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Hepatoprotective Evaluation of *Coccinia Grandis* Fruit Extract against Antitubercular Drug Induced Hepatotoxicity in Albino Wistar Rats

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ABSTRACT: The prepared extracts have been subjected to an preliminary phytochemical assessment, which became discovered via way of means of estimating the overall content material of phenol and standard flavonoids. The identity of numerous markers, particularly oleanolic acid, betulinic acid and chlorogenic acid, became finished via way of means of excessive stress layer chromatography (HPLC). It is documented withinside the literature that oxidative pressure performs a position withinside the pathogenesis of liver harm. Grandis became attributed a hepatoprotective interest with antioxidant effects. With this perspective, the grandis selected for the essential remark became first evaluated for its antioxidant potential via the usage of suitable in vitro antioxidants. This opted for the plant, *H. Coccinia grandis* (L.) Pericarp showed a definitely antioxidant interest, especially ethanol extracts. The extracts have been discovered for acute toxicity and have been properly positioned. Therefore, the leaf extracts had been subjected to in vivo hepatoprotective investigations. In the poisonous-brought on styles of acute liver toxicity (rifampicin), the extracts showed good enough liver safety in comparison to poison manage. The interest of *Coccinia grandis* (L.) Extract ethanol extracts proved to be advanced, even supposing the petroleum ether extract showed a excellent interest in each plant global in comparison to different extracts. This is in all likelihood because of the presence of numerous antioxidant and cytoprotective / hepatoprotective concepts. Based at the discovered acute hepatotoxicity, the extracts of PEFMB, EAFMB and PELAP and EOLAP have been categorized for ethanol brought about non-stop hepatitis, the ethanol extracts of *Coccinia grandis* (L.) Extracts have been categorized as essential existence in comparison to the extracts of petroleum ether of every plant. These extracts have been further examined for his or her anti inflammatory and immunomodulatory residences. In the case of a better dose degree, the plant has been proven to be thoroughly anti inflammatory and immunomodulatory. In the closing degree, cognizances of the isolation of viable phytocomponents have become 2nd nature. The promising effects of in vitro and in vivo studies for the candidate flowers supported us for the isolation of doable phytocomponents from the ethanolic extract of *Coccinia grandis* (L.). This extract became partitioned on silica gel with the solvent combination of various polarities to provide severa fractions. A overall of eight fractions of every bioactive plant extract have been prepared. These fractions have been screened for antioxidant pursuits in vitro. Fractions with suitable antioxidant potential have been decided on for comparable isolation and purification. The column became eluted with solvents of various polarities. Column fractions have been monitored via way of means of TLC and similar fractions have been combined and further processed to provide the isolation of four special compounds. These remote compounds had been characterised via way of means of the use of appropriate spectral techniques that you could use to confirm their structure. Oleanolic acid, catechin and firoxycinnamic acid have been detected withinside the ethanolic extract of *Coccinia grandis* (L.).

I. INTRODUCTION

The liver is the pivotal organ of paramount importance. It plays an essential role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. [1] It has great capacity to synthesize useful principles that regulate internal chemical environment. The spectrum of its functions include: metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly, metabolism of lipids, carbohydrates and proteins, blood coagulation and immunomodulation. [2] Liver disease is a worldwide problem. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Drugs when taken in overdoses and sometimes even when



introduced within therapeutic ranges may injure the organ. Hepatotoxic agents such as paracetamol, antitubercular drugs, CCl₄, excess consumption of alcohol etc. produces hepatotoxicity.

Many hepatotoxicants require metabolic activation, particularly by the liver CYP-450 enzymes, to form reactive, toxic metabolites, which in turn cause liver injury in experimental animals and humans. [3]

The administration of Rifampicin, the most common medication prescribed against tuberculosis, produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antitubercular drugs. These antitubercular drugs induce hepatitis by a multiple step mechanism.

leading to cell death. These bioactive metabolites are produced by a series of enzymes including CYP-450 and induce oxidative stress. CYP2E1 is involved in Rifampicin induced hepatotoxicity in humans and animals. [4,5]

Rifampicin is a potent inducer of CYP-450 system which mediates generation of toxic metabolites of drugs and their covalent binding to hepatic macromolecules. Oxidative stress is the major mechanism of rifampicin induced hepatotoxicity in experimental rats.⁹

(Vitaceae), commonly known as Hajoda. It is a shrub with quadrangular stem. Traditionally it is employed for the treatment of piles, stomachic, tonic, analgesic, anthelmintic, digestible, loss of appetite, constipation, blindness, epileptic fits, chronic ulcers, scurvy, beneficial in the drug fractures of bone and in ascites. [6,7] Phytochemical review reveals the presence of two tetracyclic triterpenoids, β -sitosterol, δ -amyrin, isopentacosanoic acid, flavonoids like quercetin, kaempferol, steroidal principles, beta carotene and vitamin C. [8-10]

II. MATERIALS AND METHODS

- Animal selection
- Housing of the animals
- List of chemicals
- List of instruments used during experiments
- Phytochemical investigations
- Extract used\
- Dose fixation
- Pharmacological screening methods
- Histopathological study

1. **Animal selection:** Albino rats (wistar) of either sex weighing 150-200 g were selected for hepatoprotective activity. They were procured from Venkat Enterprises, New Delhi.
2. **Housing of the animals:** The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C \pm 2°C under 12 hours dark / light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water ad libitum was provided. Ethical clearance for use of the animals was obtained from the institutional animal's ethical committee prior to the beginning of the project work.



List of instruments used during experiments Auto analyser (ERBA Mannheim Chem-5 Plus V2) UV-Visible Spectrophotometer
Electronic water bath Homogenizer (Genei) Hot air oven Micropipettes Micro centrifuge (Genei)

Phytochemical Investigations:

Collection and extraction of *Coccinia grandis* stem

Collection: *Coccinia grandis* stems were collected from the fields of CCS University, Meerut U.P.

Authentication: Whole plant was authenticated by Prof. (Dr.) Vijai Malik (Department of Botany) from CCS University of Meerut. India, a pattern replica of the herbarium has been saved within side the laboratory for destiny reference.

Qualitative Chemical Analysis [11,12]:

The ethanol extract of *Coccinia grandis* is subjected to qualitative chemical analysis to detect the presence of various phytoconstituents.

The various test is performed like test for glycoside, alkaloids, tannin, saponins e.t.c

Table 1 Qualitative chemical analysis of ethanol extract of *Coccinia grandis*.

Test for Phyto-constituents	Ethanol extract
Alkaloids	+
Carbohydrates	+
Glycosides	+
Flavonoids	+
Sterols	-
Triterpenoids	+
Proteins and Amino acids	-
Tannins	-
Saponins	-

Key: “+” = Present; “-” = Absent

Measurement of β -carotene in ethanol extract of CQ by HPLC analysis⁶⁸:Preparation of extract sample:

Transfer sample about 100 mg (ethanolic extract of CQ) to a 100 ml calibrated volumetric flask. Add about 60 ml ethanol (HPLC grade) to dissolve and sonicate for about 5 mins, dilute to volume with ethanol and mix.

Pharmacological Screening:

Evaluation of Hepatoprotective Activity in Rifampicin-Induced Hepatotoxicity⁴³The animals were divided into IV groups of six animals each. All the drugs were prepared in 1 % CMC for oral administration. Animals were sacrificed 24 h after the last dose by mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters. The blood so collected was centrifuged to get clear serum. Liver was dissected out and 30% of liver homogenized with phosphate buffer. After centrifugation, the clear supernatant was used for assay of various endogenous antioxidant activities by standard method. Liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Evaluation of Hepatoprotective Activity in Rifampicin-Induced Hepatotoxicity [14]

All the drugs were prepared in 1 % CMC for oral administration.

Animals were sacrificed 24 h after the last dose by mild ether anesthesia. Blood samples were collected for evaluating the serum biochemical parameters. The blood so collected was centrifuged to get clear serum. Liver was dissected out and 30% of liver homogenized with phosphate buffer. After centrifugation, the clear supernatant was used for assay of

various endogenous antioxidant activities by standard method. Liver was dissected out, blotted off blood.

Methods for estimation of biomarkers:

1. Estimation of Serum Alanine Transaminase (ALT) [13]:

Alanine Transaminase (ALT) is located in cytosol of liver cell. During liver cell inflammation and break down of liver cells, they are released into circulation due to increased permeability of cell membrane. Hence determination of ALT is an index of the extent of liver damage.

Alanine amino transferase catalyses the transfer of amino group from alanine to α -keto glutarate (α -KG) with the formation of Glutamate and Pyruvate. The liberated pyruvate reduced to lactate, by lactate dehydrogenase (LDH) in the same reaction an equivalent amount of NADH is oxidized to NAD.

Estimation of Serum Aspartate amino Transferase (AST)⁷⁰:

AST is located on the cytosol of liver. In addition, it is also found in the mitochondria and in many tissues of heart, liver, skeletal muscle and kidney. The hepatic cell damage leads to increased levels of AST in blood serum.

Serum Aspartate amino Transferase (AST) is a tissue enzyme that catalyses the exchange of amino and keto groups between alpha amino acids and alpha keto acids. AST is widely distributed in tissue principally cardiac, hepatic muscle and

kidney; injury to these tissues results in increase in the AST enzyme level into general circulation. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma and viral hepatitis will also increases serum AST levels.

Estimation of Serum Alkaline Phosphatase (ALP) [15]:

Clinical Significance

ALP is present in high concentrations in liver, bone, placenta intestine and certain tumors.

Principle:

Under alkaline condition, colorless p-nitrophenol is converted to 4- nitrophenoxide, which develops a very intense yellow color. Its intensity is proportional to the activity of alkaline phosphatase in the sample.

Estimation of Serum Bilirubin [16]:

Principle

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to bilirubin concentration. Direct Bilirubin, being water soluble directly reacts in acidic medium. However indirect or unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to Direct Bilirubin.

In-Vivo Antioxidant Activity

A. Reagents for estimation of reduced glutathione.

- TCA 10% w/v: 10 g TCA in 100 ml distilled water
- 0.2 M potassium di-hydrogen orthophosphate: 2.721 g in 100 ml distilled Water
- 0.2 M NaOH: 0.8 g in 100 ml of distilled water.
- 0.2 M Phosphate buffer (pH 8): 50 ml potassium di-hydrogen orthophosphate (0.2M) + 46.1 ml NaOH (0.2M), volume made up to 200 ml and pH adjusted to 8.
- DTNB solution: 0.4 mg DTNB in 1 ml 1% sodium citrate solution.
- 1% Sodium citrate: 1 g sodium citrate in 100 ml of distilled water.

B. Reagents for estimation of lipid peroxidation

- HCl (0.25 N): 2.125 ml of concentrated HCl.
- TCA (15%): Take 15 g of TCA.
- TBA (0.375%): Take 375 mg of TBA
- TCA-TBA-HCl reagent: Dissolve the above chemicals and make up the volume to 100 ml.

C. Reagents for estimation of superoxide dismutase

- Sodium Pyrophosphate buffer (0.052 M, pH 8.3): 2.31 g in 100 ml of distilled water adjusted to pH 8.3.
- 186 μ M Phenazine metha sulphate (PMS): 5.7 mg in 100 ml of distilled water.
- 300 μ M nitro blue tetrazolium (NBT): 2.45 mg in 1 ml of distilled water.
- 780 μ M NADH: 5.53 mg in 1 ml distilled water.
- Glacial acetic acid
- n-butanol

D. Reagents for estimation of catalase

- 0.2 M phosphate buffer (pH 8.0): 50 ml potassium di-hydrogen orthophosphate (0.2M) + 46.1 ml NaOH (0.2M), volume made up to 200 ml and pH adjusted to 8.
- 2 M Hydrogen peroxide: 22.45 ml of H₂O₂, volume made upto 100 ml.
- 5% potassium dichromate: 5 gm in 100 ml of distilled water.
- Dichromate-acetic acid reagent- 5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio.
- Methods for estimation of oxidative stress:

A. Estimation of reduced glutathione (GSH) [17]:

1 g of scrapped tissue of the liver is homogenized with 10 ml of 10% TCA in ice cold condition and centrifuged at 3000 rpm for 10 minutes. The supernatant is separated. 2 ml of 0.2 M phosphate buffer of pH 8 is added to 0.5 ml of supernatant. Add 0.2 ml of DTNB solution and take the absorbance immediately at 412 nm. The amount of glutathione is calculated using the absorption $13,600\text{M}^{-1}\text{cm}^{-1}$.

B. Estimation of lipid peroxidation (MDA) [18]:

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust. 1.0 ml of the sample extract was added with 2.0 ml of the TCA-TBA- HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10000 rpm to remove the precipitate. The absorbance was read at 535 nm and malondialdehyde concentration of the sample was calculated using extinction coefficient of $1.56 \times 10^5\text{M}^{-1}\text{cm}^{-1}$.

C. Estimation of superoxide dismutase (SOD) [19]:

The estimation will be done as per *Kakkar et al.* The scrapped tissue of the liver was homogenized by using a Potter-Elvehjem glass homogenizer for 30 sec either in ice cold 0.9% saline (5%). Briefly, to 0.4 ml of the homogenate was added

1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of 186 μ M of phenazine methasulphate (PMS), 0.3 ml of 300 μ M nitro blue tetrazolium (NBT) and

0.8 ml of distilled water was added to make up the volume up to 3 ml. The reaction was started by addition of 0.2 ml of NADH (780 μ M). It was incubated at 30°C for 60 sec. The reaction was stopped by addition of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer will be taken out. Colour intensity of the chromogen was measured against butanol at 560 nm using spectrophotometer. A system devoid of enzyme activity was defined as enzyme concentration required decreasing the rate of reaction by 50% in one min under the assay conditions. The results are expressed as units (U) of SOD activity/mg protein.

D. Estimation of Catalase (CAT) [20]:

The scrapped tissue of the liver was weighed and 30% homogenate was prepared with 0.2 M phosphate buffer pH 8.0. After centrifugation, the clear supernatant was used for assay of enzyme activity. The reaction mixture (1.5 ml, vol.) contained 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of tissue homogenate (supernatant) and 0.4 ml of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5%) potassium dichromate and glacial acetic acid was mixed in 1:3 ratios. Colour intensity was measured colorimetrically at 620 nm and expressed as μ moles of H₂O₂ consumed /min/mg protein as described by Sinha.



III. RESULTS

Tables f Hepatoprotective Activity (Rifampicin Model)

Table 2: Effect of ethanol extract of *Coccinia grandis* on liver weight (g /100 g)

Animal No.	Normal	Rifampicin	Ethanol extract 400 mg/kg	Silymarin group 50mg/kg
1	2.57	3.31	2.80	3.05
2	2.18	3.80	3.65	2.87
3	2.49	3.16	3.05	2.60
4	2.39	4.01	3.23	3.22
5	2.34	3.72	3.47	2.75
6	2.37	3.96	2.92	2.98
Mean ± SEM	2.39 ± 0.05	3.66 ± 0.14 ^b	3.18 ± 0.14*	2.91 ± 0.09 ***

Values are the Mean ± S.E.M., n=6 animals in each group

^ap< 0.01, ^bp<0.001 when compared with Control group.

*p< 0.05, **p< 0.01, ***p<0.001 when compared with Rifampicin group.

Graph 2: Showing AST level in various groups

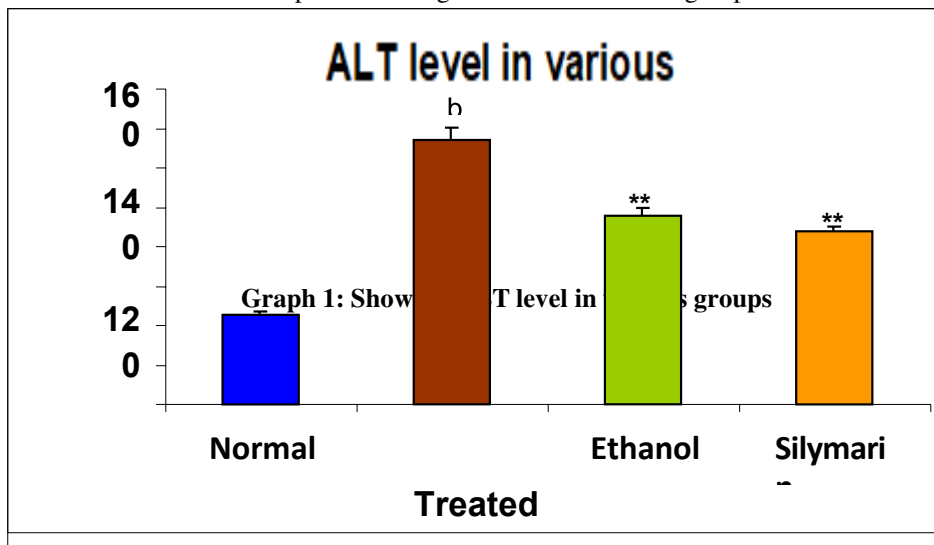
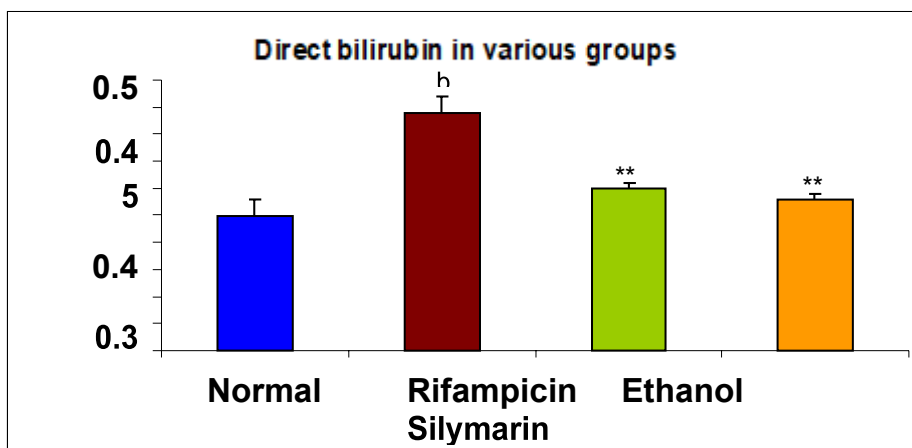




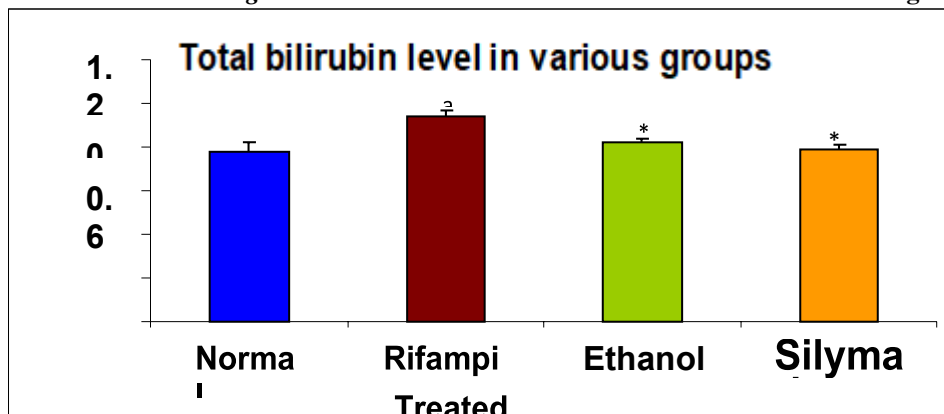
Table 3: Effect of ethanol extract of *Coccinia grandis* on enzyme ALP level in blood serum in various groups

Animal No.	Normal	Rifampicin	Ethanol extract 400 mg/kg	Silymarin group 50 mg/kg
1	122.00	273.75	194.50	168.30
2	136.24	196.09	194.01	156.31
3	129.26	308.58	252.50	160.20
4	139.34	201.81	202.20	137.13
5	126.78	254.77	185.60	147.98
6	145.76	269.97	190.44	153.70
Mean ± SEM	133.2 ± 3.60	250.8 ± 17.93	203.2 ± 10.11	153.9 ± 4.35



Graph 2: Showing Total bilirubin level in various groups

Effect of ethanol extract of *Coccinia grandis* on Direct bilirubin level in blood serum in various groups



Graph 3: Showing Direct bilirubin levels in various groups

HISTOPATHOLOGICAL STUDIES

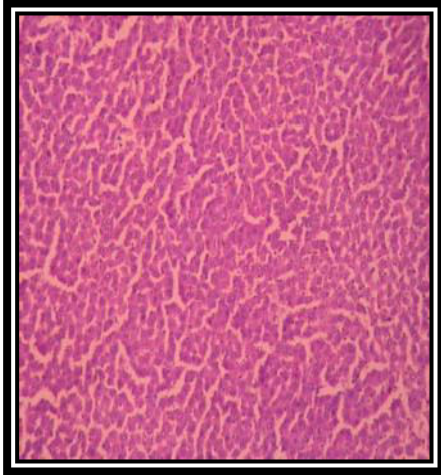


Fig 1: Normal liver

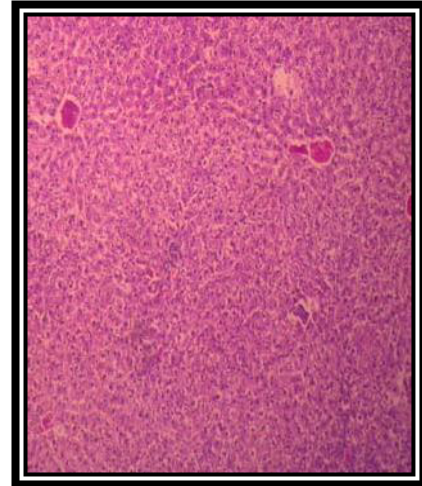


Fig 2: Rifampicin control (Inflammation, sinus congestion)

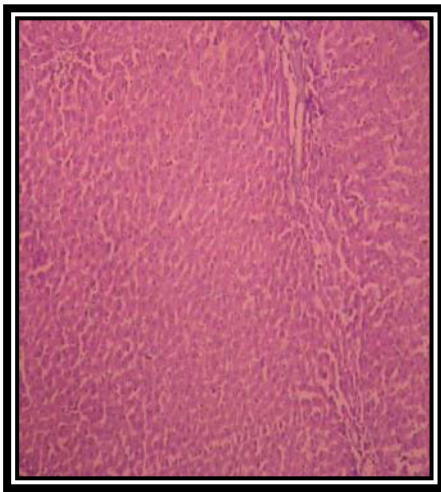


Fig 3: Ethanol extract of CQ + Rifampicin (Regeneration of hepatocytes)

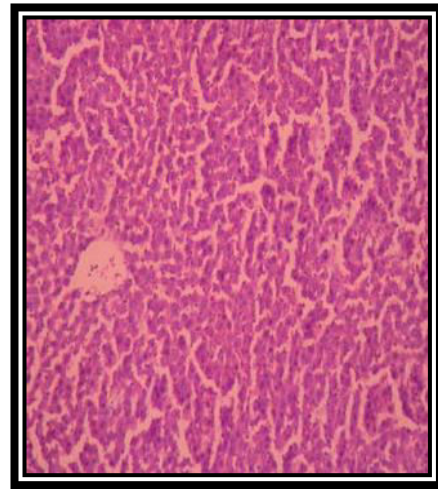


Fig 4: Silymarin + Rifampicin (Regeneration of hepatocytes)



**TABLES OF HEPATOPROTECTIVE ACTIVITY
(Rifampicin model)**

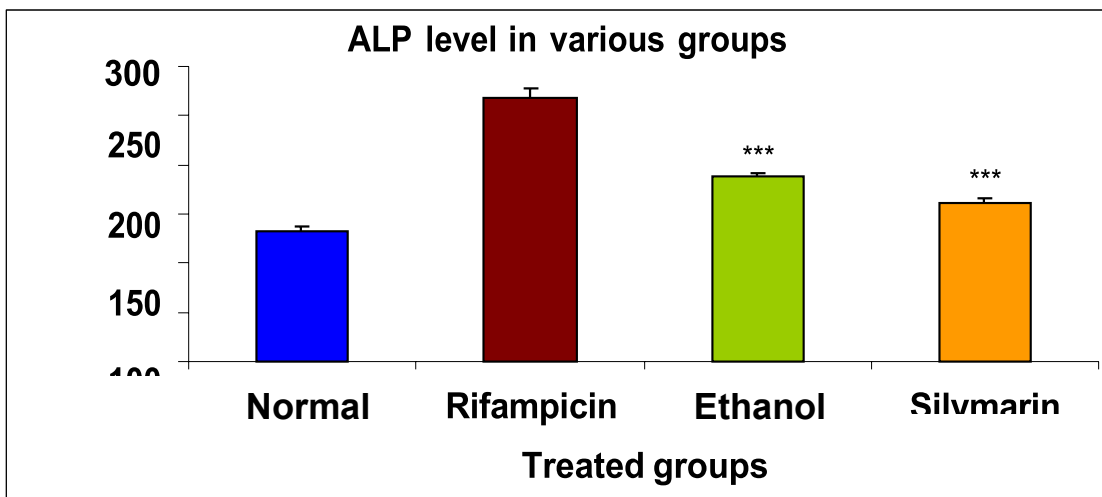
Table 12: Effect of ethanol extract of *Coccinia grandis* on liver weight (g / 100 g)

Animal No.	Normal	Rifampicin	Ethanol extract + Rifampicin	Silymarin + Rifampicin
1	2.57	3.74	2.78	2.92
2	2.18	3.59	3.35	2.51
3	2.49	3.39	3.19	2.63
4	2.39	3.74	3.03	2.70
5	2.34	4.02	2.75	2.58
6	2.37	3.97	2.88	2.65
Mean ± SEM	2.39 ± 0.05	3.74 ± 0.10 ^b	2.99 ± 0.10 ***	2.66 ± 0.06 ***

Values are the Mean ± S.E.M., n=6 animals in each group
 *p< 0.05, **p< 0.01, ***p<0.001 when compared with Rifampicin group.
^ap< 0.01, ^bp<0.001 when compared with Control group.

Table 15: Effect of ethanol extract of *Coccinia grandis* on enzyme ALP level in blood serum in various groups

Animal No.	Normal	Rifampicin	Ethanol extract 400 mg/kg	Silymarin group 50 mg/kg
1	122.00	246.33	178.56	168.12
2	136.24	251.57	203.76	151.25
3	129.26	289.25	192.34	167.87
4	139.34	241.71	185.55	154.70
5	126.78	301.47	189.23	178.54
6	145.76	275.76	175.65	149.67
Mean ± SEM	133.2 ± 3.60	267.7 ± 10.10	187.5 ± 4.14	161.7 ± 4.71



Graph 4: Showing ALP level in various groups

HISTOPATHOLOGICAL STUDIES

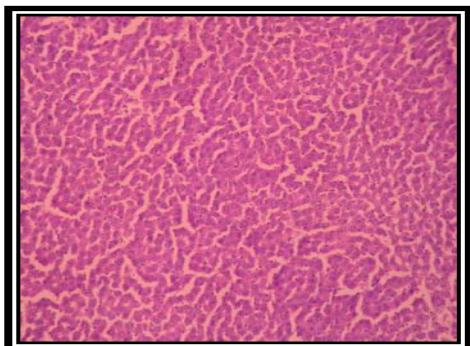


Fig 5: Normal liver

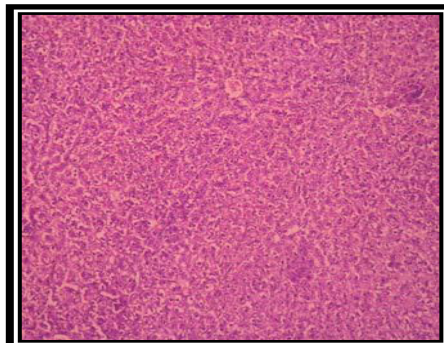


Fig 6: Rifampicin control

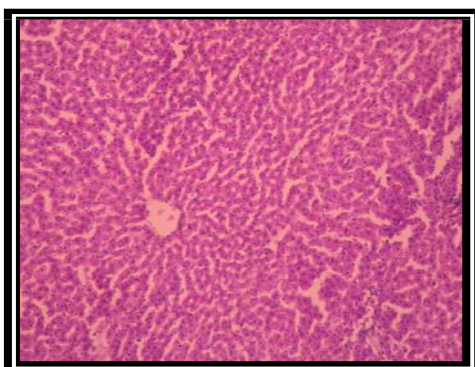


Fig6: Ethanol extract of CQ + Rifampicin

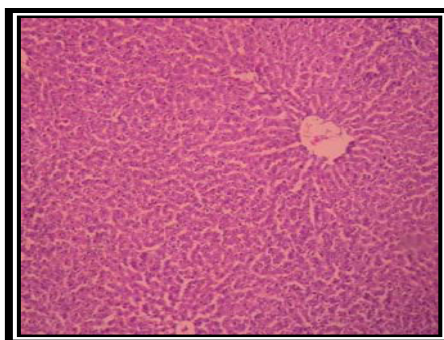


Fig 7: Silymarin + Rifampicin (Regeneration of hepatocytes)

IV. CONCLUSION

It can be concluded that the hepatoprotective effect of *Coccinia grandis* is probably due to the increase of the activities of the antioxidant enzymes and non- enzymatic antioxidants or to a counteraction of the free radicals by the presence of the electrophilic constituents present in the ethanol extract of *Coccinia grandis* or to an activated conjugation of antitubercular drugs with GSH in liver.

The hepatoprotective and antioxidant activity of *Coccinia grandis* extract was confirmed by biochemical and histopathological studies.

Hence, the ethanol extract of *Coccinia grandis* possess hepatoprotective and antioxidant activity. Further studies are needed to confirm for better understanding of the mechanism action and its anti-hepatitis activity.

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