Indian Journal of Experimental Biology
Vol. 64, November 2026, 1040-1052
DOI: 10.56042/ijeb.v64i11.32916

Anti-icteric effect mediated by hepatoprotective activity of *Amaranthus spinosus* L. evaluated through phytochemicals analysis, liver biochemical parameters, antioxidant assays and PKC pathway modulation

Faruk Alam[1], Mohidul Islam[2]*, Moksood Ahmed Laskar[2], Roshan Maiti[2] & Sajal Kumar Jha[2]

[1]Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Guwahati, Assam-781 026, India

[2]Bengal College of Pharmaceutical Technology, Dubrajpur, Birbhum, West Bengal-731 123, India

Received 22 June 2026; *revised 31 July 2026*

Protein kinase C (PKC) and its active forms, phospho-PKC (pPKC), are key mediators of Hepatic jaundice. Despite modern drugs, plant-based therapies in are preferred for safer and effective alternatives. Although *Amaranthus spinosus* L. roots is traditionally used for jaundice management in Assam, the scientific validation of hepatic anti-icteric activity by PKC pathway mechanism remains limited. This study systematically evaluated the hepatic anti-icteric activity of *A. spinosus* roots through phytochemical screening, liver biochemical analysis, antioxidant activity and PKC pathway modulation. *A. spinosus* roots fractionation was performed by Soxhalation with Chloroform, Ethyl acetate, Methanol and Hydro-alcohol (1:9). The methanol extract of *Amaranthus spinosus* roots (MEAS) showed greater bioactive compounds mainly alkaloids and phenolics and significantly higher antioxidant potential. MEAS contained 14 compounds identified by GC-MS, in which "(22E)-Ergosta-5, 22-dien-3-ol" served as a key bioactive compound with strong binding affinity (-6.20 kcal/mol) against PKC. MEAS showed Significant ($P< 0.01$) reductions in ROS and PKC/pPKC activity in HepG2 cells. MEAS at 400 mg/kg markedly ($P< 0.001$) restored serum biochemical markers and improved hepatic histoarchitecture. These findings validate the traditional use of *A. spinosus* roots and highlight its potential as a therapeutic agent for hepatic anti-icteric potential via PKC pathway mechanism.

Keywords: *Amaranthus spinosus L.,* Antioxidant effect, Hepatic anti-icteric effect, HepG2 cell, Molecular docking, Protein kinase C

Jaundice is characterized by yellowish pigmentation of the skin, mucous membrane, and sclera that causes changes in skin colour, liver inflammation, abdominal pain, fever, chills, *etc*[1]. Hepatic jaundice is the most common form other types in adults[2]. This occurs by the severe hepatic injury through mechanisms involving oxidative stress, inflammation, insulin resistance, apoptosis, and fibrosis[3]. It can be caused by oxidative stress, nuclear factor kappa B (NF-κB) activation, hepatic stellate cell activation, activation of Transforming growth factor-β (TGF-β) signaling rising extracellular matrix deposition, hepatocellular apoptosis due to mitochondrial dysfunction, *etc*[4]. The protein kinase C (PKC), a key protein that is distributed in multiple cell types and subcellular locations of the liver, plays an important role in hepatic function and disease progression[5]. The localization and function of PKC depend on its isoform, which determines its specific involvement in liver physiology and pathology. When PKC phosphorylated to its active form phospho-protein kinase C (pPKC), plays direct role in the hepatic jaundice causing liver injury[6]. The pPKC activates NF-κB, causes the release of a number of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). These further cause inflammation of hepatocytes and ultimately lead to liver injury[7]. PKC (a family of serine or threonine kinases) and its active form play crucial roles in hepatic damage through their involvement in signaling pathways related to inflammation, oxidative stress, and fibrosis[6,8].

In the liver, activation of PKC is implicated in several pathological processes such as- Oxidative Stress: enhances reactive oxygen species (ROS) production, contributing to lipid peroxidation and hepatocyte injury[9];

*Correspondence:
Phone: +91-9101659319 (Mob)
E-mail: mohidulislam11302@gmail.com

Abbreviations: DCFDA, 2′7′–Dichlorofluorescin diacetate; DMEM, Dulbecco's modified eagle medium; HepG2, Hepatocellular Carcinoma; MEAS, Methanol extracts of roots of *Amaranthus spinosus* L.; MTT- 3, [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; PDB, Protein data bank; Ppkc, Phospho Protein kinase C; TFC, Total flavonoid content

Inflammation: the isoforms (such as PKC_{α}, PKC_{β}, and PKC_{δ}) activate NF-κB and other transcription factors, promoting release of pro-inflammatory cytokine, *e.g.*, IL-6, TNF-α[10]; Insulin Resistance: PKC_{θ} and PKCε contribute to insulin resistance in hepatocytes, one of the main characteristics of nonalcoholic fatty liver disease (NAFLD)[11]; Fibrosis: stimulates activation of hepatic stellate cell, increasing extracellular matrix (ECM) deposition and leading to fibrosis[12].

While, pPKC plays more direct role in liver injury such as- Activation of Cell Death Pathways: $pPKC_{\delta}$ and $pPKC_{\zeta}$ contribute to hepatocyte apoptosis by activating caspases[13]; Mitochondrial Dysfunction: alters mitochondrial membrane permeability, leading to cytochrome c release and hepatocyte apoptosis[14]; Endoplasmic Reticulum (ER) Stress: exacerbates ER stress, which is linked to liver diseases like alcoholic liver disease (ALD) and NAFLD[15]; Hepatic Fibrogenesis: enhances TGF-β signaling, a key driver of liver fibrosis. Likewise, PKC and pPKC are central key molecules to hepatic injury. Thus, targeting these pathways could be a potential strategy for treatment of hepatic jaundice.

Despite modern medical interventions, the traditional therapies that involve the use of medicinal plants are playing an important role in their ability to restore health and well-being[16]. *Amaranthus spinosus* is one of the common plants used traditionally to treat kidney stone, jaundice in various regions of Assam[17]. Thus, the study aimed to scientifically analyze the traditional claim by evaluating the beneficial effect of *A. spinosus* root against hepatic jaundice. This research uniquely investigated by integration of experimental and computational approaches to evaluate the potential of *A. spinosus* root extract in treating hepatic jaundice via Protein Kinase C (PKC) signaling pathway.

Materials and Methods

Preparation of extracts

Roots from mature plant *A. spinosus* L. were collected by hand-pinking method from the Fulgasa, a village of the district Dhubri, Assam, India in the month of September 2020. The roots were washed thoroughly using purified water, sun dried to remove external water content. Further, air dried at room temperature and pulverized to fine powder. This powdered material was then packed in thimble, defatted with petroleum ether (40°C – 60°C) and extracted by soxhlet apparatus using solvents such as Chloroform, Ethyl acetate, Methanol and Hydro-alcohol (1:9) in their increased order of polarity[18,19]. Each extraction cycle was continued for 6-8 h, or until the solvent in the siphon arm became colorless. Each extract obtained was carefully collected and subsequently evaporated under reduced pressure until complete dryness. All solvent extracts were further analyzed by using different chemical test for the occurrence of phytoconstituents present in the extracts[18,19].

Quantitative analysis of *A. spinosus* root extracts

Determination of total alkaloid content (TAC)

A solution of 1 mg/mL of each solvent extracts were prepared. In the other hand, 50, 100, 150, 200 and 250 µg/mL solutions of atropine sulphate was prepared. 1 mL of each solution was transferred to a 25 mL volumetric flask, in which 5 mL phosphate buffer of pH 4.7 solution followed by 5 mL of bromo cresol green solutions were added. To this, 4 mL chloroform was added and shaken well. The volume was made up to the mark with chloroform. The absorbance of the resulting solution was measured by UV-visible spectrophotometer (SHIMADZU CORP., 80866) at a wavelength of 470 nm[20,21]. Finally, total alkaloid content of each extracts was calculated from the standard curve.

Determination of total flavonoid content (TFC)

A solution of 1 mg/mL of each solvent extracts were prepared. In the other hand, 10, 20, 30, 40, 50 µg/mL, 60, 70, 80, 90 and 100 µg/mL solutions of rutin was prepared. For 6 min, a mixture of 1 mL of all solution, distilled water (4 mL), and 5% sodium nitrite solution (0.3 mL) was left in a dark area. The reaction mixtures were incubated in a dark area for 5 min after adding 0.3 mL of 10% aluminum chloride. Next, 0.5 mL of distilled water and 1ml of sodium hydroxide (1M) were added. The solution's absorbance was measured at 510 nm using a UV-visible spectrophotometer (SHIMADZU CORP., 80866) after vigorously shaking the reaction mixture[20,21]. The standard curve was used to determine the total flavonoid concentration of each extract.

Determination of total phenolic content (TPC)

A solution of 1 mg/mL of each solvent extracts were prepared. In the other hand, 10, 20, 30, 40, 50 µg/mL, 60, 70, 80, 90 and 100 µg/mL solutions of gallic acid was prepared. 0.5 mL solution of extracts and each solution of gallic acid were mixed 2 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution. The reaction mixture was shaken

well and incubated at room temperature for 30 min. Absorbance of the resulting solutions was measured by UV-visible spectrophotometer (SHIMADZU CORP., 80866) at the wavelength of 765 nm[20,21]. Finally, total phenolic content of each extract was calculated from the standard curve.

Antioxidant activity of *A. spinosus* root extracts

DPPH radical scavenging assay

0.1mM DPPH (39.4mg of DPPH in 1000 mL) solution was prepared in methanol. Estimation was done on 1:1 ratio as 2.5 mL of methanol and 2.5 mL DPPH solution as the control. 2.5 mL methanol used as the blank while, 2.5 mL ascorbic acid along with the with 2.5 mL DPPH solution as the standard at different concentrations like 1, 2, 4, 8, 16 and 32 µg/mL. Similarly, 2.5 mL solution of plant extracts were mixed with 2.5 mL DPPH as sample at different concentrations as standard. There was an instant effort to keep all of these solutions out of the light. After 30 min, the absorbance was measured at 517nm using a UV-Visible spectrophotometer (SHIMADZU CORP., 80866)[22]. The IC_{50} was then determined using the calibration curve.

Hydrogen peroxide (H_2O_2) radical scavenging assay

1 mg/mL stock solutions of all solvent extracts and standard (Ascorbic acid) were prepared using distilled water. Then 0.4 M solution of hydrogen peroxide and 0.5 M phosphate buffer of pH 7.4 were prepared. Then each solution of extracts and ascorbic acid were further diluted to prepare 10, 20, 40, 80, 160, 320, 640 and 1280 µg/mL solutions. To 100µL of each solution, 600 µL of hydrogen peroxide solution and 4 mL of phosphate buffer was added and incubated for 10 min. The control was prepared by addition of 600 µL of hydrogen peroxide solution in 4 mL of phosphate buffer solution. UV-Visible spectrophotometer (SHIMADZU CORP., 80866) absorbance were recorded at 230nm utilizing phosphate buffer as the blank, and the calibration curve yielded IC_{50} values[23].

Superoxide (O2–) radical scavenging assay

1 mg/mL stock solutions of all solvent extracts and standard (Ascorbic acid) were prepared using distilled water. Then each solution of extracts and ascorbic acid were further diluted to prepare 10, 20, 40, 80, 160, 320, 640 and 1280 µg/mL solutions. To 0.1 mL of each solutions, 0.1 mL of 6 µM ethylene diamine tetra acetic acid containing sodium cyanide, 0.1 mL of 50 µM nitro blue tetrazolium and 0.05 mL of 2 µM riboflavin were added, and ultimate volume was prepared up to 3 mL using phosphate buffer of pH 7.8. The reaction mixture was then incubated at 25°C for 5 min. The control was prepared by addition of phosphate buffer solution without drug or extract solution. The absorbance was measured at 560 nm in UV-Visible spectrophotometer (SHIMADZU CORP., 80866) against phosphate buffer as the blank and IC_{50} values were calculated from the calibration curve[24].

Characterization of phytoconstituents of MEAS by GC-MS

1 gram of methanol extract of roots of *Amaranthus spinosus* L. (MEAS) was solubilized in 10 mL of solvent methanol, it was then filtered and dilute and qualitatively identified by GC-MS analysis fitted with TG-5MS capillary column (30 M × 0.25 mM × 0.25 µM), Thermo Scientific. The optimization parameters for the study of roots extract by the column alignment were as such: carrier gas hydrogen was utilized at 1 mL/min flow rate, the injection temperature was set 200°C, injection capacity was fixed as 1 µL and operation mode was the splitless (1:100). Programmer temperature was set 50°C (detained for two minute) then raised at 5°C/min that reached up to 200°C (detained for 5 min) that further increased to 320°C at 15°C/min, which detained for 5 min. Mass spectrometry detection was done by electron ionization technique in which the temperatures were set as 200°C and 250°C for ion source detector and interface correspondingly. The sort of scanning of mass to charge ratio (m/z) of ion was set 40-700 with scanning intermission of 0.5 sec at a scanning rate of 1.5 scans/sec[25]. Mass spectra obtained from the methanol extract of the plant were then matched with Mass Spectral Library (ISQ-7000) of University of Science and Technology, Meghalaya. Thus, compounds that are present in the MEAS were identified.

***In silico* molecular docking study of phytoconstituents of MEAS**

Preparation of protein

3D structures of the Protein Kinase C (PKC) (PDB ID: 1KPA), was downloaded from RCBS protein data bank (PDB) in PDB format. The proteins were then optimized by removing heteroatoms using Notepad and energy was minimized using Swiss PDB viewer. Then the protein was prepared for docking using Discovery Studio 2020 Client. All the water molecules, the drugs bound to protein and all the

chains other chain-A were deleted and finally saved in PDB file.

Preparation of ligands

The chemical structure of phytoconstituents of MEAS were drawn using ChemSketch (ACD labs) and saved as MDL mol files which were further converted to pdb files utilizing the software Open Babel GUI.

Docking and visualization of compounds

The compound was docked against PKC using Autodock Tools 1.5.6. Ligands and the protein was made visible in the software and water molecules deleted, further added Kolmann charges and assigned the atom to AD4 type and then docked against the desired protein PKC. The docking of the compounds were analyzed and visualized utilizing Discovery Studio 2020 Client Visualizer[26].

Effect of MEAS in HepG2 cells

Safety study of MEAS on HepG2 cell line by MTT assay

HepG2 Cells from National Centre for Cell Science (NCCS), Pune were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% Fetal bovine serum (FBS) and 1% penicillin and Streptomycine (100 µg/mL) in 5% carbon dioxide (CO_2) in controlled conditions. HepG2 cells were seeded at a density of 4×105 cells in 96 well plates and treated with 10-200 µg/mL of MEAS or only with 0.1% dimethyl sulfoxide (DMSO) and incubated for 24 h. Then 20 µL solution of 2.5 mg/mL concentration of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) was added to each of the well plates and incubated again for 4 h. After dissolving MTT formazan crystals in 500 µL DMSO, the optical density was measured at 570 nm using an enzyme-linked immuno sorbent assay (ELISA) plate reader [27].

Effect of MEAS on intracellular reactive oxygen species (ROS) by LPS-induced ROS- DCFDA dye fluorescent analysis

HepG2 Cells (NCCS Pune) were grown in DMEM medium supplemented with 10% FBS in 5% CO_2 in controlled conditions. More than 95% of viable cells were treated with or without lipopolysaccharide (LPS) (25 µg/mL) and different concentrations of MEAS (0-100 µg/mL) in the same controlled condition in 96 healthy plates. The level of intracellular reactive oxygen species (ROS) was estimated using 2′7′–dichlorofluorescin diacetate (DCFDA) fluorescence analysis[27].

LPS-induced PKC and phospho-PKC activity of MEAS in HepG2 cells

HepG2 Cell (NCCS Pune) were grown in DMEM medium supplemented with 10 % FBS in 5% CO_2 and controlled conditions. More than 95% of viable cells were treated with or without LPS (25 µg/mL) and different concentrations of MEAS (0-100 µg/mL) in the same controlled condition in 96 healthy plates. The Protein kinase C (PKC) and Phospho-PKC levels were estimated using a commercially available Sandwich ELISA kit (KBH0816 and KBH15203) [28]. The unknown concentration of PKC and pPKC in the treated and untreated cells were interpolated using a standard curve using linear regression analysis in Graph Pad prism, version 5, considering X=0, Y=0.

Animals

We picked healthy adult Wistar albino rats of both sexes that weighed between 140 g and 160 g. Animals were kept in clean, sterile polypropylene cages at room temperature (25 ± 2°C) with a 12 h light and dark cycle. They had unfettered access to commercial pellet food and water. The rats were randomly put into different groups and kept apart for a week to get used to their new habitat and to handle them before the experiments started[21].

Acute toxicity study

Acute toxicity study was executed in accordance to OECD Guideline number 423, adopted in 2001 in female rats of 8-12 weeks. Female rats were retained in chamber temperature maintaining 12 h of bright and 12 h of black for 5 days prior to dosing. Animals were provided normal food and drinking water. Three female rats were fasted overnight with food while drinking water was withheld for 3-4 h then administered the initial dose of MEAS as 2000 mg/kg of body weight in the volume 0.1 mL/10 g of bodyweight by oral route with oral feeding cannula. All 3 female rats were observed independently for the initial 30 min after MEAS administration, then for 4 h at 1 h interval and periodically for 24 h and then for 14 days regularly[29].

Evaluation of hepatic anti-icteric activity of MEAS in experimental rats

Rats were grouped into five groups consisting six rats in each group both male and female in the same ratio as follows;

Group-1: 0.5% Carboxy methyl cellulose (CMC), per oral (p.o.)

Group-2: Paracetamol (PCM) (2000 mg/kg) + 0.5% CMC, per oral (p.o.)

Group-3:Paracetamol (PCM) (2000 mg/kg) + Silymarin (100 mg/kg), per oral (p.o.)

Group-4: Paracetamol (PCM) (2000 mg/kg) + MEAS (200 mg/kg), per oral (p.o.)

Group-5: Paracetamol (PCM) (2000 mg/kg) + MEAS (400 mg/kg), per oral (p.o.)

The rats were administered with paracetamol (2000 mg/kg, per oral) for first five days to grow jaundice in accordance of standard protocol[21]. The rats were casually scattered into five groups (n = 6). The treatment for hepatic jaundice with Silymarin and MEAS was for 5 days and was begun from 6th day up to 10th day. Plasma samples were taken through tail veins of all groups of rats on 1st day, 5th day (6 h of paracetamol post-intake), and on the 10th day and plasma concentration of total bilirubin (TB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were determined (using kits from Span Diagnostic Ltd.) following standard procedure[21].

At the end of the therapy term, histopathological analyses were performed[21]. Carbon dioxide (CO_2) asphyxiation was used to euthanize both control and experimental animals. Livers from all groups were isolated, fixed in saline containing 10% formalin, dehydrated in ethanol, cleaned with xylol, and mounted in molten paraplast at 58°C to 62°C. Five-micron specimens were produced, dyed with haematoxylin and eosin, and analyzed with a bright-field microscope at 100X magnification for structural alterations.

Evaluation of *in vivo* antioxidant potential of MEAS in rat liver tissue

According to the standard protocol[30], 1 gram of liver tissue isolated from the rats was homogenized in 9 mL of 20 mM Tris-hydrochloric acid using a homogenizer. The homogenized liver tissue was then centrifuged for 30 min at 15000 rpm at 4°C. The supernatant liquid was then shifted to eppendorf tubes and kept at (-80°C) until the biochemical investigation was executed. lipid peroxidation (LPO) activity was measured in which absorbance values were checked at the wavelength of 412 nm. Similarly, glutathione (GSH) activity was measured and absorbance recorded at time intervals of 30 secs at the wavelength of 340 nm for 3 min. Catalase (CAT) activity was checked at the wavelength of 405 nm, and superoxide dismutase (SOD) activity at the wavelength of 560 nm using the colorimetric method based on the degree of blockage of nitroblue tetrazolium.

Statistical analysis

Data were statistically studied for determination of mean value and their standard deviation. In addition, the data were processed for one-way analysis of variance (ANOVA) followed by the Dunnett's post-hoc for conclusion of significance level.

Result

Pharmacognostic and physicochemical studies of plant materials

Physicochemical evaluation plays a crucial role in confirming the identity, purity, and quality of crude plant materials. In the present investigation, the results were found optimum and and within permissible range. The moisture content (8.03 ± 0.68 %), Water soluble extractive value (1.29 ± 0.10 % *w/w*), Alcohol soluble extractive value (2.31 ± 0.24 % *w/w*), Total ash (8.63 ± 0.32 % *w/w*), Acid insoluble ash (1.89 ± 0.12 % *w/w*) and Water soluble ash (2.72 ± 0.05 % *w/w*). The observed findings support the purity of the sample and its suitability for further pharmacological investigations.

Further, successive extraction of roots of the plant with different solvents using soxhlet apparatus, the methanol extract of roots of *Amaranthus spinosus* L. (MEAS) showed the highest yield value (3.80 % *w/w*) than other solvent extracts. The yield obtained for other solvent extracts are chloroform extract (0.45 % *w/w*), Ethyl acetate extract (2.22 % *w/w*) and hydro-alcoholic extract (0.97 % *w/w*). All the solvent extracts were analyzed by phytochemical screening and showed numerous important phytoconstituents such as alkaloids, glycosides, terpenoids, phenolic compounds, *etc* (Table 1). The MEAS contained most of them.

Quantitative analysis of *A. spinosus* root extracts

The abundance of phenolics and flavonoids suggests potential antioxidant and other phytoconstituents may further contribute to diverse biological activity. On analysis, *A. spinosus* showed the presence of significant amount of alkaloids, flavonoids and phenolic contents equivalent to atropine sulphate, rutin and gallic acid respectively when calculated from the standard calibration curves as shown in Fig. (1). Significant findings of MEAS and presence of other bioactive compounds could be responsible for the therapeutic potential.

Table 1 — Phytochemical analysis of several solvent extracts of roots of *Amaranthus spinosus* L.

Phytoconstituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Hydro-alcoholic extract
Terpenoids	-	-	-	-
Glycosides	-	-	+	+
Carbohydrates	-	-	+	-
Proteins	-	-	-	-
Alkaloids	-	-	+	-
Fixed oils and Fats	+	+	+	-
Flavonoids	-	+	-	-
Tannins & phenolic compounds	+	+	+	+

Note: "+" represents Present, "-" represents Absent.

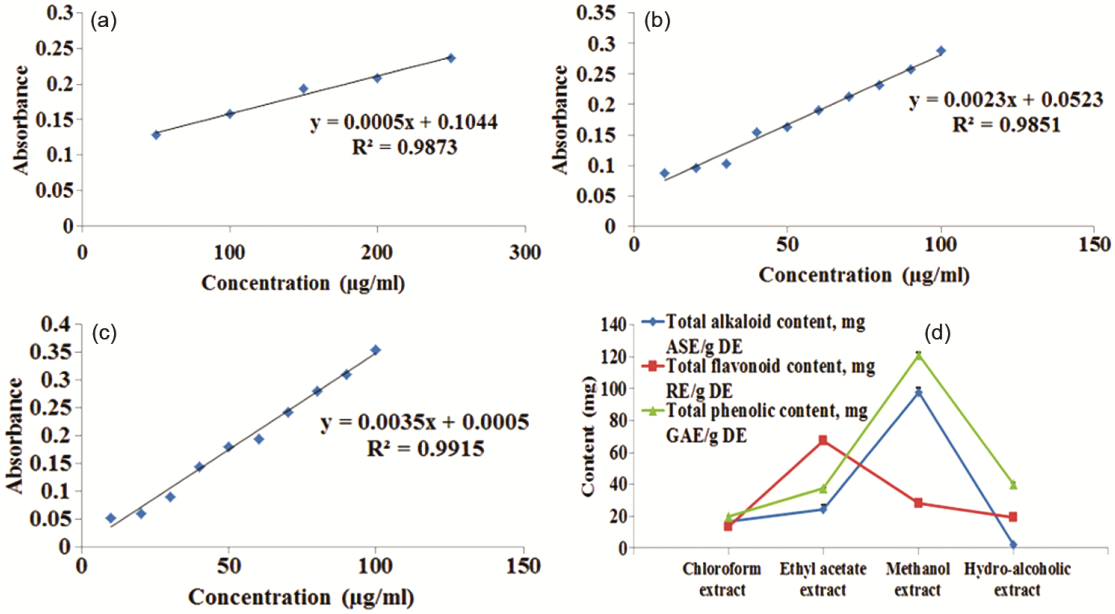


Fig. 1 — Quantitative analysis of extracts of roots of *Amaranthus spinosus* L. (a) Standard calibration curve of Atropine sulphate for estimation of total alkaloid content; (b) Standard calibration curve of Rutin for estimation of total flavonoid content; (c) Standard calibration curve of Gallic acid for estimation of total phenolic content; and (d) Total alkaloid, flavonoid and phenolic contents of extracts of roots of *Amaranthus spinosus* L. Data presented as mean ± standard deviation (SD) of three variables. "ASE"- Atropine sulphate equivalent; 'RE"- Rutin equivalent; "GAE"- Gallic acid equivalent; "DE" Dry extract

Antioxidant activity of *A. spinosus* root extracts

All solvent extracts of roots of *A. spinosus* were analyzed for their *in vitro* antioxidant efficacy by DPPH free radical scavenging assay, hydrogen peroxide scavenging assay and superoxide radical scavenging assay as in the Fig. (2). MEAS showed promising antioxidant property when compared to the standard drug Ascorbic acid. These findings reflected that MEAS is the potent extract of all other solvent extracts. Hence, MEAS could be responsible for promising activity against hepatic aundice.

Phytoconstituents of MEAS identified by GC-MS

GC-MS chromatogram obtained showed a number of peaks for the presence of chemical components as shown in the Fig. (3)., which were identified by the mass spectral library. The GC-MS spectrum identified 14 compounds in MEAS (Table 2). These compounds further subjected to docking with specific protein (PKC) responsible for hepatic jaundice to confirm the possible mechanism of action.

***In silico* molecular docking of phytoconstituents of MEAS**

On docking of all compounds of MEAS enlisted in Table 4 against PKC. The only compound "(22E)-Ergosta-5,22-dien-3-ol" showed binding affinity (-6.20 kcal/mol) towards the PKC interacting with 4 amino acids such as Val80, Lys83, Cys84 and Phe33 with carbon-hydrogen bond, alkyl and pi-alkyl bonds as in Fig. (4). While the standard drug Silymarin

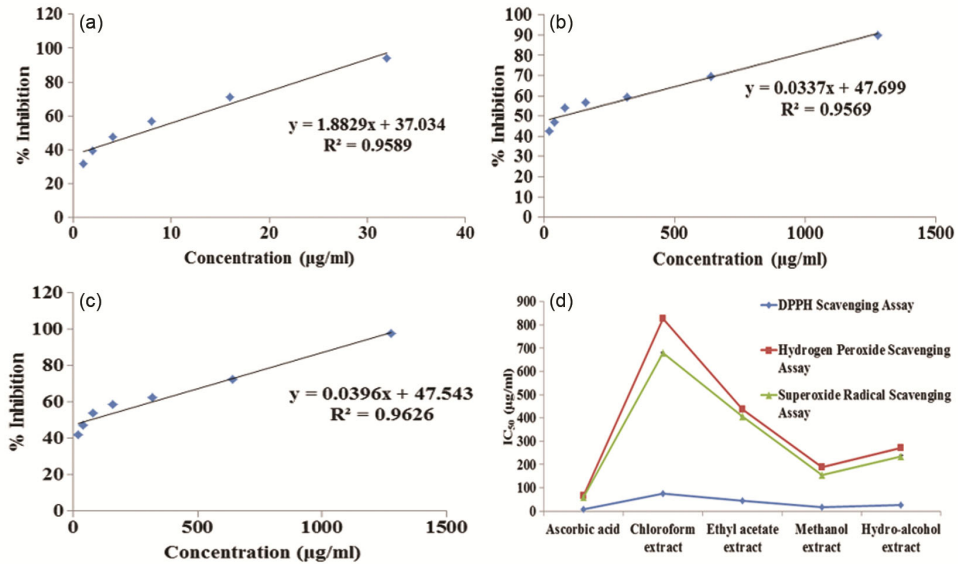


Fig. 2 — Antioxidant study of extracts of *Amaranthus spinosus* L. *in vitro*. (a-c) Standard inhibitory calibration curve by DPPH, Hydrogen peroxide and superoxide radical scavenging assay; and (d) Antioxidant property of extracts of *Amaranthus spinosus* L. Data are shown as mean ± standard deviation (SD) of three variables

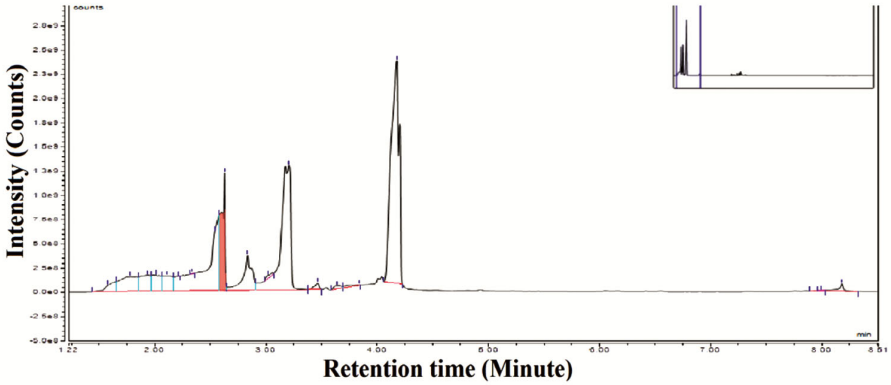


Fig. 3 — GC-MS chromatogram of MEAS

Table 2 — Phytoconstituents of MEAS identified by GC-MS analysis

S.No.	Retention time (Min)	Name of compound	Similarity index (SI)	Reverse similarity index (RSI)	Molecular formula
1	2.920	Methyltartronic acid	792	802	$C_4H_6O_5$
2	3.548	2-Methyl-1-propanol	921	930	$C_4H_{10}O$
3	4.184	1,1-Diethoxy- ethane	843	857	$C_6H_{14}O_2$
4	5.228	3,3-Dimethyl-1,2-epoxybutane	817	848	$C_6H_{12}O$
5	6.201	Ethyl butanoate	884	890	$C_6H_{12}O_2$
6	7.316	1-Butanol-3-methyl acetate	857	862	$C_7H_{14}O$
7	8.776	Ethyl hexanoate	905	905	$C_8H_{16}O_2$
8	9.626	Ethyl heptanoate	842	850	$C_9H_{18}O_2$
9	10.354	Ethyl octanoate	853	861	$C_{10}H_{20}O_2$
10	11.187	Tetradecamethyl-cycloheptasiloxane	831	851	$C_{14}H_{42}O_7Si_7$
11	12.319	2,4-Di-tert-butylphenol	883	907	$C_{14}H_{22}O_2$
12	12.789	Phthalic acid, di-(1-hexen-5-yl) ester	678	715	$C_{20}H_{26}O_4$
13	14.588	Ethyl hexadecanoate	877	884	$C_{18}H_{36}O_2$
14	15.326	(22E)-Ergosta-5,22-dien-3-ol	609	640	$C_{28}H_{46}O$

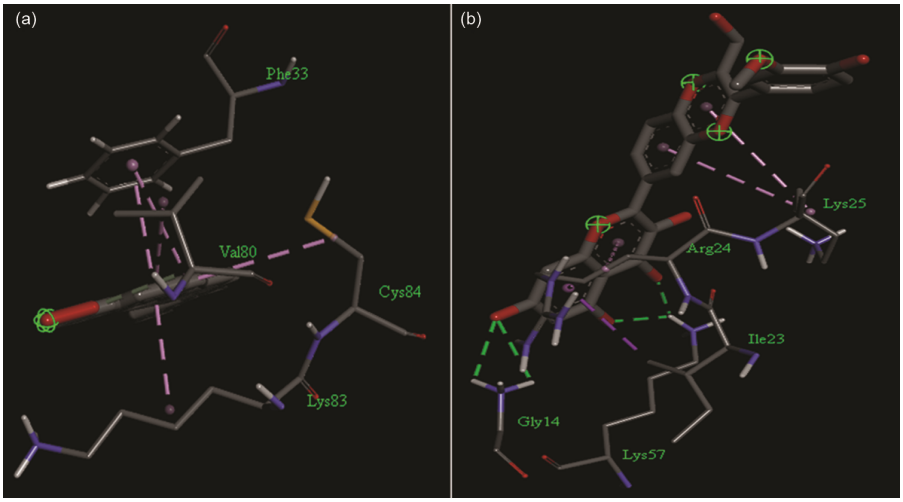


Fig. 4 — *In silico* molecular docking analysis. (a) Interaction of MEAS compound "(22E)-Ergosta-5,22-dien-3-ol" against PKC; (b) Interaction of Silymarin against PKC. Green dashed lines represent Hydrogen bond; Purple dashed lines represent π-π and π-cation interactions

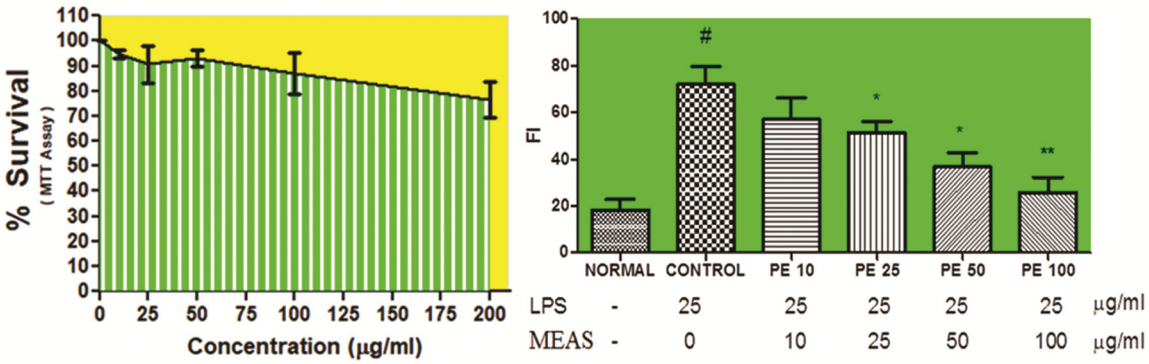


Fig. 5 — Effect of MEAS on HepG2 cell line. (a) Safety study of MEAS by MTT assay; and (b) Effect of MEAS on ROS production. Data are shown as mean ± standard deviation of four variables. "LPS"- Lipopolysaccharide; "MEAS"- Methanol extract of *Amaranthus spinosus* L.; "FI"- Fluorescence intensity. # represents $P< 0.05$ when compared with normal; * represents $P< 0.05$, ** represents $P< 0.01$, when compared with control

interacted with -5.65 kcal/mol with 5 amino acids such as Lys57, Ile23, Gly14, Arg24 and Lys25 with conventional hydrogen bond, and pi-bonds.

Effect of MEAS in HepG2 cell

The MEAS showed decreasing HepG2 cells viability with increasing concentration from 10 µg/mL to 200 µg/mL as represented in the Fig. (5). More than 70% cell survival was observed at the 200 µg/mL concentration. This represented the extract is safe for the study. Further, reactive oxygen species (ROS) were estimated in the HepG2 cell line by the LPS-induced ROS- DCFDA dye fluorescent analysis in which the MEAS showed decreased formation of ROS in the higher concentrations as in the (Fig. 5).

The Protein kinase C (PKC) and Phospho-PKC (pPKC) levels were also estimated from the standard curve using linear regression analysis by utilizing commercially available Sandwich ELISA kit. The MEAS showed a significant and promising effect inhibiting the phosphorylation of PKC as shown in the (Fig. 6).

Acute toxicity study

After the observation for 14 days rats were free from any toxic signs and symptoms and no mortality was seen. Thus, the evaluations of *in vivo* Pharmacological activities were carried out using 200 mg/kg bodyweight as low dose and 400 mg/kg bodyweight as the high dose.

Hepatic anti-icteric effect of MEAS

MEAS was administered orally to the jaundiced rats and the potency was compared using Silymarin as the

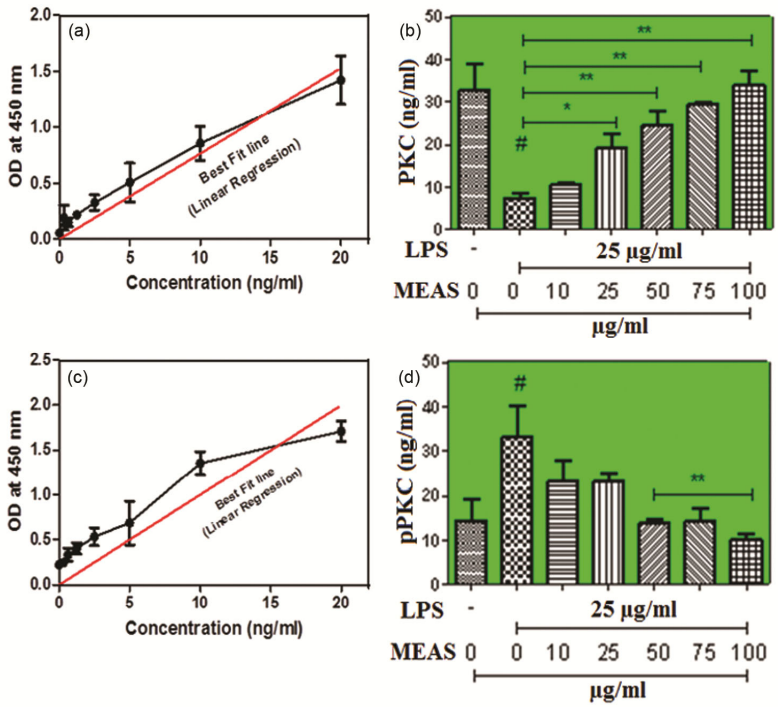


Fig. 6 — Mechanistic approach of MEAS on HepG2 cell line by Sandwich ELISA. (a) Standard curve for Protein Kinase C; (b) Effect of MEAS on Protein Kinase C (PKC); (c) Phospho-Protein Kinase C; and (d) Effect of MEAS on phospho-Protein Kinase C (pPKC). Data are shown as mean ± standard deviation of four variables. "LPS"- Lipopolysaccharide; "MEAS"- Methanol extract of *Amaranthus spinosus* L. # represents $P< 0.05$ when compared with normal (without LPS); * represents $P< 0.05$, ** represents $P< 0.01$, when compared with control (with LPS)

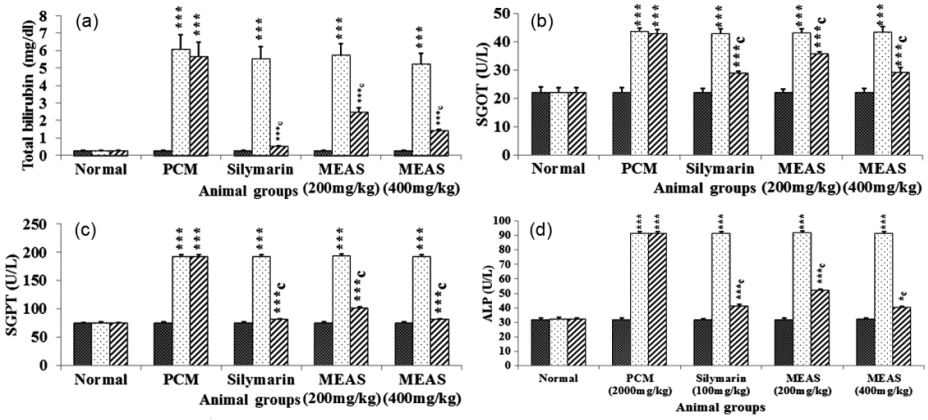


Fig. 7 — Effect of MEAS against hepatic jaundice in experimental rat model; (a) Effect of MEAS on total bilirubin level; (b) Effect of MEAS on SGOT level; (c) Effect of MEAS on SGPT level; and (d) Effect of MEAS on ALP level. Data are shown as mean ± standard deviation of six variables. PCM: Paracetamol; MEAS: Methanol extract of *Amaranthus spinosus* L.; SGOT: Serum Glutamic Oxaloacetic Transminase; SGPT: Serum Glutamic Pyruvic Transminase; ALP: Alkaline Phosphatase. '#' represents $P< 0.05$. '##' represents $P<0.01$, '###' represents $P< 0.001$, when compared with normal control; '*' represents $P< 0.05$, '**' represents $P< 0.01$, '***' represents $P< 0.001$, when compared with positive control (PCM)

standard drug determining biochemical parameters such as total bilirubin, SGOT, SGPT and ALP. The total bilirubin of jaundiced rats was significantly normalized by MEAS ($P< 0.001$). Similarly, SGOT, SGPT and ALP levels of jaundiced rats was significantly normalized by MEAS ($P< 0.001$) as shown in (Fig. 7).

Effect of MEAS on liver architectures

In the histopathological study of the isolated livers of the experimental rats under digital microscope at

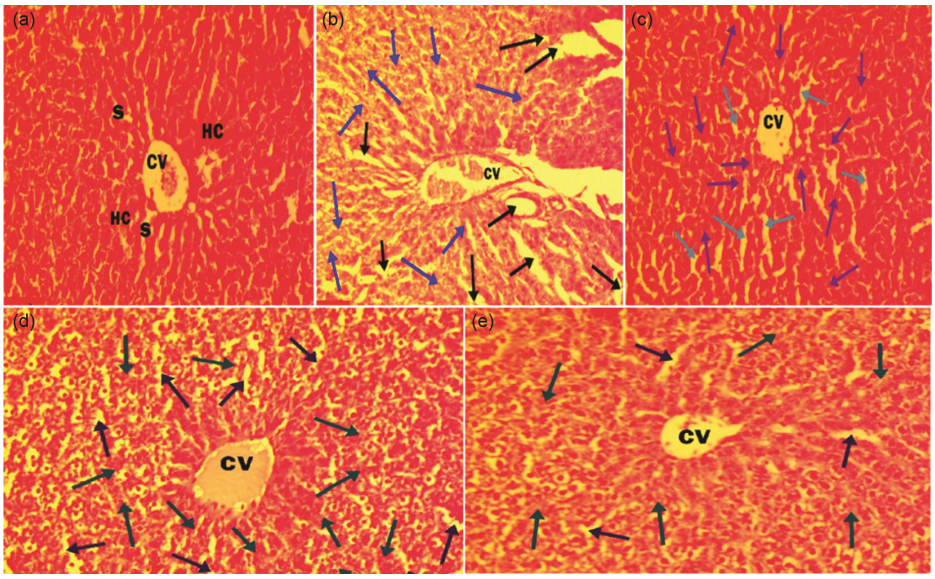


Fig. 8 — Photomicrograph of liver tissues of experimental rats. a)The untreated rats exhibited with the central vein (CV) surrounded by normal hepatic cells (HC) and sinusoids (S) among the HC. b) Jaundiced induced rats exhibited with high damage of HC characterized by the blue arrows, breakdown of the CV followed by infiltration as well as oversize of the S (black arrows). c) Treatment with the standard drug rats showed speedy recovery of the liver and exhibited with the normal HC, S and the CV. d) Treatment with the MEAS at low dose HC regeneration was observed (Dark Green arrow), recovery of S (Grey arrow) and appearance of the CV e) whereas at high dose normal like CV, HC (Dark Green arrow) and S (Dark violet arrow) was observed.

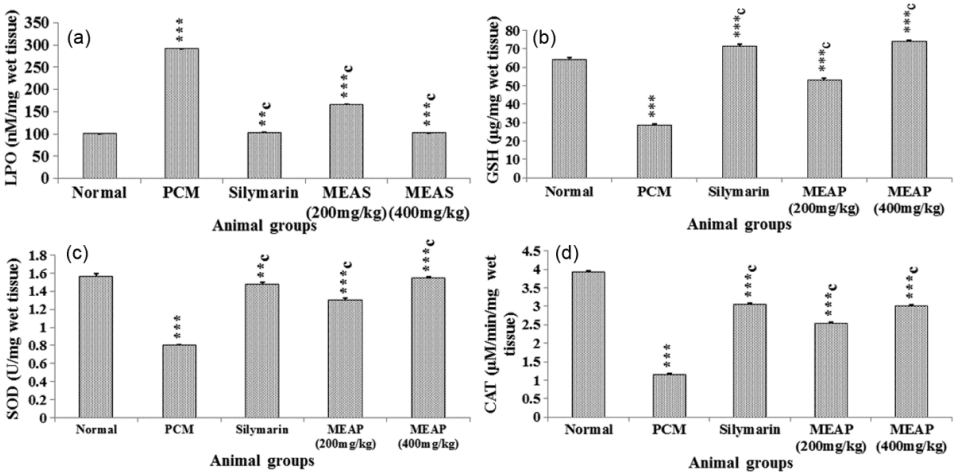


Fig. 9 — Antioxidant properties of MEAS in wet liver tissues of experimental rats. (a) Effect of MEAS on LPO level; (b) Effect of MEAS on GSH level; (c) Effect of MEAS on SOD level; (d) Effect of MEAS on CAT level. Data are shown as mean ± standard deviation of six variables. LPO: Lipid Peroxidation; GSH: Glutathione; SOD: Superoxide Dismutase; CAT: Catalase; PCM: Paracetamol; MEAS: Methanol extract of *Amaranthus spinosus* L. '#' represents p< 0.05. '##' represents *P*<0.01, '###' represents *P*< 0.0001, when compared with normal control; '*' represents *P*< 0.05, '**' represents *P*< 0.01, '***' represents *P*< 0.001, when compared with positive control (PCM)

100X magnification, MEAS showed significance recovery of the hepatic cells and other histological characteristics as shown in (Fig. 8).

Antioxidant property of MEAS on isolated liver tissue

The isolated livers of the experimental rats were analyzed for antioxidant property by determining LPO, GSH, SOD and CAT. The jaundiced animals treated with the MEAS and showed the significant recovery of the level of LPO, GSH, SOD and CAT (*P*< 0.001) as shown in (Fig. 9).

Discussion

The current work offers extensive data on the efficacy against hepatic jaundice of the methanol

extract from the roots of *Amaranthus spinosus* L. (MEAS) in the context of paracetamol-induced hepatic jaundice, including *in vitro*, *in vivo*, and *in silico* investigations. Our results corroborate the ethnomedicinal use of *A. spinosus* roots in the treatment of jaundice and elucidate the mechanisms behind its protective benefits by mitigating oxidative stress and modulating PKC signaling pathways.

Phytochemical analysis revealed that MEAS is abundant in alkaloids, flavonoids, and phenolic compounds, which are well-documented for their hepatoprotective and antioxidant effects[20,21]. The GC-MS study also found bioactive compounds, such as (22E)-ergosta-5,22-dien-3-ol, which demonstrated a robust binding affinity with PKC in docking tests, indicating a direct involvement in the modulation of PKC-mediated inflammatory and apoptotic pathways. This is important because dysregulation of PKC and its phosphorylated version (pPKC) is a major cause of damage to liver cells via the production of ROS, mitochondrial dysfunction, and the formation of fibrous tissue[6,14].

The antioxidant tests showed that MEAS was as good as ascorbic acid in getting rid of free radicals such DPPH, hydrogen peroxide, and superoxide. These findings aligned with the reported restoration of endogenous antioxidant defenses (SOD, CAT, GSH) and the decrease of lipid peroxidation in rat liver tissues, suggesting that MEAS provides hepatoprotection predominantly via reducing oxidative stress.

Cell line tests in HepG2 cells confirmed the antioxidant results, showing that MEAS dramatically reduced LPS-induced ROS production and blocked PKC phosphorylation. These findings corroborate the concept that MEAS disrupts ROS–PKC cross talk, hence inhibiting the amplification of inflammatory cascades that culminate in hepatocellular damage. This combined action of removing ROS and blocking PKC could be a good way to treat hepatic jaundice.

The safety profile of MEAS was determined using acute toxicity testing, which showed no deaths or other toxic effects up to 2000 mg/kg. This confirmed that it has a broad therapeutic range. *In vivo* research further substantiated the effectiveness of MEAS in hepatic jaundice. Oral treatment of MEAS (200 and 400 mg/kg bodyweight) significantly normalized Total bilirubin, SGOT, SGPT, and ALP levels in paracetamol-induced jaundiced rats, equivalent to the standard medication silymarin. Histopathological analysis corroborated the biochemical results, revealing that MEAS-treated groups exhibited substantial restoration of normal hepatic architecture, characterized by hepatocyte regeneration and the maintenance of central veins and sinusoids. The high dose group (400 mg/kg bodyweight) exhibited recovery patterns similar to silymarin, indicating a dose-dependent benefit in the treatment of hepatic jaundice.

Nonetheless, this research has certain limitations. The specific contributions of particular phytoconstituents were not specified. Additional bioactivity-guided fractionation, separation of active components, and validation in chronic liver damage models are necessary. Moreover, molecular investigations focusing on downstream signaling pathways (*e.g.*, NF-κB, TGF-β, mitochondrial apoptosis) may provide more comprehensive mechanistic insights.

Conclusion

The current study shows that the methanol extract of *Amaranthus spinosus* L. roots (MEAS) has significant properties against paracetamol-induced hepatic jaundice. MEAS markedly normalized biochemical indicators, reinstated antioxidant enzyme levels, decreased oxidative stress, and maintained liver histoarchitecture in rats. In HepG2 cells, MEAS efficiently decreased ROS production and lowered PKC phosphorylation. Molecular docking revealed ergosta-5,22-dien-3-ol as a possible PKC inhibitor. These results corroborate the ethnomedicinal usage of *A. spinosus* for hepatic jaundice and show its therapeutic potential as a safe and natural drug. Additional research concentrating on the separation of bioactive compounds, chronic liver disease models, and the clarification of molecular pathways is essential to facilitate its progression into a phytopharmaceutical for liver illnesses.

Acknowledgement

All the Authors express their gratitude towards the Department of Botany; Gauhati University. In this study, the plant specimen was authenticated and identified as *Amaranthus spinosus* L. with the Accession no. 19794.

The experimental procedures were permitted by Institutional Animal Ethics Committee (IAEC) of Assam down town University, Guwahati, Assam,

India with IAEC approval no. AdtU/IAEC/2022/10 on 24/09/2022.

Conflict of interest

The authors declare no conflicts of interest.

References

1 Johnston M & Ravindran RR, Jaundice. *Surgery (Oxf)*, 41 (2023) 334.

2 Alshoabi SA, Binnuhaid AA, Muslem HF, Hamid AM, Alhazmi FH, Alrehily FA, Qurashi AA, Abdulaal OM, Alshamrani AF & Omer AM, Demographic profiles, etiological spectrum, and anatomical locations of the post-hepatic obstructive jaundice in adult population in Hadhramout region in Yemen. *Diseases*, 12 (2024) 333.

3 Kisseleva T & Brenner D, Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat Rev Gastroenterol Hepatol*, 18 (2021) 151.

4 Antar SA, Ash NA, Marawan ME & Al-Karmalawy AA, Fibrosis: types, effects, markers, mechanisms for disease progression, and its relation with oxidative stress, immunity, and inflammation. *Int J Mol Sci*, 24 (2023) 4004.

5 Liu R, Qian MP & Cui YY, Protein kinases: The key contributors in pathogenesis and treatment of nonalcoholic fatty liver disease-derived hepatocellular carcinoma. *Metabolism*, 147 (2023) 155665.

6 Gandhi D, Rudrashetti AP & Rajasekaran S, The impact of environmental and occupational exposures of manganese on pulmonary, hepatic, and renal functions. *J Appl Toxicol*, 42 (2022) 103.

7 Wikan N, Tocharus J, Oka C, Sivasinprasasn S, Chaichompoo W, Suksamrarn A & Tocharus C, The capsaicinoid nonivamide suppresses the inflammatory response and attenuates the progression of steatosis in a NAFLD-rat model. *J Biochem Mol Toxicol*, 37 (2023) e23279.

8 Yang Q, Langston JC, Tang Y, Kiani MF & Kilpatrick LE, The role of tyrosine phosphorylation of protein kinase C delta in infection and inflammation. *Int J Mol Sci*, 20 (2019) 1498.

9 Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F & Peng ZY, Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid Med Cell Longev*, 2019 (2019) 5080843.

10 Neagu M & Constantin C, Signal transduction in immune cells and protein kinases. In: *Protein Kinase-Mediated Decisions between Life and Death*, (Springer International Publishing, Cham), 2021, 133.

11 Petersen MC, Samuel VT, Petersen KF & Shulman GI, Non-alcoholic fatty liver disease and insulin resistance. In: *The Liver: Biology and Pathobiology*, (John Wiley & Sons, Hoboken), 2020, 455.

12 Foglia B, Cannito S, Bocca C, Parola M & Novo E, ERK pathway in activated, myofibroblast-like, hepatic stellate cells: a critical signaling crossroad sustaining liver fibrosis. *Int J Mol Sci*, 20 (2019) 2700.

13 Wang W, Song N, Zhang H, Xie J & Wang J, 6-Hydroxydopamine upregulates iron regulatory protein 1 by activating certain protein kinase C isoforms in the dopaminergic MES23.5 cell line. *Int J Biochem Cell Biol*, 44 (2012) 1987.

14 Palanirajan SK & Gummadi SN, Phospholipid scramblase 3: a latent mediator connecting mitochondria and heavy metal apoptosis. *Cell Biochem Biophys*, 81 (2023) 443.

15 Zhang J, Li Y, Yang L, Ma N, Qian S, Chen Y, Duan Y, Xiang X & He Y, New advances in drug development for metabolic dysfunction-associated diseases and alcohol-associated liver disease. *Cell Biosci*, 14 (2024) 90.

16 Mbelebele Z, Mdoda L, Ntlanga SS, Nontu Y & Gidi LS, Harmonizing traditional knowledge with environmental preservation: sustainable strategies for the conservation of indigenous medicinal plants and their implications for economic well-being. *Sustainability*, 16 (2024) 5841.

17 Borah P & Saikia M, Ethnobotanical study of medicinal plants used by the different tribal communities in Nilakh-Sripani area of Dhemaji District, Assam. *Int J Pharm Sci Res*, 13 (2022) 4586.

18 Lahare RP, Yadav HS, Bisen YK & Dashahre AK, Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus* from Durg district, Chhattisgarh, India. *Sch Bull*, 7 (2021) 1.

19 Islam M & Alam F, *Pharmacognosy for Diploma in Pharmacy*. 1st ed. (AITBS Publishers, Delhi), 2022.

20 Kokate CK, Purohit AP & Gokhale SB, *Pharmacognosy*. 45th ed. (Nirali Prakashan, Pune), 2010.

21 Sinaga E, Fitrayadi A, Asrori A, Rahayu SE, Suprihatin S & Prasasty VD, Hepatoprotective effect of *Pandanus odoratissimus* seed extracts on paracetamol-induced rats. *Pharm Biol*, 59 (2021) 31.

22 Sharma D, Shrestha T, Rapal N, Poudel SC, Shrestha S, Sharma S, Poudel KP & Lamichane J, Screening and bioactivity measurement of high altitude medicinal plants of Nepal. *Vegetos*, 29 (2016) 41.

23 Hussen EM & Endalew SA, In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complement Med Ther*, 23 (2023) 146.

24 Lim S, Choi AH, Kwon M, Joung EJ, Shin T, Lee SG, Kim NG & Kim HR, Evaluation of antioxidant activities of various solvent extracts from *Sargassum serratifolium* and its major antioxidant components. *Food Chem*, 278 (2019) 178.

25 Erida G, Saidi N, Hasanuddin H & Syafruddin S, Herbicidal effects of ethyl acetate extracts of billygoat weed (*Ageratum conyzoides* L.) on spiny amaranth (*Amaranthus spinosus* L.) growth. *Agronomy*, 11 (2021) 1991.

26 Ahmad S, Ali MD, Alotibi AM, Aljedaie SM, Alotaibi KS, Alam N, Ali MS, Haider N, Alam MS, Hussain S & Ansari MS, Phytochemical profiling and anti-inflammatory, analgesic activity of *Ficus religiosa* L. and molecular docking study against iNOS and TNF-α enzymes. *Pak J Pharm Sci*, 36 (2023) 1467.

27 Zhang Q, Bao J & Yang J, Genistein-triggered anticancer activity against liver cancer cell line HepG2 involves ROS generation, mitochondrial apoptosis, G2/M cell cycle arrest and inhibition of cell migration. *Arch Med Sci*, 15 (2019) 1001.

28 Maurya AK & Vinayak M, Anticarcinogenic action of quercetin by downregulation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) via induction of p53 in hepatocellular carcinoma (HepG2) cell line. *Mol Biol Rep*, 42 (2015) 1419.

29 Organisation for Economic Co-operation and Development (OECD), Test No. 423: Acute oral toxicity–acute toxic class method. In: *OECD Guidelines for the Testing of Chemicals*, (OECD Publishing, Paris), 2001.

30 Santiago KH, López-López AL, Sánchez-Muñoz F, Altamirano JLC, Alfaro-Rodríguez A & Bonilla-Jaime H, Sleep deprivation induces oxidative stress in the liver and pancreas in young and aging rats. *Heliyon*, 7 (2021) e06466.