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PROTECTIVE EFFECT OF *CROSSANDRA UNDULAEFOLIA* ON PARACETAMOL INDUCED HEPATOTOXICITY IN ALBINO RAT

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ABSTRACT

Many hepatoprotective herbal preparations have been recommended in alternative system of medicine for the treatment of hepatic disorders. No systemic study has been done on protective efficacy of *Crossandra Undulaefolia* belonging to family Acanthaceae to treat diseases. The Hydro-Ethanollic extracts of aerial parts of *Crossandra Undulaefolias* (ECU). The animals were weighed each and divided in group of five. Liver damage was achieved by injecting Paracetamol (2 g/kg). The treatment groups pretreated with ECU Group-IV (200mg/kg) and Group-V (400mg/kg). Silymar in was used as reference standard drug. At the end of 7 days, blood was collected, liver extracted, weighed, processed for histopathological assessments and alteration in the levels of biochemical markers of hepatic damage like serum transaminases (AST,ALT), alkaline phosphatase (ALP) , bilirubin cholesterol, high density lipoprotein (HDL) and tissue GSH were tested in all the groups. The ECU exhibited a significant hepatoprotective effect by lowering the elevated serum levels of AST, ALT, ALP, total and direct serum bilirubin, cholesterol and significantly ($p<0.05$) increased HDL and moderately increased total protein and albumin in a dose dependent manner These biochemical observations were supplemented by treatment of liver toxicity in traditional medicine. Histopathological examination of liver sections. These findings suggest the use of this plant for its Hepatoprotective effects.

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INTRODUCTION

The analgesic paracetamol causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. The initial phases of toxicity were described in Dr. Gillette's laboratory in the 1970s. These findings indicated that Paracetamol was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that repletion of GSH prevented the toxicity. Liver is a vital organ of the body. It plays a pivotal role in the metabolism, secretion and storage. Any type of the injury or impairment of its functions may leads to many type of complication in one s health. Unfortunately, Hepatic dysfunction due to ingestion or inhalation of hepatotoxins is increasing Worldwide. Management of liver disease is still a challenge to the modern medicine. Due limited therapeutic options and disappointing therapeutic success of the modern medicine, uses of herbal drugs has increased worldwide numerous medicinal plants and their formulations used for liver disorders in ethanomedical practices and in traditional system of medicine in India. In this modern age it is very important to provide scientific proof to justify the medicinal uses of herbs. Efficacy of the drugs should be tested by standard experimental methods and there should be adequate data from studies to validate the therapeutic potential. In the present study, in order to search for a new natural remedy for hepatic disorder, the *Crossandra undulaefolia*, plants was assessed in terms of growth and flowering of the angiosperm crossandra belonging to family acanthaceae was

chosen for its possible hepatoprotective activity. The plant is also known for its use in folk medicines, traditionally, the plant has been used as Antifungal, antipyretic and analgesic activity. The phytochemical investigation examination of this plan has shown the presence of triterpenoids, of searching for hepatoprotective agents from medicinal plants, the hydro-ethanolic extract of aerial part of *Crossandra undulaefolia*.¹

MATERIALS AND METHODS:

Plant materials and preparation of extracts:

Crossandra Undulaefolia whole plant was obtained from Abhirami botanicals, Tuticorin, Tamilnadu. The plant material was identified and authenticated by resident botanist, Dr. S. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai. The voucher specimen was submitted at Dept of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai, and T.N.

Preparation of Plant Extracts: The aerial parts was chopped to small pieces and dried in shade. The dried root was powdered and a weighed quantity of the powder (650 g) was passed through sieve number 20 and subjected to hot solvent extraction in a soxhlet apparatus using aqueous EtOH (50:50), at a temperature range of 60-70°C. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The aqueous EtOH (50:50) extract yielded a brown semi-solid, weighing 78.0g (12.0%) and the extract was preserved in a refrigerator for its usage^{2,3}.

Animals: Inbred adult Wister albino rats, weighing 180-220 g of either sex were obtained from animal

house of C.L.Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided *ad libitum* through out experimentation period. Animals were acclimatized to laboratory conditions one week prior to beginning of experiments. Fasting refers to that the animals were deprived of food for 16 hours but were allowed to free access for water. The project was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) through its reference no: **IAEC/XII/02/CLBMCP/2008-2009**, **dated: 24/11/2008**.

Drugs and Chemicals: PARACETAMOL and Silymarin were obtained as gift sample from Alkem Laboratories, Navi Mumbai. SGOT, SGPT, ALP, kits were procured from span dignostics, Surat. LDH, GGT kits were procured from all other chemicals and reagents used were of analytical grade.

Toxicological Evaluation:

Acute Oral Toxicity Study (OECD 423)⁴: The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). This procedure helps in the use of minimal number of animals while allowing for acceptable data based scientific conclusion. This method makes use by defined doses (5, 50, 500, 2000mg/kg body weight) and the results allow a

substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity

Experimental Design:

Rats were divided into five groups, each group consisting of six animals.

Group I: Controls received the vehicle viz. normal saline (2 ml/kg).

Group II: Received Paracetamol (750 mg/kg p.o.) at every 72 hrs for 10 days.

Group III: Received silymarin 50 mg/kg p.o. for 10 days and simultaneously administered 750 Paracetamol mg/kg body wt. every 72 hrs,

Group IV: Received alcohol extract of ECU 200 mg/kg p.o. for 10 days and simultaneously administered Paracetamol 750 mg/kg body wt. every 72 hrs.

Group V: Received alcohol extract of ECU 400 mg/kg p.o. for 10 days and simultaneously administered Paracetamol 750 mg/kg body wt. every 72 hrs.

At the end of experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters

Assessment Of Liver Function:

Biochemical parameters i.e., aspartate amino transferase (AST)⁵, alanine aminotransferase (ALT), alkaline phosphatase (ALP)⁶, glutamate transpeptidase (GGTP)⁷, total bilirubin and total protein were analyzed according to reported

TABLE 1

Effect of the aqueous EtOH (50:50) extract of *Crossandra undulaefolia* (ECU) on biochemical parameters in paracetamol-induced hepatic injury in rats.

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
AST (U/ml)	157.66±4.6	227.5±6.8	151.4±6.63	217.5±4.64	174.25±6.42
ALT(U/ml)	74.2±2.92	176±4.7	89.2±3.6	122±2.5	107.25±5.42
ALP(U/ml)	188.4±3.16	578±8.9	228.4±5.42	414±12.89	299.75±1.89
Total Bilirubin(mg/dl)	0.8±0.05	1.1±0.08	0.72±0.03	0.65±0.06	0.7±0.4
Total protein (mg/dl)	8.13±1.4	6.35±0.35	8.12±0.56	9.22±0.31	8.62±0.9
GGTP (U/ml)	26.01±1.01	62.1±2.48	35.3±1.7	39.6±1.08	31.5±3.61

The values are expressed as Mean±SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. **p<0.01, *p<0.05.

FIGURE 1

Effect of the aqueous EtOH (50:50) extract of *Crossandra Undulaefolia* (ECU) on biochemical parameters in paracetamol-induced hepatic injury in rats.

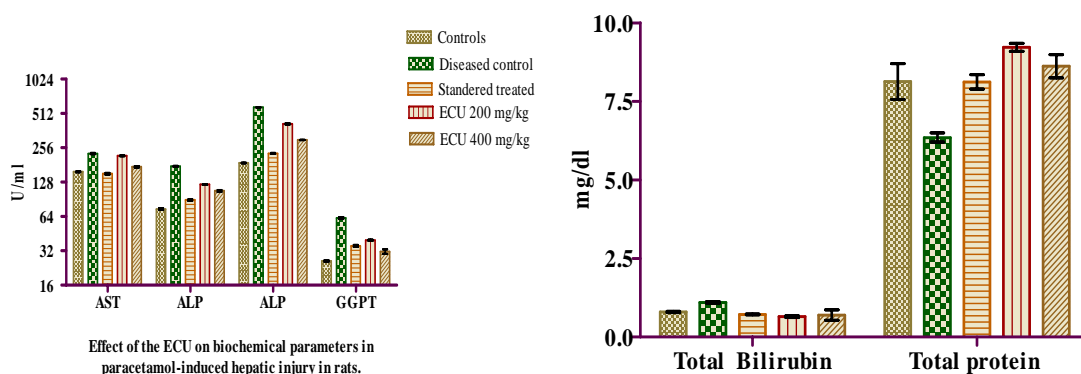


TABLE 2

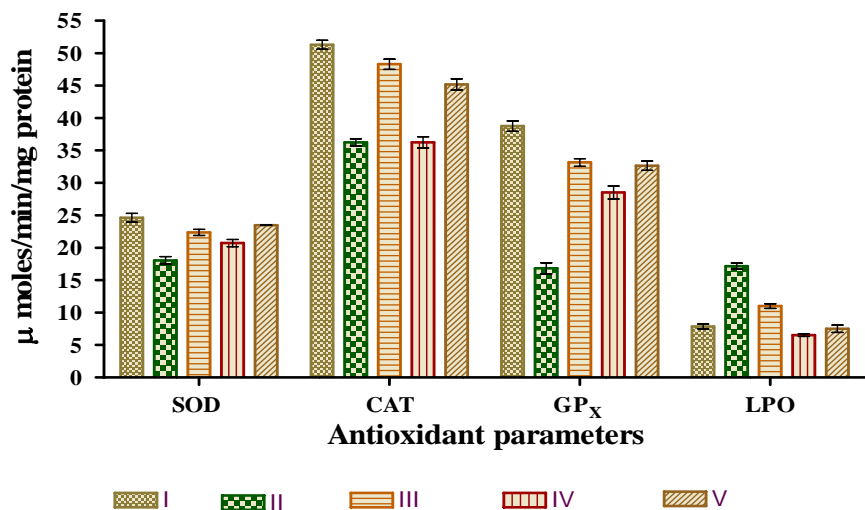
Effect of the aqueous EtOH (50:50) extract of *Crossandra Undulaefoli* (ECU) on antioxidant parameters in paracetamol-induced s in paracetamol-induced hepatic injury in rats

TREATMENT	Dose /kg.	SOD	CAT	GP _x	LPO
Group I	-	24.61±1.68	51.29±1.67	38.75±1.96	7.85±0.92
Group I	750	18.05±1.45	36.24±1.35	16.82±2.10	17.17±1.14
Group I	50	22.36±1.18	48.29±1.92	33.14±1.45	11.01±0.87
Group I	200	20.72±1.39	36.24±2.10	28.52±2.45	6.54±0.51
Group I	400	23.48±0.047	45.17±2.10	32.64±1.76	7.51±1.38

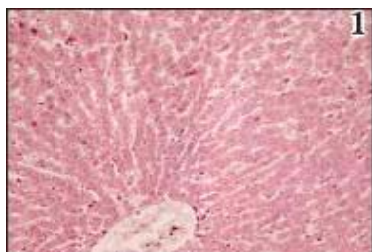
The values are expressed as Mean±SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. **p<0.01, *p<0.05

FIGURE 3

Effect of the aqueous EtOH (50:50) extract of *Crossandra Undulaefoli* (ECU) on antioxidant parameters in paracetamol-induced s in paracetamol-induced hepatic injury in rats

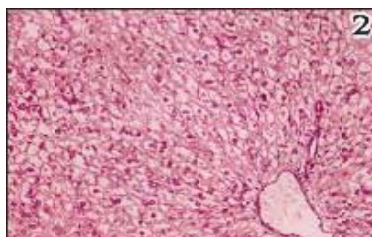


CAT = μ mole of H₂O₂ consumed/ min/mg protein.
 SOD = Units/min/mg protein
 GP_x = μ moles of GSH oxidized/min /mg protein.
 LPO = μ moles of MDA/ min/mg protein.



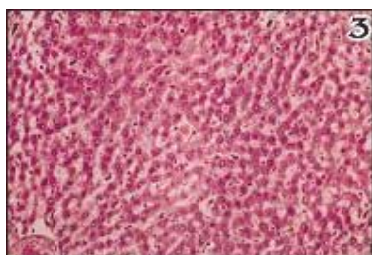
Group I: Controls received the vehicle

[1] liver from rat treated with saline shows normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein



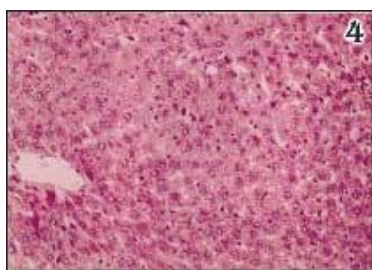
Group II: Received Paracetamol (750 mg/kg p.o.) at every 72 h for 10 days.

[2] Liver from rat treated with Paracetamol exhibited severe hepatocyte degeneration and necrosis



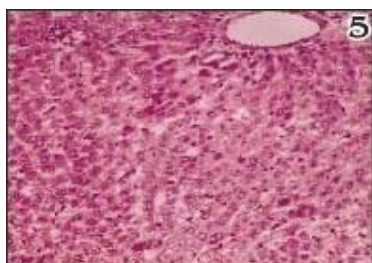
Group III: Received silymarin 50 mg/kg p.o. for 10 days and simultaneously administered 750 Paracetamol mg/kg body wt. every 72 h.

[3] Liver treated with silymarin (50 mg/kg, p.o.) plus Paracetamol shows normal architecture with mild hepatocyte degeneration.



Group IV: Received alcohol extract of ECU 200 mg/kg p.o. for 10 days and simultaneously administered Paracetamol 750 mg/kg body wt. every 72 h.

[4] Liver treated with ECU (200 mg/kg,) p.o. plus Paracetamol shows mild hepatocyte degeneration



Group V: Received alcohol extract of ECU 400 mg/kg p.o. for 10 days and simultaneously administered Paracetamol 750 mg/kg body wt. every 72 h.

[5] Liver treated with ECU (400 mg/kg,) p.o. plus Paracetamol shows mild hepatocyte degeneration

method⁸. The liver was removed, weighed and morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO)⁹, superoxide dismutase (SOD)¹⁰, catalase and glutathione peroxidase (GPx)^{11,13}.

Histopathological Study:

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin. Histological observations were made under light microscope^{14, 15}.

Statistical Analysis:

Statistical analysis were carried out by one way analysis variation (ANOVA) followed by Dunnet's test. *p< 0.05, **p< 0.01, ns - non significant. The values are expressed as mean \pm SEM.

RESULT AND DISCUSSION

Paracetamol has enhanced the levels of aminotransferases (AST,ALT), bilirubin (both total and direct bilirubin levels), Alkaline phosphatase level (ALP), total cholesterol, Treatment with silymar in and 200 mg/kg and 400

mg/kg of ECU (aqueous EtOH (50:50) extract of *Crossandra Undulaefoli*) has significantly brought down the elevated levels of AST,ALT, ALP, bilirubin and glutamate transpeptidase (GGTP) significantly (p<0.05) (**Table 1, figure 1**). Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.001) increase in the acetaminophen treated rats. Treatment with ECU (200 mg/kg and 400 mg/kg) significantly (P<0.001) prevented the increase in LPO level which was brought to near normal. The effect of ECU was comparable with that of standard drug silymarin (**Table 2, figure 2**). Paracetamol is normally eliminated mainly as sulfate and glucuronide. Only 5% of the paracetamol is converted into N-acetyl-p- benzoquinimine. However, upon administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinimine (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently

binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leading to increased lipid peroxidation and liver damage there is elevation in the levels of various biochemical markers of hepatic damage like AST, ALT, bilirubin, and cholesterol. Treatment with silymarin and ECU (aqueous EtOH (50:50) extract of *Crossandra Undulaefoli*) has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels which was significant ($p < 0.05$) in group V compared to Group IV. It may be concluded that the hepatoprotective effect of aqueous EtOH (50:50) extract of *Crossandra Undulaefoli* is due to the prevention of the depletion in the tissue GSH levels. The ECU contains terpenoids have quercetin-3-rutinoside and other flavonoids which are still present in the methanolic extract. Therefore there is a possibility that the root extract may possess antioxidant property, which may be involved in the hepatoprotective property. In addition it is necessary to carry-out further studies to rule out if treatment with ECU is able to inhibit oxidation of paracetamol to highly reactive NAPQI. The results were further substantiated with the histopathological study, the reduced hepatic damage or improvement in the hepatic in a dose dependent manner.

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