Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2015; 7(4): 239-245

ISSN- 0975 1556

Research Article

Hepatoprotective Activity of *Canthium dicoccum* In Isoniazid and Rifampicin Induced Hepatotoxicity

Bhaargavi Vuyyuri[1*], Bhagyalakshmi A[2], Rajyalakshmi R[3], Jagadeeswari S[2]

[1]*Maharajah`s college of pharmacy, Phool Baugh, Vizianagaram, Andhra Pradesh, India*
[2]*Department of Pathology, Andhra medical college / King George hospital, Visakhapatnam, Andhra Pradesh, India*
[3]*Department of Pathology, Rangaraya Medical College / Government general hospital, Kakinada, Andhra Pradesh, India*

Available Online: 15th June, 2015

ABSTRACT

Objectives: The present study was designed to study the hepatoprotective activity of ethanolic extract of *Canthium dicoccum* (ECD) whole plant against the standard Silymarin in isoniazid (INH) and rifampicin (RIF) induced hepatotoxicity. Methods: ECD was administered orally for 21 days (150 and 300mg/kg body weight). Hepatotoxicity was induced by oral administration of INH (50mg/kg) and RIF (100mg/kg) on every 72hrs for a period of 21days. Silymarin (50mg/kg p.o) was used as standard. Liver function parameters were evaluated to test the activity of *Canthium dicoccum*. Sleeping time was measured by an intraperitonial injection of 40mg/kg Thiopental Sodium. Results: INH and RIF insult significantly increased the serum levels of SGPT, SGOT, ALP, T-CHO, T-BIL and significantly decreased serum T-PRO when compared to normal control group at $P<0.05$. Treatment with ECD significantly attenuated the INH and RIF induced elevated serum levels of SGPT, SGOT, ALP, and T-CHO. Also the plant extract significantly improved the serum levels of T-PRO when compared to INH and RIF induced toxic group. The plant extract at 300mg/kg dose level proved to be comparable to the standard silymarin and is considered to be more active than silymarin in reducing the serum elevated SGOT levels and improving the serum T-PRO levels.Conclusions: It can be thus concluded that Ethanolic Extract of *Canthium dicoccum* whole plant exhibited hepatoprotective activity against Isoniazid and Rifampicin induced hepatotoxicity.

Keywords: Hepatoprotective activity, *Canthium dicoccum*, Silymarin, Isoniazid, Rifampicin

INTRODUCTION

Canthium dicoccum the Ceylon boxwood also known as nalla balusu in telugu, belongs to the family Rubiaceae[1]. In India its bark is used for fever, and decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain[2,3]. The plant is proved for its anti-inflammatory activity[1], antidiabetic and nephroprotective activity[4]. Bark contains sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin[5]. Leaves contain ursolic acid, quercetin, rutin, 7-O-(6-O-benzoyl-β-glucopyranosyl)-rutin, spathulenol (20.76 %), caryophyllene oxide (19.25 %), cedren-13-ol (10.62 %) and ledene oxide (5.24 %)[2]. As liver being the central organ of drug metabolism hepatotoxicity is the major adverse drug reaction with many of the drugs, and it is a major health problem which is responsible for 50% of all acute liver failures[6]. Modern medicines have very little to offer for alleviation of various hepatic diseases and it is chiefly the plant based preparations which are used for their treatment (Orhan et al., 2007)[7], because the available synthetic drugs that are used to treat liver disorders further damage the liver cells[8]. The antioxidant property of the herbal plants plays an important role in inhibiting and scavenging the free radicals and thus provides protection against infections and degenerative diseases. In order to investigate the INH and RIF induced hepatotoxicity, albino rats were used to assess the hepatoprotective activity of ECD against the standard silymarin. Rats show a similar genetically determined acetyltransferase activity as in humans and are more sensitive to INH induced hepatotoxicity due to a high amidase activity, which results in the release of large amount of acetylhydrazine, which induces hepatotoxicity[9]. As there are no previous reports on the hepatoprotective activity, the present work is aimed at studying the hepatoprotective activity of ethanolic extract of *Canthium dicoccum* whole plant in INH and RIF induced hepatotoxicity.

MATERIALS AND METHOD

Chemicals

Ethanol, diethylether (rankem ltd, New Delhi), Silymarin (micro labs ltd, Bangalore), Isoniazid (macleods pharmaceutical ltd, Mumbai), Rifampicin (lupin ltd, Aurangabad), Thiosal i.v (neon laboratory ltd, Mumbai). All the drugs were obtained from local market.

Collection of Plant Material and Preparation of Extract

The whole plant *Canthium dicoccum* was collected from the Chittoor district of Andhra Pradesh. The plant was identified, confirmed and authenticated by botanist Dr. K. Madhava Chetty, Asst. prof, department of Botony, Sri

**Author for Correspondence*

Venkateswara University, Tirupati. The dried material was then pulverized into coarse powder by a mechanical grinder and extracted in a soxhlet apparatus at 50°C with ethanol.

Animals

All the animals which were used in this study were taken care of ethical consideration, with an approval from the institutional ethical committee, SICRA preclinical lab, Andhra Pradesh (registration number: 769/2011/ CPCSEA).

Acute toxicity study

The acute toxicity of ECD was determined according to OECD guidelines (guideline 423, adopted on 17th December 2001). Rats were fasted overnight and were randomly divided into 4 groups (A-D) of six rats in each. Animals were observed individually after dosing once during the first 30 minutes, 4hrs, 24 hours, and then daily thereafter, for a total of 14 days.

Group A: 10 ml/kg p.o distilled water
Group B: 300 mg/kg p.o of ECD
Group C: 1000 mg/kg p.o of ECD
Group D: 2000 mg/kg p.o of ECD

Repeated dose toxicity study

The repeated dose toxicity of ECD was determined according to OECD guidelines (guideline 407, adopted on 27th July 1995). Rats were randomly divided into 4 groups (A-D) of six rats per group. All animals were treated (daily, single dose) for a period of 14 days. After dosing all animals were observed for toxic signs. Hematological, biochemical and histopathological analysis was carried out.

Group A: orally 10 ml/ kg distilled water
Group B: orally 100 mg/kg ECD
Group C: orally 250 mg/kg ECD
Group D: orally 500 mg/kg ECD

Hepatoprotective activity

Animals

Rats were randomly divided into 5 groups (A-E) with six in each and maintained under standard conditions (temp 23±2°C, and 12 hours light dark cycle), and fed with standard diet and water ad libitum during the study period.

Method

All the preparations were made using 2% tween 80.

Group A: Received standard diet and 10ml/kg vehicle for 21days
Group B: Received INH and RIF (50mg/kg and 100mg/Kg p.o) at every 72hrs till 21days
Group C: Received the standard silymarin 50 mg/kg p.o. for 21 days and simultaneously administered INH & RIF (50 & 100 mg/kg p.o.) for every 72 h.
Group D: Received ECD 150 mg/kg p.o for 21 days and simultaneously administered INH & RIF (50mg/kg & 100 mg/kg p.o.) for every 72 h.
Group E: Received ECD 300 mg/kg p.o. for 21 days and simultaneously administered INH & RIF (50mg/kg & 100 mg/kg p.o.) for every 72 h.

Thiopental sleeping time

Thiopental sodium (40 mg/kg, i.p) was injected and the sleeping time was recorded immediately in all the animals on the 21st day 1hr after the dose administration.

% Hepatoprotection = {1- (treated- normal control/toxicant-normal control)} × 100

Biochemical analysis

The Serum was analyzed for total cholesterol, SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase), total proteins, alkaline phosphatase (ALP), and total bilirubin using biochemical kits.

Histopathological studies

The liver was excised quickly and fixed in 10% formalin and stained with Hematoxylin and eosin and then observed under the microscope for pathological changes.

RESULTS

Results are expressed as mean ± SEM. Data was analysed using the one-way Analysis of Variance (ANOVA) followed by the Dunnette`s test using graphpad prism software. The P value < 0.05 was considered significant.

Acute toxicity study

No toxic signs were observed at all the three test doses, except for the first 30min and 4h of drug administration diarrhoea was observed in group D i.e. 2000mg/kg dose level. All the animals survived at the end of the experiment i.e. 14days at all the three dose levels, indicating the cut off LD_{50} to be greater than 2000mg/kg.

Repeated dose toxicity study

There was a slight reduction in hemoglobin and W.B.C count at all the three dose levels but the difference is not statistically significant at P<0.05 (table 1). There was an increase in SGOT level at high dose and increase in SGPT level for both 250mg/kg and 500mg/kg dose level, and increase in T-CHO at high dose level, but these increments were not statistically different from group A at P<0.05 (Table 2). No mortality was observed at any dose level indicating the three doses i.e. 100mg/kg, 250mg/kg and 500mg/kg to be safe. There were no significant changes in the body weight of rats that survived at the end of the experiment (fig 1). It was observed that there was slight enlargement in weight of the kidneys in group D but was statistically not significant at P<0.05 compared to group A (table 3).

Histopathology of organs in the repeated dose toxicity study (fig 2)

Group A (normal control) = liver section shows structure of liver with normal lobular architecture with prominent nucleus and mild congestive sinusoids. Kidney section shows normal structure of kidney with corticomedullary differentiation and mild congested blood vessels. Spleen section shows structure of spleen with normal architecture.

Group B (100mg/kg ECD) = liver section shows structure of liver with normal lobular architecture with prominent nucleus. Kidney section shows normal structure of kidney with normal glomeruli and tubules. Spleen section shows structure of spleen with normal architecture.

Group C (250mg/kg ECD) = liver section shows structure of liver with normal lobular architecture with prominent nucleus. Kidney section shows normal structure of kidney with normal glomeruli and tubules. Spleen section shows structure of spleen with congested blood vessels.

Table 1: Effect of ECD on haematological parameters of rats in the repeated dose toxicity study (n=6)

Groups	Dose (mg/kg p.o)	Haemoglobin (g/dl)	R.B.C (10^6cu.mm)	W.B.C (10^3cu.mm)
A	10 ml/kg vehicle	14.13±0.398	8.442±0.404	8.847±0.343
B	100 mg/kg	13.93±0.084	8.538±0.372	8.122±0.493
C	250 mg/kg	13.67±0.412	8.288±0.332	8.363±0.352
D	500 mg/kg	13.7±0.562	8.428±0.342	8.458±0.288

Values are expressed as mean±SEM (Standard Error Mean)

Table 2: Effect of ECD on liver function test in the repeated dose toxicity study (n=6)

Groups	Dose (mg/kg p.o)	SGOT (IU/L)	SGPT (IU/L)	T-PRO (g/dl)	T-CHO (mg/dl)
A	10ml/kg vehicle	60.38±4.14	46.77±3.83	7.3±0.31	65.23±2.75
B	100 mg/kg	64.76±4.09	55.5±4.66	7.33±0.36	65.83±2.86
C	250 mg/kg	67.31±4.20	53.9±3.86	6.95±0.26	71.65±2.79
D	500 mg/kg	68.45±2.78	53.04±2.77	6.93±0.23	68.58±4.55

Values are expressed as mean±SEM (Standard Error Mean)

Table 3: Effect of ECD on organ weights in the repeated dose toxicity study

Groups	Dose (mg/kg p.o)	Liver (g/100g)	Spleen (g/100g)	Right kidney (g/100g)	Left kidney (g/100g)
A	10ml/kg vehicle	4.05±0.10	0.396±0.04	0.518±0.06	0.491±0.04
B	100 mg/kg	4±0.14	0.405±0.05	0.515±0.01	0.486±0.05
C	250 mg/kg	4.167±0.1	0.403±0.06	0.516±0.05	0.468±0.08
D	500 mg/kg	3.95±0.09	0.395±0.08	0.525±0.05	0.503±0.04

Values are expressed as mean±SEM (Standard Error Mean)

Table 4: Effect of ECD on liver function tests (n=6)

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	T-PRO (g/dl)	T-BIL (mg/dl)	T-CHO (mg/dl)	Liver weight (g/100g)	% Increase liver weight
A	58.4±2.6^{a}	47.15±2.3^{b}	114.6±7.4^{c}	7.338±0.3^{b}	0.396±0.0^{b}	51.71±2.4^{b}	3.63±0.07**	-
B	185.4±5.7	161.2±10.0	218.8±11.4	3.633±0.6	1.273±0.1	81.36±4.1	4.833±0.04	33.1%
C	142±2.3^{c}	73.86±6.7^{c}	136.7±13.5^{c}	7.1±0.2^{d}	0.76±0.0^{c}	54.02±2.2^{c}	3.82±0.09*	5.2%
D	154.7±4.0^{d}	116.9±7.5^{d}	182.7±4.2^{d}	6.433±0.4	1.072±0.0	69.39±4.5	4.257±0.13	17.2%
E	133.4±3.8^{c}	87.77±7.4^{c}	157.1±13.2^{d}	7.367±0.2^{d}	0.89±0.0^{d}	56.61±3.3^{c}	3.88±0.09*	6.8%

Values are expressed as mean±SEM (Standard Error Mean); $^{d}P < 0.05$ indicating significant, $^{c}P < 0.01$ indicating very significant, $^{b}P < 0.001$ indicating extremely significant and $^{a}P < 0.001$ indicating extremely significant, when compared to group B. A=normal control, B=toxicant control, C=standard, D= test low dose, E=test high dose, $^{*}P < 0.05$ indicating significant and $^{**}P < 0.01$ indicating very significant, when compared to group B

Table 5: Effect of ECD on thiopental induces sleeping time of isoniazid and rifampicin intoxicated rats (n=6)

Groups	Sleeping time (min)	% Hepatoprotection
A	28.5±3.63****	-
B	173.5±5.79	-
C	100.8±4.57***	50.1%
D	134±2.46**	27.2%
E	90.5±3.80****	57.2%

Values are expressed as mean±SEM (Standard Error Mean); $^{**}P < 0.01$ indicating very significant and $^{***}P < 0.001$ indicating extremely significant and when compared to group B; % hepatoprotection = {1- (treated- normal control/toxicant-normal control)} × 100

Group D (500mg/kg ECD) = liver section shows structure of liver with normal lobular architecture with prominent nucleus. Kidney section shows normal structure of kidney with normal glomeruli and tubules with mild congested blood vessels. Spleen section shows structure of spleen with normal architecture.

Hepatoprotective activity

The results of the experiment indicate that the high dose 300mg/kg ECD is comparable to 50mg/kg Silymarin in reducing the serum elevated levels of SGPT, ALP, and T-CHO (table 4). The extract is considered to be more active than Silymarin in reducing the serum elevated SGOT levels and improving the serum total protein levels.

Effect of ECD pretreatment against isoniazid and rifampicin intoxication on serum marker Enzymes

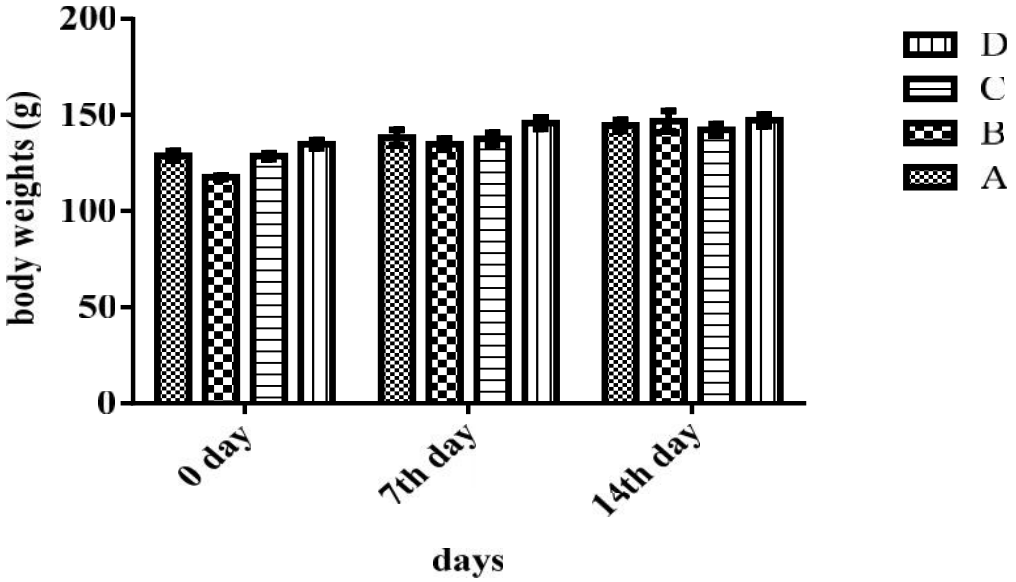

Fig 1: Effect of ECD on body weights of rats in the repeated dose toxicity study

- The ranking for the ability to reduce the elevated SGOT can be given as:
  ECD 300mg/kg > silymarin > ECD 150mg/kg
- The ranking for the ability to reduce the elevated SGPT can be given as:
  Silymarin > ECD 300mg/kg > ECD 150mg/kg
- The ranking for the ability to reduce the elevated ALP can be given as:
  Silymarin > ECD 300mg/kg > ECD 150mg/kg
- The ranking for the ability to increase the serum total protein level can be given as:
  ECD 300mg/kg > Silymarin > ECD 150mg/kg
- The ranking for the ability to reduce the elevated T-BIL can be given as:
  Silymarin > ECD 300mg/kg > ECD 150mg/kg
- The ranking for the ability to reduce the elevated T-CHO can be given as:
  Silymarin > ECD 300mg/kg > ECD 150mg/kg

Histopathological analysis of liver after 21 days (fig 3)

Group A (normal control) = Section shows structure of liver with normal lobular architecture having prominent nucleus with few inflammatory infiltration, which could be due to pre-existence inflammation.

Group B (toxic control) = Section shows structure of liver with prominent nucleus with necrosis, severe inflammatory infiltration.

Group C (standard) = Section shows structure of liver with normal lobular architecture having prominent nucleus with mild degeneration. There are only a few inflammatory cells when compared to toxic control group.

Group D (low dose ECD) = Section shows moderate inflammation with degeneration.

Group E (high dose ECD) = Section shows liver with normal lobular architecture having prominent nucleus. Very few inflammatory cells are observed when compared to toxic control group.

Hepatoprotective activity of ECD was also assessed by measuring sleeping time induced by thiopental sodium (40mg/kg i.p). Thiopental sodium a lipid soluble ultra short acting barbiturate (20min) binds to plasma proteins and is not easily eliminated by kidneys. Hence it must be biotransformed by the liver, and eliminated by the kidney. So liver damage by the toxins will affect the biotransformation of thiopental and prolong its anaesthetic time. INH and RIF caused significant increase in duration of sleep in group B. While treatment with silymarin, 150mg/kg and 300mg/kg ECD significantly reduced duration of sleep by 50.1%, 27.2% and 57.2% respectively in comparison to group B (table 5). From the results it is clear that 300mg/kg ECD offered more hepatoprotection when compared to the standard silymarin. The ranking for the ability to reduce the increased duration of sleep can be given as:
ECD 300mg/kg > Silymarin > ECD 150mg/kg.

DISCUSSIONS

Subacute or chronic treatment with INH has been reported to induce hepatotoxicity in man (Mitchell JR et al, 1976)[10]. The metabolite of INH, hydrazine plays an important role in INH induced hepatotoxicity[11.] Biotransformation of RIF into its active metabolite, 25-desacetyl rifampicin reduces the drug metabolizing enzymes and specifically binds to RNA polymerase which inhibits the nucleic acid and protein synthesis responsible for hepatotoxicity (Saraswathy S et al, 2001)[12]. RIF a powerful inducer of mixed function oxidase system, increases the hepatotoxicity of INH by enhancing the production of toxic metabolites from acetylhydrazine (Ellard et al, Kalra et al., 2007)[13.] ECD contains quercetin and ursolic acid which are proved to be hepatoprotective constituents. Quercetin, by multiple mechanisms demonstrates hepatoprotective effect on liver-injury by increasing the antioxidant system activities against oxidative stress and lowering the expressions of proinflammation cytokines[14]. Ursolic acid improves the antioxidant status by decreasing the levels of lipid

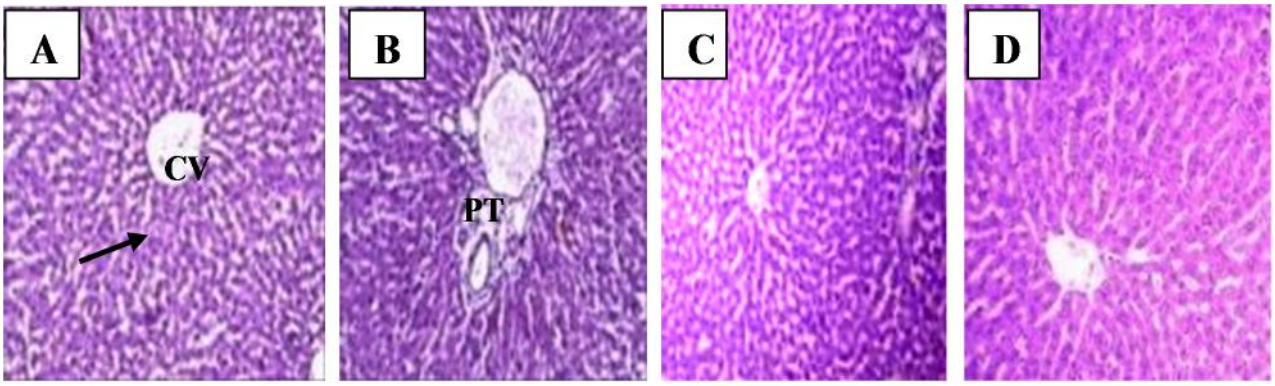

Fig 2a: histopathological studies in the repeated dose toxicity studies (rat liver)
A = normal control, B = 100mg/kg ECD, C = 250mg/kg ECD, D = 500mg/kg ECD, Black arrow = congested sinusoids, CV = Central vein, PT = Portal triad

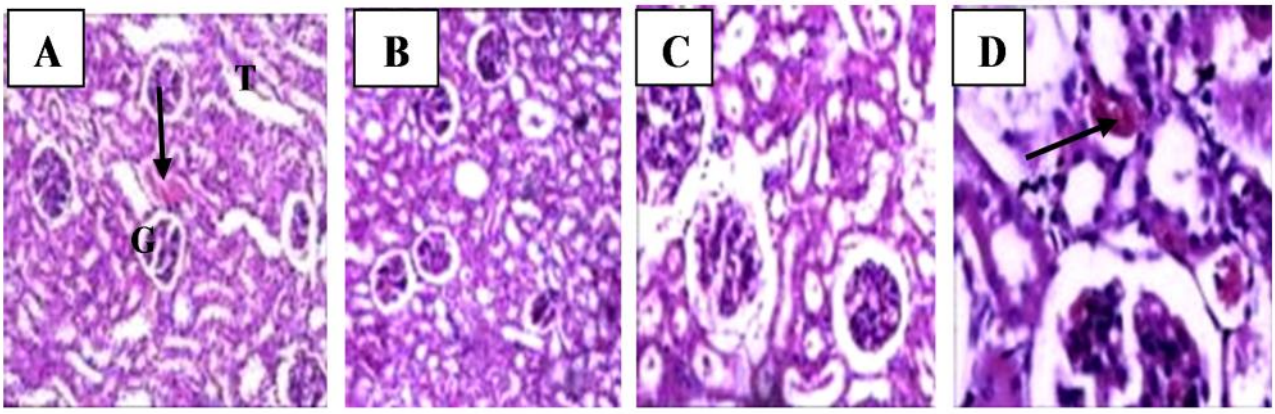

Fig 2b: histopathological studies in the repeated dose toxicity studies (rat kidney)
A = normal control, B = 100mg/kg ECD, C = 250mg/kg ECD, D = 500mg/kg ECD
Black arrow = congested blood vessel, T = tubule, G= glomerulus

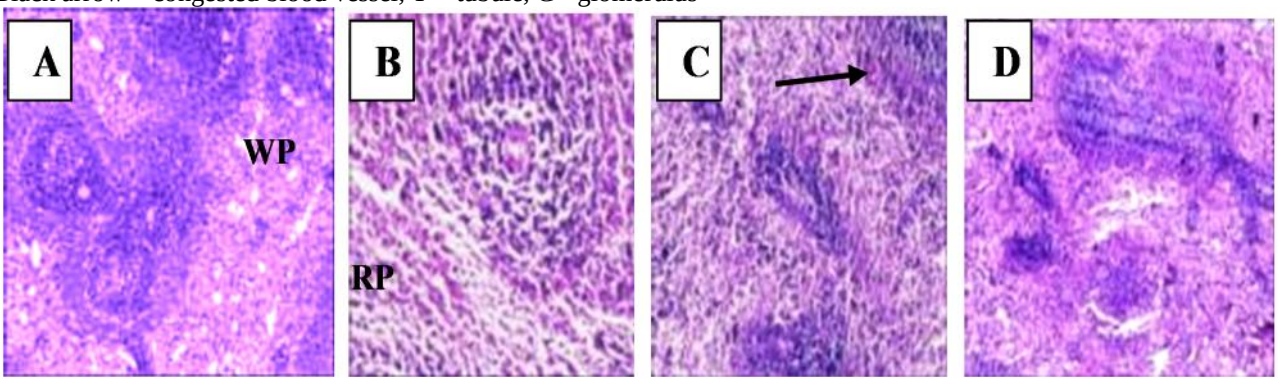

Fig 2b: histopathological studies in the repeated dose toxicity studies (rat spleen)
A = normal control, B = 100mg/kg ECD, C = 250mg/kg ECD, D = 500mg/kg ECD, Black arrow = congestion, WP = White pulp, RP = Red pulp

peroxidation markers in plasma (thiobarbituric acid reactive substances and lipid hydroperoxides) and increasing the levels of circulatory antioxidants such as reduced glutathione, ascorbic acid and α-tocopherol[15].

Liver enzyme biomarkers are enzymes that are concentrated mainly in the liver and upon damage to the liver enter in to the circulation. A measure of their levels in plasma indicates the extent of liver damage. SGOT, SGPT, and AST were significantly higher in INH-RIF treated group. But these higher levels were reduced when treated with ECD especially with 300mg/kg indicating its protective action. A total protein in the plasma was lowered in the INH-RIF only treated group due to the inability of the liver to synthesize proteins. In the INH-RIF and ECD treated groups, the extract mitigated the damage done by the anti-tubercular drugs, and resulting in higher protein level in the plasma. Bilirubin concentration was higher in the INH-RIF only treated group, this is due to the inability of the liver to conjugate bilirubin with glucoronide thereby causing an accumulation of unconjugated bilirubin in the blood. Co-administration of ECD with the anti-tubercular drugs mitigated hepatic damage, hence improved bilirubin-glucoronide conjugation in the liver.

Thus it can be concluded that the ECD has significant protective effect on serum hepatic marker enzyme

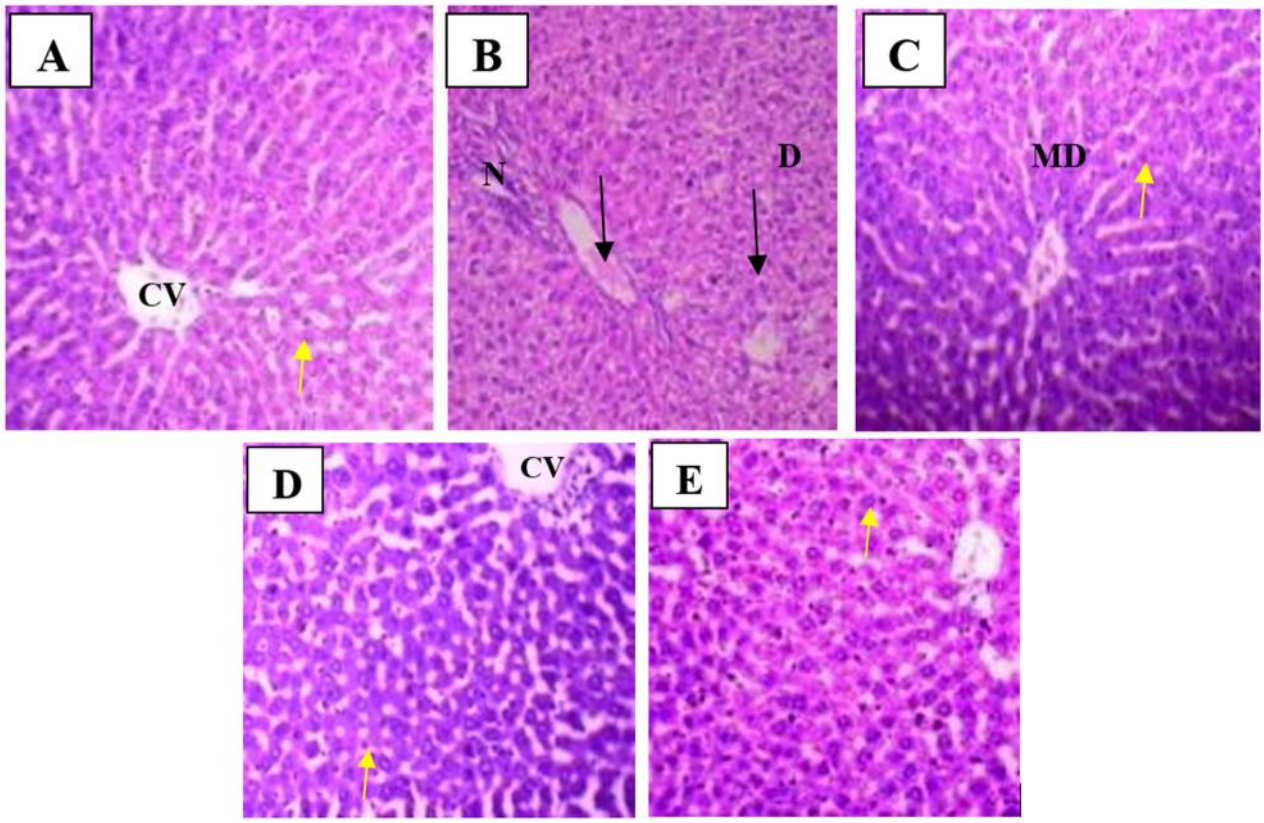

Fig 3: Histopathology of rat liver in the hepatoprotective activity
A = normal control, B = toxic control, C = standard, D = 150mg/kg ECD, E = 300mg/kg ECD. CV = central vein, N = necrosis, MD = mild degeneration, D= degenaration, black arrow = congestion, yellow arrow = inflammatory cells,

activities and bilirubin level as well as improving the serum total protein levels due to INH and RIF induced hepatotoxicity.

ACKNOWLEDGEMENTS
The authors of the present study are grateful to Dr. C. Ramesh, Director of SICRA labs Hyderabad for providing the necessary facilities to carry out the research work.

REFERENCES

1. Bhaargavi V, Reshma T, Jyotsna G, Firasat Ali. Anti-inflammatory activity of ethanolic extract of *Canthium* dicoccum. International Journal of Pharmaceutical and Phytopharmacological Research 2013; 3: 226-230.
2. Raja Rajeswari N, Ramalakshmi S, Muthuchelian K. GC-MS Analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum* (Gaertn.) Teijsm & Binn. Journal of Chemical and Pharmaceutical Research 2011; 3: 792-798.
3. Neelima M, Prasad GP, Sudarsanam G, Penchala PG, Jothi B. Ethnobotanical studies in rapur forest division of Nellore district in Andhra Pradesh. Life Sciences leaflets 2011; 11: 333-345.
4. Santhan S, Janarthan M, Zuber ali M. Evaluation of Antidiabetic and Nephroprotective activity of 95% ethanol extract of *Canthium dicoccum* whole plant by using albino rats. Journal of chemical and pharmaceutical sciences 2013; 6: 218-222.
5. Herath WH, Sultanbawa MUS, Wannigama GP, Andre C. Alkaloidal and other constituents of *Uncaria elliptica* and *Canthium dicoccum*. Phytochemistry 1979; 18: 1385-1387.
6. Samuel SA, Solairaj P, Subramonium A. Effectiveness of ellagic acid on isoniazid-rifampicin induced liver damage in rats. Journal of Pharmacology and Pharmacotherapeutics 2013; 4: 60-62.
7. M.Muthulingam. Antihepatotoxic effects of *Boerhaavia diffusa* L. on antituberculosis drug rifampicin induced liver injury in rats. Journal of Pharmacology and Toxicology 2008; 3 (2): 75-83.
8. Phaneendra P, Rupesh KM, Surendra B, Fasalu R, Tamizmani T. Hepatoprotective herbs: an overview. International journal of pharmaceutical research and development 2011; 3: 105-111.
9. Ravi V, Patel SS, Verma NK, Dutta D, Saleem TSM. Hepatoprotective Activity of *Bombax ceiba* Linn against Isoniazid and Rifampicin-induced Toxicity in Experimental Rats. International Journal of Applied Research in Natural Products 2010; 3: 19-26
10. Karthikeyan S. Hepatotoxicity of isoniazid: A study on the activity of marker enzymes of liver toxicity in serum and liver tissue of rabbits. Indian Journal of Pharmacology 2004; 36: 247-9
11. Jiang Y, Ren-xiu P, Jing Y, Rui KG, Juan L. CYP 2E1 mediated isoniazid-induced hepatotoxicity in rats. Acta Pharmacologica Sinica 2004; 25: 699-704

12. Vishal BJ, Vishnu N, Thakare, Anupama AS, Avinash DD, Suresh RN. Hepatoprotective activity of *Luffa acutangula*. Indian journal of experimental biology 2010; 48: 822-829.
13. Bhupinder SK, Sarita A, Nita K, Usha G. Effect of cimetidine on hepatotoxicity induced by isoniazid-rifampicin combination in rabbits. Indian journal of gasteroenterology 2007; 26: 18-21
14. Chen X. Protective effects of quercetin on liver injury induced by ethanol. Pharmacognosy Magazine 2010; 6:135-41.
15. Sarvanan R, Viswanathan P, Pugalendi KV. Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. Life Sciences 2006; 78: 713-718