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Research Article

Antioxidant and Hepatoprotective Studies on Methanolic Extract of Caryopses of *Echinochloa frumentacea* Link

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ABSTRACT

Background: The present study was aimed to evaluate the methanolic extract of caryopses of *Echinochloa frumentacea* (EFME) for its *in-vitro* antioxidant activity and *in-vivo* hepatoprotective activity against paracetamol and antihepatotoxic activity against D-Galactosamine induced hepatic damage in rats. Materials and Methods: The flavonoid and phenolic contents, of EFME were estimated and the antioxidant studies were conducted for the extract, EFME using various methods such as DPPH, superoxide, nitric oxide, hydroxyl radical, hydrogen peroxide scavenging activities and reducing power assay. Liver damage was induced by administering paracetamol (3g/Kg) and the hepatoprotective 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 taking Silymarin (100mg/kg) as the standard. Among the three test doses of EFME, 100 mg/kg was found to be the effective. Hence, EFME 100 mg/kg was selected to assess its antihepatotoxic activity against D-Galactosamine (400 mg/kg) induced hepatic damage in rats. Results: The flavonoid and phenolic contents of the extract, EFME were found to be 7.56±1.29mg and 138.53±2.11mg of rutin and gallic acid equivalents per gram of extract respectively. The extract exhibited antioxidant activity and significant ($p<0.01$) hepatoprotective activity. Conclusion: The study revealed that the extract EFME at 100 mg/kg has shown significant hepatoprotective and antihepatotoxic activity against paracetamol and D-Galactosamine induced hepatic damage in rats respectively.

Keywords: Antioxidants, D-Galactosamine, *Echinochloa frumentacea,* Hepatoprotective activity.

INTRODUCTION

Plants are one of the important sources for medicines used in the prevention and cure of diseases and also for rejuvenation. Herbal medicine is a major component in traditional medicine of all indigenous people and a common element in Ayurveda, Siddha, Amchi, Unani, Homeopathic, Naturopathic and Native American Indian medicine[1]. Although herbal medicines are effective in treatment of various ailments such as Cancer, AIDS, Tuberculosis, Diabetes and Liver disorders etc., very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science[2]. Therefore there is a need for identification of such plants for scientific pharmacological investigation.

Echinochloa frumentacea Link of Family Graminae is a stout annual plant from 90-150 cm high. The seed is 2-3 mm long and 1.5-2 mm wide. The flowers are hermaphrodite and are pollinated by wind. It occurs widely in tropical Asia as a cereal and millet. This millet is widely grown as a cereal in India, Pakistan and Nepal. Traditionally the grains of the plant are used to treat biliousness, constipation, in ascites, obesity and diabetes[3]. In view of its traditional claim in the treatment of liver disorders, the present investigation was aimed to evaluate its hepatoprotective and antihepatotoxic activities against paracetamol and D-Galactosamine induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection and preparation of methanolic extract:

The plant *Echinochloa frumentacea* Link was collected in the month of September 2014 from rice fields of Warangal, Telangana, India, after the authentication of the plant by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal. A voucher specimen of the plant is being maintained in the herbarium of Department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal. The grains of *Echinochloa frumentacea* were made free from the adherent foreign material and air dried. Then the air dried material (600g) was coarsely powdered and macerated with methanol, filtered and concentrated under reduced pressure [Rotavapour, Switzerland] to yield a green semisolid mass (6g). It was given a code EFME. The so obtained extract was kept in a dessicator to remove moisture and stored properly until used.

Drugs and chemicals:

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Table 1: In-vitro antioxidant studies on the extract, EFME.

Free radical	IC50 value of the extract EFME in µg/mL	IC50 value of the extract standard in µg/mL
DPPH	51.49 ± 1.8	0.39 ± 0.12 (Rutin)
Superoxide	589.67 ± 7.7	0.34 ± 0.06 (Rutin)
Nitric Oxide	47.89 ± 3.9	6.14 ± 0.24 (Ascorbic acid)
Hydroxyl radical	450.21 ± 5.1	3.12 ± 0.12 (Mannitol)
Hydrogen peroxide	410.8 ± 3.25	3.18 ± 0.05 (Ascorbic acid)

Data expressed as mean ± SD, n=3

Table 2: Effect of EFME on serum biochemical parameters and prothrombin time against paracetamol induced hepatotoxicity in rats.

Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dl)	DB (mg/dl)	TP (mg/dl)	PT Sec
CONTROL	250± 3.9	49.5± 3.2	278.56± 5.1	0.38± 0.3	0.20± 0.13	7.34± 0.98	16±2.22
TOXIC (paracetamol)	523.36± 5.2**	127.6± 4.02**	592.37± 5.34**	2.22± 0.2**	0.59± 0.07**	4.10± 1.11**	45.4± 3.01**
STANDARD (Silymarin 100mg/kg)	292± 5.22 ** (69.2%)	53.12± 6.2** (82.83%)	318± 5.5** (83.09%)	1.01± 0.14** (94.85%)	0.23± 0.01** (90.47%)	7.69± 1.04** (96.34%)	23.2± 1.2** (70.2%)
EFME 100mg/kg	294.4± 3.92** (76.21%)	59.3± 6.3** (84.46%)	324.15± 6.1** (88.52%)	1.05± 0.17** (89.71%)	0.33± 0.02** (78.09%)	7.29± 2.21** (87.31%)	27.1± 3.1** (70.8%)
EFME 200 mg/kg	309± 3.9** (56.4%)	62.3± 6.2** (54.02%)	333.2± 6** (67.90%)	1.12± 0.15** (70.85%)	0.2± 0.1** (61.90%)	6.65± 1.23** (64.65%)	35.2± 1.2** (30.9%)
EFME 400 mg/kg	334.26± 5.8** (45.33%)	79± 6.15** (69.26%)	354.12± 4.2** (62.2%)	1.4± 0.2** (50.28%)	0.28± 0.04** (52.38%)	5.58± 1.18** (56.34%)	43.41± 4.0** (25.5%)

Data expressed as mean ± SD, values in parenthesis indicate percentage recovery
P value-control Vs other groups; P<0.01**

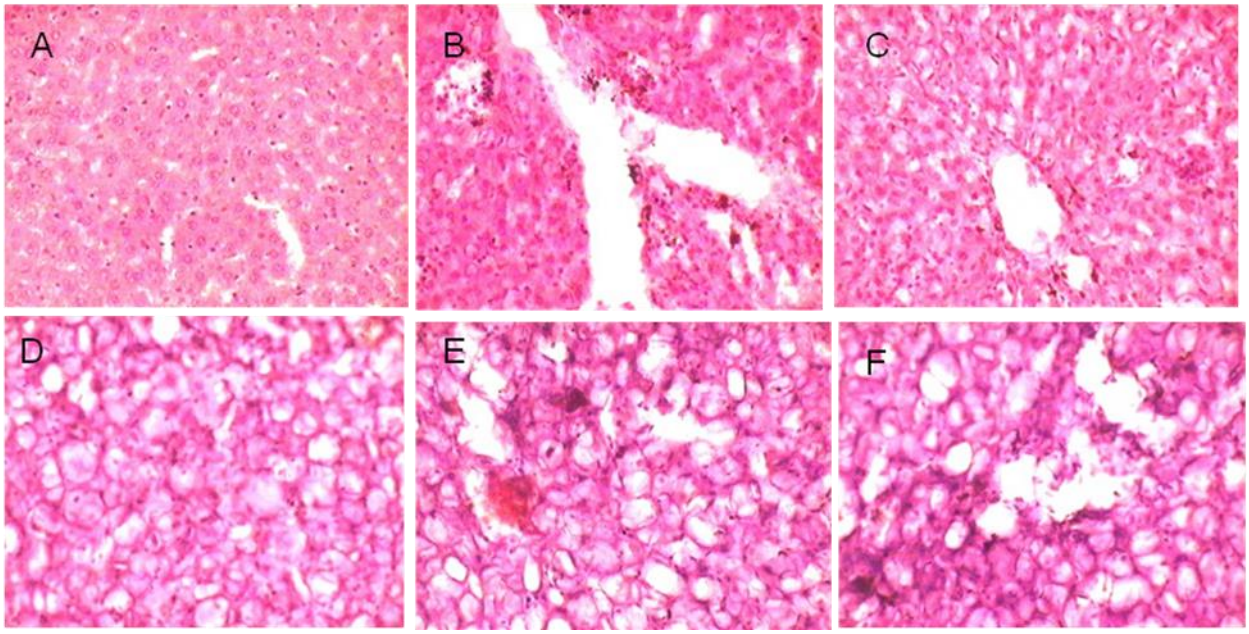

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Figure 1: Effect of EFME on histopathological changes in liver of rats in paracetamol induced hepatotoxicity. A: Normal; B: Toxic; C: Silymarin 100mg/kg; D: EFME 100mg/kg; E: EFME 200mg/kg; F: EFME 400mg/kg

Table 3: Effect of EFME on serum biochemical parameters and prothrombin time against D-Galactosamine induced antihepatotoxicity in rats.

Dose (mg/kg)	GLU (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dl)	DB (mg/dl)	TP (mg/dl)	PT (sec)
Control	80.53± 2.15	66.7± 2.23	66.2± 2.59	448.56± 8.25	0.18± 0.03	0.02± 0.001	8.2± 0.35	18± 2.22
Toxic (D-GaIN 400 mg/kg)	43.21± 1.25**	188.6± 5.21**	227.6± 5.68**	792.7± 12.54**	2.2± 0.13**	1.59± 0.02**	4.1± 0.05**	145.4± 4.02 **
Standard (Silymarin 100mg/kg)	68.34± 2.46** (67.5%)	80.5± 2.15** (88.6%)	85.12± 6.45** (86.2%)	518.35± 14.32** (79.6%)	0.35± 0.05** (67.5%)	0.23± 0.005** (85.6%)	7.61± 0.58** (85.1%)	29.2± 4.1** (75.2%)
EFME 100mg/kg	59.65± 3.21** (43.2%)	98.2± 3.14** (73.7%)	102.5± 6.34** (77.2%)	574.12± 12.38** (63.3%)	0.67± 0.01** (60.31%)	0.43± 0.01** (73.8%)	7.21± 0.79** (75.6%)	37.05± 3.1** (43.8%)

Data expressed as mean ± SD, values in parenthesis indicate percentage recovery. P value-control Vs other groups; P<0.01**

Paracetamol was obtained as gift sample from Natco pharma Limited, Hyderabad, India. All other drugs and chemicals were purchased from various companies and the details are as follows: Silymarin, D-galactosamine (D-GaIN)- Sigma Aldrich, Spruce Street, St. Louis, China; biochemical analytical kits [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total Bilirubin (TB), Direct Bilirubin (DB), Glucose(GLU), Albumin (ALB), and Total Protein (TP)] and Trichloro acetic acid- Merck Specialities Private Limited, Mumbai, India; 1,1 Diphenyl-1-picryl hydrazyl (DPPH), Thiobarbituric acid (TBA)-Himedia, Mumbai, India; All other chemicals and solvents used were of analytical grade.

Preliminary phytochemical screening:

The methanolic extract of *Echinochloa frumentacea* (EFME), was subjected to chemical tests for detection of various phytoconstituents such as saponins, steroid/triterpenoidal, flavonoidal compounds and their glycosides, alkaloids, phenolics and tannins[4].

Determination of total phenolic and flavonoid contents:

The total phenolic and flavonoid contents in EFME was determined using Folin Ciocalteu reagent and aluminium chloride assay methods respectively as described in the literature[5] with minor modifications. The total phenolic and flavonoid contents in EFME were carried out in triplicate and expressed as Gallic acid equivalents (GAE) and rutin equivalents (RE) in mg per gram of extract.

Determination of in-vitro antioxidant activity of EFME:

The test extract, EFME was screened to assess its antioxidant property by DPPH radical[6], superoxide[7], nitric oxide[8], hydroxyl[9], hydrogen peroxide[10] radical assay methods and also by reducing power assay[11].

Acute toxicity study:

Acute toxicity study was carried out for methanolic extract of *Echinochloa frumentacea* according to the Organization for Economic Co-operation and Development (OECD) 420 guidelines. All animals were observed for toxic symptoms and mortality for 72 hours.

Evaluation of protective effect of EFME in paracetamol induced hepatotoxicity in rats:

The experiment was performed according to the method given in the literature[12]. The rats were divided into six groups comprising six in each. 5% gum acacia was used as vehicle for suspending the standard drug and extract. Group I was kept as control received a single daily dose of vehicle (5% gum acacia 1 ml/kg.b.w.p.o.) for seven days. Groups II, III, IV and V were given orally a single daily dose of vehicle, Silymarin (50 mg/kg b.w.), EFME (100 mg/kg b.w) and EFME (200 mg/kg b.w), EFME (400 mg/kg b.w) for seven days respectively. On 8th day a single dose of paracetamol (3 g/kg.b.w.p.o) was administered to the animals of all groups leaving group I. Then blood and liver samples were collected from the animals of all groups 24hrs after administration of paracetamol for estimation of various biochemical parameters (ALT, ALP, TB, DB, GLU, TP) and histopathological studies. Prothrombin time was determined on 8th day by collecting blood in normal capillary tubes and breaking it into pieces until a thread is observed. Time was noted between the collections of blood to the appearance of thread.

Evaluation of antihepatotoxic effect of EFME on D-galactosamine induced hepatotoxicity in rats:

The antihepatotoxic activity of extract against D-GaIN induced hepatotoxicity was carried out according to the procedure given in the literature with minor modifications[13]. The rats were randomly divided into four groups of six animals each. Group I served as normal and received the vehicle (1mL/kg orally of 2% gum acacia in water) for 3 days. On the first day, D-GaIN (400 mg/kg i.p) was given to groups II, III and IV. Vehicle (2%gum acacia 1 mL/kg), Silymarin (100 mg/kg) and extract (100 mg/kg) were given to the animals of Groups II, III and IV respectively for three times at the time point of 2 hours, 24 hours, 48 hours after the administration of D-GaIN. The blood and liver samples were collected from the animals 1 hour after the last treatment for estimation of various biochemical parameters and histopathological studies respectively. Prothrombin time was determined on 4th day by collecting blood in normal capillary tubes and breaking it into pieces until a thread is observed. Time

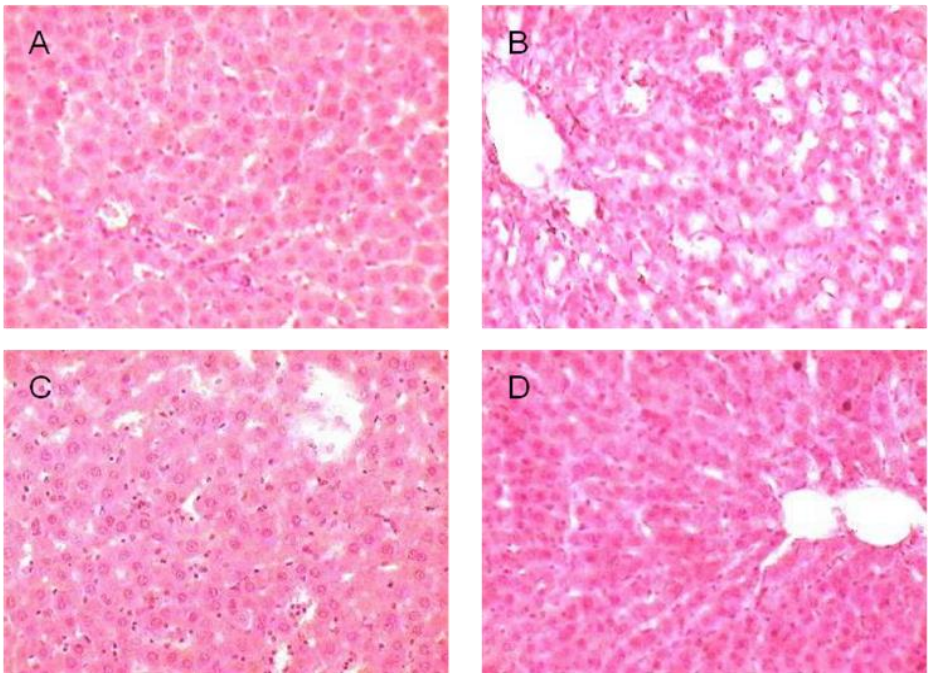

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Figure 2: Effect of EFME on histopathological changes in liver of rats in D-Galactosamine induced antihepatotoxicity. A: Normal; B: Toxic; C: Silymarin 100mg/kg; D: EFME 100mg/kg.

was noted between the collections of blood to the appearance of thread.

Histological studies:

Histological studies were done by staining the fine section of liver isolates and examining under the microscope. The liver samples collected from the rats of the study were washed with normal saline (0.9%). Then, 2-3 pieces of approximately 6cu.mm size were cut and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5µm thickness of liver tissue were cut and stained with haemotoxylin-eosin stain.

Statistical analysis**:**

The data obtained were analyzed by one-way of variance (ANOVA) followed by Durnett all Vs control for the significant interrelation between the various groups using Graph pad prism-3 computer software. $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

The preliminary investigation on the methanolic extract of caryopses of *Echinochloa frumentacea* (EFME) revealed that it contains chemical constituents of pharmacological significance such as steroidal/triterpenoidal and flavonoidal glycosides, saponins and phenolic compounds.

The total phenolic and flavonoidal contents:

The flavonoidal and phenolic compounds play an important role in culminating the oxidative stress produced by reactive oxygen species and hydroxyl radicals generated in liver diseases[14]. The total phenolic and flavonoidal contents of the extract, EFME were found to be 138.53±2.11 mg and 7.56±1.29 mg of gallic acid and rutin equivalents per gram of extract respectively.

Determination of in-vitro antioxidant activity of EFME:

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, liver disorders, cancer and ageing. Cells in the body use oxygen to break various nutrients and give energy. During this processes highly reactive free radicals are generated, however the higher quantities of such radicals like superoxide anion, hydrogen peroxide radical, nitric oxide radical, and hydroxyl ion radical may interact with cell membranes and cause damage[15]. The test extract, EFME shown a concentration dependent *in-vitro* free radical scavenging activity. The IC_{50} of EFME and the standard are shown in Table 1. The extract also showed a concentration dependent reducing power. The reducing power of the extract EFME is expressed in terms of ascorbic acid equivalents and was found to be 25.13±1.91mg.

Acute toxicity study:

The extract, EFME did not cause any adverse effects and mortality up to a dose level of 2000 mg/kg b.w. Hence, three graded doses of the extract i.e, 100, 200 and 400mg/kg b.w. were chosen for hepatoprotective and anti-hepatotoxic studies.

Evaluation of protective effect of EFME in paracetamol induced hepatotoxicity in rats:

The results of the study are presented in Table 2. Paracetamol intoxication in normal rats significantly ($P<0.001$) elevated the level of hepatospecific enzymes (AST, ALT, ALP), bilirubin in serum, and PT and decreased the level of TP in serum, indicating acute hepatocellular damage and biliary obstruction. The rats treated with EFME 100, 200 and 400 mg/kg b.w.p.o. and

Standard (Silymarin 100mg/kg b.w) showed a significant reversal of the level of these parameters. Based on the results, the hepatoprotective effect of EFME 100 mg/kg b.w.p.o. was well comparable to that of reference drug, Silymarin (100 mg/kg). Histopathological photographs of liver section of rats of the study are shown in Fig. 1. Histopathological examination of the liver sections of control rats showed normal hepatocytes where as paracetamol intoxicated rats revealed remarkable changes in the normal liver architecture of rats showing necrosis, fatty changes, dilatation of sinusoidal space, and bleeding in hepatic lobes. The liver section of rats pretreated with EFME 100, 200 and 400 mg/kg and Silymarin (100 mg/kg b.w) showed a significant recovery from the paracetamol induced hepatic damage with little dilatation of sinusoidal space and moderate accumulation of fatty lobules. Thus the histopathological studies support the findings of serum biochemical parameters of the study, revealing the hepatoprotective activity of the extract, EFME against paracetamol intoxication in rats.

Evaluation of antihepatotoxic effect of EFME on D-Galactosamine induced hepatotoxicity in rats: The results are presented in Table 3. D-Galactosamine induced hepatotoxicity experimental model in rat resembles viral hepatitis in humans, in both morphological and functional points of view[16]. Galactosamine has a great specificity towards hepatocytes as they consist of high levels of galactokinase and galactose 1-uridyl transfarase. It causes hepatic injury with spotty hepatocytes necrosis and marked portal and paranchymal infiltration[17]. It also causes depletion of uridine di phosphate by increasing the formation of UDP sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration[18]. D-GaIN intoxication in normal rats significantly increased the level of hepatic enzymes (AST, ALT, ALP), TBL and decreased the level of TP in serum. The rats treated with EFME 100 effective dose mg/kg b.w.p.o. and Standard (Silymarin 100mg/kg b.w) showed a significant reversal of the level of these parameters. The microscopic photographs of the liver sections of the animals of the study are shown in Fig 2. The intoxication of rats with D-GaIN caused severe liver damage involving remarkable changes in the normal liver architecture of rats showing necrosis, degeneration and deformation of hepatocytes, dailation in sinusoidal spaces, bleeding in hepatic lobes. The liver section of the rats treated with EFME 100 mg/kg and Silymarin (100 mg/kg b.w) showed a significant recovery from the D-GaIN induced hepatic damage. Based on results, the hepatoprotective effect of EFME 100 mg/kg b.w.p.o. was close to that of reference drug, Silymarin (100 mg/kg).Thus the histopathological studies support the serum biochemical parameters of the study, revealing the antihepatotoxic activity of the extract.

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CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest.

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