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Research Article

Hepatoprotective Activity of Ethanolic Extract of *Cissus quadrangularis linn* Fruits in Alcohol-induced Liver Damage in Rats

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ABSTRACT

Reactive oxygen species are one of the major reasons for liver damage. The study investigates the hepatoprotective activity of ethanolic extract of *Cissus Quadrangularis* (EECQ) *Linn* of fruits against ethanol-induced hepatotoxicity. Fruit Powder of *Cissus Quadrangularis* was successively extracted with ethanol. Preliminary phytochemical tests were done. The hepatoprotective activity of the AST, ALT, ALP, ALB, BIT and BID were assessed in alcohol-induced hepatotoxicity rats. The EECQ showed the presence of alkaloids, carbohydrates, steroids, saponins and triterpenes, while alkaloids, carbohydrates and saponins were present Ethanol produced significant changes in physical (increased liver weight and volume), biochemical (increase in serum alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, total bilirubin, cholesterol, triglycerides and decrease in total protein and albumin level) Pre-treatment with EECQ extract significantly prevented the physical, biochemical, histological and functional changes induced by alcohol in the liver. The present study indicates that EECQ extract possessed potential hepatoprotective activity. This activity may be attributed due to the rich phytoconstituents of the plant extract.

Introduction

Reactive oxygen species (ROS) are continuously generated during metabolic processes to regulate several physiological functions essential to the body.[1] ROS are prone to withdraw electrons from biological macromolecules such as proteins, lipids, nucleic acids to gain stability in the biological system. When the production of ROS exceeds the capability of the body to detoxify these reactive intermediates, oxidative stress would be generated.[2] This may lead to drastic harm to the body such as membrane damage, mutations due to attenuation of deoxyribonucleic acid (DNA) molecules, and disruption to various enzymatic activities in the metabolism of the body.[3-5]

Alcohol, a natural product that has been available for human consumption for thousands of years, is a common cause of ROS insult in the liver.[6] Despite the claim that a small amount of alcohol consumption may be beneficial for preventing and reducing the mortality rate of coronary heart diseases and ischemic stroke, it should also be noted that alcohol is toxic to almost every organ of the body.[7] Metabolism of alcohol in the liver generates excessive free radicals and increased peroxisomal oxidation of fatty acid, which would ultimately affect the functionality of the antioxidant systems to eliminate ROS in the body.[6] Therefore, the mechanism to restore hepatic injuries caused by alcoholic oxidative stress is tightly regulated by the antioxidant status of a living system.

Many plants that portrayed good antioxidant activity are also associated with hepatoprotection potential. Some good examples include *Myristica malabarica* L.,[8] *Calotropis*

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gigantea,[9] *Acanthus ilicifolius,*[10] *Momordica dioica,*[11] and *Phyllanthus niruri.*[12]. Members from the Elephantopus family, including *Elephantopus scaber* Linn, *Elephantopus mollis* Kunth., and *Elephantopus tomentosus* have also been shown to possess hepatoprotective activities in rats.[13–15] Additionally, the use of *E. scaber* liver protective purposes was also observed in folk medicinal practices. Back in Brazil, the root juice of E. scaber had already been consumed for years to heal liver troubles and hepatitis.[16] In China, a traditional herbal drink, which is made up of a few herbal products including *E. scaber,* had been claimed to protect the liver against cancer, hemangioma, fatty accumulation, and cirrhosis as well as for anti-hepatitis B.[17] This formulation was also named "Yi-Gan-Yin," which means "drink that is beneficial to the liver" to relate to its function.

Cissus quadrangularis is an evergreen climber growing to 5 m (16 ft) by .5 m (1.6 ft) at a fast rate. It is hardy to zone (UK) 10. Suitable for: light (sandy), medium (loamy) and heavy (clay) soils, prefers well-drained soil and can grow in nutritionally poor soil. Suitable pH: acid, neutral and basic (alkaline) soils and can grow in very acid and very alkaline soils. It cannot grow in the shade. It prefers dry or moist soil and can tolerate drought.

From the literature, it is evident that *C. quadrangularis* is used in the herbal formulation for the management liver health along with other herbs.[18-19] The stems are reported to possess potent hepatoprotective activity against toxic chemicals and antibiotics, especially Rifampicin[20] and Isoniazide.[21] The stems are also proven to protect against heavy metals such as lead.[22]

The liver plays a pivotal role in the biological system that is responsible for the metabolism and clearance of drugs and xenobiotics, including ROS. The liver has become the central organ for detoxification as the liver cells (hepatocytes), the main components that make up the organ, contain a majority of enzymes responsible for the drug metabolism of the entire body. However, when the amount of drugs or xenobiotics encountered has exceeded the liver's maximum metabolic capability, the damaging effect of the toxins may lead to various liver ailments. Overconsumption of alcohol had been associated with a spectrum of liver injuries with varying degrees of severity, with some common pathology including steatosis, foamy degeneration, steatonecrosis, venous lesion, and cirrhosis.[23-25]

Since the liver is the first targeted organ for any drug molecules, there is a need to develop novel hepatoprotective agents and formulations to revert the normal physiology and functioning of the liver affected by xenobiotics, pollution, and alcohol consumption. The current investigation is taken up to evaluate the hepatoprotective potential of the fruits of *C. quadrangularis* in an alcohol-induced hepatotoxicity model in rats.

Material and Methods

Materials

Experimental Animals

Adult Wistar albino rats of either sex, weighing 150-200g, inbred in the institutional animal house were used for the study. Animals were housed in polypropylene cages in a controlled environmental condition (22 ± 3°C, 55 ± 5% humidity, and a 12 hours light/dark cycle). The animals were fed with a standard rodent diet and water ad libitum. They were allowed to acclimatize to these conditions for one week.

Methods

Plant Collection and Authentication

C. Quadrangularis Linn Fruits *and Michelia Champaea Leaves* were obtained from the local places of Tirupati, AP. *C. Quadrangularis Linn* Fruits was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Extraction by Maceration

Fresh leaves of *M. Champaea and fruits of C. Quadrangularis Linn* were washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried leaves and fruits were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and is immersed in 95% ethanol and kept for maceration for 7 days with occasional stirring. On the 8th day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40^{o}C. The resultant extract was put in a desiccator to remove any ethanol left in it. The dried ethanolic extract of *M. Champaea* (EEMC) and EECQ were packed in an air-tight bottle and put in a dry place for further studies.

Qualitative Evaluation of Phytoconstituents

The EECQ was screened for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, etc.

Hepatoprotective Activity

Evaluation of Hepatoprotective Activity in Alcohol-induced Hepatotoxicity

Albino rats (Wistar Strain) of either sex weighing 150-200 g were selected and divided into five groups of six animals each.

Group I: Vehicle-treated rats were kept on a normal diet and controlled for 15 days.

Group II: Rats orally received 30% alcohol (1.5 mL/rat/twice a day) for 15 days.
Group III: Rats orally received Silymarin (25 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group IV: Rats orally received *EECQ* (100 mg/kg b.w/day) and alcohol as group II, for 15 days.
Group V: Rats orally received *EECQ* (200 mg/kg b.w/day) and alcohol as group II, for 15 days.

During this period of treatment, the rats were maintained under normal diet and water. The blood was collected from the retro-orbital plexus of the rats of all groups 24 hours after the last dose administration, under light anesthetic ether. The blood samples are centrifuged at 3000 rpm for 30 minutes to separate the serum. The serum was analyzed for various biochemical parameters such as AST, ALT, ALP, ALB, BIT and BID.[26-27]

Their percentage protection was calculated using Auto analyzer. The liver was dissected out and subjected for morphological studies such as liver weight and liver volume of each animal. Further, the liver was placed in a 10% formalin solution for histopathological study.

In vivo Anti-oxidant Studies

Under *In vivo* anti-oxidant studies, Lipid peroxidase (LPO) activity, Reduced Glutathione

Catalase (CAT) activity was estimated.

Table 1: Results of Phytochemical screening of EECQ

S. No	*Name of the Phytochemical*	*EECQ*
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
	Alkaloids	+
	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
	Phenolic compounds	+
10.	Tannins	+
11.	Steroids	-
12.	Gums	-

Where, + means positive and - means negative.

Table 2: Effect of EECQ on Liver weight and liver volume in alcohol induced hepatotoxicity in rats

Group	*Liver weight gm/100gm*	*Liver Volume ml/100gm*
Control	3.68±0.08	6.92±0.15
Toxic control	4.82±0.10	8.92±0.05
Silymarin	3.81±0.08**	7.22±0.12**
EECQ (100mg/kg)	4.21±0.13*	7.58±0.11**
EECQ (200mg/kg)	4.11±0.13**	7.48±0.10**

Values are expressed as mean ± SEM; n=6
* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

Results

Preliminary Phytochemical Screening

Results of phytochemical screening were elucidated in Table 1.

The preliminary phytochemical screening showed various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in EECQ.

Alcohol-induced Hepatotoxicity

Liver Weight and Liver Volume

Alcohol treatment in rats resulted in enlargement of the liver, which was evident by increased liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume, whereas test groups treated with EECQ and EEMC significantly affected liver weight and liver volume compared to the toxic control group. Results were shown in Table 2 and Figs. 1 & 2.

Effect of EECQ on SGOT, SGPT and ALP levels in Alcohol-induced Hepatotoxicity in Rats

Rats treated with alcohol developed significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and ALP compared to normal control. Treatment with

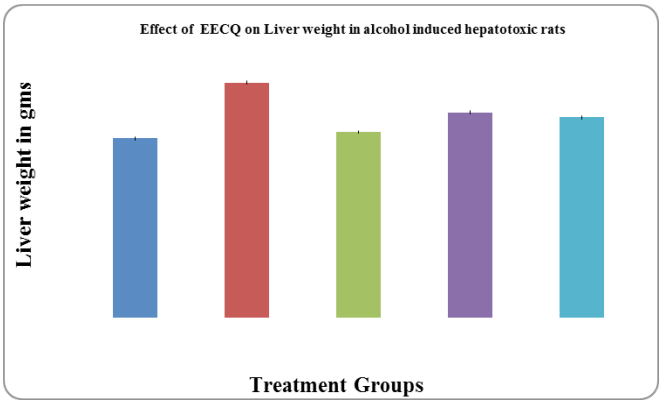

Fig. 1: Effect of EECQ on Liver weight in alcohol induced hepatotoxicity in rats

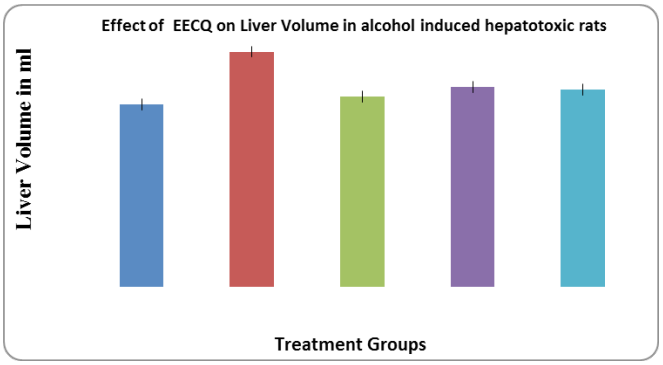

Fig. 2: Effect of EECQ on Liver Volume in alcohol induced hepatotoxicity in rats Bio chemical parameters:

Silymarin had shown good protection against alcohol-induced toxicity to the liver. Groups treated with EECQ and EEMC showed a significant effect which can be comparable with toxic control. Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals. The results are shown in Table 3 and Fig. 3.

Effect on Total Bilirubin

The total bilirubin concentration was found to increase in animals with liver damage by alcohol. In the standard group, Silymarin administration reduced total bilirubin and animals treated with EECQ have exhibited dose-dependent significant reduction in total bilirubin compared to the toxic control group.

Effect on Direct Bilirubin

Alcohol-treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed a good reduction in bilirubin level in respective groups. The results are shown in Table 4.

Effect on Albumin

Induction of liver damage by administration of alcohol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown a significant increase while EECQ has shown dose-dependent increase in serum albumin level compared to the toxic control group. Table 4 and Fig. 4.

In vivo Antioxidant Studies of EECQ

Values are represented as Mean ± SEM. Statistical analysis was done by one-way ANOVA followed by post hoc Dunnett's multiple comparison tests. $^{***}p < 0.0001$, $^{**}p < 0.001$, and $^{*}p < 0.05$ vs Disease control

Antioxidant parameters like LPO, GSH, and CAT were also evaluated. Reduced activity of CAT and levels of GSH after treatment with Gentamicin suppresses endogenous enzymatic antioxidant machinery. Treatment with

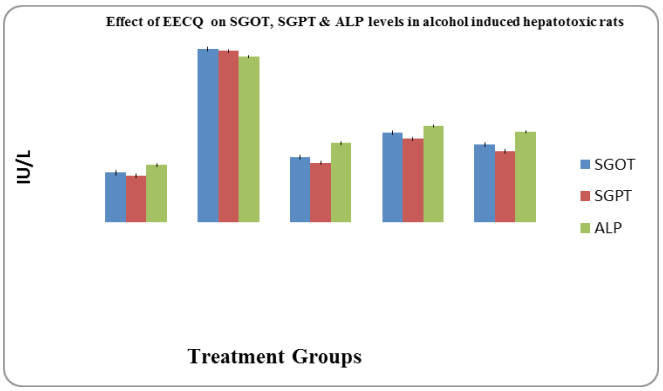

Fig. 3: Effect of EECQ on SGOT, SGPT and ALP levels in alcohol induced Hepatotoxicity in rats

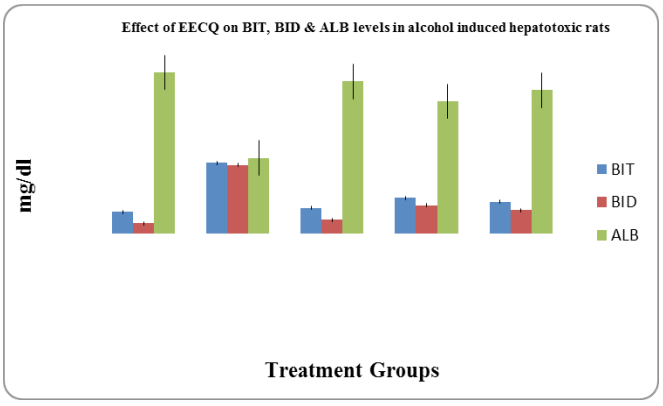

Fig. 4: Effect of EECQ on BIT, BID and ALB levels in alcohol induced hepatotoxicity in rats

Table 3: Effect of EECQ on SGOT, SGPT & ALP levels in alcohol induced hepatotoxicity in rats

Group	*SGOT (IU/L)*	*SGPT (IU/L)*	*ALP (IU/L)*
Control	113.15 ± 5.05	106.11 ± 4.13	130.75 ± 3.97
Toxic control	396.93 ± 0.93	391.77 ± 7.87	378.62 ± 3.59
Silymarin	148.22 ± 1.79**	135.39 ± 6.88**	181.04 ± 11.49**
EECQ(100 mg/kg)	205.24 ± 3.56**	190.47 ± 8.11**	220.35 ± 10.05**
EECQ (200 mg/kg)	177.30 ± 9.56**	162.25 ± 3.67**	206.69 ± 2.18**

Values are expressed as Mean ± SEM; n=6
* p≤0.05, **p≤0.01 and ***P<0.001. Comparison with toxic control

Table 4: Effect of EECQ on LPO, GSH, and CAT

S.No	*Treatment groups*	*Lipid peroxidation (in µM/mg tissue)*	*Reduced glutathione (in µM of GSH/mg tissue)*	*Catalase (in units/mg protein)*
1.	Normal control	2.853 ± 0.087	4.227 ± 0.104	0.720 ± 0.049
2.	Disease control	5.233 ± 0.214	2.907 ± 0.136	0.450 ± 0.031
3.	Standard control	2.965 ± 0.1^{64*}**	3.610 ± 0.083***	0.637 ± 0.022**
4.	EECQ 100mg/Kg	3.303 ± 0.0^{82*}**	3.550 ± 0.109***	0.598 ± 0.032*
5.	EECQ 200mg/Kg	3.590 ± 0.08^{5**}*	3.410 ± 0.083**	0.603 ± 0.052*

EECQ increased the activity of CAT and levels of GSH significantly compared to the disease control animals. It was also observed that an increase in LPO in the disease group is due to altered antioxidant machinery and higher susceptibility towards oxidative damage. However, EECQ lowered LPO levels in EECQ treated groups. As per the findings, the secondary metabolites, flavonoids and phenolic compounds are present in the antioxidant plants.

Discussion

Alcohol treatment in rats resulted in enlargement of the liver, which increased the liver weight (4.82 ± 0.1) and volume (8.92 ± 0.05). The groups treated with Silymarin showed good restoration of liver weight (3.81 ± 0.08) and liver volume (7.22 ± 0.12) whereas test groups treated with EECQ showed statistically significant (**p ≤ 0.01) effect on liver weight and (4.11 ± 0.13) and liver volume (7.48 ± 0.10).

Rats treated with alcohol and paracetamol developed significant hepatic damage observed as elevated serum levels of hepatic specific enzymes like SGPT, SGOT and ALP when compared to normal control. Treatment with Silymarin had shown good protection against alcohol and paracetamol-induced toxicity to the liver. Groups treated with EECQ showed significant (**p ≤ 0.01) effects.

The total bilirubin and direct bilirubin concentration were found to increase whereas serum albumin levels were decreased in disease control. Silymarin and EECQ administration reduced bilirubin and direct bilirubin concentration in a dose-dependent manner and significantly increased serum albumin levels.

From the above results, it can be drawn that the fruits of *C. Quadrangularis* are good enough to restore the disrupted liver functions caused by environmental or drug induced liver damage.

Conclusion

The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity. Some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. Reactive oxygen species are an important cause for liver damage, ethanolic extraction of *C. Quadrangularis* with potent antioxidant properties, showing good hepatoprotective action. The chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like tannins, reducing sugars and proteins present in the extract, may be responsible for such activity.

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