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Research Article

HEPATOPROTECTIVE ACTIVITY OF *ECBOLIUM VIRIDE (FORSK.) ALST.* (ACANTHACEAE) ON EXPERIMENTAL LIVER DAMAGE IN RATS

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ABSTRACT
The Methanol extract of *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), was tested for hepatoprotective activity against carbon tetrachloride and paracetamol-induced hepatotoxicity in rats. *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), exhibited significant hepatoprotective activity by reducing carbon tetrachloride and paracetamol-induced change in bio-chemical parameters that was evident by enzymatic examination. The plant extract may interfere with free-radical formation, which may conclude in hepatoprotective action. Acute toxicity studies revealed that the LD_{50} value is more than the dose of 5 g/kg body wt.

KEY WORDS: *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), Carbon tetrachloride; Paracetamol

INTRODUCTION

All parts of the plant are used for gout and dysuria. Decoction of the leaves is given for stricture. Roots are used for jaundice[1], menorrhagia and rheumatism. Roots and leaves together are used to treat tumours. EtOH (50%) extract of the plant possesses cardiovascular effects [2]. Leaves, roots and flowers contain orientin, vitexin, isoorientin, isovitexin. They also contain glycoflavones and other flavones[3]. They involve in the many pathological conditions like cardiac, neuro, hepatodisorders, cancer and diabetes[4]. Current research into free radicals has confirmed that food rich in antioxidants play an essential role in prevention of cardiovascular diseases and cancer [5]. *Ecbolium viride* (forsk).Alston commonly known as Green Shrimp Plant and its synonym is '*Ecbolium ligustrinum'* It is

known as "Nakka toka" in Telugu, "Sahachara" in Sanskrit, "Nilambar" i in Tamil belongs to the family Acanthaceae and the roots of the plant are reported to be used for jaundice, menorrhagia and rheumatism[6,7]. The roots are reported to contain glycoflavones such as Orientin, Vitexin, Isoorienten, and Isovitexin [8].Based on the uses and phytoconstituents, the plant was selected for this study to prove its hepatoprotective potential.

MATERIALS AND METHODS

Plant material: The whole plant of *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), used in this study were collected from Tirupati during the month of April-May where a voucher specimen has been preserved for future identification. The plants were shade dried and powdered. 1Kg of the powdered plant were extracted with Methanol and filtered. The filtrate was dried by vacuum rotary evaporation to yield a solid methanolic extract of 55g.

Animals: Wistar albino rats (150-200 mg each) of either sex, maintained under standard animal housing conditions (12 h light and dark cycle), were used for all sets of experiments performed on eight rats each. The rats were allowed standard laboratory feed and water ad libitum.

Acute toxicity: Acute toxicity studies were performed for extracts according to the toxic classic method as per OECD guidelines. Female albino rats were used for the acute toxicity study. The animals were kept fasting overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher dose i.e., 2000 mg/kg, 2500mg/kg, 3000mg/kg.

Hepatoprotective activity

Carbon tetrachloride-induced experimental liver damage: The aqueous extract of *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), methanol extract at doses of 100 mg and 200 mg/kg body wt. and silymarin at a dose of 100mg/kg body wt. were administered orally to rats of the respective groups three times at 12 h intervals. Control animals received vehicle. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in a dose of 1ml/kg body wt. for 2 days to all animal groups except for control [9]. Animals of the untreated group received only CCl_4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. After 36 h of

carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital plexus.

Serum was separated by centrifugation at 2500 rpm at 37^0C for 15 min and analyzed for various biochemical parameters.

Paracetamol-induced experimental liver damage: In case of paracetamol-induced hepatotoxicity, the *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), extract (at doses of 100 and 200 mg/kg body wt.) and silymarin (100 mg/kg) were given orally to respective groups once daily for 3days. On the third day, paracetamol at 3 g/kg body wt.was administered to all groups except for control, 30 min after the respective treatment with *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), methanolic extract, silymarin and vehicle [10] .One groups received only paracetamol to assist in assessing the severity of toxicity produced by paracetamol at 3 g/kg body wt. After 48 h of paracetamol administration, blood was collected from all groups, including control, and serum was separated and analyzed for various biochemical parameters as in the case of carbon tetrachloride-induced liver damage.

Assessment of liver functions: Biochemical parameters, such as serum glutamic oxaloacetate Transaminase, serum glutamic pyruvate transaminase [11], alkaline phosphate [12], total bilirubin, direct bilirubin[13] and liver glutathione [14], were analyzed according to the standard method.

Statistical analysis: The mean value [±] SEM was calculated for each parameter. Results were statistically analyzed by student's' test [15],1967). $P < 0.01$ indicates significant differences between group means.

RESULTS

The methanol extract of whole plant of *Ecbolium viride (Forsk.) Alst.* (Acanthaceae),, was found to be practically nontoxic

Table 1. Effect of methanolic extract of *Ecbolium viridae* on carbon tetrachloride induced hepatotoxicity in rats.

Design of treatment	SGOT	SGP	ALKP	T. Bil	D. Bil	GSH
Control	129.52±2.32	65.12± 1.76	141.54±6.11	1.04±0.20	0.18±0.01	10.64±0.76
Carbon tetra-chloride	826.47±7.31(a)	37.00± 8.60 (a)	448.61±8.22(a)	3.56±0.37(a)	1.65±0.31(a)	6.76±0.58(a)
Silymarin	154.22±3.08(b	67.34±2.00 (b)	173.81±4.28(b)	0.99±0.05(b)	0.34±0.02(b)	8.83±0.63(b)

Ecbolium viridae (100 mg/kg body wt.)	294.42±8.21(b)	208.41±5.16(b)	226.52±7.65(b)	1.86±0.22(b)	0.62±0.04(b)	8.08±0.51(b)
Ecbolium viridae (200 mg/kg body wt.)	271.78±6.98(b	201.56±6.78(b)	212.91±6.28(b)	1.63±0.56(b)	0.51±0.04(b)	8.56±0.32b)

Values are mean [±] S.E.; n = 8; (a) $p < 0.01$ compared to control; (b) $P < 0.01$ compared to carbon tetrachloride.

Table 2. Effect of methanolic extract of *Ecbolium viridae* on paracetamol-induced hepatotoxicity in rats.

Design of treatment	SGOT	SGPT	ALKP	T. Bil	D. Bil	GSH
Control	132.69±6.18	59.06±2.67	132.36±6.12	1.14± 0.15	0.24±0.02	10.38±0 .89
Paracetamol	366.24±3.0(a)	284.18±3.18(a)	328.31±1.50 (a)	3.76±0.20(a)	0.80±0.04(a)	3.91±0.76(a)
Silymarin	116.46±12.34(b)	45.08± 1.08(b)	101.2±3.72 (b)	1.09±0.08(b)	0.36±0.01(b)	8.12±0.40(b)
Ecbolium viridae (100 mg/kg body wt	231.26± 4.23 (b)	57.16± 6.10(b)	152.09±5.09 (b)	1.33±0.17(b)	0.37±0.04(b)	7.02±0.61(b)
Ecbolium viridae (200 mg/kg body wt	213.21± 6.24 (b)	51.31± 5.14(b)	141.22±4.75 (b)	1.54±0.16(b	) 0.36±0.02(b)	7.8±0.44(b)

Values are mean [±] S.E.; n = 8; (a) $p < 0.01$ compared to control; (b) $P < 0.01$ compared to paracetamol.

Hepatoprotective activity of Ecbolium viride (Forsk.) Alst. (Acanthaceae), On Experimental liver damage in rats

When administered orally to rats and its LD_{50} value was found to be higher than 4 g/kg body wt.Administration of carbon tetrachloride and paracetamol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pretreatment of rats with *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), aqueous extract exhibited marked protection against carbon tetrachloride and paracetamol induced hepatotoxicity, which is shown in Tables 1and 2, respectively. The aqueous extract of *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), showed significant hepatoprotective activity against carbon tetrachloride and paracetamol, comparable with the standard silymarin.

DISCUSSION

Hepatic cells appear to participate in a variety ofenzymatic metabolic activities and both carbon tetrachloride and paracetamol produced marked liver damage at the given doses as expected [16,17]. administration of carbon tetrachloride elevated the serum levels of SGOT, SGPT,ALKP and bilirubin significantly, due to its enzymatic activation of CCl_3 free radical, which in turn alters the structure and function of liver cells [18] . Pretreatment with *Ecbolium viride (Forsk.) Alst.* (Acanthaceae), methanolic extract showed a dose dependent protection against the injurious effects of carbon tetrachloride that may result from the interference with cytochrome P_{450} resulting in the hindrance of the formation of hapatotoxic free radicals [19,20]. Paracetamol in larger doses produces liver necrosis after undergoing bioactivation to a toxic electrophile, N-acetyl-p-benzoquinone- imine (NAPQI)by cytochrome P_{450} monooxygenase [21]. NAPQI binds to macromolecules and cellular proteins, and also oxidizes lipids and alters homeostasis of calcium after depletion of glutathione.Pretreatment with methanolic extract of Ecbolium viridae restored the depleted GSH Concentration near normal and also brought down the elevated levels of SGOT, SGPT, ALKP and bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome P_{450} or/and promotion of its glucuronidation [22,23]. Further studies are in progress to isolate the active constituents and also to evaluate the exact mechanism of action.

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