Hepatoprotective And Antioxidant Evaluation of Extract of Cissus Quadrangularis

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ABSTRACT: Liver, an important organ that is responsible for metabolism of various drugs. The main enzyme responsible for such function is CYP450 and its subclass. However, if these enzymes are inhibited by metabolites of drugs, it results in various liver diseases. Hence, to diminish such effects, use of natural products are highly encouraged. In our study, we have studies the hepatoprotective as well as anti-oxidant function of Ciccus Quadrangularis. From the results obtained, the ME-CQ extract was found to have hepatoprotective function as it has reduced the liver tissue damage caused/induced by isoniazid and rifampicin and have also shown the regeneration of hepatocytes. Further studies are required to determine its mechanism of action.

KEYWORDS: Liver diseases, CYP450, Cissus Quadrangularis, anti-oxidatant property, hepato-protective action

INTRODUCTION:

Liver is among the most vital organ as it plays vital role in regulating various metabolic pathways within the body such as metabolism, secretion and storage.[1] It also participates in detoxification of chemicals (xenobiotics) directly or indirectly via Phase I, phase II and phase III reactions resulting in formation of metabolites due to the action of several subclasses of CYP450 enzymes of the liver.[2] Inhibition of liver enzymes, especially CYP450 by endo- or exo-genous compounds may result in decreased activity of the enzyme function resulting in various liver diseases such as cirrhosis, hepatitis, cholestasis, etc.[3] To diminish serious problems that occur due to synthetic drugs, most of the patients adhere to traditional medication which is alternate form of medication such as unani, ayurveda, etc.[4] Such systems of medication are proven to have efficient treatment for various illnesses with lesser side effects by the use of various medicinal plants.[5] One among such plants is Cissus Quadrangularis.[6] From the literature survey, the alcoholic extract was shown to possess anti-protozoal activity as it has killed earthworms in 17-30mins.[7] The ethanolic extract was proved for its anti-osteoporotic activity.[8] In the further study, it also was found to possess anti-inflammatory as well as analgesic activity.[9, 10] However, there was no report concerning to liver protection, hence, the present research work is focused to assess hepato-protective action by inducing hepatotoxicity.

MATERIAL AND METHODS:

Animal selection and storage

Albino rats weighing 150-200g were chosen for hepato-protective action and were procured from Venkateshwara enterprises, Bangalore.

Plant identification and extraction

The plant was identified and authenticated by Prof Mahmood, HOD, Department of Botany, Osmania University, Hyderabad, India. The stems were size reduced and stored in a sealed container which were later powdered coarsely and subjected for soxhlation to obtain extract with ether followed by ethanol and concentrated to obtain pure extract.

Identification of chemical constituents

The concentrated extract was subjected for various qualitative tests for the presence of various phyto-constituents such as carbohydrates, glycosides, alkaloids, tanins etc.

2Pharmacological screening

ME-CQ was reported to show gastroprotective effect at 500mg/kg body weight, hence, it was fixed for our study. The animals were isolated and divided into four different groups which includes six animals each and treated with A1 1% CMC, Isoniazid, ME-CQ with isoniazid and silymarine with isoniazid in each group respectively and estimated for the presences of various biomarkers such as ALT, AST, ALP, etc.

The liver tissues were taken and homogenized and various solutions were prepared to determine the anti-oxidant activity estimating reduced glutathione, lipid peroxidation and superoxide dismutase followed by histopathological studies to observe the changes in the liver tissue upon treating with ME-CQ

RESULTS AND DISCUSSION

From the qualitative analysis of phyto-chemicals in the extract, the results depict presence of flavonoids, triterpenoids, alkaloids, glycosides and carbohydrates as shown in table 1. The yield obtained was >6.124%. The absorbance of extract at 546nm or 630 nm against blank reagent has shown a peak at the retention time 15.36min which is similar to that of β-carotene 15.5min which confirms its presence.

TABLE 1: PHYTOCHMICAL SCREENING OF ME-CQ

Phyto-constituents test	Methanolic extract
Alkaloids	++
Glycosides	++
Carbohydrates	++
Flavonoids	++
Proteins and Amino acids	--
Triterpenoids	++
Sterols	--
Saponins	--
Tannins	--

Hepatoprotective action

Effect on body weight

Weights of liver of all the rats and their difference with other groups was noted. Increased liver weights were observed in isoniazid groups (3.67±0.14g) while the control was 2.37±0.05g. Group administered with extract at 500mg/Kg, has remarkably decreased the weight (3.18±0.14g) closer to silymarin group (2.90±0.09g).

Similarly, increased weights were observed in rifampicin group (3.73±0.10g) which is greater than the control. On the other hand, animals administered with extract have shown reduced weight (2.98±0.10g) closer to silymarin group (2.65±0.06g). The results of the effect on body weight is tabulated in table 2 and depicted in figure 1.

TABLE 2: EFFECT ON BODY WEIGHT BY ME-CQ

Animal No.	Normal	Isoniazid	CH3OH extract 500mg/kg	Silymarin group 50mg/kg	Normal	Rifampicin	CH3OH extract 500mg/kg	Silymarin group 50mg/kg
1	2.57	3.31	2.8	3.05	2.57	3.73	2.77	2.92
2	2.18	3.8	3.65	2.87	2.18	3.59	3.35	2.51
3	2.49	3.46	3.05	2.6	2.49	3.39	3.19	2.62
4	2.37	4.01	3.23	3.22	2.37	3.73	3.03	2.70
5	2.34	3.72	3.47	2.75	2.34	4.02	2.75	2.58
6	2.37	3.96	2.92	2.98	2.37	3.97	2.88	2.65
Mean ±SEM	2.371 ±0.05	3.671 ±0.14	3.18±0.14	2.900 ±0.09	2.37±0. 05	3.73±0.10	2.98±0.10	2.65±0.06

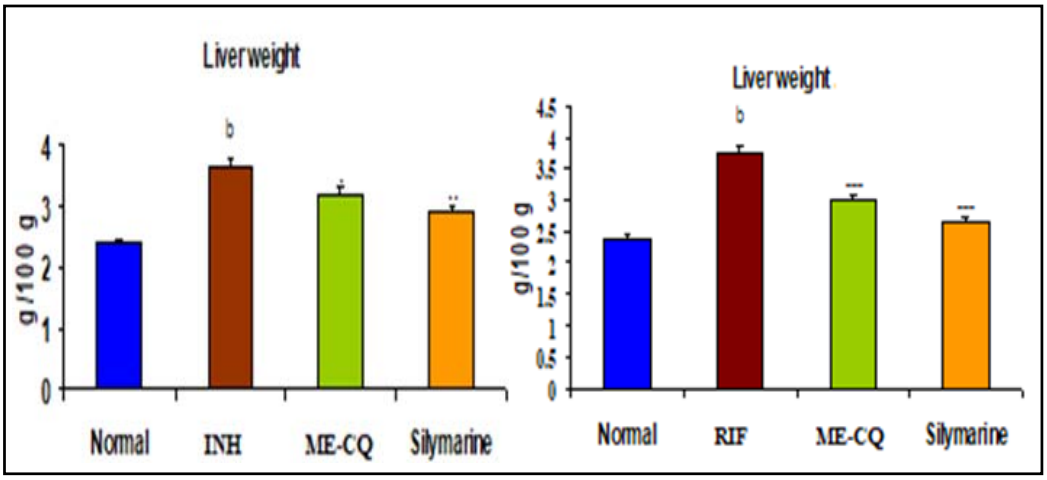

Figure 1: Effect of body weight by ME-CQ

Effect on biomarkers of liver

From the obtained results, it is evident that, in case of isoniazid as well as in rifampicin group, the levels of AST, ALT, ALP, total and indirect bilirubin levels were increased indicating toxicity was induced and the results obtained from the animals treated with the extract were observed to show reducing the levels indicating that the extract has a significant effect on the liver. Toxicity induced and recovery from the extract results are summarized in the following table 3 and depicted in figure 2.

TABLE 3: EFFECT ON BIOMARKERS BY ME-CQ

	Normal Control	**INZC (54mg/kg p.o.)**	**INZ + ME-CQ (54mg/kg p.o + 500mg/kg p.o.)**	**INZ + Silymarin (54mg/kg p.o + 50mg/kg p.o.)**	**Normal Control**	**RIFC (54mg/kg p.o.)**	**RIF + ME-CQ (54mg/kg p.o + 500mg/kg p.o.)**	**RIF + Silymarin (54mg/kg p.o + 50mg/kg p.o.)**
AST	53.62±3.06	93.72±3.36	63.51±3.10	60.23±2.53	53.62±3.06	90.84±3.39	75.68±2.62	69.98±2.40
ALT	45.23±2.20	134.3±6.04	95.79±4.02	87.73±2.5	45.23±2.20	97.62±3.98	71.02±3.71	64.01±2.65
ALP	133.19±3.60	250.79± 17.93	203.21±10.11	153.88±4.35	133.19±3.60	267.7±10.10	187.5±4.14	161.7± 4.71
TB	0.79±0.04	0.94±0.03	0.819±0.02	0.79±0.02	0.79±0.04	1.08±0.10	0.84 ±0.02	0.79±0.01
DB	0.24±0.03	0.43±0.03	0.30± 0.01	0.28±0.01	0.24±0.03	0.52±0.03	0.37±0.02	0.27±0.01

INZC = Isoniazid control; RIFC = Rifampicin control; TB = Total bilirubin; DB = Direct bilirubin

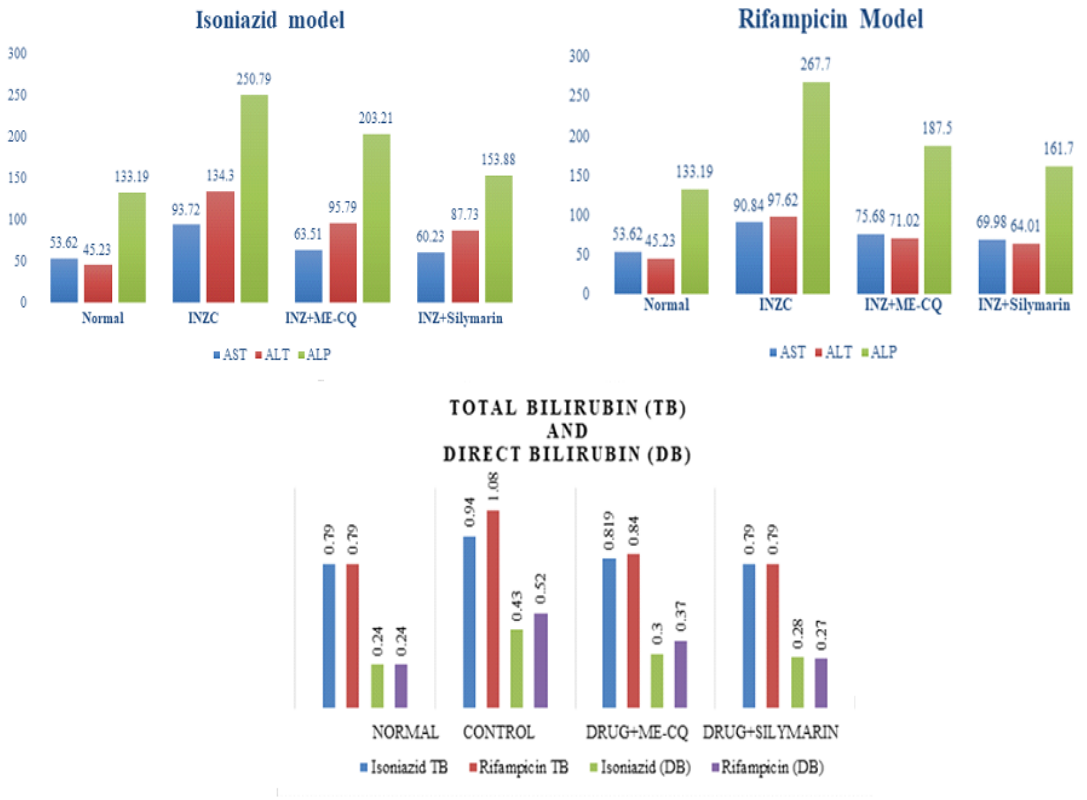

Figure 2: Biomarker levels in isoniazid and rifampicin groups

Anti-oxidant effect

Isoniazid as well as rifampicin groups has shown increased lipid peroxidation while decreasing the levels of superoxide dismutase, glutathione and catalase activity comparatively to normal group as can be seen in table 4 and depicted in figure 3. Rats were observed to undergo high lipid peroxidation in case of isoniazid (4.67± 0.35) and rifampicin (4.10±0.16) when compared with the normal control. However, the ME-CQ (mg/kg dose) has shown better action by decreasing the peroxidation levels in isoniazid (3.08±0.18) as well as rifampicin (3.15±0.14) models respectively which is closer to silymarin. Further, it observed to cause decreased GSH levels in case of isoniazid (3.67±0.35) and rifampicin (3.51±0.52) when compared with the normal control (6.97±1.06). However, the pre-treatment of the ME-CQ (500mg/kg dose) has shown better action by increasing the GSH levels in isoniazid (6.47±0.66) as well as rifampicin (6.39±0.50) models respectively which is closer to silymarin.

SOD levels are basically low in case of liver disorders. Hence, SOD levels in this study were observed to be low in case of isoniazid (6.36±1.08) and rifampicin (6.01±1.09) comparative to the normal control (12.76±1.36). Pre-treatment of the ME-CQ (mg/kg dose) has shown better action by increasing the SOD levels in isoniazid (10.68±0.99) as well as rifampicin (10.13±0.43) models respectively which are closer to silymarin action in isoniazid (10.74±1.08) and rifampicin (11.00±1.37) control model.

Similar to SOD levels, activity of CAT is also observed to be low in case of liver disorders. Hence, CAT activity levels in this study were observed to be lower in isoniazid (46.68±2.18) and rifampicin (53.30±2.57) comparative to the normal control (71.96±2.13). Pre-treatment of the ME-CQ (mg/kg dose) has shown increasing activity of CAT in isoniazid (60.64±2.64) as well as rifampicin (62.62±2.17) models respectively which are closer to silymarin action in isoniazid (67.13±1.59) and rifampicin (66.71±2.04) control model.

TABLE 4: ANTI-OXIDANT ACTIVITY BY ME-CQ

	Isoniazid model				Rifampicin model			
	Normal Control	**INZC (54mg/kg p.o.)**	**INZ + ME-CQ (54mg/kg p.o + 500mg/kg p.o.)**	**INZ + Silymarin (54mg/kg p.o + 50mg/kg p.o.)**	**Normal Control**	**RIFC (54mg/kg p.o.)**	**RIF + ME-CQ (54mg/kg p.o + 500mg/kg p.o.)**	**RIF + Silymarin (54mg/kg p.o + 50mg/kg p.o.)**
LPO (nmol MDA/g)	2.27±0.22	4.67±0.35	3.08±0.18	2.77±0.18	2.27±0.22	4.10±0.16	3.15±0.14	2.55±0.23
GSH (µmol/g)	6.97±1.06	3.67±0.35	6.47±0.66	6.83±0.66	6.97±1.06	3.51±0.52	6.39±0.50	6.48±0.76
SOD (U/mg)	12.76±1.36	6.36±1.08	10.68±0.99	10.74±1.08	12.76±1.36	6.01±1.09	10.13±0.43	11.00±1.37
CAT (U/mg)	71.96±2.13	46.68±2.18	60.64±2.64	67.13±1.59	71.96±2.13	53.30±2.57	62.62±2.17	66.71±2.04

LPO = Lipid peroxidation; GSH = Glutathione reduction; SOD = Superoxide dismutase; CAT = Catalase; INZC = Isoniazid control; RIFC = Rifampicin control

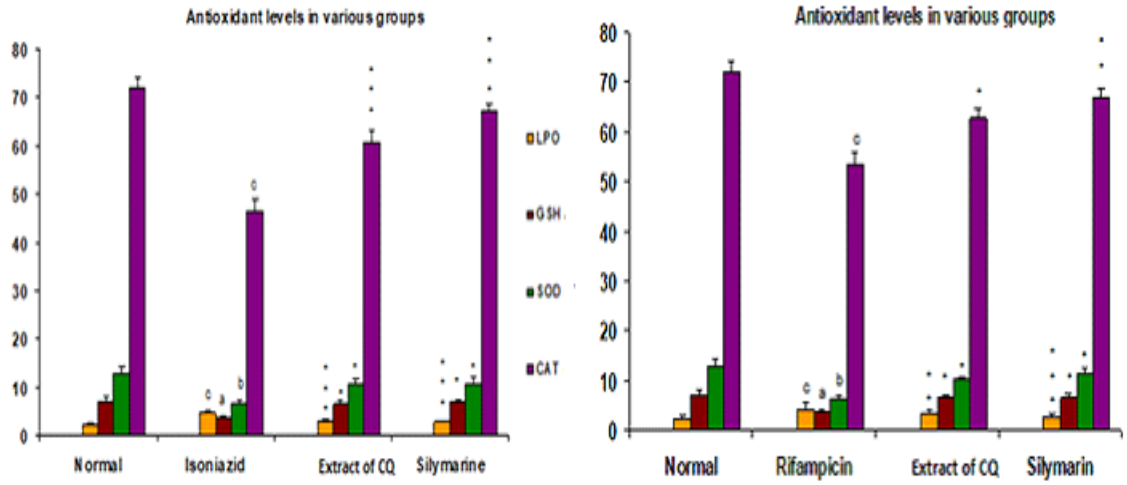

Figure 3: Anti-oxidant effect in isoniazid and rifampicin groups

Histopathological studies

Histopathological findings show the focal haemorrhage, inflammation, necrosis and degeneration in isoniazid group, on the other hand, focal haemorrhage, inflammation; spotty, bridging and centrilobular necrosis in rifampicin group. Pre-treatment with methanolic extract reduced inflammation, focal haemorrhage, centrilobular and decreasing necrosis in isoniazid as well as rifampicin treated groups respectively.

In normal control group, hepatic globular structure, central vein as well as portal triad appeared normal as shown in figure 4a. In isoniazid treated group, sections of liver have shown focal haemorrhage, centrilobular, inflammation, degeneration, centrilobular necrosis and sinus congestion (figure 4b, 4c). In 500mg/kg ME-CQ treated group, focal haemorrhage, centrilobular, inflammation, degeneration, sinus congestion and centrilobular necrosis was reduced (figure 4d, 4e). In 500mg/kg Silymarin treated group reveals regeneration hepatocytes. The hepatic-globular architecture was normal (figure 4f, 4g).

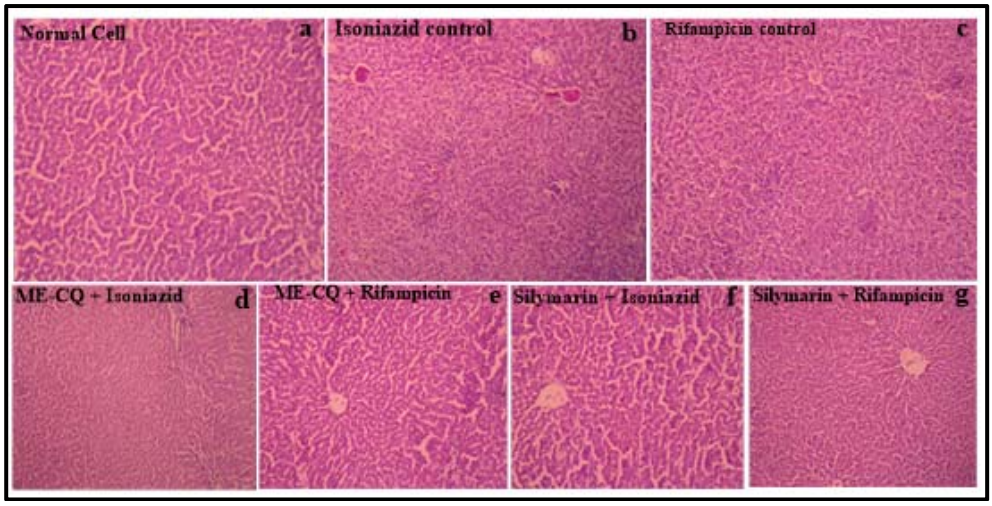

Figure 4: Histopathological study of liver tissue

Treatment with ME-CQ has neutralized isoniazid as well as rifampicin induced biochemical and histopathological changes, therefore it suggests the protective action against isoniazid as well as rifampicin challenge is probably because of radical scavenging activity and also prevention of lipid peroxidation

CONCLUSION

It can be concluded that the hepato-protective effect of *C. Quadrangularis* is due to elevated action of antioxidant enzymes as well as non-enzymatic antioxidants or preventing the free radical formation by the presence of the electrophilic by constituents present in the ME-CQ or by activation of conjugation of anti-tubercular drugs with GSH in liver.

Hepato-protective as well as anti-oxidant action of *ME-CQ* was confirmed by biochemical as well as histopathological studies. Hence, it possesses hepato-protective and anti-oxidant activity. Further studies are necessary to confirm for better understanding the mechanism of anti-hepatitis activity.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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