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Research Article

EFFECT OF EUPHORBIA MILII EXTRACT ON CYTOTOXICITY AND HEPATOPROTECTIVE ACTIVITY AGAINST PARACETAMOL-INDUCED HEPATIC DAMAGE IN WISTAR RATS

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ABSTRACT

Objectives: The objective of the study is to evaluate the hepatoprotective potential of the alcoholic extract of Euphorbia milii (AEEM) (family: *Euphorbiaceae*) against paracetamol (PCM)-induced acute liver damage, in support of its traditional use in Indian medicine.

Methods: An *in vitro* cytotoxicity test was conducted following ISO guidelines to assess the safety profile of the extract. Hepatotoxicity was induced in experimental models using PCM, and the protective effects of the Euphorbia milii (EM) extract were assessed by measuring liver function markers, including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum bilirubin (SB). Levels of lipid peroxidation (LPO) and glutathione (GSH) were also evaluated. Histopathological examination of liver tissues was performed to corroborate the biochemical findings.

Results: The *in vitro* cytotoxicity of AEEM reveals that the % viability of test item concentration observed as 105.06%, 103.12%, 100.05%, and 83.75% at 12.5%, 25%, 50%, and 100%, respectively. The % viability of positive control concentration observed as 47.11%, 8.92%, 0.51%, and 0.71% at 12.5%, 25%, 50%, and 100%, respectively. The test compound (AEEM extract) % viability was observed >70% of the blank, so the test item is concluded as "Non-Cytotoxic". EM alcoholic extract exhibited dose-dependent hepatoprotective effects. The high dose (Group III) significantly improved liver enzyme profiles and bilirubin levels, almost matching the standard treatment (Group VI), indicating its strong therapeutic potential against PCM-induced liver damage. The extract also enhanced hepatic antioxidant status by reducing LPO and increasing GSH levels. Histopathological analysis supported these findings by demonstrating preserved liver architecture and reduced cellular damage in extract-treated groups. This confirms the protective action of AEEM against experimentally induced liver damage in rats.

Conclusion: The present study confirms the hepatoprotective potential of the AEEM against PCM-induced liver damage. Preliminary phytochemical screening revealed the presence of bioactive constituents such as glycosides, proteins, terpenoids, phenols, and flavonoids, which may contribute to its pharmacological activity. The *in vitro* cytotoxicity assay demonstrated more than 70% cell viability, indicating the extract is non-cytotoxic and safe for biological use. The extract significantly attenuated elevated serum markers (AST, ALT, ALP, and SB) and preserved normal liver architecture in histopathological evaluations, comparable to the standard drug silymarin. These findings support the traditional use of EM in the management of hepatic disorders and suggest its potential as a natural hepatoprotective agent with antioxidant properties. Further studies are warranted to isolate the active constituents and elucidate the precise mechanisms of action.

Keywords: Cytotoxicity, *Euphorbia milii*, Flavonoids, Hepatic, Paracetamol, Silymarin.

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INTRODUCTION

Euphorbia milii (EM) (Family: *Euphorbiaceae*) is also known as corn of thorns, a species of flowering plant. It is a shrub grown in warmer climates, and this medicinal plant is widely distributed throughout the tropical regions of India, China, and Pakistan [1]. EM has been reported to possess different pharmacological activities such as analgesic, anti-inflammatory, diuretic, anthelmintic, and immune-modulatory activity [2,3]. The various species of Euphorbia are implicated as a folk medicine for the treatment of different ailments such as warts, eczema, cancer, antifungal, liver disorders, and molluscicidal properties [4,5]. Although a plethora of evidence regarding the therapeutic efficacy of EM has been published, the knowledge of bioactive phytochemicals, owing to its hepatoprotective efficacy, is quite limited.

With a view that sterols, flavonoids, and triterpenes found in medicinal plants are used as hepatoprotective drugs, it was thought worthwhile to conduct hepatoprotective studies on the flowers of the EM in a scientific manner to validate its use in the traditional system of medicine. Furthermore, histological reviews had been carried out to prove the effectiveness of EM in a preventive and healing function against paracetamol (PCM) induced toxicity of liver histopathology in Wistar rats.

METHODS

Chemicals

0.1% zinc diethyldithiocarbamate polyurethane film (positive control), 10% fetal bovine serum (FBS) was used as a vehicle control, cell line L-929 cells (test system), minimum essential medium (MEM) with 10% FBS (growth media), Temperature: 37±1°C, Carbon dioxide: 5% (growth conditions).

Acetaminophen (PCM) 500 mg API was purchased from Sigma, while silymarin and saline were procured from a local pharmacy. The following biochemical parameters of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin were estimated through specifications kits obtained from Span Diagnostics, Surat, India. Other chemicals and reagents for this investigation had been of diagnostic grade.

Plant materials

EM plant material was collected from Rangareddy District, Telangana. The

plant specimen was identified by Prof. Rana Kausar, Department of Botany, Osmania University, Hyderabad, Telangana State. A specimen was deposited in their herbarium. Then after the flowers were washed thoroughly, dried in shade at room temperature, and ground to an optimal coarse powder.

Extraction
EM flower was collected, shade-dried, and powdered. Each powder was subjected to Soxhlet extraction three times at ambient temperature (50–60°C) with 90% methanol. During the extraction with solvents, the solvent was changed every 24 h. The solvents from the pooled extracts were removed by rotary evaporator under reduced pressure at 50–60°C to create crude extracts of alcoholic extract of EM (AEEM). The extracts were subjected to preliminary phytochemical investigation and subjected to the biological activity screening tests [6,7].

Preliminary phytochemical studies
The extract of EM was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, mucilage, proteins, steroids, tannins, and terpenoids.

Cytotoxicity
The cytotoxicity of EM extract was studied using the MTT assay using L-929 cells (NCTC clone 929: CCL 1, American Type Culture Collection [ATCC]) and the study was conducted according to ISO 10993-5:2009 (E): Tests for *in vitro* cytotoxicity [8].

Each concentration of the EM extract and reference items (negative and positive control) was added (100 µL) per well of cell culture 96-well plates for the treatment. The test compound, reference item, was handled aseptically throughout the experiment. The dilution of the test compound and reference item was prepared shortly before the treatment. On the day of treatment, each plate was examined under a phase contrast microscope to ensure that cell growth was relatively even across the 96-well microtiter plates. The spent culture medium was aspirated from the cells. After aspiration, all the test items, negative and positive control extract dilutions were added (100 µL) to the respective wells of the treatment plate of the cells. Along with these test compounds, a reference item, vehicle control also added to respective wells, and it is considered a blank. Plate (cells) was incubated for 24 h at 37°C, 5% CO_2. After 24 h treatment, each plate was examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Changes were recorded in the morphology of the cells due to the cytotoxic effects of the test sample extract.

Inclusion criteria
1. L-929 cell line (NCTC clone 929: CCL 1, ATCC) was used as the test system
2. MEM supplemented with 10% FBS
3. Cells exhibiting normal growth and morphology at seeding
4. Only those experimental plates with uniform cell distribution and a blank OD570 ≥0.2 were included
5. Healthy Wistar albino rats of either sex (male/female), aged approximately 2 months
6. Animal body weight between 150 and 200 g.

Exclusion criteria
1. Plant parts other than the flowers of EM
2. Unauthenticated or improperly identified plant samples
3. Cell cultures with contamination, poor viability, or inconsistent growth patterns
4. Plates with OD570 <0.2 or blanks showing >15% deviation in OD from the mean
5. Animals showing signs of illness, abnormal behavior, or injury before experimentation
6. Animals with body weight outside the 150–200 g range
7. Animals not receiving full treatment protocol (e.g., missed dose or incomplete PCM induction)
8. Samples collected after deviation from the scheduled time post-treatment.

Hepatoprotective activity and grouping
An experimental study was carried out on Wister albino rats of either sex (M/F) rat's aged 2 months. Their body weights ranged from 150 to 200 g. Divided into 6 groups of 6 animals per cage. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24 h). The food in the form of dry pellets and water is provided *ad libitum*. All the animals were approved by the ethics approval committee of the institute (Reg. No. 1636/PO/Re/S/12/CPCSEA).

Randomization
Based on body weight.

Experimental setup
- Group I: Vehicle-treated rats (1 mg/kg b.wt)
- Group II: Control (PCM 100 mg/kg b.wt)
- Group III: PCM+Silymarin (100 mg/kg b.wt)
- Group IV: Rats treated with AEEM (100 mg/kg b.wt)
- Group V: Rats treated with AEEM (250 mg/kg b.wt)
- Group VI: Rats treated with AEEM (500 mg/kg b.wt).

The PCM was diluted with saline (vehicle) before oral administration (o.p). To enhance the acute liver damage in animals of groups II, III, IV, V, and VI, food was withdrawn 12 h before PCM administration. Animals were sacrificed 24 h after administration of PCM. Blood samples were collected by puncturing the retro-orbital plexus under light ether anesthesia and allowed to coagulate for 30 min at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters [9,10].

Antioxidant activity
Free radical scavenging activity
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of the extract of AEEM were carried out based on the procedure laid by Ohkawa *et al*. Briefly, 0.2 mL of the sample solutions of various concentrations was added to 1 mL of 0.1 mm of freshly prepared DPPH solution. The reaction mixture was shaken forcefully, and the absorbance at 517 nm was determined after 20 min at room temperature. A control sample was prepared containing the same volume without test compounds or reference antioxidants, while dimethyl sulfoxide was used as a blank. The reference antioxidant butylated hydroxytoluene (BHT) was used as the positive control in all the assays. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated as follows:

$$\text{Scavenging effect}(\%) = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\right]$$

Where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the extract or fractions or standard.

Assay of $FeCl_3$ power
The ferric reducing antioxidant power (FRAP) assay was determined by the technique of Benzie and Strain with minor adjustments [11,12]. It depends on the capacity of the sample to reduce the ferric tripyridyltriazine complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intense blue color, which can be understood by writing at 575 nm. The stock solutions consist of 300 mm acetate buffer (pH 3.6), 10 mm 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mm of HCl, and 20 mm ferric chloride solution. The new working solution was ready by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3 \cdot 6H_2O$, and the temperature was maintained at 35°C earlier before use. The various concentrations of extract, fractions, and BHT (10–50 µg/mL) were allowed to react with 2 mL of the FRAP solution for 30 min in dark conditions. The absorbance was recorded at 575 nm. The results are spoken in µMFe (II)/g and were estimated using aqueous $FeSO_4 \cdot 7H_2O$ (20–100 µM) as a standard for calibration.

The in vitro lipid peroxidation (LPO) inhibition activity in rat liver homogenate [13]

A cold thiobarbituric acid (TBS), a 5% rat liver-homogenate was prepared, and 50 µL of that was used in the assay. Fresh plant tissue (0.5 g) in 1 mL of cold TBS was correctly weighed and homogenized. 50 µL of it were used in the assay. To cause oxidation, ferrous sulphate was applied to the assay media at a final concentration of 10 µmoles. The final volumes were made with cold TBS in the test tubes at up to 500 µL. For each sample, tests were prepared containing the respective plant extract (50 µL), liver homogenate (50 µL), and TBS to make up the final volume to 500 µL. The control tubes were not filled with prooxidant. Furthermore, a blank containing no plant extract, no homogeneous liver but only $FeSO_4$ and TBS was prepared to make a final volume of 500 µL. By adding all the other constituents except the plant extract, an assay medium corresponding to 100% oxidant was prepared, and the volume with cold TBS was made up to 500 µL. The auto-oxidation-related experimental medium included only the liver homogenate and TBS to make up the final volume to 500 µL. All the tubes were incubated for 1 h at 37°C. Following the incubation period, to avoid the reaction, 500 µL of 70% alcohol was applied to all the tubes. 1 mL of 10% TBA was added to all the tubes, followed by boiling for 20 min in a hot water bath. The tubes were centrifuged after they had cooled to room temperature. To each supernatant, 500 µL of acetone was added, and the formation of TBARS was quantified spectrophotometrically at 535 nm.

Reduced glutathione (GSH) estimation [14]

Through homogenizing 0.5 g of the sample in 2.5 mL of 5% trichloroacetic acid (TCA), a 20% homogeneous solution was obtained. To which 0.5 mL of homogeneous tissue was added to precipitate the protein, 125 µL of 25% TCA. The precipitated protein had been centrifuged for 10 min at 1000 rpm. The homogenate was cooled on ice, and the supernatant was taken to 0.1 mL for estimation. The supernatant was buffered with 0.2M sodium phosphate (pH 8.0) for up to 1 mL. 2.0 mL of freshly prepared DTNB solution was added to the tubes, and after 10 min, the yellow color intensity was formed and measured at 412 nm in a spectrophotometer.

Assessment of liver functions

The hepatoprotective impact of the extract was assessed by the measurement of liver potential, biochemical parameters, for design ALT, AST, ALP, and Total serum bilirubin (SB), LPO as malondialdehyde (MDA), and GSH as per commonplace protocols.

Histopathological study

Histopathological investigation of the liver was done according to the Chowdhury *et al*. Method [15].

Statistical analysis

On each set of data Bartlett's test was conducted to ensure that the variation of the set is homogeneous. In the case of a homogeneous set of results, analysis of variance was conducted to assess the treatment effects, and using Origin Pro 7.6 statistical software, Dunnett's test was used as applicable. This was transformed using the correct transformation in the case of heterogeneous data. The variance was measured at a meaning level of 5% and the values were expressed as mean±standard error of the mean (SEM), and $p<0.05$ was considered as statistically significant.

RESULTS

Extraction

Fresh EM plants were collected under shade and dried. Each plant powder was subjected to Soxhlet extraction three times at ambient temperature (50–60°C) with 90% methanol. During the extraction with solvents, the solvent was changed every 24 h. The solvents from the pooled extracts were removed by rotary evaporator under reduced pressure at 50–60°C to create crude extracts of AEEM. The percentage yields (15.5%) of the extractives of the plant AEEM. The extracts were subjected to preliminary phytochemical investigation and subjected to the biological activity screening tests.

Preliminary phytochemical studies

The phytochemical studies show the presence of glycosides, steroids, proteins, flavonoids, terpenoids, and tannins.

***In vitro* cytotoxicity**

A decrease in the number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of blue–violet formazan formed, as monitored by the optical density (OD) at 570 nm. To calculate the reduction of viability compared to the blank, the following equation will be used:

$$\text{Viability \%} = \left[\frac{100 \times OD_{570e}}{OD_{570b}}\right]$$

Where,

- $OD_{570}e$ is the mean value of the measured OD of the 100% extracts of the test sample
- OD_{570b} is the mean value of the measured OD of the blanks
- The lower the viability % value, the higher the cytotoxic potential of the test item is
- The absolute value of OD, OD_{570e}, obtained in the untreated blank, indicates whether the 1×10^4 cells seeded per well have grown exponentially with normal doubling time during the 2 days of the assay
- A test meets the acceptance criteria if the mean OD_{570} of untreated blanks is ≥0.2
- A test meets the acceptance criteria if the left and the right means of the blanks do not differ by more than 15% from the mean of all blanks.

Microscopic observations

At the end of the treatment (24 h), the cultures were examined to assess the general morphology, vacuolization, detachment, color change of the media, and turbidity. Cell morphology was fibroblast. No vacuolization was observed in vehicle control, negative control, and test item concentrations; vacuolization was observed in the positive control. No cell detachment was observed in vehicle control, negative control,

Table 1: Results of Average OD value for Test, Negative and Positive control

Group	VC1	NC1	T1	T2	T3	T4	PC1	PC2	PC3	PC4	NC2
Average	0.849	0	0.912	0.923	0.854	0.61	0.386	0.073	0.004	0.006	0
SD	0.027	0	0.044	0.043	0.04	0.031	0.024	0.008	0.002	0.011	0

Table 2: Summary of results for % viability and % inhibition

GROUP	VC	NC	T1	T2	T3	T4	PC1	PC2	PC3	PC4
%VIABILTY	100	0	105.06	103.12	100.05	83.75	47.11	8.92	0.51	0.71
%INHIBITION	0	0	-5.6	-3.12	-0.05	16.25	52.89	91.08	99.49	99.29

VC=Vehicle Control; NC=Negative Control; TI=Test item concentration (T4=Highest Concentration; T1=Lowest Concentration);PC=Positive control(PC4=Highest Concentration; PC1=Lowest Concentration)

Table 3: Effect of AEEM on ALT, AST, ALP, and SB in PCM-induced liver toxicity in rats

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	SB (mg/dL)
Group-I	57.00±1.02	52.00±2.84	103.33±6.42	0.55±0.01
Group-II	220.66±2.52[a]	190.33±3.02[a]	248.16±5.38[a]	2.03±0.15[a]
Group-III	172.00±1.54***	133.50±3.48***	198.5±3.63***	0.93±0.05***
Group-IV	191.83±5.12***	142.16±3.51***	212.16±5.51*	0.99±0.05***
Group-V	180.25±1.52	135.78±4.12	198.26±2.42	0.95±0.08
Group-VI	169.72±4.13	130.56±2.15	188.25±4.10	0.92±0.02

AEEM: Alcoholic extract of *Euphorbia milii,* ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SB: Total serum bilirubin, PCM: Paracetamol. Each value represents the mean±standard error of the mean. n=6 number of animals in each group. [a]p<0.001 versus vehicle control, *p<0.05, **p<0.01,***p<0.001, Compared to respective PCM treated control groups

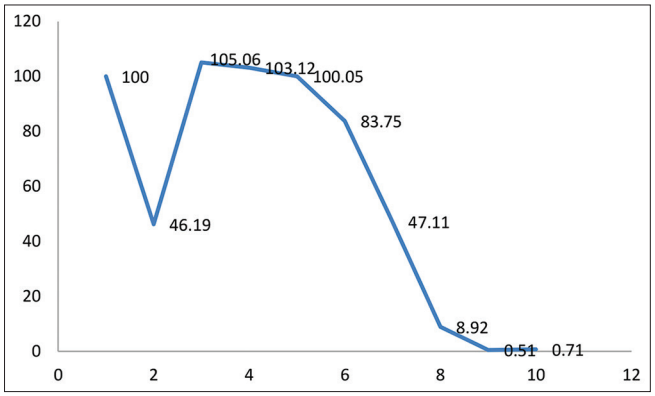

Fig. 1: Comparison of % viability of test samples with control. T=Test item concentration (T1=12.5% T2=25%, T3=50% and T4=100%); PC: Positive control concentration (P1=12.5% P2=25%, P3=50% and P4=100%); VC: Treated with plain culture medium with cells NC: Negative control

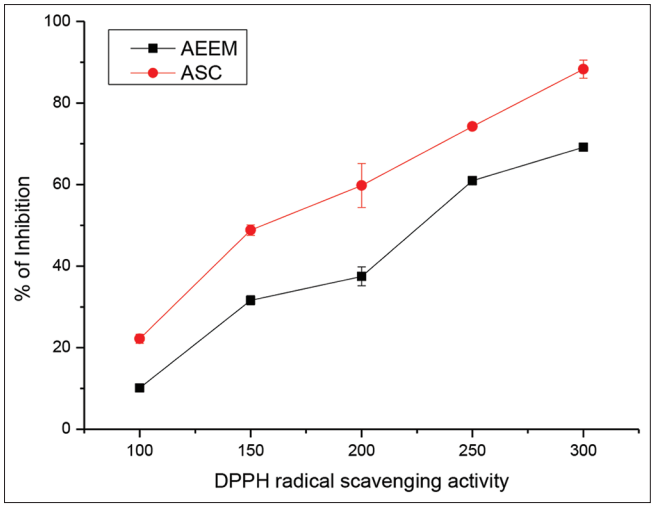

Fig. 2: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of alcoholic extract of *Euphorbia milii*. (ascorbic acid): Standard antioxidant; data represented as means±standard deviation (n=3)

and test compound concentrations; cell detachment was observed in positive control. No color change of the media was observed in the test compound and control groups. No turbidity was observed in the test compound and control groups.

Reading the absorbance

The mean OD of the blank was observed as 0.819. The observed blank OD meets the acceptance criteria (at least ≥0.2) of the MTT assay. The % viability and % inhibition of test, negative and positive control were

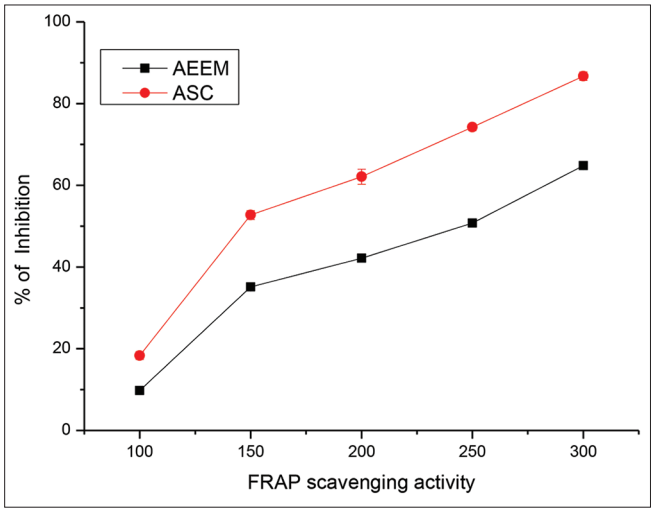

Fig. 3: Ferric reducing antioxidant power scavenging activity of alcoholic extract of *Euphorbia milii*

calculated by using average OD values (Table 1) and summary of results is given in Table 2, and a summary of results is given in Table 3. The % viability of test item concentration observed as 105.06%, 103.12%, 100.05%, and 83.75% at 12.5%, 25%, 50%, and 100%, respectively. The % viability of positive control concentration observed as 47.11%, 8.92%, 0.51%, and 0.71% at 12.5%, 25%, 50%, and 100% respectively. The negative control OD values were observed as 46.19% at 100% concentration (Fig. 1).

Free radical scavenging activity

DPPH radical evaluation

The substances are called antioxidants when they can reduce the stable radical (purple) DPPH to the non-radical form DPPH-H (yellow) and thus serve as radical scavengers because of their hydrogen donation capabilities. Fig. 2 presents the results of DPPH scavenging activity for all the test samples. With an increase in sample concentration (100–500 µg/mL), scavenging activity of AEEM extract and ascorbic acid (ASC) grew. AEEM and ASC found the IC_{50} values at 111.93 µg/mL (Y=0.294×−17.036) and 144.82 µg/mL (Y=0.315×−4.366). From these data obtained, the AEEM have been considered an effective free-radical inhibitor as well as the primary antioxidants, which can limit free-radical damage in the body.

Antioxidant power reduction assay (FRAP)

The FRAP assay is widely used in dietary polyphenols for assessing the antioxidant component. The reduction properties are usually related to the presence of compounds that exert their action through the donation of a hydrogen atom, breaking the free-radical chain. Fig. 3 shows the results of plant extracts reduction potential relative to ASC, a well-known antioxidant data. AEEM extract and ASC IC_{50} values showed 159.88 µg/mL (Y=0.2516×−9.774) and 143.93 µg/mL

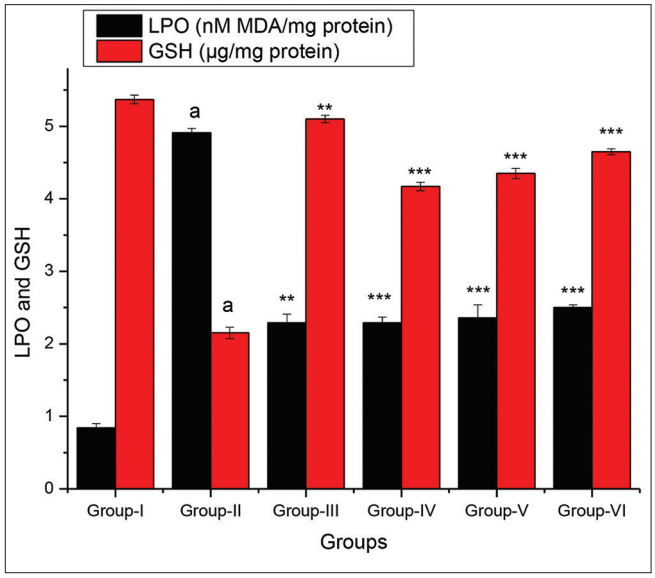

Fig. 4: Effect of alcoholic extract of *Euphorbia milii* on lipid peroxidation and glutathione, paracetamol (PCM) induced hepatic damage in rats. Each value represents the mean±standard error of the mean. n=6 number of animals in each group. [a]p<0.001 versus vehicle control, *p<0.05, **p<0.01, ***p<0.001, compared to respective PCM treated control groups.

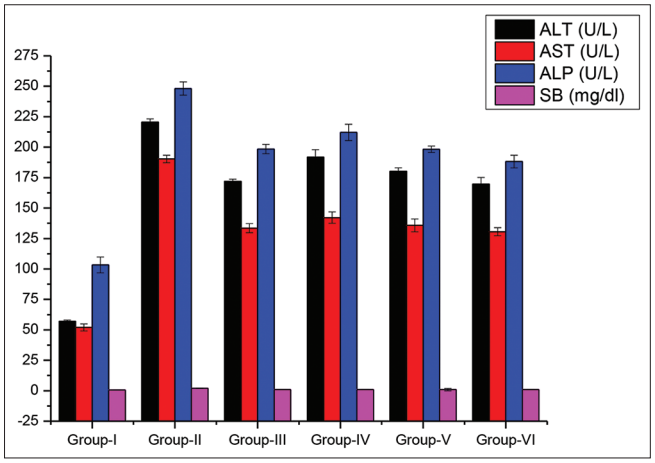

Fig. 5: Effect of alcoholic extract of *Euphorbia milii* on alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and serum bilirubin in paracetamol induced liver toxicity in wistar rats

(Y=0.3164×−4.46) of these.

The in vitro LPO inhibition activity in rat liver homogenate

There has been a critical increase in MDA substance and decrease in PCM Inebriated animals' GSH activities. Pre-treatment with silymarin (100 mg/kg b.wt) and AEEM (100, 250 and 500 mg/kg b.wt) effectively kept the expansion at MDA levels and transmitted them close to the typical level, while GSH levels were increased overall (p<0.01), along these lines giving assurance against toxicity to PCM. Results are shown in Fig. 4.

Hepatoprotective activity

PCM-induced liver toxicity

As part of the study, crude extraction of AEEM safeguarded the auxiliary uprightness of the hepatocellular film in a subordinate measurement manner, as clear from the assurance given by Silymarin (100 mg/kg b.wt; po), a well-known hepatoprotective specialist. PCM is known to affect liver damage through the activity of its dangerous metabolite, n-acetyl-p-benzoquinone imine, delivered by cytochrome P-450. This metabolite causes GSH exhaustion, which prompts the passage of cells.

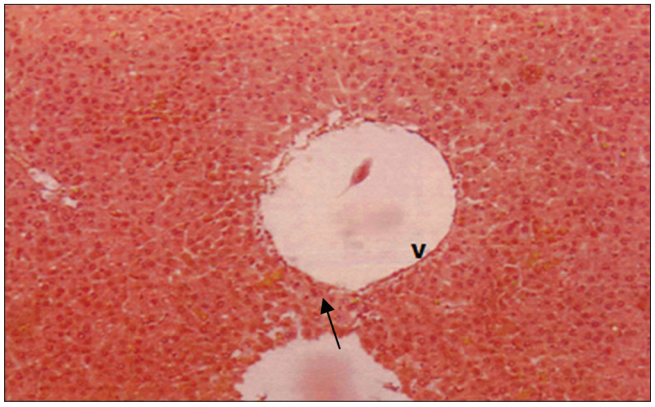

Fig. 6: Liver tissues of control animal showing normal histology. Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and liver ducts (arrow head). Stain H and E, grossing ×100 (Group I)

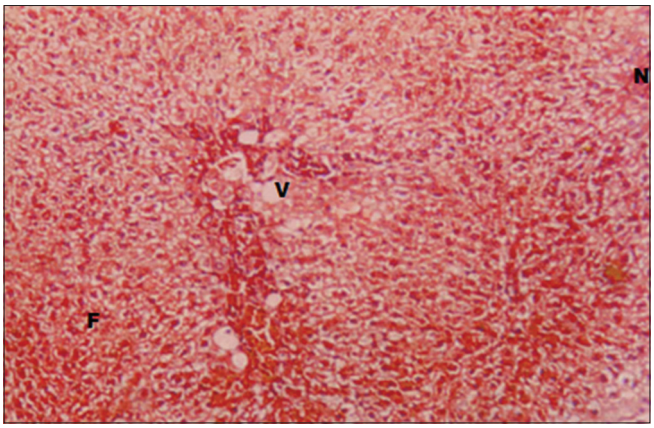

Fig. 7: Liver tissue of animal treated with paracetamol (PCM) showing necrosis. Liver tissue section of the animal treated with PCM showing necrosis (N), fatty vacuole (F) and central vein (v). Stain H and E, magnification ×100 (Group II)

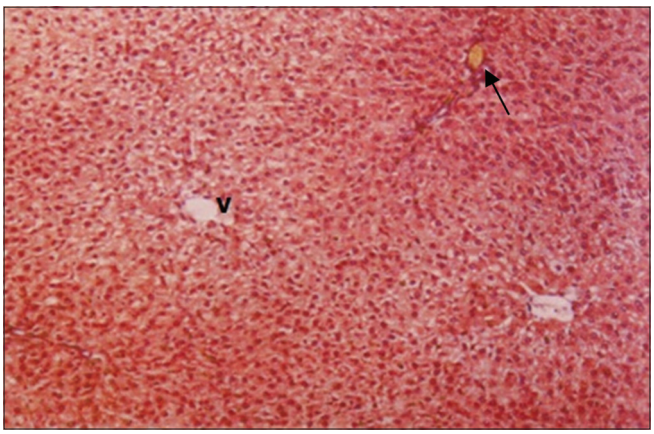

Fig. 8: Liver tissue of paracetamol (PCM)+silymarin treated animals showing normal hepatocytes. Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, ×100 magnification (Group III)

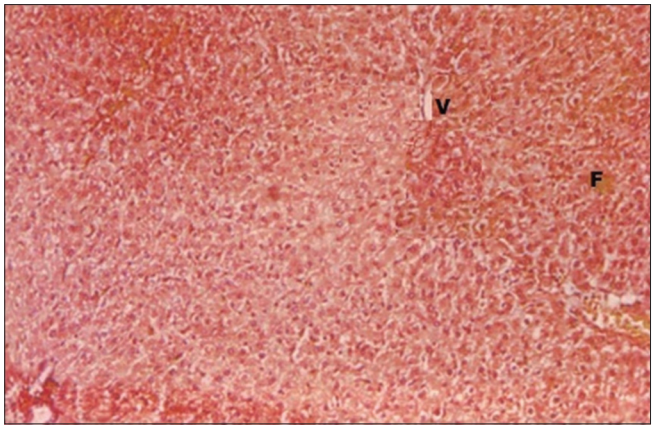

Fig. 9: Liver tissue of paracetamol (PCM)+100 mg/kg b.wt, po alcoholic extract of *Euphorbia milii* showing normal arrangement of hepatocytes. Liver tissue section of PCM+100 mg/kg b.wt, po cell enhancement and lymphocyte stimulation treated animals showing normal pattern of hepatocytes around the portal vein (V), lack of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, ×100 magnification (Group IV)

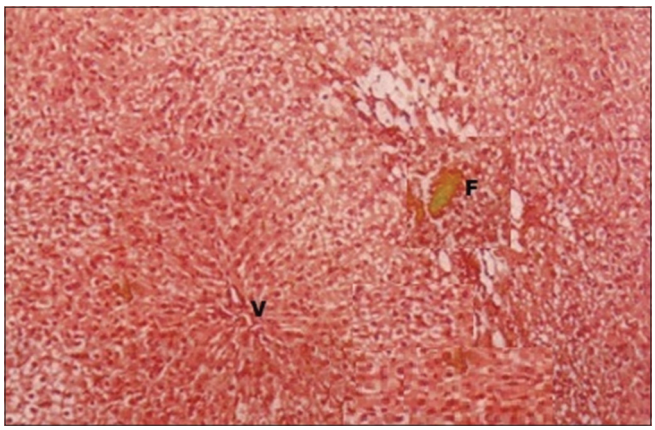

Fig. 10: Liver tissue of paracetamol (PCM)+250 mg/kg b.wt, po alcoholic extract of *Euphorbia milii* (AEEM) showing normal arrangement of hepatocytes. Liver tissue section of PCM+250 mg/kg b.wt, po AEEM-treated animals showing normal pattern of hepatocytes around the portal vein (V), lack of necrosis, and moderate accumulation of fatty vacuoles (F). Stain H and E, ×100 magnification (Group-V)

The concentration of AEEM will decrease all the hissed levels of AST, ALT, ALP, and total SB toward the ordinary standard means that plasma layer adjustment and also hepatotoxin-induced repair of hepatic tissue harms.

Table 3 (Fig. 5), the similar viability of the concentrates tried results for their hepatoprotective movement.

Percentage of inhibition = 100 × (value of toxic control - value of test sample)/(value of toxic control - value of control).

Histopathological examination of rat livers

The test animals were later collected on the 9th day, puncturing the retro-orbital plexus under mellow ether anesthesia, then sacrificing the animals and collecting liver tissues. Histopathological observation of the liver was performed in this study to further support proof of the biochemical examination. The model collection revealed the most extreme harm of all groups; microscopic view of Silymarin's liver tissue and alcoholic extraction of AEEM on ALT, AST, ALP, and SB in PCM affected liver lethality in rats. Histological changes in the liver

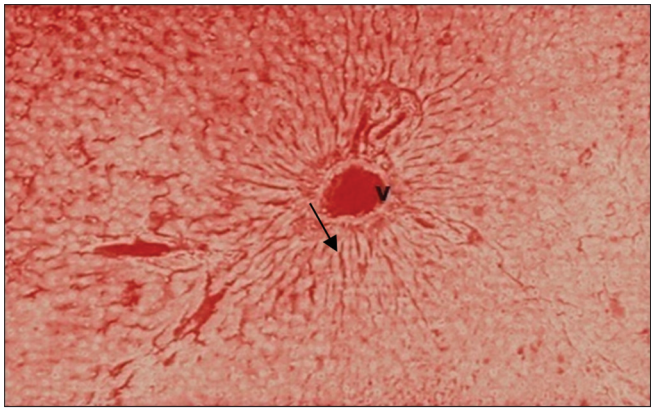

Fig. 11: Liver tissue of paracetamol (PCM)+500 mg/kg b.wt, po alcoholic extract of *Euphorbia milii* (AEEM) showing normal arrangement of hepatocytes. Liver tissue section of PCM+500 mg/kg b.wt, po AEEM treated animals showing normal hepatocyte arrangement around the portal vein (V), the portal artery (arrow), and the hepatic ducts (arrow head). Stain H and E, ×100 magnifications (Group-VI)

tissue from collections that were treated at 100, 250, and 500 mg/kg (Figs. 6-11) as well as possible.

Microscopic view of liver tissue of alcoholic extract of AEEM on ALT, AST, ALP, and SB in PCM-induced liver toxicity in rats.

DISCUSSION

The EM extract has been reported to contain different types of phytochemical constituents such as flavonoids, phenols, and terpenoids. A number of compounds belonging to the class of polyphenols have been suggested to possess antioxidant activity. The *in vitro* cytotoxicity test of EM and the comparison of % viability of test compound extract, negative control, and positive control with vehicle control observed that, in the positive control the % viability was very less. In the negative control, the % viability was observed as 46.19%. In the case of the test item, the % of viability at highest concentration (100%) was observed as 83.75%. In control and test items, the % viability was more than 70%. These results meet the acceptance criteria of the MTT assay. The pre-treatment of animals with AEEM and silymarin prevented the PCM- induced rise in serum level of transaminases and total SB, confirming the protective effects of AEEM against PCM-induced hepatic damage. The hepatoprotective activity of AEEM (500 mg/kg) was compared with the activity of standard silymarin (100 mg/kg). However, there was no effect on the rise in serum ALP levels by the test extract and silymarin. Extensive liver damage by PCM itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P450 or depletion of hepatic GSH is a prerequisite for PCM-induced toxicity. The AEEM reduced the elevated stages of all the biochemical parameters through PCM. PCM-induced liver necrosis was once inhibited significantly using AEEM, which confirms the protective action of AEEM against experimentally induced liver damage in rats. ALT, AST, ALP, and SB are the most sensitive tests employed in the diagnosis of hepatic disease. It can be concluded from this investigation that AEEM possesses hepatoprotective activity. Further, detailed studies are warranted to confirm the utility profile of this drug.

CONCLUSION

The results of the present study demonstrate that the preliminary phytochemical investigation reveals the presence of glycosides, proteins, terpenoids, phenols, and flavonoids. The *in vitro* cytotoxicity study of the test compound (AEEM extract) % viability was more than 70% of the blank, so the test item is concluded as "Non-Cytotoxic". The alcoholic extract of *Euphorbia milii* demonstrated significant biological

activity, particularly antioxidant properties, suggesting its potential as a folk medicinal drug. Pre-treatment with *Euphorbia milii* extract and silymarin markedly prevented the PCM-induced alterations in biochemical parameters (serum AST, ALT, ALP, and total bilirubin) as well as histopathological changes in liver tissues. This study confirms its use as hepatoprotective as per the ethno pharmacological claims.

AUTHORS' CONTRIBUTIONS

Kasireddy Paul Babu: Literature review, data curation, writing original draft, and evaluation; Shanmugasundaram P: Review and editing, supervision, evaluation.

CONFLICT OF INTEREST

None.

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