

HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *COCCINIA GRANDIS* ROOT EXTRACT AGAINST PARACETAMOL INDUCED HEPATIC OXIDATIVE STRESS IN WISTAR ALBINO RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective and antioxidant activity of *Coccinia grandis* root extract against paracetamol induced hepatic oxidative stress in wistar albino rats. The ethanolic extracts of *Coccinia grandis* (200mg/kg and 400mg/kg) were administered orally to the animals and hepatotoxicity induced by paracetamol (750mg/kg). The extracts were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. Silymarin (25mg/kg) was given as reference standard. The ethanolic extract of *Coccinia grandis* roots produced a significant ($P<0.01$) decrease in SGOT, SGPT, SALP, Total bilirubin and Direct bilirubin and it also produced a significant ($P<0.01$) increase in Total protein when compared to paracetamol treated group indicating hepatoprotective action. The ethanolic extract of *Coccinia grandis* root produced a significant ($P<0.01$) increase in SOD, CAT and GSH activity when compared to paracetamol treated group and it also produced significant ($P<0.01$) increase in activity of Px and GPx at 400mg/kg dose, indicating antioxidant activity. But it produced less significant in Px at 200mg/kg dose and it showed no significant activity in GPx at 200mg/kg dose. The histopathological study of liver section of rat treated with ethanolic extract of *Coccinia grandis* (200 and 400 mg/kg) showed mild hepatocyte degeneration. It was concluded from the result that ethanolic extract of *Coccinia grandis* possesses hepatoprotective and antioxidant activity against paracetamol induced hepatotoxicity in wistar albino rats.

KEYWORDS: *Coccinia grandis*, Hepatoprotective, Paracetamol, Carboxy methyl cellulose.

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INTRODUCTION

The liver disorder are one of the serious health problem, throughout the world more than 350 million peoples were affected with chronic hepatitis infection and India about 20,000 deaths found every year due to liver disorder. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently inadequate, so far not get any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects¹. The liver is an important organ for metabolizing and detoxifying drugs. However, drugs and their metabolites can damage the liver². According to the United States Acute Liver Failure Study Group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury

triggered by other drugs (13%). Drugs are an important cause on liver injury. Approximately 75% of the idiosyncratic drug reaction results in liver transplantation or death³.

Coccinia grandis (L) voigt belongs to the family Cucurbitaceae commonly known as Ivy Gourd in English, Kovai in Tamil, Kovakka in Malayalam and Kundru in Hindi. Description of the plant are Scandent shrubs, flowers white, Leaves alternate, Fruits bright red in colour. It mainly present in Africa and Asia. All the parts of the plant used in traditional system of medicine and it used for Liver diseases, Diabetes mellitus, Antimicrobial, Asthma, Ulcer, Urinary tract diseases, Allergy, Bronchitis⁴.

Since no previous attempts have been made to examine the hepatoprotective and antioxidant activity of *Coccinia grandis* root extract on experimental animal.

MATERIALS AND METHODS

Plant material

The fresh roots of *Coccinia grandis* were collected from Vengara, Malappuram district, Kerala during the month of July 2010. The plants materials were identified and authenticated by Dr.P.Satyanarayana, Scientist 'D' in-charge, Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore, Tamilnadu and the voucher specimen No.BSI/SRC/5/23/10/Tech-867.

Extraction

The plant material dried in shade under room temperature and pulverized to a coarse powder using motor grinder. The finely powdered roots of *Coccinia grandis* (80gm) was defatted with petroleum ether (60-80°C) around 7 hrs and then extracted with 95% ethyl alcohol (500 ml) at (60-80°C) using soxhlet apparatus till exhaustion for about 32 hr. The ethanolic extract was concentrated using heating mantle (60°C) to get the residues. The percentage yield of ethanolic extract was found to be 10.75% (w/w).

Phytochemical analysis

The extract was subjected to preliminary screening for various active phytochemical constituents such as alkaloids, glycosides, flavonoids, protein, tannins, carbohydrates, terpenoids and fixed oils.

Experimental animals

Wister albino rats of either sex (150-200gm) procured from the animal house, Nandha College of Pharmacy, Erode, Tamilnadu. The animals were placed randomly in poly propylene cages with paddy husk as bedding and housed at standard condition maintained a temperature of 24±2°C and relative humidity of 30-70%. A 12 hours lights and 12 hours dark cycle were strictly followed. The animals had a free access to standard animal pellet diet (Pranav agro industries Ltd, Pune) and water ad libitum. A research proposal were submitted according to the guidelines of CPCSEA and approved by Institutional Animal Ethical Committee (IAEC) of Nandha College of Pharmacy and Research Institute, Erode-52 (Proposal No.NCP/IAEC/PG/2010-17).

Experimental design

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

Group I: (Normal control) which receives the 0.5% (CMC) carboxy methyl cellulose solution (1ml/kg)

Group II: (Negative control) which receives paracetamol (750 mg/kg p.o) at every 72 h for 10 days.

Group III: (Positive control) which receives silymarin 25 mg/kg p.o. for 10 days and simultaneously administered paracetamol 750 mg/kg body wt. every 72 h.

Group IV: (Test 1) which receives ethanolic extract of *Coccinia grandis* 200mg/kg p.o. for 10 days and simultaneously administered paracetamol 750 mg/kg body wt. every 72 h.

Group V: (Test 2) which receives ethanolic extract of *Coccinia grandis* 400mg/kg p.o. for 10 days and simultaneously administered paracetamol 750 mg/kg body wt. every 72 h.

Estimation of biochemical parameters

At the end of experimental period, all the animals were anaesthetised by using diethyl ether and blood sample collected by retro orbital puncture. The blood sample allowed to clot, serum was separated by centrifuging at 2500 rpm for 15 minutes (Model C24- BL, Remi Industries, Mumbai), and analysed for various biochemical parameters like SGOT, SGPT, SALP, Total protein, Total bilirubin, Direct bilirubin with auto analyser (MERCK, microlab 300).

Antioxidant activity study

At the end of the experimental period the rats were sacrificed by cervical decapitation. The livers were removed and washed thoroughly with 0.9% NaCl, weighed and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of the phosphate buffer. The homogenate was centrifuged at 2500 rpm for 15 min and supernatant was used for estimation of enzymatic antioxidant like superoxide dismutase (SOD), catalase (CAT), peroxidase (Px), and glutathione peroxidase (GPx), and the non-enzymatic antioxidant reduced glutathione (GSH).

Histopathological study

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. A small portion of the liver was fixed in 10% neutral buffered formalin. Thin section of 4-5 µm were taken, stained with Haematoxylin and Eosin and finally mounted in Diphenylxylene. The sections were observed under a microscope for histopathological changes in liver architecture and their photomicrograph was taken.

Statistical analysis

The collected data were subjected to appropriate statistical tests including one-way ANOVA, followed by an appropriate Dunnett's t-test. P values of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using Graph pad prism software of version 4.

RESULTS**Phytochemical analysis**

Phytochemical studies indicate that ethanolic extract of *Coccinia grandis* contains alkaloids, glycosides, flavonoids, tannins, carbohydrates and it showed negative results in proteins, terpenoids and fixed oils (Table no.1).

Estimation of biochemical parameters

The ethanolic extract of *Coccinia grandis* roots produced a significant ($P < 0.01$) decrease in hepatic serum enzyme SGOT, SGPT, SALP and it also produced a significant ($P < 0.01$) increase in Total protein when compared to paracetamol treated group indicating hepatoprotective action. The extract of *Coccinia grandis* produced a significant ($P < 0.01$) decrease in Total bilirubin and Direct bilirubin when compared to paracetamol treated group. Silymarin treated group also showed significant ($P < 0.01$) result compared to paracetamol treated group (Table no.2).

Antioxidant activity study

The ethanolic extract of *Coccinia grandis* root produced a significant ($P < 0.01$) increase in SOD, CAT and GSH activity when compared to paracetamol treated group and it also produced significant ($P < 0.01$) increase in activity of Px and GPx at 400 mg/kg dose, indicating antioxidant activity. But it produced less significant in Px at 200 mg/kg dose and it showed no significant activity in GPx at 200 mg/kg dose. Silymarin treated group also showed significant ($P < 0.01$) result compared to paracetamol treated group (Table no.3).

Histopathological study

Histopathological examination of liver section of normal rats showed normal cellular architecture with distinct hepatic cells, sinusoidal space and a central vein (fig.1). Liver section of rat intoxicated with paracetamol showed severe hepatocyte degeneration and necrosis (fig.2). Liver section of rat treated with silymarin and intoxicated with paracetamol showed normal architecture with mild hepatocyte degeneration (fig.3). Liver section of rat treated ethanolic extract of *Coccinia grandis* (200 and 400 mg/kg) showed mild hepatocyte degeneration (fig.4 and 5).

DISCUSSION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver disease are inadequate and sometimes can have serious side effects. Herbal drugs play a role in the management of various liver disorders in addition to other natural healing processes of the liver. Previous studies have demonstrated the use of paracetamol to successfully induce hepatotoxicity in experimental animals. Which produce hepatic necrosis at

higher doses. This hepatotoxicant is primarily metabolized by sulfation and glucuronidation to reactive metabolites and then activated by the enzyme cytochrome P-450 system to produce liver injury. Its mode of action is by covalent binding of its toxic metabolite, n-acetyl p-benzo quinone-amine to tissue macromolecules, resulting in cell necrosis.

A reduction in the levels of SGOT and SGPT towards normal value is an indication of revival of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. A reduction of ALP levels with concurrent decrease in the raised bilirubin level suggests the stabilization of biliary function which had been adversely affected by injury due to paracetamol. Protein level was raised by the extract suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by the ethanolic extract was similar to that due to silymarin treatment.

The decreased levels of antioxidants SOD, CAT, GPx activities may be due in part to an overwhelming oxidative modification of the enzymatic proteins by excessive ROS generation. More so, reduction in the activities of these enzymes may stem from decrease in their rate of synthesis. The observed increase of SOD activity suggests that the *Coccinia grandis* extract has an efficient protective activity in response of ROS. These findings also indicate that *Coccinia grandis* may be associated with decreased oxidative stress and free radical mediated tissue damage.

CAT is a key component of the antioxidant defence system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage. Excessive production of free radicals may result in alteration of the biological activity of cellular macromolecules. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of *Coccinia grandis* increase the activities of catalase in paracetamol induced liver damaged rat to prevent the accumulation of excessive free radicals and protects the liver from paracetamol induced toxication.

CONCLUSION

The above study has been concluded that the extract of roots of *Coccinia grandis* offers protective effect against paracetamol induced hepatotoxicity in experimental rats. Further investigation should be made to elucidate the mechanism of *Coccinia grandis* and its role in hepatoprotective and antioxidant effect. The mechanism of action is yet to be investigated but may be due to the

antioxidant effect of flavonoids found to be present in the roots.

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Table 1. Phytochemical analysis of *Coccinia grandis* root extract

Phytochemical constituent	Presence of phytochemical constituent
Alkaloids	+
Flavonoids	+
Glycosides	+
Terpenoids	-
Proteins	-
Carbohydrates	+
Tannins	+
Fixed oils	-

+ve Present
-ve Absent

Table 2. Estimation of rats blood serum profile of *Coccinia grandis* roots extract on paracetamol intoxicated rats

Groups and Treatment	Dose(mg/kg)	SGOT(IU/L)	SGPT(IU/L)	SALP(IU/L)	Total Protein(g/dl)	Total Bilirubin(mg/dl)	Direct Bilirubin(mg/l)
Group I Normal control	2ml/kg	68.72±1.426* *	56.12±1.700* *	138.47±2.230**	8.72±0.060**	0.53±0.009**	0.12±0.003**
Group II Paracetamol control	750mg/kg	162.91±2.623	142.44±2.167	277.13±3.689	5.33±0.069	0.94±0.012	0.20±0.004
Group III Silymarin + paracetamol	25mg/kg+ 750mg/kg	89.36±2.293* *	70.43±1.425* *	152.43±2.380**	8.15±0.013**	0.57±0.005**	0.14±0.002**
Group IV 200 mg/kg extract + paracetamol	200mg/kg + 750mg/kg	131.27±2.783 **	108.61±1.860 **	193.01±2.350**	6.64±0.136**	0.78±0.012**	0.18±0.002**
Group V 400 mg/kg Extract+ paracetamol	400mg/kg + 750mg/kg	108.78±2.495 **	89.69±1.189* *	165.72±2.485**	7.50±0.095**	0.64±0.015**	0.16±0.003**

Values are mean ±SEM; n=6 animals in each group;*p<0.05, **P<0.01, ***p<0.001 when compared to paracetamol control group

Table 3. Antioxidant activity of ethanolic extract of *Coccinia grandis* root on paracetamol intoxicated rats

Groups and treatment	Dose(mg/kg)	SOD	CAT	Px	GPx	GSH
Group I (Normal Control)	2 ml/kg	6.19 ± 0.192**	176.76 ± 0.825**	62.02 ± 1.923**	42.65 ± 1.694**	39.12 ± 0.633**
Group II (Paracetamol control)	750 mg/kg	2.79 ± 0.213	60.37 ± 1.260	91.42 ± 1.519	17.76 ± 1.444	16.43 ± 0.547
Group III (Silymarin + paracetamol)	25 mg/kg + 750 mg/kg	5.46 ± 0.161**	157.29 ± 1.084**	70.39 ± 2.144**	35.40 ± 2.428**	31.72 ± 0.692**
Group IV (200 mg/kg extract + Paracetamol)	200 mg/kg + 750 mg/kg	3.73 ± 0.149**	106.50 ± 0.915**	83.06 ± 1.972*	23.06 ± 1.896 ^{ns}	20.49 ± 0.614**
Group V (400 mg/kg extract + paracetamol)	400 mg/kg + 750 mg/kg	4.76 ± 0.168**	148.21 ± 1.048**	77.70 ± 1.491**	29.15 ± 2.137**	27.93 ± 0.762**

Values are mean ±SEM; n=6 animals in each group;*p<0.05, **P<0.01, ***p<0.001 when compared to paracetamol control group
SOD=units/min/mg protein, CAT=μmoles of H₂O₂ consumed/min/mg protein, Px=μmoles/min/mg protein, GPx=μmoles of GSH oxidized/min/mg protein, GSH=μmoles/min/mg protein.

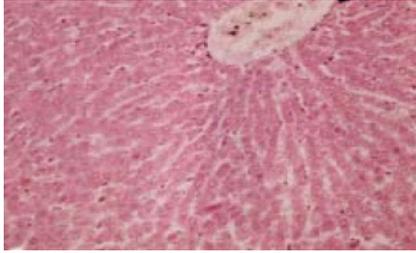


Fig.1. Normal rat liver section.

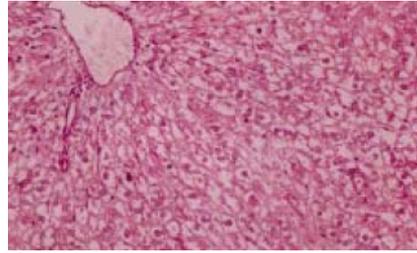


Fig.2. Liver section of rat intoxicated with paracetamol

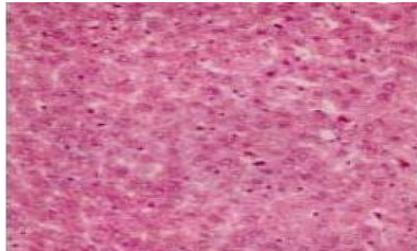


Fig.3. Liver section of rat treated with Silymarin and intoxicated with paracetamol

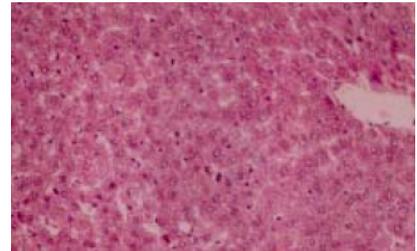


Fig.4. Liver section of rat treated with 200 mg/kg ethanolic extract and intoxicated with paracetamol

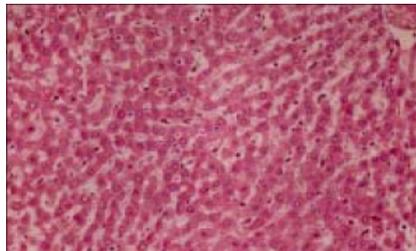


Fig.5. Liver section of rat treated with 400 mg/kg ethanolic extract and intoxicated with paracetamol

HISTOPATHOLOGICAL EXAMINATION

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