

Hepatoprotective Activity of *Wedelia calendulacea* L. Against Acute Hepatotoxicity in Rats

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Abstract: The hepatoprotective activity of ethanolic extract of *Wedelia calendulacea* L. (Family: Asteraceae) was studied against CCl₄ induced, acute hepatotoxicity in rats. Hepatoprotective activity of the ethanolic-leaf extract of *W.calendulacea* (EEWC) was studied by estimating serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), protein and bilirubin. The treatment with EEWC showed a dose-dependent reduction of CCl₄ induced elevated serum levels of enzyme activities with parallel increase in total protein and bilirubin, indicating the extract could preserve the normal functional status of the liver. The weight of the organs such as liver, heart, lung, spleen and kidney in CCl₄ induced experimental animals administered with EEWC showed an increase over CCl₄ control group.

Key words: Hepatoprotective activity, *Wedelia calendulacea*, hepatotoxicity, enzyme activity, biochemical studies

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, energy provision and reproduction^[19]. Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol and prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease^[15,18]. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis^[16]. Many Ayurvedic herbs, such as *Wedelia calendulacea* have a long history of traditional use in revitalizing the liver and treating liver dysfunction and disease^[6]. Many of these herbs have been evaluated in pharmacological studies and are currently being investigated phytochemically to better understand their actions.

Wedelia calendulacea L. (Family: Asteraceae) is a traditional Ayurvedic herb and used for the treatment of several ailments, including respiratory infections and pain. Due to the widespread use of this plant by the rural

communities to treat several diseases, the objective of the present study was framed to determine the effect of ethanolic leaf extracts of *W.calendulacea* on circulating liver enzyme levels, serum bilirubin and protein at liver injury, during the earliest phases of implantation in rats induced with CCl₄ hepatotoxicity.

MATERIALS AND METHODS

The leaves of *Wedelia calendulacea* L. belonging to the family Asteraceae were collected from wild habitats in and around of Pattukkottai, Tamil Nadu, India. The confirmation of plant identification was done with the help of local floras^[4,11]. A voucher specimen has been preserved in our laboratory. The leaves of *W.calendulacea* were air dried and powdered. The powdered plant leaves were extracted with ethyl alcohol using Soxhlet apparatus and concentrated *in-vacuo*. Approximately, 0.5 g of dried ethanolic extract of *W.calendulacea* leaves (EEWC) was obtained from 10 g of dried leaf material (5%). The extract was suspended in 5% gum acacia and used for studying hepatoprotective activity.

Albino rats weighing between 150 and 175 g were used in this study. The rats were divided into five groups of six rats each. Hepatoprotective activity of *W.calendulacea* was evaluated using CCl₄ induced model. Group I: served as control; Group II: received CCl₄ (0.2 ml/100 g) by orally, Group III and IV: received

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EEWC (25 mg/100 g and 50 mg/100 g respectively) daily for a period of 10 days and Group V: received silymarin (2.5 mg/100 g) daily for a period of 10 days. On the tenth day, CCl₄ was given orally 30 min after the administration of silymarin and EEWC after 36 hours of CCl₄ administration.

After the treatment period, the rats were anaesthetized with pentobarbital sodium and sacrificed by cervical decapitation. The organs such as heart, lung, liver, spleen and kidney and blood were collected. The serum was separated by centrifugation and used for studying various biochemical parameters. Biochemical parameters like serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by standard methods [21]. The activity of alkaline phosphatase [12], levels of total bilirubin [10] and protein [9] were estimated. Statistical significance was determined using one-way analysis of variance (ANOVA) by comparing the results obtained for each experimental group i.e., normal control, CCl₄ control, silymarin and EEWC administered albino rats. The difference in results between groups was considered as significant if $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the effect of *W.calendulacea* on CCl₄ induced hepatotoxicity in rats. Rats induced with CCl₄ showed a significant reduction ($P < 0.05$) in the levels of protein and significant decrease in the levels of bilirubin and also the activities of AST, ALT and ALP. Treatment with alcoholic extract of *W.calendulacea* for a period of

10 days significantly altered these changes. Effect of EEWC on body weight, liver, kidney, left lung and spleen are shown in Table 2. Rats induced with CCl₄ showed a significant reduction ($P < 0.05$) in the organ weights such as liver, heart, left lung, spleen and kidney without any significant change in the body weight. Treatment with ethanolic extract of *W.calendulacea* significantly altered these organs weight.

Carbon tetrachloride has been widely used to induce experimental hepatic damage [20]. It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration [2,17]. The body weight of the animals treated with ethanolic extract once a day during 10 days did not show any significant change when compared with the control group, although had a tendency to decrease body weight. This decrease can be associated with the decrease of liver weight at the dose of 200 mg/kg on comparison with the control group. The macroscopic examination of the organs of treated animals (liver, lung, heart, spleen and kidney) showed significant decrease in weight when compared with the control group, but showed increase over CCl₄ control group.

Liver is rich in transaminases, which increase in patients with acute hepatic diseases. AST, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver disease [14]. ALT, AST and ALP are the specific markers to assess hepato-cellular damage. Estimation of the serum total bilirubin, protein and alkaline phosphatase activity is one of the most widely used means of measuring hepato-cellular injury. In this

Table 1: Effect of ethanolic leaf extracts of *Wedelia calendulacea* (EEWC) on bilirubin, AST, ALT, ALP and protein of CCl₄ intoxicated rats.

Parameters	Normal control	CCl ₄ control	EEWC (100 mg/kg)	EEWC (200 mg/kg)	Silymarin (25 mg/kg)
Bilirubin (mg/dl)	0.9±0.45 ^a	2.6±0.3	1.9±0.18 ^c	1.3±0.2	1.2±0.2 ^d
AST (U/L)	61.1±6.2 ^a	178.3±14.8	144.0±12.0	102.0±10.0	95.2±5.6 ^c
ALT (U/L)	48.2±4.8 ^a	102.1±10.9 ^b	80.0±7.9	63.6±4.2	59.0±5.6
ALP (U/L)	129.1±10.6 ^a	352.9±22.2	215.1±14.0	176.5±12.6	163.2±0.8 ^c
protein (g/dl)	7.0±0.5 ^a	5.4±0.4	6.2±0.6	6.9±0.6	6.5±0.7

Values are mean ± SD; Values followed by same superscript are not significant at $P < 0.05$; CCl₄ control group compared with normal control group $P < 0.001$.

Table 2: Effect of oral administration of ethanolic leaf extract of *W. calendulacea* (EEWC) on the weight organs in rats.

Organs	Control	CCl ₄ (0.2 ml/100g)	Silymarin (25 mg/kg)	EEWC (100 mg/kg)	EEWC (200 mg/kg)
Liver (g)	4.50±0.20	3.10±0.40	3.50±0.30	3.90±0.20	4.20±0.20
Heart (g)	0.544±0.05	0.415±0.04	0.468±0.02	0.483±0.10	0.512±0.50
Left lung (g)	0.313±0.03	0.191±0.01	0.235±0.02	0.252±0.02	0.288±0.03
Spleen (g)	0.196±0.02	0.156±0.01	0.165±0.01	0.176±0.03	0.185±0.02
Kidney (g)	0.465±0.02	0.357±0.02	0.175±0.03	0.171±0.03	0.174±0.02

Values are Mean ± SD;

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, EEWC administration possesses significant effect on CCl₄ induced hepatotoxicity. Decrease in the levels of serum bilirubin, the activities of AST, ALT and ALP with significant increase in protein after treatment with EEWC indicated the effectiveness of the extract against CCl₄ induced hepatotoxicity. The synergistic effects of active compounds in a related species, *Wedelia chinensis* and their potential in prostrate cancer prevention and therapy were demonstrated^[3]. Other related species, *Wedelia paludosa* and *Wedelia trilobata* contain the diterpene (kaurenoic acid), eudesmanolide lactones and luteolin with a variety of biological activities (in leaves and stems)^[8,11]. The use of plants, *Epaltes divaricata* (aqueous extract), *Launaea pinnatifida* (ethanolic extract) and *Ficus carica* (methanol extract) as hepatoprotective agents using animal model of hepatotoxicity induced by carbon tetrachloride, has been well explicated the potentiality of plant compounds in the protection of induced hepatotoxicity^[5, 13, 71]. So the hepatoprotective effect of *W.calendulacea* may be due to its chemical contents. The investigation on the chemical contents of *W.calendulacea* is needed to justify with evidenced effect of the results of the study.

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