Hepatoprotective studies on methanolic extract of caryopses of *Echinochloa colona* Link

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Abstract: The caryopses (seeds) of *Echinochloa colona* Link of family Poaceae are traditionally used for the treatment of jaundice. The methanolic extract of caryopses of *Echinochloa colona* (ECME) was evaluated for its hepatoprotective activity in paracetamol (3g/kg per oral) and ethanol (5g/kg per oral) intoxicated rats while its antihepatotoxic activity against D-galactosamine (400mg/kg body weight intra peritoneal). The activity of the extract was assessed on the basis of improvement in the altered level of various serum biochemical parameters and in the changes occurred in the histology of liver of the rats. The extract was also investigated for its antioxidant potential by employing different *in vitro* methods. The extract exhibited ferrous ion reducing power, 1,1 Diphenyl-1-picryl hydrazyl (DPPH), superoxide, nitric oxide and hydroxyl radical scavenging activities. The significant ($p<0.001$) hepatoprotective and antioxidant activities exhibited by the extract ECME, in different *in vivo* models and *in vitro* studies respectively may be attributed to the flavonoids and phenolic compounds present in the extract.

Keywords: Antioxidants, ethanol, galactosamine, *Echinochloa colona*, paracetamol.

INTRODUCTION

Oxidative stress causes several diseases to quote a few, diabetes, cardiovascular diseases, liver cirrhosis, (Bharat *et al*, 2011) nephrotoxicity, cancer, aging etc (Lien A.P *et al*., 2008). Antioxidants counteract the oxidative stress and give protection from degenerative disorders induced by many hepatotoxins. The search for new naturally occurring antioxidants has been on for the past few decades (Sundararajan *et al*., 2006; Kosecik *et al*., 2005). Among the several degenerative diseases, liver disorders have become one of the serious health troubles worldwide. Liver damage occurs due to oxidative stress i.e., either by extensive use of some drugs or exposure to some chemicals or by excess consumption of alcohol. The three features of herbal drugs such as safety, cost effectiveness and efficacy, made herbal drugs to gain high popularity and importance (Vakiloddin *et al*., 2015). Since the traditional medicinal plants are potential source of new drugs and drug leads, the present investigation was taken up on *Echinochloa colona*, a medicinal plant which is stated to be useful in the treatment of many liver disorders, to prove its hepatoprotective activity scientifically by different methods.

Echinochloa colona (L.) Link of grass family (Poaceae) is an annual crop (jungle rice, awnless barnyard grass) cum cop-mimic (weed) in northern Telangana (Ramana *et al*., 2009). It is a major weed of many crops, including rice, corn, sorghum, sugarcane, cotton, etc. in India. It is found throughout tropical Asia and Africa. The caryopses of the plant are traditionally used in diabetes, biliousness and constipation (Madhavachetty *et al*., 2008). In the present study, the methanolic extract of caryopses of *Echinochloa colona* was evaluated for its *in vitro* antioxidant potential and *in vivo* hepatoprotective activity.

MATERIALS AND METHODS

Animals

Wistar albino rats weighing 150-200 g were purchased from Sainath agencies, Hyderabad, Telangana, India with a prior permission from our institutional animal ethical committee (1820/GO/Re/S/15/CPCSEA, Date: 01-09-2015) and used for the studies. The animals were caged under constant environmental and nutritional conditions (12:12 hour light and dark cycle; at an ambient temperature of 25±5^{o}C; 35- 60% of relative humidity). They had free access to food and water *ad libutum*.

Ethical approval

Wistar albino rats were used in the studies after taking prior permission from our institutional animal ethical committee (1820/GO/Re/S/15/CPCSEA, Date: 01-09-2015)

Collection and preparation of extracts

The seeds of the plant, *Echinochloa colona* Link were collected in July 2012, from paddy fields of Madikonda, Warangal, Telangana State, India. The plant was authenticated by Prof. V.S. Raju, taxonomist, Kakatiya University, Warangal. A voucher specimen of the plant

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(KU/UCPSC/49) is being maintained in the herbarium of Department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

The caryopses were dried thoroughly under shade, powdered coarsely and macerated with methanol in a round bottomed flask for 7days with stirring at regular intervals and filtered after seven days. It is then concentrated under reduced pressure using rotavapour evaporator, (Buchi rotavapour Switzerland) to yield a semisolid mass (7.8%) and coded as ECME.

Acute toxicity study

The study was carried out for methanolic extract of *E.colona* according to the Organization for Economic Co-operation and Development (OECD) 423 guidelines using female wistar albino rats. The rats were not fed with diet for overnight (12 h), and divided into five groups of three animals in each group. Group I was given vehicle (2% gum acacia orally) and other groups (Group- II, III, IV and V) were orally fed with ECME 300, 1000, 1500 and 2000 mg/kg body weight. All the animals were kept under observation for 72hours for any toxic symptoms.

Assessment of hepatoprotective activity of ECME against paracetamol induced hepatotoxicity

The rats were divided into six groups of six each, control, toxic, standard, and three test groups. The procedure was followed from Sabeena and Ajay, 2013 with minor modifications.

Group I (Control group): Treated with vehicle, (1 ml/kg b.w. of 2% gum acacia in water) daily for seven days.
Group II (Toxic group): Treated with vehicle (1 ml/kg b.w of 2% gum acacia in water) daily for seven days followed by paracetamol on the eighth day.
Group III (Standard group), Group IV (ECME 100), Group V (ECME 200) and Group VI (ECME 400) were treated with Silymarin (100 mg/kg b.w), ECME 100, 200, 400 mg/kg b.w. respectively for seven days followed by paracetamol on eighth day.

The blood and liver samples were collected from the animals of all groups 24h after administration of paracetamol, for estimation of various serum biochemical parameters and histological studies respectively.

Assessment of hepatoprotective activity of ECME against ethanol induced hepatotoxicity

The rats were divided into six groups and pretreated for ten days with single daily dose of vehicle, Silymarin, and the extract ECME in different doses as described in paracetamol induced hepatotoxicity experiment. On 10^{th} day one hour after the daily treatment, the animals of all the groups leaving group I, intoxicated with an acute oral dose of ethanol (5g/kg.b.w) in distilled water (6:4 v/v). 18hr after administration of ethanol, the blood and liver samples were collected under ether anesthesia, for numerical estimation of various serum biochemical parameters and histological studies respectively (Srivastava *et al*., 2006).

Assessment of antihepatotoxic activity of ECME against D-galactosamine induced hepatotoxicity in rats

It was done according to Karan *et al*. (1999) the rats were divided into four groups of six animals each. Among the three test doses, ECME at 200 and 400mg/kg exhibited a remarkable protection against both drug and ethanol induced hepatic damage which is evident from both serum biochemical parameters and histological profile of liver of rats. Though the two test doses have shown good activity, the extract ECME at 200mg/kg was selected as the effective dose as the percentage protection offered by the double of the dose (400mg/kg b.w) was very close. Hence smaller dose is preferred for the further studies. Group I served as normal and is given the vehicle i.e., 2% gum acacia in water (1mL/kg b.w p.o) for 3 days. On the first day, D-galactosamine (400 mg/kg intra peritoneal) was given to groups II, III and IV. Vehicle (2% gum acacia 1mL/kg b.w p.o), Silymarin (100 mg/kg b.w.) and ECME (200mg/kg b.w) were given to the animals of groups II, III and IV respectively for three times at the time point of 2h, 24h, 48h after the administration of D-galactosamine. The blood and liver samples were collected from the animals 1h after the last treatment for determination of various serum biochemical parameters and histological studies respectively.

Histological studies

The liver samples isolated from the rats of the study were cleaned properly with normal saline (0.9%). Then, 2-3 pieces of nearly 6 mm^3 size were sliced and fixed in phosphate buffered 10% formaldehyde solution. Thin sections of 5μm thickness of liver tissue were cut after embedding in paraffin wax and stained with hemotoxylin-eosin stain.

Determination of Prothrombin time (PT)

The prothrombin time was determined by collecting blood from animals in normal capillary tubes and the capillaries were broken down into pieces until a thread was observed, and the clotting time was noted (Glover and Kuzell, 1961).

Total phenolic content

The total phenolic content of the extract, ECME was determined using the Folin-Ciocalteu colorimetric method (Samatha *et al*., 2012). The total phenolic content was calculated with respect to the standard Gallic acid and is expressed as Gallic acid equivalents (GAE) in mg per gram of extract.

Total flavonoid content

The total flavonoid content of the extract, ECME was measured using the aluminium chloride colorimetric method (Samatha *et al*., 2012). It was expressed as rutin equivalents in mg per gram of extract.

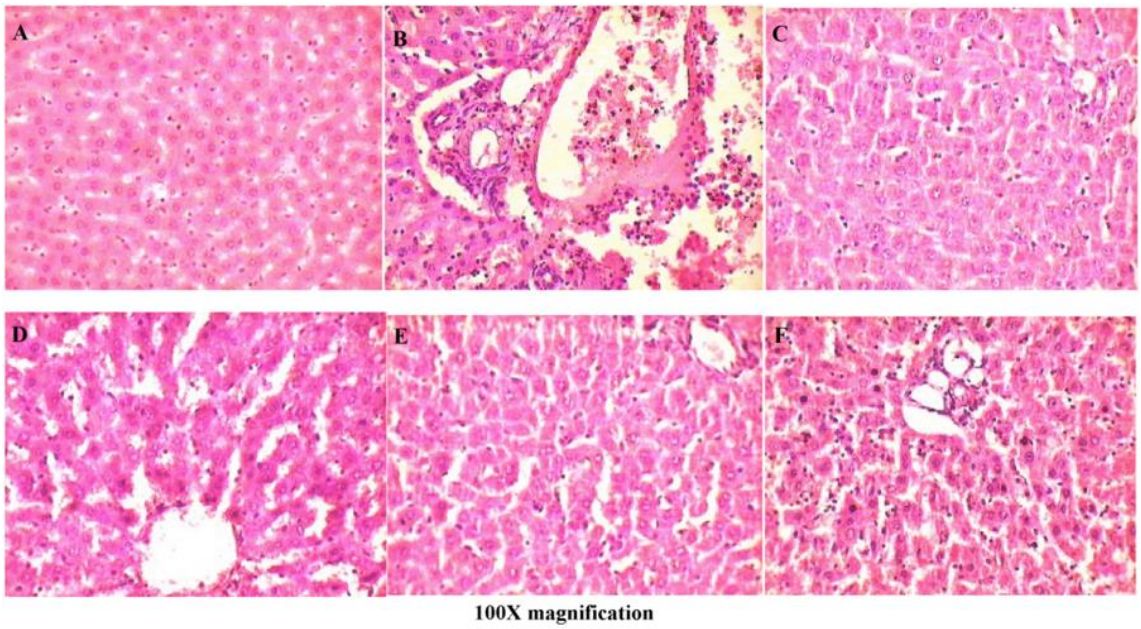

Fig. 1: Effect of Methanolic extract of *Echinochloa colona* (ECME) on liver pathologic analysis by hematoxyline-eosin stain after Paracetamol treatment in rats. ECME and Paracetamol were given as described in "Methods". (A) Normal control group; (B) Paracetamol -treated toxic group; (C) Paracetamol and 100mg/kg silymarin-treated group; (D) Paracetamol - and 100mg/kg ECME-treated group; (E) Paracetamol and 200mg/kg ECME-treated group; (F) Paracetamol - and 400mg/kg ECME-treated group. (Magnification 100X)

In vitro antioxidant studies

The test extract, ECME was screened to assess its antioxidant property by DPPH radical scavenging assay (Saketh *et al*., 2013), superoxide scavenging activity (Jiang *et al*., 2013), nitric oxide scavenging activity (Garratt, 1964), hydroxyl radical scavenging activity (Hazra *et al*., 2008) and reducing power assay (Bursal and Koksal, 2011).

STATISTICAL ANALYSIS

The data obtained were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism version 3 (GraphPad Software, La Jolla California USA).

RESULTS

Acute toxicity study

Administration of the extract, ECME up to a dose of 2000 mg/kg was found to be safe as there were no signs of toxic symptoms. As there is no evidence of acute toxicity of ECME in rats 3 doses i.e., 100, 200 and 400 mg/kg body weight were selected for the experimental study.

Assessment of hepatoprotective activity of ECME against paracetamol induced hepatotoxicity

The rats treated with overdose of paracetamol (3g/kg) caused significant liver damage which was evident from the changes in serum biochemical parameters and histology of liver of rats**.**The results are shown in table 2 and fig. 1. The level of hepatospecific enzymes such as [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP)], Total Bilirubin (TBL), Direct Bilirubin (DBL) and Lactate dehydrogenase (LDH) were increased, and the level of Albumin (ALB) and Total Protein (TP) in serum were decreased, indicating persistent liver cell damage and obstruction of bile ducts which was endorsed by the histological examination of the liver sections of rats. The rats which were given ECME 100, 200 and 400mg/kg body weight per oral (b.w.p.o) and Standard (Silymarin 100mg/kg) exhibited a significant ($P<0.001$) protection against paracetamol induced hepatic damage by reversal of the altered level of serum biochemical parameters and by minimising the histopathological abnormalities. Among all the test doses, percentage protection shown by ECME at 200 and 400 mg/kg body weight per oral (b.w.p.o.) was comparable to that of standard drug, Silymarin 100 mg/kg b.w.

Assessment of hepatoprotective activity of ECME against ethanol induced hepatotoxicity

The results are presented in table 3, fig. 2. In ethanol induced hepatotoxicity in rats, the extract, ECME also exhibited remarkable hepatoprotective activity which was evident from the reversal of altered serum biochemical parameters and histopathological features of liver of rats. The percentage protection shown by ECME at 200 and 400 mg/kg was found to be good and the effect was very close to that of the reference standard, Silymarin 100 mg/kg b.w.

Table 1: Effect of ECME on different serum biochemical parameters in Paracetamol induced hepatotoxicity in rats.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dL)	DB (mg/dL)	TP (g/dL)	ALB (g/dL)	LDH (U/L)	PT (seconds)
Normal	63.19 ±0.75	59.7 ±3.7	524±2.4	0.42 ±0.01	0.2± 0.005	6.5± 0.25	3.78± 0.2	111.7 ±1.6	12.44 ±1.71
Toxic	167.08 ±1.04	172.1 ±5.4	926±2.8	2.73 ±0.15	1.47± 0.01	2.4± 0.1	1.99± 0.1	236.31 ±1.95	151.16 ±2.8
Standard	78.2 ±3.3[c] (85.5%)	72.35 ±4.2[c] (88.5%)	598.77 ±4.7[c] (81.6%)	0.68 ±0.03[c] (88.7%)	0.31± 0.01[c] (91.3%)	5.9± 0.1[c] (85.3%)	3.51± 0.04[c] (84.92%)	130.1 ±0.9[c] (84.8%)	35.55 ±3.7[c] (83.4%)
ECME 100mg/kg	94.8±2.[c] (69.4%)	98.52 ±6.8[c] (65.4%)	651.3 ±2.05[c] (68.8%)	0.94 ±0.04[c] (77.4%)	0.48± 0.005[c] (77.96%)	4.96± 0.16[c] (62.4%)	3±0.15[c] (56.43%)	161.3 ±1.2[c] (60.2%)	49.29 ±0.7[c] (73.4%)
ECME 200mg/kg	86.2 ±1.16[c] (77.7%)	80.5 ±2.2[c] (81.2%)	620.3 ±4.9[c] (76.1%)	0.89 ±0.64[c] (79.6%)	0.42± 0.01[c] (82.6%)	5.68± 0.1[c] (80%)	3.36± 0.05[c] (78.19%)	142.9 ±0.6[c] (75.1%)	38.4 ±1.72[c] (81.3%)
ECME 400mg/kg	84.6 ±1.6 [c] 79.8%)	77.2 ±4.8[c] 84.08%)	609.3 ±7.9[c] (78.8%)	0.86 ±0.02[c] (80.9%)	0.39± 0.005[c] (85.04%)	5.81± 0.16[c] (83.18%)	3.45± 0.07[c] (81.3%)	137.3 ±0.74[c] (79.19%)	39.4 ±1.55[c] (80.5%)

Table 2: Effect of ECME on different serum biochemical parameters in Ethanol induced hepatotoxicity in rats.

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dL)	DB (mg/dL)	TP (g/dL)	ALB (g/dL)	LDH (U/L)	PT (seconds)
Normal	22.23 ±2.02	53.33 ±1.12	345.2 ±4.82	0.113 ±0.005	0.04 ±0.005	7.6 ±0.1	4.73 ±0.3	200.01 ±1.5	16.6 ±2.5
Toxic	118.67 ±2.09	159.66 ±2.4	792.17 ±3.58	2.3± 0.05	0.76 ±0.01	3.33 ±0.05	2.46 ±0.3	562.6 ±2.4	113.6 ±3.0
Standard	39.63 ±1.62[c] (81.96%)	63.3 ±2.4[c] (93.48%)	392.97 ±10.14[c] (89.3%)	0.34 ±0.01[c] (89.6%)	0.12 ±0.05[c] (88.9%)	7.0 ±0.3[c] (81.9%)	4.3 ±0.2[c] (81.06%)	231.67 ±2.05[c] (91.26%)	21.6 ±3.2[c] (94.8%)
ECME 100 mg/kg	66.93 ±2.32[c] (53.65%)	99.43 ±1.9[c] (56.65%)	478.9 ±1.34[c] (70.3%)	0.82 ±0.19[c] (67.6%)	0.23 ±0.01[c] (74%)	6.53 ±0.23[c] (74.95%)	3.63 ±0.05[c] (51.5%)	301.5 ±3.98[c] (72.01%)	48.4 ±2.32[c] (67.3%)
ECME 200 mg/kg	45.76 ±2.09[c] (76.05%)	70.56 ±2.1[c] (83.05%)	417.1 ±5.72[c] (83.9%)	0.54 ±0.04[c] (80.4%)	0.18 ±0.01[c] (80.5%)	6.83 ±0.15[c] (81.9%)	4.13 ±0.05[c] (73.55%)	263.7 ±1.83[c] (82.6%)	28.11 ±2.24[c] (88.14%)
ECME 400 mg/kg	40.66 ±2.15[c] (80.89%)	66.10 ±1.77[c] (84.89%)	403.8 ±1.82[c] (86.3%)	0.41 ±0.02[c] (86.4%)	0.15 ±0.01[c] (84.7%)	6.93 ±0.15[c] (84.3%)	4.22 ±0.11[c] (77.5%)	245.37 ±1.16[c] (87.5%)	23.43 ±4.1[c] (92.6%)

Data expressed as mean ± SD, n=6, values in parenthesis indicate percentage recovery. *p* value-Ethanol Vs vehicle; *p* value Ethanol Vs treatments- [a]<0.05; [b]<0.01; [c]<0.001.Methanolic extract of *Echinochloa colona* (ECME)

Table 3: Effect of ECME on different serum biochemical parameters in D-galactosamine induced hepatotoxicity in rats.

Groups	Glucose (U/L)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dL)	DB (mg/dL)	TP (g/dL)	ALB (g/dL)	LDH (U/L)	PT (seconds)
Normal	84±3	65.7±3.0	69.2±2.3	452.7±4.2	0.15±0.03	0.02±0.01	8.8±0.26	3.9±0.19	199.0±8.9	18.5±2.25
Toxic	15.6 ±2.51	199.9 ±4.4	263.07 ±6.2	1282.6 ±11.6	2.73± 0.15	1.84± 0.06	4.2±0.3	1.44± 0.06	373.17 ±6.3	157.09 ±5.39
Standard	64.33 ±3.5[c] (71.2%)	81.46 ±2.0[c] (88.2%)	87.83 ±2.07[c] (90.4%)	537.8 ±13.1[c] (87.7%)	0.58± 0.01[c] (83.3%)	0.22± 0.005[c] (90.5%)	7.96± 0.15[c] (80.4%)	3.27 ±0.16[c] (74.4%)	227.01 ±5.6[c] (83.9%)	29.77 ±4.39[c] (92.1%)
ECME 200	40.3 ±2.5[c] (36.1%)	99.43 ±3.6[c] (77.8%)	109.3 ±7.2[c] (80.1%)	762.5 ±21.5[c] (62.6%)	0.8± 0.01[c] (74.8%)	0.28 ±0.03[c] (85.7%)	7.83 ±0.25[c] (78.2%)	3.03 ±0.20[c] (64.6%)	265.5 ±4.16[c] (62.1%)	46.61 ±6.45[c] (79.8%)

Data expressed as mean ± SD, n=6, values in parenthesis indicate percentage recovery. *p* value - D-galactosamine Vs vehicle ; *p* value D-galactosamine Vs treatments-[a]<0.05; [b]<0.01; [c]<0.001. Methanolic extract of *Echinochloa colona* (ECME)

Table 4: *In vitro* antioxidant studies on the extract, ECME.

Free radical	IC50 value of the extract ECME in µg/mL	IC50 value of the extract standard in µg/mL
DPPH	64.5±3.2	0.39±0.12 (Rutin)
Superoxide	574.4±4.9	3.42±0.06 (Rutin)
Nitric Oxide	70.23±5.5	6.14±0.24 (Ascorbic acid)
Hydroxyl radical	431.9±3.2	3.41±0.12 (Mannitol)

Data expressed as mean ± SD, n=6

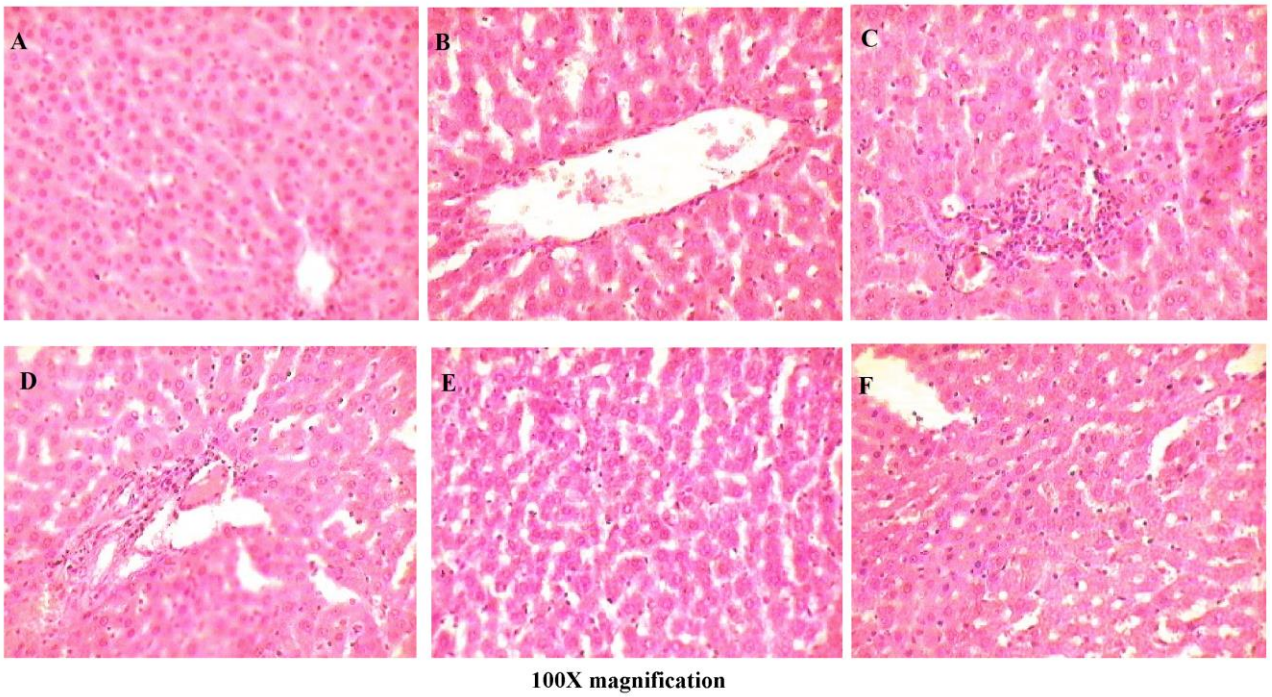

Fig. 2: Effect of Methanolic extract of *Echinochloa colona* (ECME) on liver pathologic analysis by hematoxyline-eosin stain after Ethanol treatment in rats. ECME and Ethanol were given as described in "Methods". (A) Normal control group; (B) Ethanol -treated toxic group; (C) Ethanol and 100mg/kg silymarin-treated group; (D Ethanol - and 100mg/kg ECME-treated group; (E) Ethanol - and 200mg/kg ECME-treated group; (F) Ethanol and 400mg/kg ECME-treated group. (Magnification 100X)

Assessment of Antihepatotoxic activity of ECME in D-Galactosamine induced hepatotoxicity in rats

The results of the study are presented in table 4 and fig. 3. Pretreatment of rats with D-galactosamine caused hepatotoxicity in rats. The hepatotoxicity of D-Galactosamine was confirmed by estimating the hepatic enzymes, bile pigments, proteins and glucose (GLU) in serum and in the histology of liver of rats. Treatment with reference standard (Silymarin 100mg/kg) and the extract, ECME (200mg/kg b.w.p.o.) showed a significant ($p<0.001$) protection, against D-galactosamine induced liver damage with good recovery from the altered level of aforesaid serum biochemical parameters and by curtailing the histological abnormalities.

In vitro antioxidant studies of ECME

ECME has shown a concentration dependent in *vitro* free radical scavenging activity. The IC_{50} of ECME compared to standards is shown in table 4.

Total flavonoid and phenolic contents

The total flavonoid content in the ECME extract corresponds to 55.9 mg of rutin equivalents per gram of extract (SD is 1.37, 6 measurements). The total phenolic content in the ECME extract corresponds to 13.65 mg of gallic acid equivalents per gram of the extract (SD is 0.49, 6 measurements).

Reducing power assay

The results are expressed in terms of ascorbic acid equivalents and were found to be 31.86±0.67mg of ascorbic acid equivalents per gram of extract.

DISCUSSION

As the caryopses of the plant *Echinochloa colona* is traditionally claimed to be useful in the treatment of liver disorders and the preliminary chemical investigation on the caryopses of the plant indicated the presence of phytoconstituents of pharmacological importance such as

flavonoids, phenolic compounds, steroids, saponins etc., the present investigation was undertaken to assess scientifically the hepatoprotective potential of the plant.

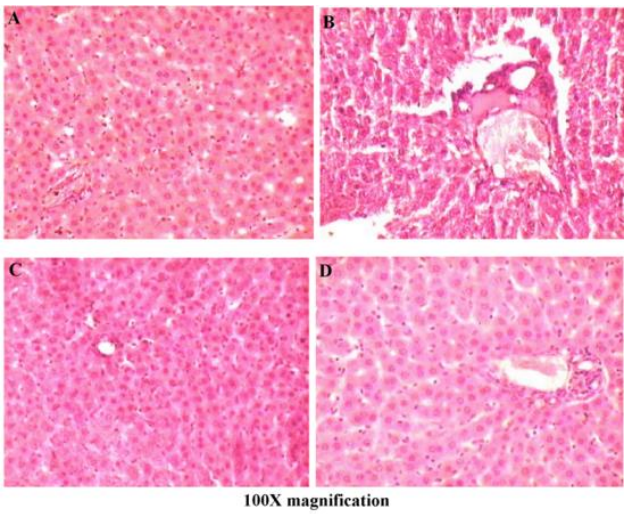

Fig. 3: Effect of methanolic extract of *Echinochloa colona* (ECME) on liver pathologic analysis by hematoxyline-eosin stain after D-galactosamine treatment in rats. ECME and D-galactosamine were given as described in "Methods". (A) Normal control group; (B) D-galactosamine -treated toxic group; (C) D-galactosamine and 100mg/kg silymarin-treated group; (D) D-galactosamine and 200mg/kg ECME-treated group. (Magnification 100X)

Paracetamol, a widely used analgesic and antipyretic drug and is harmful to liver when taken in larger doses due to the formation of a toxic metabolite N-acetyl-parabenzo quinoneimine, which cause damage to plasma membrane of hepatocytes leading to liver necrosis (Gazzard *et al*., 1974). In excess consumption of alcohol, the cytochrome P450 isoenzyme participates in ethanol oxidation and generates free radicals and culminates into various liver associated diseases (Kumar and Vasudevana, 2007). Paracetamol or ethanol intoxication in rats lead to the change in the level of hepatic enzymes ALT, AST, ALP and LDH in serum due to damage to the liver cells (Arshed *et al*., 2012). In liver injury, other biochemical parameters such as TB, DB and TP, ALB, PT were also significantly ($p<0.001$) altered due to obstruction of bile ducts and inhibition of protein synthesis respectively. Pretreatment with ECME with respect to intoxication with paracetamol or ethanol controlled the levels of hepatic enzymes and other biochemical parameters when compared with the toxic group. This might be due to its capability to preserve the normal hepatic physiological mechanisms and to reduce the toxic effects produced by the toxicants. The histological profile of the liver in paracetamol and ethanol treated rats, revealed the noticeable alterations in its histoarchitecture showing fatty changes, ballooning degeneration, centrilobular necrosis, increase in sinusoidal spaces, proliferation of kupffer cells and damage and bleeding in hepatic lobes. The extract, ECME has shown a definite sign of protection against injury at all test doses. Among the three test doses, ECME at 200 and 400mg/kg exhibited a remarkable protection from alteration of serum biochemical parameters and histoarchitecture of the liver. Hence smaller dose i.e. ECME 200mg/kg is preferred to assess the curative effect against D-Galactosamine induced hepatotoxicity.

The hepatotoxicity induced by D-Galactosamine (D-GaIN) resembles that of human viral hepatitis both in metabolic and morphological aberrations that always caused peri-portal inflammation (Keppler *et al*., 1968) and hepatocyte apoptosis (Decker and Keppler, 1974). The hepatotoxicity induced by D-GaIN is due to the acquisition of UDP-GaIN derivatives in the liver and depletion of hepatic UTP, thus resulting in the inhibition of mRNA and protein synthesis leading to activation various signaling pathways consequently causing apoptotic cellular death (Catal and Bolkent, 2008). Treatment with ECME 200mg/kg decreased the level of serum ALT, AST, ALP and LDH in serum, suggesting extract's ability to scavenge reactive oxygen species generated from D-GaIN intoxication and hence prevent hepatic cellular enzymes from leaking into the blood. Increase in serum bilirubin level in D-GaIN treated rats is due to abnormal excretion of bile by the liver. Administration of ECME 200mg/kg decreased the serum bilirubin level indicating the extract's ability to repair the damaged hepatocytes. In addition it also increased the reduced levels of TP and ALB in serum which may be attributed to the extract's ability to stabilize endoplasmic reticulum and trigger protein synthesis. The extract also increased the serum GLU level by eliminating the toxic metabolite and accelerating the formation of hepatic UTP. Liver synthesizes different clotting factors such as I, II, V, VII, IX and X (Suttie and Jackson, 1977). Apart from the effect on other hepatospecific parameters, the extract, ECME also showed a significant ($p<0.001$) recovery in PT which indicates the improved synthetic capacity of the liver.

The histological examination of the liver of rats treated with D-GaIN showed inflammation of portal tract, hyperplasia of kupffer cells, vacuolization of hepatocytes and bleeding in midzonal areas. Treatment with ECME 200mg/kg has shown significant recovery against the damage, which may be due to prevention of accumulation of UDP-GaIN in liver cells there by inhibiting various signaling pathways leading to apoptotic cell death.

The free radical scavenging activity of ECME was performed in DPPH experiment. The extract has shown concentration-dependent DPPH radical scavenging activity which was evident through the formation of yellowish-coloured diphenyl-1-picryl hydrazine molecule. The endogenous free radicals such as superoxide anion, hydroxyl radical and nitric oxide are very harmful and

toxic to tissues as they can cause oxidative damage to DNA, lipids, proteins and are capable of damaging almost every molecule found in living cells (Naskar, 2010). In the present study the extract ECME, scavenged these free radicals and exhibited reducing power in a concentration dependent manner. The reducing ability of the extract, ECME may be due to the formation of reductants which would react with free radicals and terminate the radical chain reaction. And the antioxidant activity exhibited by the extract, ECME may be attributed to the flavonoids and phenolic compounds present in it.

CONCLUSION

The pretreatment with the extract, ECME has shown a significant protection against paracetamol and ethanol induced hepatic damage which might be due to increased hepatic cell regeneration and increase in synthetic capacity of liver. The extract also showed remarkable recovery against D-Galactosamine induced hepatic damage indicating the ability of the extract in accelerating the detoxification of foreign chemicals. The activity of the extract may be attributed to the improved defence of the hepatocytes against the reactive oxygen species. The histological studies also substantiate the activity of the drug. Therefore, the study scientifically supports the traditional usage of this plant in the treatment of liver disorders.

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