Effect of Euphorbia milii extract on Cytotoxicity and Hepatoprotective activity against paracetamol induced hepatic damage in wistar rats

Kasireddy Paul Babu[1]*, P. Shanmugasundaram[2]

[1]Research Scholar, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-117, Tamil Nadu, India.

[2]School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-117, Tamil Nadu, India.

***Corresponding author**

Kasireddy Paul Babu, M.Pharm.

Research Scholar, Department of Pharmaceutical Sciences,

Vels Institute of Science, Technology and Advanced Studies (VISTAS),

Pallavaram, Chennai-117, Tamil Nadu, India.

Mob. No. 8121396887

E-Mail: paulkasireddy19@gmail.com

DOI:10.48047/ecb/2023.12.si4.702

Abstract: *Euphorbia miliihas* been traditionally used in Indian medicine as a result of its curative results of hepatitis, gonorrhea and diabetes; it is probably not proof-founded. However folklore has given us many powerful therapies, based on plant sources. So claims which can be made for the protective efficacy of *Euphorbia milii* (family: Euphorbiaceae) to treat hepatic diseases. The present study focused on investigating the role of alcoholic extract of *Euphorbia milii*for its antioxidant activity such as DPPH and FRAP scavenging activity. The *in vitro* cytotoxicity test was performed to determine the toxicity effect as per ISO guideline and found to be non-toxic. The plant extract (AEEM) appreciably prevented the increased in serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and total serum bilirubin (SB) level in acute liver damage by paracetamol and elevated the activities of lipid peroxidation (LPO) and glutathione (GSH) in the liver. Histopathological observation of the liver used to be additionally performed to further support the evidence from the biochemical analysis. The observation that these significant protective effect against acute hepatotoxicity induced by paracetamol of *Euphorbia milii.*

INTRODUCTION

Euphorbia milii (EM) (Family: Euphorbiaceae) is also known as corn of thorns, is a species of flowering plant. It is a shrub grown in warmer climate and this medicinal plant widely disturbed throughout the topical regions of India, China and Pakistan[1]. *E. milii* has been reported to possess different pharmacological activities like analgesic, anti-inflammatory,

diuretic, anthelmintic and immune-modulatory activity[2, 3]. The various species of euphorbia being implicated as a folk medicine for 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 *E. milii* has been published, the knowledge of bioactive phytochemicals owing its hepatoprotective efficacy is quite limited.

With a view that sterols, flavonoids, and triterpens found in medicinal plants are used as hepatoprotective drugs, it was thought worthwhile to conduct hepatoprotective studies on the flowers of the *E.milii* 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*Euphorbia milii* in apreventive and healing function against paracetamolinducedtoxicity of liver histopathology in wistar rats.

MATERIALS AND METHODS

Chemicals

0.1% ZDEC Polyurethane Film (positive control), 10% Fetal bovine serum 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 (Paracetamol) 500 mg tablets, Silymarin, saline. The following biochemical parameters of AST, ALT, 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

Euphorbia milii plant material was collected from RangareddyDistrict; Telangana.The plant specimen was identified by Prof.RanaKausar, Dept. Of Botany, Osmania University, Hyderabad, Telangana State. A specimen was deposited in their herbarium. Then after the flowers were washed thoroughly, and dried in shade at room temperature and ground to optimal coarse powder.

Extraction:

Euphorbia milii flowerwas collected, shade dried and powdered. Each powder was subjected to by 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 *Euphorbia milii* (AEEM).The extracts were subjected to preliminary phytochemical investigation and subjected for the biological activity screening tests[6, 7].

Preliminary Phytochemical Studies

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

Each concentration of the Euphorbia milii extract and reference items (Negative & Positive control) were added (100µL) 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 test compound, reference item were prepared shortly before the treatment. On the day of treatment each plate was examined under a phase contrast microscope to ensure that cell growth is relatively even across the 96 well microtitre plates. The spent culture medium was aspirated from the cells. After aspiration, all the test item, negative and positive control extract dilutions were added (100µL) to respective wells of treatment plate of the cells. Along with these test compound, reference item, vehicle control also added to respective well and it considered as 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 cytotoxic effects of the test sample extract.

Hepatoprotective activity and grouping:

An experimental study was carried out on Wister rats of either sex (M/F) rat's age two months. Their body weights ranged from 150 to 200 g. Divided into 6 groups of 6 animals per cage was used. Animals were maintained under standard laboratory aseptic conditions (12-h light/dark cycle, 24hrs). 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)

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 paracetamol (PCM) was diluted with saline (vehicle) before oral administration (p.o). To enhance the acute liver damage in animals of groups II, III, IV, V and VI, food were 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[9-11].

Antioxidant Activity:

DPPH Radical Evaluation

The DPPH radical scavenging activities of extract of AEEMwere tried by Moron et al.[12]Briefly, 0.2mL of the sample solutions of various concentrations was added to 1mL of 0.1mM of freshly prepared DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution. The reaction mixture was shaken forcefully and absorbance at 517 nm was determined after 20min at room temperature. Control sample was prepared contain the same volume without test compounds or reference antioxidants, while DMSO (Dimethyl Sulfoxide) was used as blank. The reference antioxidant BHT (butylatedhydroxyltoluene) was used as the positive control in all

the assays. The radical scavenging activity was measured as a decrease in the absorbance of DPPHand calculated as follows:

$$scavenging\ effect(\%) = \left[\frac{A\ control - A\ sample}{A\ control} \times 100\right]$$

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 FRAP assay was determined by the technique of Benzie and Strain with minor adjustments[13, 14]. It depends on the capacity of the sample to reduce the Fe (III)-TPTZ (ferric tripyridyltriazine) complex to ferrous tripyridyltriazine Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue color which can be understand writing at 575nm. The stock solutions consist of 300mM acetate buffer (pH 3.6), 10mM TPTZ (2, 4, 6 tripyridyl S triazine) in 40mM of HCl, and 20mM ferric chloride solution. The new working solution was ready by mixing 25mL of acetate buffer, 2.5mL of TPTZ, and 2.5mL of $FeCl_3 \cdot 6H_2O$ and the temperature was maintain to 35^0C earlier than use. The various concentrations of extract, fractions, and BHT (10–50 μg/mL) were allowed to react with 2mL of the FRAP solution for 30 min in the dark condition. The absorbance was record at 575 nm. The results are spoken in μMFe (II)/g and were estimated using aqueous $FeSO_4 \cdot 7H_2O$ (20–100μM) as standard for calibration.

The *In-vitro* Lipid Peroxidation Inhibition Activity in Rat Liver Homogenate[15]

A cold TBS (Thiobarbituricacid) a 5 percent rat liver-homogenate was prepared and 50µl of that was used in the assay. Fresh plant tissue (0.5g) in 1ml of cold TBS was correctly weighed and homogenised. 50µl of it aliquots were used in the assay. To cause oxidation, ferrous sulphate was applied to the assay media at a final concentration of 10µmoles. The final volumes were made with cold TBS in the test tubes at up to 500µl. For each sample, tests were prepared containing the respective plant extract (50µl), liver homogenate (50µl) and TBS to make up the final volume to 500µl. The control tubes were not filled with pro oxidant. Also a blank containing no plant extract, no homogeneous liver but only FeSO4 and TBS was prepared to make a final volume of 500µl. By adding all the other constituents except the plant extract, an assay medium corresponding to 100 percent oxidant was prepared, and the volume with cold TBS was made up to 500µl. 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 hour at 37°C following the incubationperiod, to avoid the reaction, 500µl of 70 percent alcohol was applied to all the tubes. 1ml of 10 percent TBA was added to all the tubes, followed by boiling for 20 minutes in a hot water bath. The tubes were centrifuged after they had cooled to room temperature. 500µl of acetone was added to the clear supernatants collected into tubes, and the TBARS was measured in a spectrophotometer at 535nm.

Reduced glutathione estimation

Through homogenizing 0.5g of sample in 2.5 ml of 5%TCA, a 20 percent homogeneous was obtained. To which 0.5 ml of homogeneous tissue to precipitate the protein 125 µl of 25 per cent TCA. The precipitated protein had been for 10 minutes centrifuged at 1000rpm. The homogenate was cooled on-ice, and the supernatant took 0.1 ml for estimation. The

supernatant was buffered with 0.2M sodiumphosphate (pH 8.0) for up to 1 ml. 2.0 ml of freshly prepared DTNB solution was added to the tubes, and after 10 minutes the yellow color intensity was formed and measured at 412nm in a spectrophotometer.

Assessment of Liver Functions

The hepatoprotective impact of extract was assessed by the measure of liver potential, biochemical parameters, for design, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Total Serum Bilirubin (SB), Lipid Peroxidation (LPO) as Malondialdehyde (MDA) and Glutathione (GSH) as per commonplace protocols[9, 12].

Histopathological study

Histopathological investigation of the liver was done according to the standish et al. method[14].

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, ANOVA 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 correct transformation in the case of heterogeneous data. The variance was measured at a meaning level of 5 percent and the values expressed as mean ± SEM, and the statistically significant $P<0.05$ was considered.

RESULT

Extraction:

Fresh *Euphorbia milii* plants were collected under shade and dried. Each plant powder was subjected to by 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 extracts *Euphorbia milii(*AEEM). The percentage yields (15.5%) of the extractives of the plant AEEM. The extracts were subjected to preliminary phytochemical investigation and subjected for the biological activity screening tests.

Preliminary phytochemical studies:

The phytochemical studies shows the presence of glycosides, steroids, proteins, flavonoids, terpenoids and tanins.

***In vitro* Cytotoxicity**:

A decrease in 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 at 570nm. To calculate the reduction of viability compared to the blank the following equation will be used:

$$\text{Viability } \% = \frac{100 \text{ X } \text{OD570e}}{\text{OD570b}}$$

Where,

- $OD_{570}e$ is the mean value of the measured optical density of the 100% extracts of the test sample.
- OD_{570b} is the mean value of the measured optical density of the blanks.

- The lower the Viability % value, the higher the cytotoxic potential of the test item is.
- The absolute value of optical density, OD_{570e}, obtained in the untreated blank indicates whether the 1 X 10^4 cells seeded per well have grown exponentially with normal doubling time during the two 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 mean of the blanks do not differ by more than 15% from the mean of all blanks.

Microscopic observations

At the end of the treatment (24h), the cultures were examined to assess the General morphology, Vacuolization, Detachment, color change of the media and turbidity. Cells morphology was fibroblast. No Vacuolization was observed in Vehicle control, Negative control and test item concentrations, Vacuolization was observed in Positive control. No cell Detachment was observed in Vehicle control, Negative control and test compound concentrations, cell detachment was observed in Positive control .No color change of the media was observed in test compound and Controls groups. No turbidity was observed in test compound and control groups.

Reading the absorbance

The mean optical density (OD) of Blank was observed as 0.819. The observed blank OD was meets the acceptance criteria (at least ≥ 0.2) of MTT assay. The % viability and % inhibition of test, negative and positive control were calculated by using respective OD values. The % viability of test item concentration observed as 111.66 %, 112.68 %, 105.49 % and 86.70 % at 12.5% 25%, 50% and 100% respectively. The % viability of positive control concentration observed as 47.13 %, 8.95 %, 0.52% and 0.73 % at 12.5%, 25%, 50% and 100% respectively. The negative control OD values observed as 46.19 % at 100 % concentration (Fig 1).

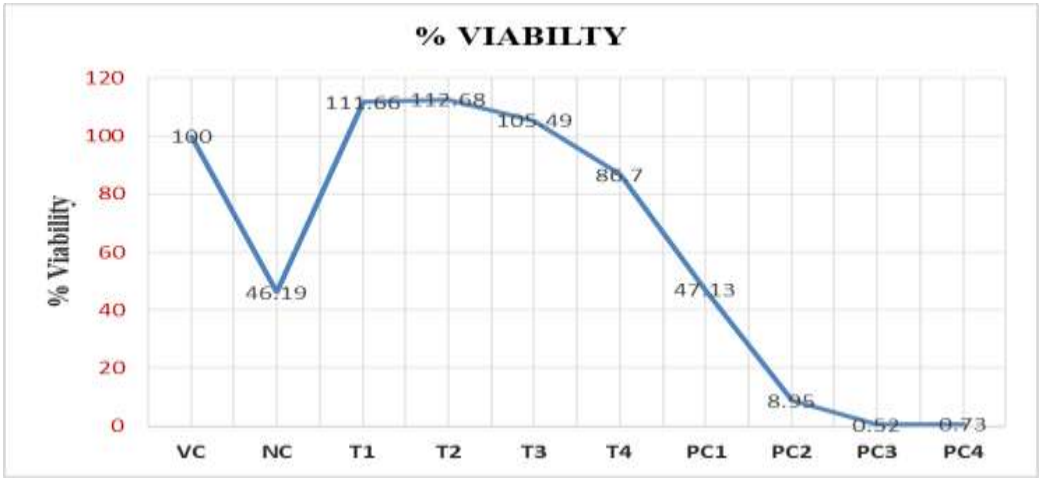


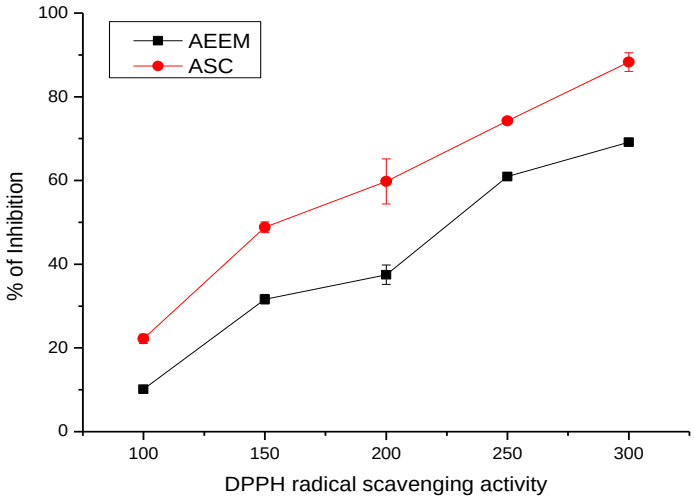
Figure 1: comparison of % viability of test samples with control

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

Antioxidant 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. Figure-2 presents the results of DPPH scavenging activity for all the test samples. With an increase in sample concentration (100-500µg mL^{-1}), scavenging activity of AEEMextract and ascorbic acid (ASC) grew. AEEM and ASC found the IC_{50}values at 111.93µg mL^{-1} (Y= 0.294x-17.036) and 144.82µg mL^{-1} (Y= 0.315x-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.



(ASC): Standard antioxidant; data represented as means ± SD (n = 3).

Figure 2: DPPH radical scavenging activity of AEEM

Antioxidant Power Reduction Assay (FRAP)

The ferric reduction / antioxidant power (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. Figure-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 159.88µg mL^{-1} (Y= 0.2516x-9.774) and 143.93µg mL^{-1} (Y= 0.3164x-4.46) both of these.

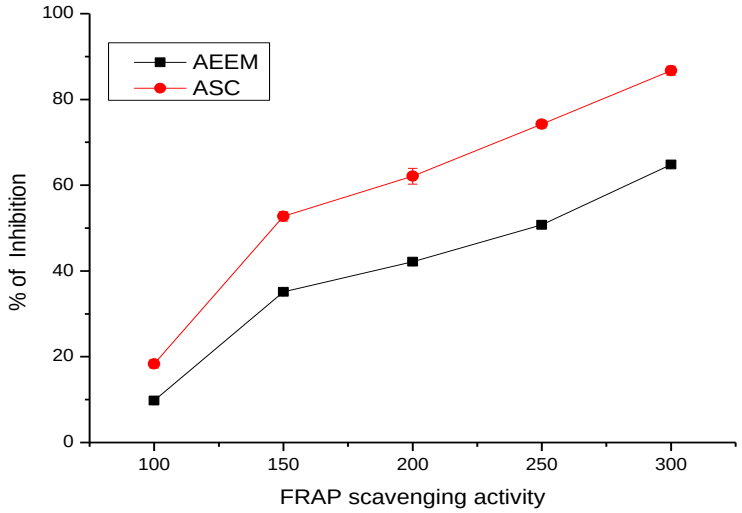
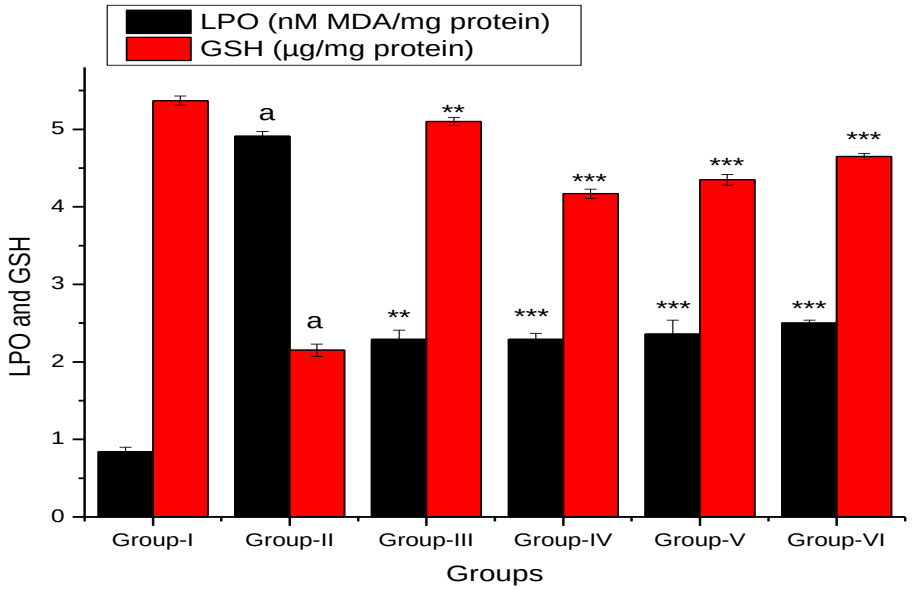


Figure 3: FRAP scavenging activity of AEEM

The *In-vitro* Lipid Peroxidation 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 paracetamol. Results are shown in Figure -4



*Each value represents the mean ± SEM. n =6 number of animals in each group. [a]P<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective PCM treated control groups*

Figure 4: Effect of AEEMon LPO and GSH, PCM induced hepatic damage in rats

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^{-1}b.wt; po), a well-known hepatoprotective specialist. PCM is realized to affect liver damage through the activity of its dangerous metabolite, N-acetyl-P-benzoquinoneimine, delivered by cytochrome activity P_{-450}. This metabolite causes glutathione (GSH) exhaustion which prompts the passage of cells. It is clear that the concentrate of AEEMwill decrease all the hissed levels of AST, ALT, ALP and Total serum bilirubin towards the ordinary standard means that plasma layer adjustment and also hepatotoxin-induced repair of hepatic tissue harms.

Given Table-1 (Figure -5), the similar viability of the concentrates tried results for their hepatoprotective movement.

Percentage of inhibition = 100 X (value of toxic control – value of test sample)/ (value of toxic control – value of control).

Table 1: Effect of AEEMon 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

*Each value represents the mean ± SEM. n =6 number of animals in each group. [a]P<0.001 vs vehicle control, *P<0.05, **P<0.01, *** P<0.001, Compared to respective PCM treated control groups*

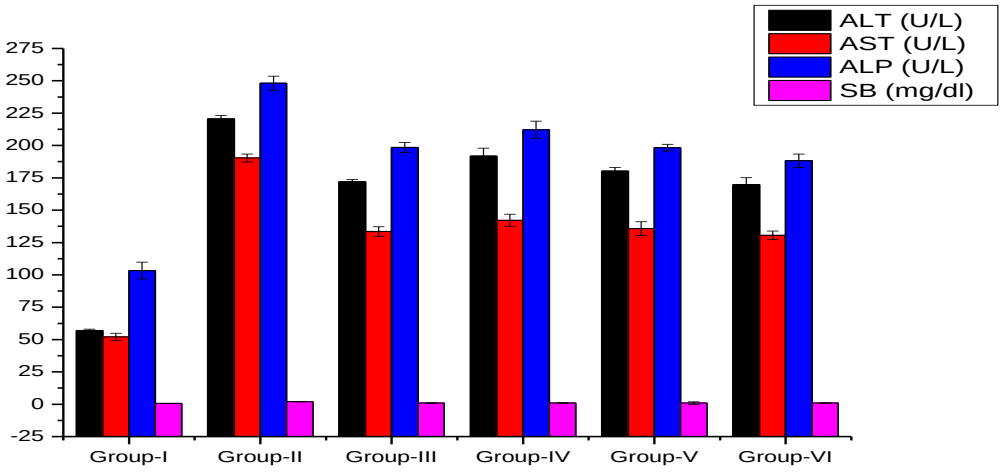


Figure 5: Effect of AEEMon ALT, AST, ALP and SB in PCM induced liver toxicity in wistar rats

Histopathological examination of rat livers

The test animals were later collected on the ninth day, puncturing the retro-orbital plexus under mellow ether anesthesia, then sacrificing 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 tissue from collections that were treated at 100, 250 and 500 mg kg-1 (Figure -6 to 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

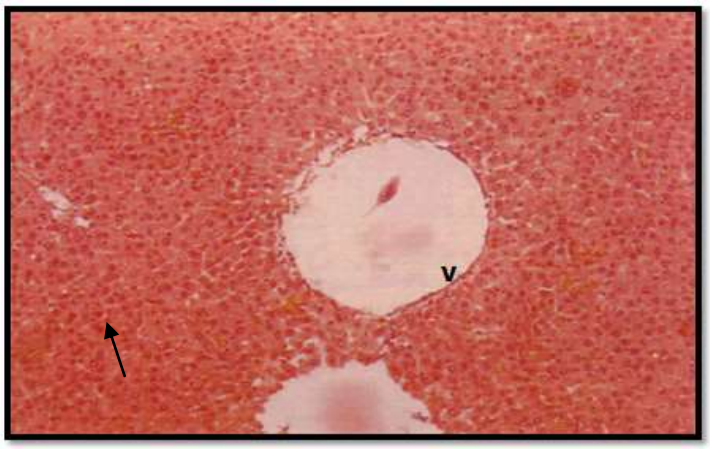


Figure 6: Liver tissues of control animal showing normal histology

Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and liver ducts (arrow head). Stain H and E, grossing100X (Group I)

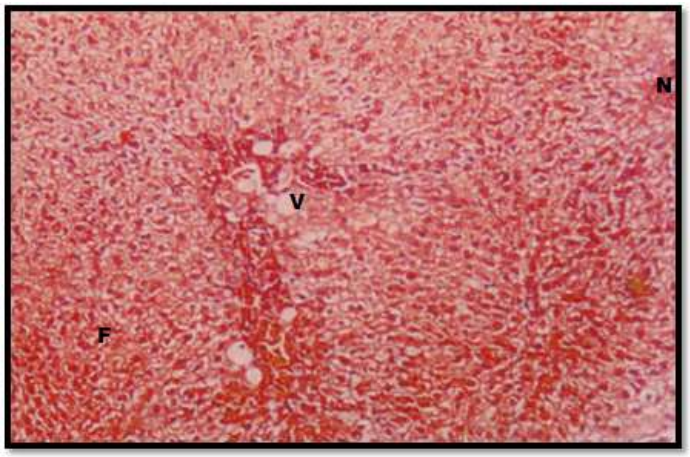


Figure 7: Liver tissue of animal treated with PCM showing necrosis

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

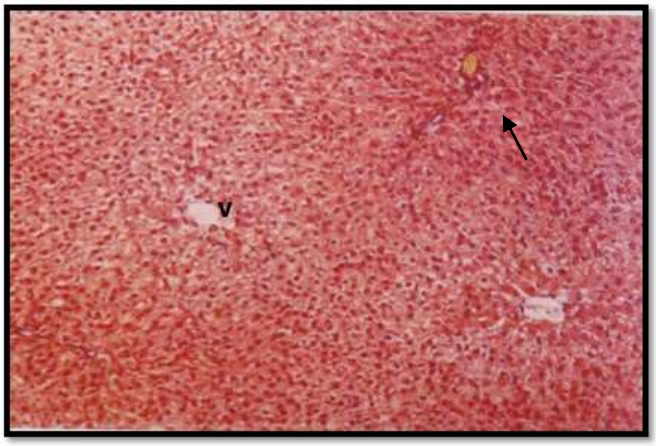


Figure 8: Liver tissue of PCM + Silymarin treated animals showing normal hepatocytes

Normal liver tissue section with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, 100X magnification (Group III)

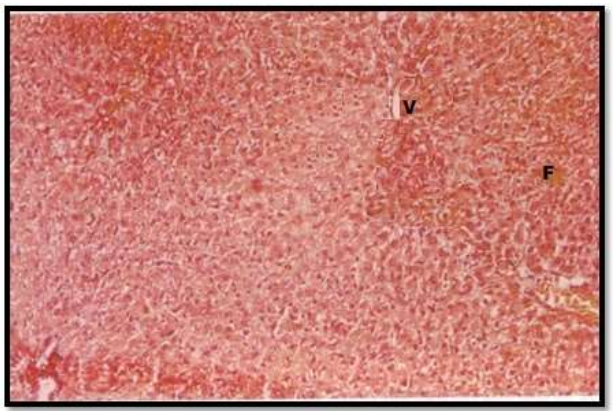


Figure9: Liver tissue of PCM + 100 mg/kg b.wt, p.oAEEMshowing normal arrangement of hepatocytes.

Liver tissue section of PCM + 100 mg / kg b.wt, p.o 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, 100X magnification (Group IV)

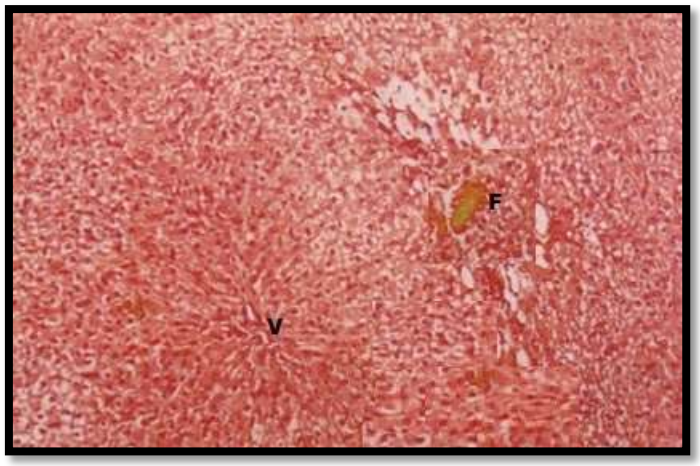


Figure10: Liver tissue of PCM + 250 mg/kg b.wt, poAEEM showing normal arrangement of hepatocytes.

Liver tissue section of PCM + 250 mg / kg b.wt, poAEEM 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, 100X magnification (Group-V)

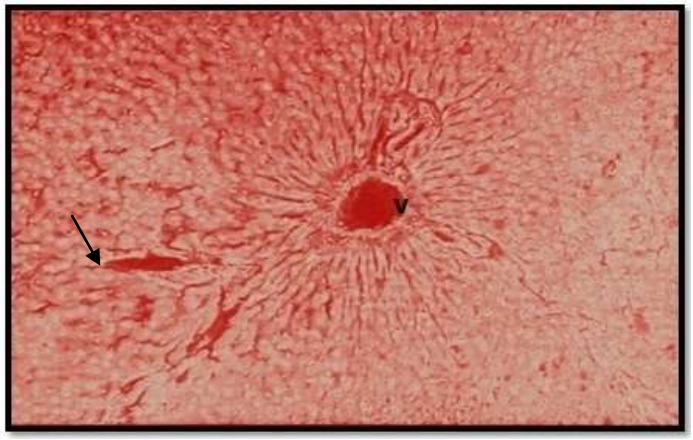


Figure 11: Liver tissue of PCM + 500 mg/kg b.wt, poAEEM showing normal arrangement of hepatocytes.

Liver tissue section of PCM + 500 mg / kg b.wt, poAEEM 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 X magnifications (Group-VI).

DISCUSSION

The *Euphorbia milii* extract has beenreported to contain different types of phytochemical constituents such as flavonoids, phenols, and terpenoids. A number of compoundsbelonging to the class of polyphenol have been suggested topossess antioxidant activity.The in vitro cytotoxicity test of *Euphoria milii* and the comparison of % viability of test compound extract, negative control and positive control with vehicle control observed that, in positive control the % viability was very less. In negative control the % viability was observed as 46.19 %. In case of test item the % of viability at highest concentration (100%) observed as 86.70 %. In control and test item the % viability was more than 70%. These results meet the acceptance criteria of MTT assay. The pre-treatment of animalswith AEEMandsilymarin prevented the paracetamol induced rise inserum level of transaminases and total serum bilirubin, confirming the protective effects of AEEMagainst PCM induced hepatic damage. The hepatoprotective activity of AEEM(500mg/kg) was comparedwith the activity of standard silymarin (100mg/kg).However, there was no effect on rise in serum alkalinephosphatase levels by the test extract and silymarin.Extensive liver damage by paracetamol itselfdecreases its rate of metabolism and other substrates forhepatic microsomal enzymes. Induction of cytochrome-P_{450} or depletion of hepatic glutathione is a prerequisite for PCM -induced toxicity. The AEEM reduced the elevatedstages of all the biochemical parameters through PCM. PCM induced liver necrosis was once inhibitedsignificantly by using AEEMwhich confirms the protective action of AEEMagainstexperimentally induced liver damage in rats. ALT, AST, ALPand SB are the most sensitive tests employed in thediagnosis of hepatic disease. It can be concluded from thisinvestigation that AEEMpossess hepatoprotective activity. Further, detailed studiesare warranted to confirm the utility profile of this drug.

CONCLUSION

The results of the present study clearly demonstrate thatthe preliminary phytochemical investigation reveals the presence of glycosides, proteins, terpenoids, phenols and flavonoids. The in vitro cytotoxicity study of test compound (AEEM extract) % viability was observed more than 70% of the blank so test item is concluded as "Non-Cytotoxic".The biological activity such as antioxidant properties of alcoholic extract of *Euphorbia milii* could be the potential drug of folk medicine. The various biochemical parameters (Serum AST, ALT, ALP and SB)histopathological transformations produced byPCM within the serum and tissue were reservedsignificantly by the pre-treatment of extracts of *Euphorbia milii* and Silymarin. This study confirms itsuse as hepatoprotective as per the ethno pharmacologicalclaims.

REFERENCES

[1] David S, Hamilton JP. Drug-induced Liver Injury. (2010). US GastroenterolHepatol Rev, 1;6:73-80.

[2] Rauf, A., Khan, A., Uddin, N., Akram, M., Arfan, M., Uddin, G., &Qaisar, M. (2014). Preliminary phytochemical screening, antimicrobial and antioxidant activities of Euphorbia milli. *Pakistan journal of pharmaceutical sciences*, *27*(4).

[3] Zheng, F., Luo, Y., Wei, X., & Wang, B. (2009). Non-terpenoid constituents from the seeds of Euphorbia lathyris. *Journal of Tropical and Subtropical Botany*, *17*(3), 298-301.

[4] Tonoli, G. H. D., Teixeira, E. M., Corrêa, A. C., Marconcini, J. M., Caixeta, L. A., Pereira-da-Silva, M. A., &Mattoso, L. H. C. (2012). Cellulose micro/nanofibres from Eucalyptus kraft pulp: preparation and properties. *Carbohydrate polymers*, *89*(1), 80-88.

[5] Chaman, S., Khan, F. Z., Khokhar, R., Maab, H., Qamar, S., Zahid, S., &Hussain, K. (2019). Cytotoxic and antiviral potentials of Euphorbia milli var. splendens leaf against Peste des petits ruminant virus. *Tropical Journal of Pharmaceutical Research*, *18*(7), 1507-1511.

[6] Sunil J, Krishna JY, Bramhachari PV. Hepatoprotective activity of HolostemmaadaKodienshcult, extract against paracetamol induced hepatic damage in rats. European Journal of Medicinal Plants. 2015;1:45-54

[7] Gopinathan S. Molecular mechanisms of liver injury induced by hepatotoxins. European Journal of Biomedical. 2016;3(11):229-37.

[8] Van Meerloo, J., Kaspers, G.J.L., Cloos, J. (2011). Cell Sensitivity Assays: The MTT Assay. In: Cree, I. (eds) Cancer Cell Culture. Methods in Molecular Biology, vol 731. Humana Press.

[9] Ghanem CI, Pérez MJ, Manautou JE, Mottino AD. (2016). Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. Pharmacological research, 109:119-31.

[10] Arun kumar Sanapala and K.Eswar Kumar. (2016). Evaluation of Hepatoprotective Activity of Pachyrhizus Erosus, extract against Paracetamol Induced Hepatic Damage in Rats., Indo Am. J. P. Sci,, 03(12).

[11] Doss A. (2009). Preliminary phytochemical screening of some Indian medicinal plants. Ancient science of life, 29(2):12.

[12] M.S. Moron, J.E. Bepierre, and B.M. Vik, (1979). "Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver," *BiochemBiophysActa*, 582: 3170-3185.

[13] PK. Haldar, S. Adhikari, S. Bera, S. Bhattacharya, SP. Panda, CC. Kandar (2011). "Hepatoprotective Efficacy of Swietenia Mahagoni L. Jacq. (Meliaceae) Bark against Paracetamol-induced Hepatic Damage in Rats," *Indian jounral Pharm Educ reserach*, 45(2): 108–13.

[14] Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. (2006). An appraisal of the histopathological assessment of liver fibrosis. Gut. 2006;55(4):569-78.

[15] Crocenzi FA, Pellegrino JM, Pozzi EJ, Mottino AD, Garay EA, Roma MG. (2000). Effect of silymarin on biliary bile salt secretion in the rat. Biochemical pharmacology, 59(8):1015-22.