and membrane bound glucose 6-phosphatase (Saxenaet al., 1993). Another pharmacokinetic study also revealed that wedelolactone possesses a potent antihepatotoxic property better than silymarin and recommended in prolong paracetamol therapy (Sagaret al., 2006).

Decline in total protein content can also be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases (Ajith and Janardhanan, 2002).Precisely, the present studies indicated that purified wedelolactone and demethylwedelolactone possess highantihepatotoxic activity as compared to various extracts.

As reported that E. albacrude extracts are used as an ayurvedic drug and has been found to be quite beneficial for rejuvenating the hepatic disorders like jaundice in children (Dixit and Achar, 1981).Other explanations have been considered for hepatoprotective activity of E. alba like superoxide radical and nitric oxide radical scavenging activities of its methanol extract. The significant hepatoprotective affect exhibited by the plant may probably be mediated through its significant antioxidant activity (Ajith and Janardhanan, 2002). Finally the wedelolactone showed good antihepatotoxic effects and cured the animals as expressed by alleviating biochemical (enzymatic) parameters.

The curative effect exhibited by wedelolactone at dose level of 100mg/kg was comparable with the standard drug silymarin hence, recommended in prolong hepatic therapy. The Ayurveda offers a host of new phytochemicals that can be used both preventively and clinically to manage a spectrum of liver related imbalances (Scott, 1998).

Conclusion

Hence proving the loss of hepatic by CCl_4 was significantly restored by various extracts of E. alba and restoration was bests shown by wedelolactone.The hepatoprotective potential observed in case of wedelolactone was better than the standard drug silymarin.

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