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HEPATOPROTECTIVE ACTIVITY OF *JATROPHA GOSSYPIFOLIA* AGAINST CARBON TETRACHLORIDE- INDUCED HEPATIC INJURY IN RATS

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The extract of aerial part of *Jatropha gossypifolia* were screened for its hepatoprotective activity in carbon tetrachloride induced liver damage in Wister albino rats. The extracts at dose of 200 mg/kg were administered orally once daily. The substantially elevated serum enzymatic levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total bilirubin, SOD and catalase were restored towards normalization significantly by the extracts. Silymarin was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. The results of this study strongly indicate that *Jatropha gossypifolia* Linn. have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. The present study also indicates that among all these three extracts, petroleum ether extract shown to possess maximum protectivity and methanolic extract showed the minimum activity.

Keywords : Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, catalase, serum alkaline phosphatase.

INTRODUCTION

The liver is the key organ of metabolism, secretion and excretion which is continuously and widely exposed to xenobiotics, environmental pollutants and chemo therapeutic agents because of its strategic location in the body. Liver disease is a worldwide problem.[1-4] Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

The plant *Jatropha gossypifolia* (family: *Euphorbiaceae*) is a bushy gregarious shrub, grows wildly almost throughout India. It possesses significant anticancer and pesticidal activity[5-6]. The leaf decoction of *Jatropha gossypifolia* is used for bathing wounds.[7] The stem sap stops bleeding and itching of cuts and scratches.[8-9] The roots are employed against leprosy, as an antidote for snakebite and in urinary complaints. A decoction of the bark is used as an emmenagogue and leaves for stomachache, venereal disease and as blood purifier.[10-11] *Jatropha gossypifolia* leaves contain histamine, apigenin, vitexin, isovitexin and tannins. The bark contains the alkaloid "jatrophine" and a lignan "jatrodien" is found in its stems.[12-13] The latex of *Jatropha gossypifolia* yielded two cyclic octapeptides i.e. cyclogossine A and B.[9, 14-15] The aerial parts contain a new lignan, gossypiline.[16] The present study was undertaken to find out the possible actions of aerial parts of *Jatropha gossypifolia* for its hepatoprotective activity.

Materials and Methods

Plant materials

Aerial parts of *Jatropha gossypifolia* Linn were collected in the month of November from Pharmacognosy garden of Department of Pharmaceutical Science, Utkal University, Bhubaneswar, Orissa, India. The plant was identified with the help of available literature and authenticated by Regional Research Laboratory, Bhubaneswar. The plants were dried in shade for 15 days and then the aerial parts of the plants were taken for the study.

Preparation of extracts

Powdered aerial parts (500 g) were packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was extracted with two liters of petroleum ether by soxhlet apparatus and then extracted material successively extracted with methanol and water and finally maceration at room temperature, then these extracts were dried by rotary vaccum dryer. The percentage of yield of petroleum ether extract, methanolic extract and aqueous extract were 4.2 %, 5.6 %, 6.0 % respectively. The extracts were given at the dose of 200 mg/kg/day of body weight per day were selected range from 1/6 to 1/15 of LD_{50} based on the preliminary study conducted at our laboratory and data are not shown in this paper.

Animal

Wister albino rats (120-200 g) of either sex were used. The animals housed under standard laboratory conditions

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TABLE- 1 Effect of various extracts of aerial parts of *Jatropha gossypifolia* on various parameters of blood serum of CCl_4 induced rats

TREATMENT	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	BILIRUBIN (mg/dl)		CHOLESTEROL (mg/dl)	PROTEIN (gm/dl)
				TOTAL	DIRECT		
Group I	127.9±4.3	150.6±4.6	417.25±10.05	0.43±0.05	0.12±0.03	81.90±5.23	6.93±0.26
Group II	391.0±9.1[a]	400.7±4.9[a]	944.10±41.20[a]	2.01±0.09[a]	0.58±0.03[a]	45.26±2.47[a]	5.16±0.20[a]
Group III	262.2±8.0[b]	181.7±6.2[b]	678.40±11.49[b]	0.55±0.04[b]	0.25±0.04[b]	77.93±3.35[b]	6.91±0.24
Group IV	161.1±5.4[b]	162.2±6.9[b]	418.10±39.68[b]	0.52±0.05[b]	0.25±0.04[b]	72.53±2.28[b]	5.55±0.32[b]
Group V	295.4±9.3[b]	228.8±26.9[b]	447.88±43.99[b]	0.65±0.05[b]	0.35±0.05	54.16±1.62	6.18±0.26[b]
Group VI	235.8±6.4[b]	189.16±19.5[b]	424.60±33.97[b]	0.56±0.05[b]	0.27±0.03[b]	77.03±2.55[b]	5.93±0.33

Values are mean ± SEM; and P<0.05, [a] compared to Group I, [b] compared to Group II.

TABLE- 2 Effect of various extracts of aerial parts of *Jatropha gossypifolia* on various parameters of liver tissue.

TREATMENT	SOD (U/mg protein)	CATALASE (nkat/mg protein)	PROTEIN%
Group I	13.06±0.97	85.64±3.02	4.6±0.15
Group II	4.04±0.19[a]	34.6±3.43[a]	2.67±0.17[a]
Group III	10.48±0.54[b]	97.86±7.08[b]	5.03±0.09[b]
Group IV	9.30±0.91[b]	83.20±5.90[b]	3.59±0.14[b]
Group V	8.62±0.60[b]	53.20±5.27	3.18±0.09
Group VI	8.13±0.34[b]	77.19±7.00[b]	3.47±0.14

Values are mean ± SEM; and P<0.05, [a] compared to Group I, [b] compared to Group II.

maintained at 25 ± 1^{0}C and under 12 / 12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India [Reg. No (678 / 02 / a / CPCSEA)].

CCl_4 induced-hepatotoxic activity

Animals were divided into six groups viz. Group A received 1 ml of 30 % PEG orally as a control group, Group B received 1 mL/kg body weight of CCl_4 subcutaneously for 7 days as a toxic group, Group C received Silymarin (100mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) of body weight for 7 days, Group D received petroleum ether extract (200mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days, Group E received methanolic extract (200mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days and Group F received aqueous extract (200mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days. Animals were sacrificed after 24 h of the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out. Liver was dissected out and used for biochemical determinations.

The biochemical parameters like serum enzymes: aspartate aminotransferase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total bilirubin cholesterol and protein were assayed[17-20] using assay kits (E-Merck and Agappe diagnostic). The

TABLE- 3 Effect of various extracts of aerial parts of *Jatropha gossypifolia* on percentage protectivity

TREATMENT	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	SOD (U/mg prote	CATALASE (nkat/mg protein)	PROTEIN %	CHOLESTE-ROL (mg/dl)	PROTEIN (gm/dl)	BILIRUBIN (mg/dl)	
									TOTAL	DIRECT
Group IV	87.41	95.38	99.81	58.31	92.21	40.58	74.42	79.4	94.93	77.12
Group V	36.33	68.76	94.18	50.77	34.67	16.47	24.31	41.6	86.45	49.56
Group VI	59.0	84.60	98.69	45.34	83.34	33.52	86.80	55.5	92.15	66.80

hepatoprotective activity expressed as percentage of protectively (H) was calculated as follows:

$$H = \{1-(T-V/C-V)\}$$

Estimation of SOD and Catalase

Grouping and dosing schedule in rats was followed similarly as mentioned in CCl_4 induced hepatotoxicity. After 7 days all animals were sacrificed by cervical dislocation. Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was used for the estimation of superoxide dismutase and catalase levels.[21-22]

Statistical analysis

Values expressed are mean ± SEM (Standard error of mean); using Student's t-test. $P < 0.05$ were considered as significant.

Result

The results of petroleum ether, aqueous and methanolic extracts of aerial parts of *Jatropha gossypifolia* on liver-injury induced by CCl_4 are summarized in Table -1-3. In the CCl_4 intoxicated group (II) SGOT, SGPT, ALP, total bilirubin and direct bilirubin were increased to 391.0 U/L, 400.7 U/L, 944.1 IU/L, 2.01 mg/dL and 0.58 mg/dL respectively, whereas these values were showed 127.9 U/L, 150.6 U/L, 417.25 IU/L, 0.43 mg/dL and 0.12 mg/dL in control group (I), respectively. The elevated levels of these parameters were significantly reduced in the animals groups treated with various extracts. Treatment with petroleum ether extract showed highly significant activity ($P<0.05$) with maximum inhibition. So, petroleum ether extract treated group was superior to the other extracts and as effective as the silymarin. Petroleum ether extract showed maximum protectively 58.31%, 92.21%, 40.58% in SOD, Catalase, protein of liver tissue, where as methanolic extract showed the minimum protective activity (Table-2).

Discussion

In the present study; petroleum ether, aqueous and methanolic extracts of aerial parts of *Jatropha gossypifolia* were evaluated for the hepatoprotective activity using hepatotoxicity induced by CCl_4 in rat model and find out the therapeutically better efficacious extract. An attempt was made to find out the correlation between antioxidant and hepatoprotective activity. This study also gives some scientific evidences on effect of extraction solvents and method of extraction. CCl_4 is being used extensively to investigate hepatoprotective activity on various experimental animals.[23] A major defense mechanism involves the antioxidant enzymes, including SOD, catalase and glutathione peroxidase, which convert active oxygen molecules into non-toxic compounds.

Liver damage was assessed by biochemical studies (SGOT, SGPT, ALP and total bilirubin) and by histopathological examinations. $CC1_4$ produces an experimental damage that histologically resembles viral hepatitis.[24] Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures.[25] The toxic metabolite $CC1_3$ radical is produced which further reacts with oxygen to give trichloromethyl peroxy radical. Cytochrome P450 2E1 is the enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In this view, the reduction in levels of SGOT and SGPT by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This effect is agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and

regeneration of hepatocytes.[26] Alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow.[27] CCl_4 induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The petroleum ether extract induced suppression of the increased SALP activity with the concurrent depletion of raised bilirubin suggest the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury with CCl_4. Thus, administration of petroleum ether, aqueous and methanolic extracts of aerial parts revealed hepatoprotective activity of *Jatropha gossypifolia* against the toxic effect of CCl_4.

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