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Original Article

EVALUATION OF ANTI-OXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *COUROUPITA GUIANENSIS* LEAVES

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Abstract: The present study was conducted to evaluate the hepatoprotective activity of ethanolic leaf extract of Couroupita guianensis against CCL_4 induced liver damage in rats. Anti-oxidant activity was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide scavenging. Ascorbic acid was used as the standard antioxidants for comparison. Healthy male Wistar albino rats of either sex (150-200g) were used for the in-vivo investigations. Liver damage was induced by administration of 30% CCl_4 suspended in olive oil (1ml/kg body weight). Activities of liver marker enzymes, serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), asparate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) at doses of 150mg/kg and 300 mg/kg showed a significant hepatoprotective effect in comparison with the standard (silymarin). The present study demonstrates that the extract of Couroupita guianensis has hepatoprotective effect against CCl_4 induced hepatotoxicity.

Key words: *Couroupita guianensis*, hepatoprotective effect, Silymarin.

INTRODUCTION

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals. Excessive consumption of alcohol, high doses of paracetamol, drugs, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, insecticides, pesticides industrial and environmental pollutant are numerous cause of liver diseases. Now-a-day's due to inadequacy of liver protective agents, researchers and traditional medicine practioners concentrate in herbal based remedies for various liver disorders. Modern medicines have little to offer for alleviation of hepatic disorders. There was neglible safe hepatoprotective drugs are available for the treatment of liver disorders. Therefore, many folk remedies from plant source are used for the protection of hepatic damages from ancient time [1].

One such plant that has been used widely in traditional medicine is *Couroupita guianensis* belonging to the family Lecythidaceae [2]. It is grown in gardens as an ornamental tree. It is native to South India and Malaysia and is commonly known as Nagalinga pushpam in Tamil [3]. The plant contains several chemical constituents, like eugenol, linalool, fernesol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, á, â-amirins, carotenoids, sterols and some acidic and phenolic compounds [4-7]. Traditionally, the leaves of this plant have been used in the treatment of skin diseases, while the flowers are used to cure cold, intestinal gas formation and stomachache. Hence the present work was undertaken to scientifically prove the hepatoprotective nature of *Couroupita guianensis*.

MATERIALS AND METHODS

Plant material: The leaves of *Couroupita guianensis* were collected from the Alipiri forest,

Tirupati and authenticated by Taxonomist Prof Madhava Chetty. The collected leaves were shade dried and pulverized to fine powdered of particle size (#) 40. Dried leaves powder 200 gm was defatted with petroleum ether (60-80^{0}C) and extracted with 95 % ethanol by Soxhelt extractor for 48 h. The extracts were concentrated to dryness under reduced pressure in vacum. The yield of ethanol extract was found to be 15 gm. Extract was kept in a dessicator till experimentation. The preliminary phytochemical screening of ethanol and aqueous extracts were performed to identify the presence of triterpenoids, flavonoids and tannins [8].

ANTI-OXIDANT ACTIVITY

DPPH radical scavenging: The free radical-scavenging activity of *Couroupita guianensis* ethanol extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH [9]. In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decrease at 515-528nm until the absorbance remains constant. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1-20 ìg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values (Fig. 1).

Hydrogen peroxide radical scavenging: Ability of *Couroupita guianensis* extract to scavenge H_2O_2 radical was determined according to the method of Ruch, et al. [10]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). TP and ML in different concentrations (25-75 µg/mL) in 3.4 mL phosphate buffer were added to a H_2O_2 solution (40 mM, 0.6 mL). The absorbance of the reaction mixture was recorded at 230 nm against phosphate buffer without H_2O_2 as blank. The percentage of H_2O_2 scavenging of TP, ML and standard compounds was calculated using the above formula mentioned in DPPH method. All the tests were performed in triplicate and the graph was plotted with the mean values (Fig. 2).

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HEPATOPROTECTIVE ACTIVITY

Animals: Albino rats of either sex weighing between 150 to 200 gms were selected. Animals were kept in polypropylene cages and fed on standard laboratory diet and water ad libitum, maintained at an ambient temperature of 25 ± 2^o C and exposing them to 12 h light/dark cycle. The ethical clearance obtained by the institutional animal ethics committee before the experiment.

Acute toxicity study: The acute toxicity of ethanol extract and aqueous extract was evaluated. The animals were fasted prior to the acute toxicity study. Different groups of animals were orally administered with ethanol and aqueous extract at 0.5, 1.0, 1.5 and 2.0 gm/kg p.o. respectively. Control group received only propylene glycol (vehicle). Drug treated and control groups were placed in polypropylene cages with free access of food and water. Mortality and general behavior of the animals were observed continuously for initial 4 h and intermittently for next 6 h and then again at 24 h and 48 h after dosing. The parameters observed and recorded were sedation, hyperactivity, grooming, loss of lightening reflex, respiratory rate and convulsions. 1/10th of the lethal dose was taken as the screening dose.

Evaluation of hepatoprotective activity [11]: Wistar albino rats were divided into five groups (Gr) of six animals each. The carbon tetrachloride (1ml/kg) was administered to all groups of animals by subcutaneous injection except Gr-I. Gr-I served as control received normal saline (10 ml/kg i.p) only. Gr-II received CCl_4 (1ml/kg. i.p). Gr-III received the reference drug, Silymarin (25 mg/kg i.p). Gr-IV and Gr-V received ethanolic extract in a dose of 150 and 300 mg/kg respectively, one hour after the administration of carbon tetrachloride (0.2 mg/kg/0.2ml in olive oil), for 2 days. All animals were sacrificed at the end of 6th day. The blood sample

Fig. 1: DPPH radical scavenging activity of the ethanolic leaf extract of *Couroupita guianensis*

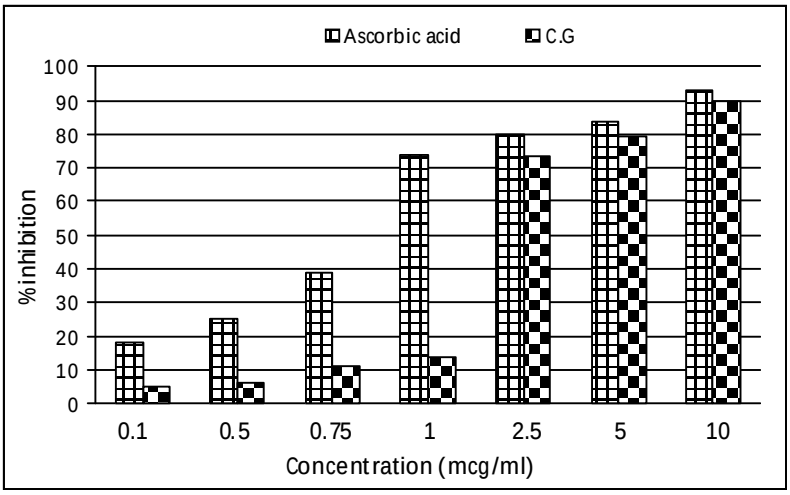

Fig. 2: Hydrogen peroxide radical scavenging activity of the ethanolic leaf extract of *Couroupita guianensis*

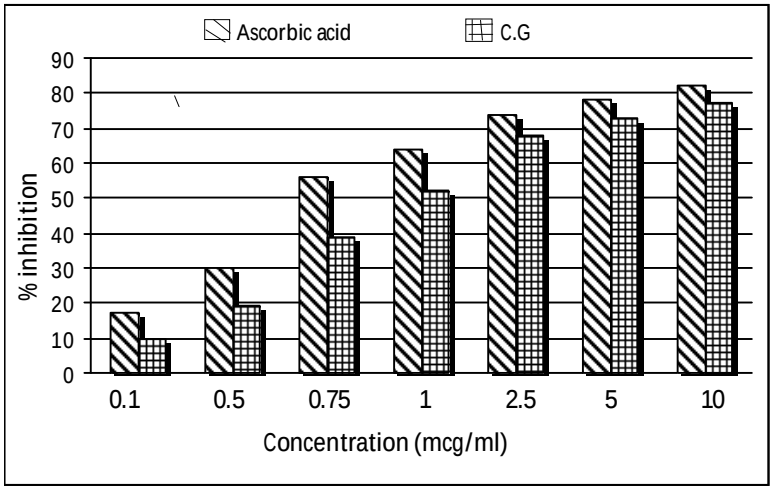

Table 1: Effect of *Couroupita guianensis* ethanolic leaf extract on DPPH and Hydrogen Peroxide Radical scavenging method

DPPH radical scavenging activity			Hydroxyl radical scavenging activity	
Concentration (mcg/ml)	Mean (%) inhibition ± SD		Mean (%) inhibition ± SD	
	C.G	Ascorbic acid	C.G	Ascorbic acid
0.1	5.61 ± 0.21	18.05 ± 0.60	10.81 ± 0.24	17.85 ± 0.23
0.5	6.56 ± 0.18	25.03 ± 0.82	19.51 ± 0.53	30.32 ± 0.37
0.75	14.01 ± 0.32	74.3 ± 1.19	39.02 ± 0.44	56.53 ± 0.21
1	46.03 ± 0.28	78.77 ± 1.02	52.77 ± 0.12	64.17 ± 0.53
2.5	73.79 ± 0.27	80.63 ± 0.68	68.39 ± 0.24	74.08 ± 0.13
5	79.78 ± 0.38	84.05 ± 0.61	73.23 ± 0.12	78.16 ± 0.0
10	90.61 ± 0.16	93.15 ± 0.82	77.05 ± 0.21	82.46 ± 0.44

Table 2: Effect of ethanolic leaf extract of *Couroupita guianensis* on CCl_4 induced hepatotoxicity in rats.

Group name	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	AST (U/L)	TOTAL PROTEIN	TOTAL BILLIRUBIN	IRECT BILLIRUBIN
Control	48.21±0.12	68.34±0.81	29.50±0.64	60.2±1.08	0.78±0.32	0.8±0.52	0.31±0.12
Toxic control	122.31±0.23	134.12±0.92	82.31±0.32	156.1±11.62	0.41±0.41	1.6±0.41	0.76±0.33
Standard (Silymarin)	51.46±0.41	74.62±0.71	36.46±0.23	70.2±5.60	1.42±0.25	0.8±0.72	0.32±0.23
C.G (150mg/kg)	86.24±0.52	96.14±0.62	61.44±0.12	128.4±4.45	0.96±0.34	1.4±0.93	0.64±0.41
C.G (300mg/kg)	55.61±0.32	76.24±0.14	40.02±0.22	96.9±4.78	1.24±0.37	1.1±0.23	0.41±0.52

of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 3000 rpm for 10 min and was subj-ected to biochemical investigation viz., serum glut-amate oxaloacetate transaminase [12], serum glutamic pyruvic transaminase [13], total and direct bilirubin [14], total protein [15], serum alanine transa-minase [16], aspartate transaminase [16] and alkaline phosphatase [17]. Results of biochemical estimations were reported as mean ± S.E of six animals in each group. The data was subjected to one way ANOVA followed by Post-hoc Dunnett's test. $p < 0.05$ was considered as statistically significantly.

RESULTS

Phytochemical analysis of *C. guianensis* extract demonstrates the presence the presence of anthroquinones, carbohydrate, flavonoids and tannins in ethanolic extract. Ethanol extract exhibited marked DPPH free radical scavenging and hydrogen peroxide radical scavenging in a dose dependent manner. The IC_{50} value of *C. guianensis* was found to be 20.01µg/mL and 42.2µg/mL respectively. The results were found to be statistically significant ($P<0.01$) at the concentration of 10 mg/mL (Table 1). The acute toxicity evaluation of ethanolic extract revealed no mortality when administered orally up to a maximum dose 3gm/kg. At this dose there were no gross behavioral changes. The 1/10th of lethal dose was taken as the screening dose. The administration of CCl4 to the animals resulted in a marked increase in marker enzymes like SGOT, SGPT, ALP, AST, total protein and bilirubin content. The toxic effect of CCl_4 was controlled in the animals treated with the methanolic extract by the way of restoration of the levels of the liver function biochemistry similar to that of standard drug Silymarin (Table 2).

DISCUSSION

The CCl_4 has been used as a tool to induce hepatotoxicity in experimental animals. This toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum enzymes, AST, ALT and ALP was the clear indication of cellular leakage and loss of functional integrity of the cell membrane. Administration of methanolic extract of *C. guianensis* showed significant hepatoprotective activity, which was comparable to that of standard Silymarin. Phytochemical analysis revealed that the methanolic extract of the plant was found to contain flavonoids and phenolic compounds further it has been reported that the flavonoid constituents of the plant possess antioxidant properties was found to be useful in the treatment of liver damage.

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