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Research Article

HEPATOPROTECTIVE ACTIVITY OF LEAVES EXTRACT OF *EICHHORNIA CRASSIPES* AGAINST CCL4 INDUCED HEPATOTOXICITY ALBINO RATS

G. Dineshkumar[1], R. Rajakumar[1], P. Mani[2*] and T.M.M. Johnbastin[2]

[1] Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College, Poondi-613 503, Thanjavur District, Tamil Nadu, India
[2] Department of Biotechnology, T.U.K. Arts and Science College, Thanjavur-613 002, Tamil Nadu, India

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of methanolic extract of *Eichhornia crassipes* against CCl4 induced albino rats. The methanolic extract of *E. crassipes* (500 mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl4 (1 ml /kg). Silymarin (100 mg/kg) was given as reference standard. The plant extract was effective in protecting the liver against the injury induced by CCl4 in rats. This was evident from significant reduction in serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the methanolic extract of *E. crassipes* possesses hepatoprotective activity against CCl4 induced hepatotoxicity in rats.

Key words: *E. crassipes*, CCl4, hepatoprotetive, hepatotoxicity.

INTRODUCTION

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc.). In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations (Hikino and Kiso, 1988; Handa *et al.*, 1989; Sharma *et al*., 1991; Evans, 1996). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides and alkaline phosphatase are elevated.

Eichhornia crassipes (Pontederiaceae) is a free-floating perennial aquatic plant which is native to tropical and sub-tropical South America. Broad, thick, glossy and ovate leaves of water hyacinth may rise above the surface of the water as much as 1 meter in height. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks. The plant is used as a carotene-rich table vegetable in Taiwan, the flowers are used for medicating the skin of horses and leaves used for hepatoprotective activity in traditional therapy (Duke and Wain, 1981). Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial and anticancer agents (Jayanthi *et al.,* 2013). In Chhattisgarh, *E.*

*Corresponding author e-mail: master.maniji@gmail.com, Tel: 91 + 9976918228

crassipes is being used as styptic. The fresh juice of this weed is used to treat fresh wounds as the tribes believe that it stops further spread of infection. Rice farmers consider this as a best first aid remedy for minor injuries. Along with vinegar, it is being used in treatment of septic wounds, but scientifically there are no valid reports available on hepatoprotective and curative activity of this plant. Hence, the present study was aimed to investigate the hepatoprotective properties of this plant against CCl4-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Experimental animal

Male Albino rats weighing between 150-220 gm were procured from the Animal House, Department of Biotechnology, Periyar Maniammai University, Vallam 613 403, Thanjavur District for the present study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC. Animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals CPCSEA/265).

Plant material

The fresh plants of *E. crassipes* were collected from Vadavaru river, Karanthai, Thanjavur, Tamil Nadu, India. The plant was identified by a botanist, and voucher specimen was deposited in the Department of Botany, St. Joseph College, Tiruchirappalli. After authentication, the plants were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of plant extract

The coarse powder plant material was extracted with methanol by using soxhlet apparatus. The solvent were removed under reduced pressure to get semisolid mass. Standard methods were used for preliminary phytochemical screening of the extract, which was performed to know the phytoconstituents in the extract (Harborne, 1984). It was found that the extract contains alkaloid, flavonoids, glycosides, steroids and tannins

Hepatoprotective activity

A total of 36 animals were equally divided into 6 groups which contains six animals each. Group I served as normal control received 0.5% (CMC) carboxy methyl cellulose solution (1 ml/kg) once daily for 3 days. Group II served as CCl4 control, administered with CCl4 (1 ml/kg) as single dose on day 3. Group III, IV and V received, *E. crassipes* extract (100, 200 and 400 mg/kg) once daily for 3 days. Group IV served as reference control, received Silymarin (25mg/kg) once daily for 3 days. Group III to VI received CCl4 (1ml/kg) as single dose on day 3, thirty minutes after the administration of *E. crassipes* and Silymarin respectively. All the test drugs and CCl4 were administered orally by suspending in 0.5% CMC solution. After 48h of CCl4 feeding, the blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated for the estimations of Serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) alkaline phosphatase (ALP) (Kind and King, 1954; Reitman and Frankel, 1957) and bilirubin (Jendrassik and Grof, 1938).

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by Student's t-test. P values were calculated using SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS

The results of body weight and hepatoprotective activity of methanolic extract of *E. crassipes* on CCl4 treated rats are shown in Table 1 and Table 2. The hepatic enzymes SGPT, SGOT, ALP and bilirubin in serum were significantly ($P < 0.001$) increased in CCL4 treated animals when compared to control. The methanolic extract of *E. crassipes* treatments significantly ($P < 0.01$) reversed the levels of SGPT, SGOT and ALP and bilirubin, 400 ($P < 0.01$) and ALT ($P<0.001$) when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in AST ($P<0.01$), ALT, ALP and bilirubin ($P<0.001$) levels when compared to CCl4 alone treated rat.

Table 1. Effect of *E. crassipes* on body weight and liver weigh of rats against CCl4 induced toxicity.

Group	Treatment	Body weight (g) (BW)	Liver weight (g) (LW)
Group I	Control	255.9±21.61	6.73±0.61
Group II	CCl4 1 ml/kg (i.p.)	207.0±15.12	11.02±1.21
Group III	*E.crassipes* 100 mg/kg + CCl4	215.1 ± 2.1*	10.67 ± 0.10*
Group IV	*E.crassipes*200 mg/kg + CCl4	231.7±15.15*	10.41±0.79*
Group V	*E.crassipes*400 mg/kg + CCl4	265.3±8.51**	10.44±0.62**
Group VI	Silymarin 100 mg/kg + CCl4	258.0±23.95***	7.75±2.58***

Values are given as Mean ± SEM (n=6 rats).
* Significant at P<0.01 when CCl4 treated compared with control group.
* *Significant at P<0.001 when CCl4 treated compared with control group.

Table 2. Effect of *E.crassipes* on liver function marker enzymes of rats against CCl4 induced toxicity.

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/Dl)
Group I	Control	138 ± 18	64± 11	201± 58	0.05± 0.03
Group II	CCl4 1 ml/kg (i.p.)	576 ± 193	747± 201	344± 93	0.23± 0.09
Group III	*E.crassipes* 100 mg/kg + CCl4	314 ± 121*	352± 103*	255± 91*	0.15± 0.05*
Group IV	*E.crassipes* 200 mg/kg + CCl4	304 ± 82*	287± 162*	221± 81*	0.06± 0.05*
Group V	*E.crassipes* 400 mg/kg + CCl4	293 ± 114**	238± 121**	169± 31***	0.05± 0.05*
Group VI	Silymarin 100 mg/kg + CCl4	190 ± 55***	151± 116***	202± 24**	0.05± 0.05*

Values are given as Mean ± SEM (n=6 rats).
* Significant at P<0.01 when CCl4 treated compared with control group.
* *Significant at P<0.001 when CCl4 treated compared with control group.

DISCUSSION

CCl4 is one of the most important and commonly used hepatotoxic agents in the experimental study of liver related disorders. The hepatotoxic effects of CCl4 are largely due to its active metabolite, trichloromethyl radical (Lesiuk *et. al*., 1999). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (PUFA) (Srivastava *et al*., 1990). This process leads to excessive formation and accumulation of lipids in tissues such as liver. Lipids from peripheral adipose tissue are translocated to liver for accumulation (Bessems *et al.,* 2001). Recent hepatoprotective drugs have the ability to inhibit the aromatase activity of cytochrome P450 thereby favouring liver regeneration (Brent and Rumack, 1993).

Normally, SGOT and SGPT are present in high concentration in liver mainly due to the hepatocyte necrosis or abnormal membrane permeability; these enzymes are released from the cells and their levels in the blood increases. SGPT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. SGPT is more selectively a liver paranchymal enzyme than SGOT (Shah *et al*., 2002). Assessment of liver function can be made by estimating the activities of serum SGPT, SGOT, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi *et al*., 2005). The elevated level of these marker enzymes observed in the group II CCl4 treated rats. The elevations of these enzymes are due to the extensive liver damage induced by toxin. The reduced concentrations of SGPT, SGOT and ALP as a result of plant extract administration observed during the present study might be due to the presence of

flavonoids. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte. Decrease in serum bilirubin after treatment with the extract in liver damage induced by CCl4, indicated the effectiveness of the extract in normal functional status of the liver. Liver protective herbal drugs contain a variety of chemical constituents like phenols, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes (Gupta and Misra, 2006).

CONCLUSION

The methanolic extract has shown the ability to maintain the normal functional status of the liver. From the above preliminary study, we conclude that the methanolic leaf extract of *E. crassipes*, is proved to be one of the herbal remedies for liver ailment.

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