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HEPATOPROTECTIVE ACTIVITY OF WEDELIA CHINENSIS AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

The ethanolic extract of *Wedelia chinensis* whole plant was evaluated for its efficacy against carbon tetrachloride (CCl_4) induced hepatotoxicity in Wister albino rats. The ethanolic extract of *Wedelia chinensis* (EEWC) at doses of 250 mg/kg p.o and 500 mg/kg p.o were administered to CCl_4 treated rats and the hepatoprotective activity was assessed using biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), total bilirubin and total protein along with histopathological studies of liver tissues. The treatment with EEWC showed dose dependent reduction of CCL_4 induced elevated levels of serum enzymes and total bilirubin along with parallel increase in total protein indicating the recovery of hepatic cells. Histopathological study of EEWC treated animals showed normal hepatic cords without any cellular necrosis and fatty infiltration. The phytochemical screening revealed the presence of phytoconstituents such as flavonoids, terpinoids and tannins which might offer hepatoprotection. Hence the above finding shows that the ethanolic extract of *Wedelia chinensis* whole plant exhibits significant hepatoprotective activity.

KEYWORDS: Hepatoprotective activity, *Wedelia chinensis*, Ethanolic extract, CCl_4, Silymarin.

INTRODUCTION

Liver disease remains one of the serious health problems and in allopathic medicine, no hepatoprotective medicine available. Plant drugs are known to play vital role in the management of various liver disorders. A numbers of plants possess hepatoprotective property. *Wedelia chinensis* is most commonly used medicinal herb in the Indian traditional system for various ailments, belongs to the family Asteraceae and commonly known as Guntagatagara in Telugu. The plant is distributed throughout India in wet places and coastal areas. All parts of the plant possess medicinal uses and have been reported to have beneficial effects on several ailments. The herbal juice contained oil soluble black dye, tannin, carotene, saponin, phytosterol, waxy compound and resin. The leaves of plant are used for dyeing grey hair and for promoting the growth of hair. They are considered tonic, alternative and useful in cough, cephalalgia, skin diseases and alopecia. The decoction of herb is used in uterine hemorrhage and menorrhagia (Nilesh Mahata *et al.*, 2010). Review of literature revealed that this rare medicinal plant remained unexplored for many of its claimed pharmacological activities. In the present study, an effect has been made to evaluate the hepatoprotective activity of ethanolic extract of *Wedelia chinensis* whole plant.

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MATERIALS AND METHODS

Plant Materials

The plant of *Wedelia chinensis* was collected in and around Kadapa district, Andrapradesh, India, during the month of August 2011. The conformation of plant identification was done with the help of local floras. The voucher specimen has been preserved in our pharmacological department for future reference (VIPS/MP-25/2011). The whole plant was shade dried and powdered with a mechanical grinder the powdered plant material was then passed through sieve No 40 and stored in an air tight container for future use.

Preparation of plant extract

About 1 kg of dried powder plant material was extracted with ethanol (64.5 -65.5 ^{0}C) by continuous hot percolation method using soxhlet apparatus for 72 hrs. The ethanolic extract was filtered and filtered extract was then concentrated to dryness in bushi rotary evaporator under reduced pressure at temperature of 40 ^{0}C. The resultant black colored residue was stored in desiccator for use in subsequent experiments. The yield of the extract was 4 % w/w.

Animals

Wister albino rats of either sex weighing about 180-200 g were procured from CPCSEA approved animal breeder, Bangalore and maintained under standard housing conditions. The animals were fed with commercial diet (Hindustan lever limited, Bangalore) and water ad libitum. Experiments on animals were performed by the following guidance of Institutional Animal Ethical committee (IAEC).

Chemicals and Reagents

CCl_4 was obtained from S.D.Fine-chemicals Ltd, and silymarin from Indena spa, Milan, Italy. All other chemicals were obtained from local sources and were of analytical grade.

Phytochemical studies

Preliminary phytochemical studies were performed according to the standard method (Khandelwar K.R *et al.,* 2004).

Hepatoprotective activity

Experiment animals were divided in five groups (n-5) of three rats each. Group I served as normal control which received vehicle orally for 7 days. Group II served as toxicant control and received CCl_4 (0.2 ml/100 mg p.o) on 1st day. Group III served as standard and received CCl_4 (0.2 ml/100 mg p.o) 1st day and silymarin (25 mg/kg p.o) daily for 7 days. Group IV and Group V rats were treated with CCl_4 (0.2 ml/100 mg p.o) on 1st day and with the ethanolic extract of EEWC at the doses of 250 mg/kg, and 500 mg/kg in 5% gum acacia daily for 7 days respectively. The entire animals were sacrificed on the 7th day under light ether anesthesia. The blood sample from each animal was collected separately in sterilized dry centrifuge tubes by retro arbital puncture and allowed to coagulate for 10 minutes at 37 ^{0}C. The clear serum was separated at 2500 rpm for 10 minutes and subjected to biochemical estimations like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) total bilirubin and total protein (Srivastava *et al*., 1990).

Histopathological examination

After collection of blood sample, the liver was excised from the animals and washed with the normal saline. The livers were fixed in 10 % buffered neutral formalin for 48 hrs and processed separately for histopathiological observation.

Statistical analysis

The results of biochemical estimations were expressed as mean±SEM. The data of hepatoprotective activity were analyzed by one way analysis of variance (ANOVA). P<O.05 was considered statistically significant.

RESULTS

The preliminary phytochemical studies of ethanolic extract of *Wedelia chinesis* indicated the presence of carbohydrates, phytosterols, saponin, tannins, alkaloid, terpinaoids and flavonoids. Table 1 shows the effect of ethanolic extract of *Wedelia chinesis* on CCl_4 induced liver damage in rats. Treatment with ethanolic extract of *Wedelia chnesis* at dose of 250mg/kg and 500mg/kg showed significant protection against CCl_4 induced liver damage in animals. It was evident form remarkable reduction in serum enzyme aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) and total bilirubin along with parallel increased in total protein level as compared to CCl_4 treated group. Hence both the doses of ethanolic extract of *Wedilia chinesis* offer dose dependent significant liver protection which was almost comparable to that of silymarin treated group.

Histopathological examination

Histopathological studies demonstrated that various pathological changes like cellular necrosis and fatty infiltration observing CCl_4 treated rats which were prevented to a normal level in group treated with plant extracts and silymarin. In control animals, liver sections showed normal hepatic cells with well preserved cytoplasm, nucleus and central vein (figure 1). In CCl_4 treated animals, liver sections showed cellular necrosis with fatty infiltration in central zone (figure 2). In CCl_4

and EEWC treated animals, the sections showed mild fatty change and mild sinusoidal congestion (figures 3, 4). In CCl_4 and silymarin treated animals, the sections showed mild fatty change and mild central venous congestion (figure 5).

Table 1. Effect of ethanolic extracts of *Wedelia chinenis* on CCl_4 induced liver damage in rats

S.NO	GROUP (n)	DOSE (p.o)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	TOTAL PROTEIN (gm %)	TOTAL BILIRUBIN (mol/L)
1	Control	Vehicle	47.93±0.63	60.83±0.36	128.9±0.60	6.8±0.33	0.79±0.04
2	CCl_4 control	2ml/kg	102.4±0.63	174.5±2.16	345.4±7.36	5.4±0.2	2.33±0.06
3	silymarin	100 mg/kg	57.7±0.50	71.93±1.83	157.6±0.66	6.5±0.13	1.26±0.06
4	EEWC	250mg/kg	73.8±1.66**	144.1±0.60**	205.2±1.63**	6.0±0.1*	1.73±0.06**
5	EEWC	500mg/kg	58.73±0.33**	80.66±0.27**	161.5±1.0**	6.46±0.6**	1.23±0.03**

Values are expressed as Mean±SEM, n=5,*****p < 0.05, ******p<0.01 compared to CCl_4 control (group 2)

Figure 1. Control group

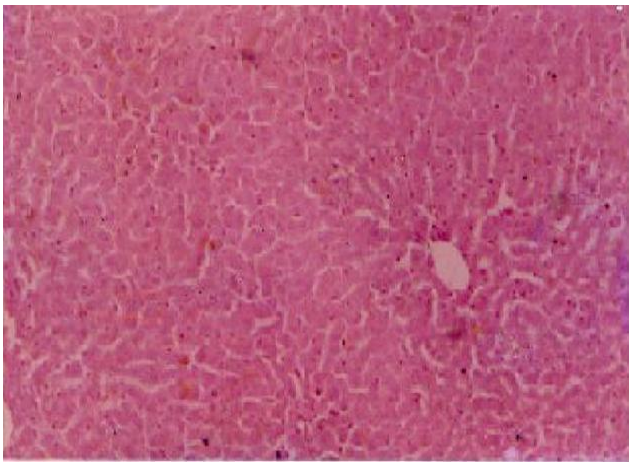


Figure 2. CCl_4 treated group

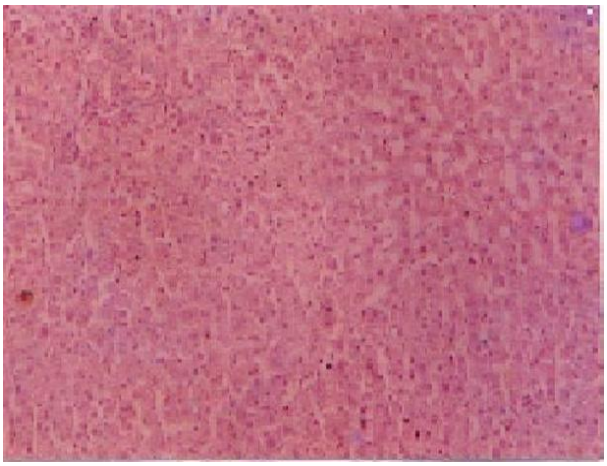


Figure 3. CCl_4 and EEWC 250 mg/kg treated group

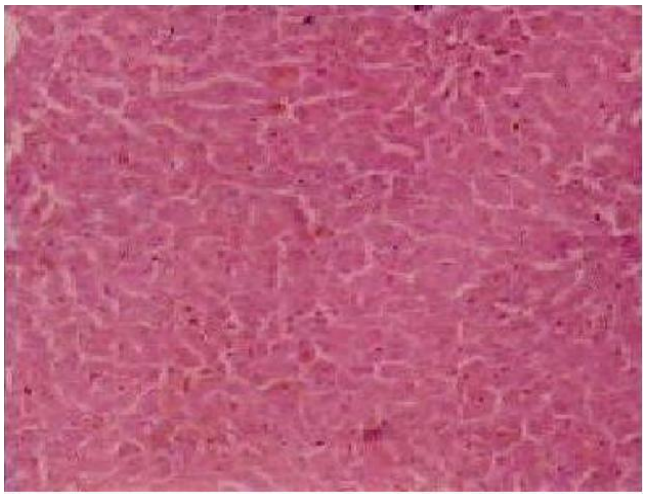


Figure 4. CCl_4 and EEWC 500 mg/kg treated group

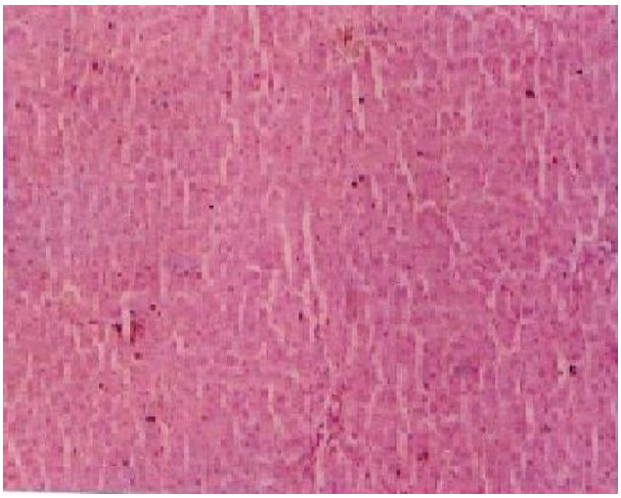
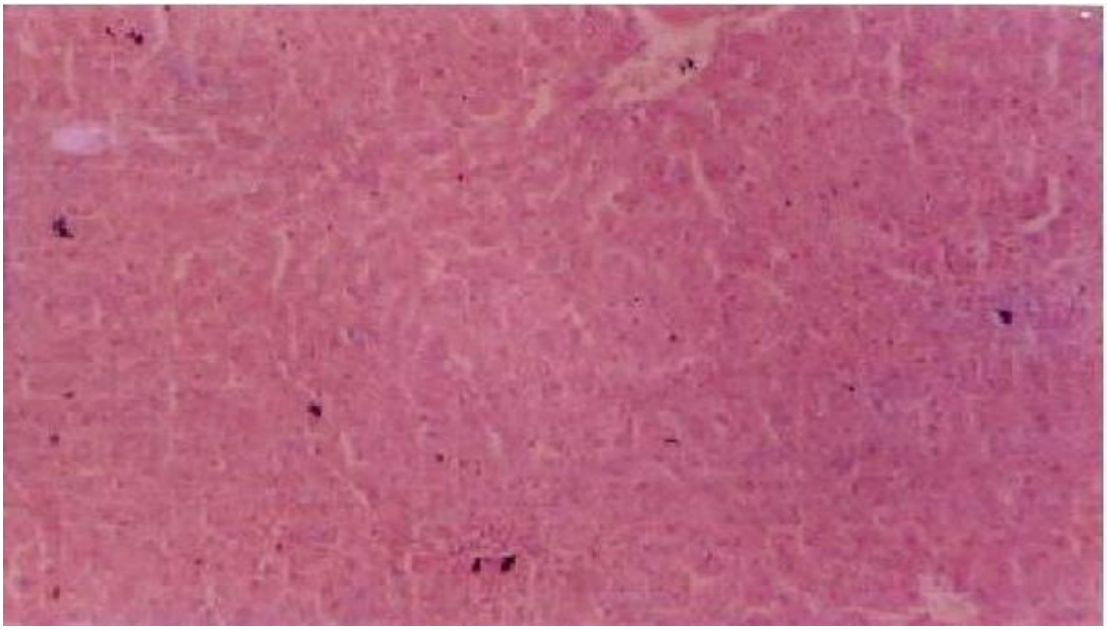


Figure 5. CCl_4 and Silymarin 100 mg/kg treated group



DISCUSSION

The present investigation revealed the ethanolic extract showed significant protection against CCl_4 induced hepatotixicity in rats. CCl_4 is widely used as hepatotoxin in the experiment studies (Johnson E and Krocning C, 1998). The CCl_4 is biotransformed by cytochrome p450 system to produce the trichloro methyl free radicals, (Akare SC, Sahare AY, 2009; Hanaa A Hassan *et al*., 2009) which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation. The changes associated with CCl_4 induced liver damages are similar to that of acute viral hepatitis (Srivastava *et al.,* 1990). The evaluation of marker enzymes level in CCl_4 treated group indicates that CCl_4 induced damage to the liver. A significant reduction was observed in AST, ALT, ALP, total bilirubin and increased in total protein levels in the groups treated with both doses of ethanolic extract of *Wedelia* chinensis were compared to that of standard (silymarin) drug treatment. Histopathological examination of the liver section of the rats treated with toxicant showed various pathological changes like cellular necrosis (Ross and Willson, 2006) and fatty infiltration which were prevented to a normal level in groups treated with plant extracts and silymarin. Further, it has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl4 or by inhibition of lipid peroxidation induced by CCl4. The exact hepatoprotective mechanism of this herbal plant is not known. But it can be attributed to the presence of the major phytochemicals such as flavonoids, terbinoids and tannins that were detected in our preliminary phytochemical studies. These result also commensurate with earlier phytochemicals of this plant (Shanmugam *et al.,* 2006). Thus present finding provides scientific evidence to the medicinal use of this rare plant.

CONCLUSION

The result of our study indicated that ethanolic extract of *Wedelia chinesis* whole plant could protect liver against CCl_4 induced liver damage in rats. Further detailed study for hepato protective is currently under way to isolate the characterized the active principle of the plant extract.

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