

Hepatorestorative Potential of *Amomum Subulatum* Roxb. Against CCl₄-Induced Hepatotoxicity in Albino Wistar Rats

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Abstract

Among the plants listed for ethnomedicinal usage to protect from or cure liver injuries, *Amomum subulatum* Roxb. (Commonly known as black cardamom), is yet not explored for scientific verification of its in vivo and in vitro hepatorestorative potential. This member of family Zingiberaceae, is widely grown in moist tropical countries. The present study was carried out to assess the hepatoprotective/hepatorestorative potential of methanolic fraction of rhizome and rhizome callus of *Amomum subulatum* Roxb against CCl₄ (1.5ml/kg bw/day, for 14 days)-induced hepatic-injury in albino wistar rats. CCl₄ produced significant changes in biochemical parameters such as increase in the level of serum glutamate oxaloacetic transaminase (SGOT), glutamate pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) and decrease in the level of total protein (TP) along with changes in histopathology (damaged hepatocytes). Treatment with methanolic extract of rhizome/rhizome callus (180mg/kg bw for 14 days) and silymarin (100mg/kg bw for 14 days) significantly ($p < 0.001$) restored normalcy in CCl₄-induced biological and histopathological changes at par with normal untreated control. GC-MS profiling and phytochemical screening of antioxidant status (total phenol, total flavonoid content, DPPH and FRAP) also confirmed the hepatoprotective (restorative) potential of rhizome and rhizome callus of *Amomum subulatum* through reduction of reactive oxygen species leading to maintenance of membrane and hepatocyte integrity.

Keywords: Amomum subulatum, Callus, TP, TF, DPPH, FRAP, GC-MS

Introduction:

Phenolic compounds, known for their endless potential in health benefits, are widely distributed in the plant kingdom, synthesized by the phenylpropanoid and shikimic acid pathway (Koksal *et al.* 2016). They could be classified into various subgroups such as flavonoids, phenolic acid, quinones, tannins, lignans, coumarins, stilbenes and curcuminoids. These compounds show various biological activities, such as, anti-thrombotic, antimicrobial, antipyretic, anti-inflammatory, antioxidant, vasodilatory and hepatoprotective activities (Puupponen-Pimia *et al.* 2001). Since the root cause of most of the critical ailments is production of Reactive Oxygen Species (ROS) exhibiting oxidative stress (Liguori *et al.* 2018), it is necessary to identify the new compounds with antioxidant activities. Earlier studies have also indicated

antioxidant phytochemicals to be potential candidates for cosmetic products, cancer management, cardiovascular disease treatment and against neurodegenerative disorders (Boudet, 2007). Liver is a vital digestive organ of the human body associated with metabolic as well as detoxification pathways, it is very sensitive to liver diseases. The major cause of hepatic injury could be drugs, pollutants, alcohol, free radicals, and food additives, leading to cirrhosis and jaundice. Lipid peroxidation is a dominant source of hepatic cells damage, consequently antioxidant potential of molecules might be majorly in protection of liver from damage. Carbon tetrachloride (CCl₄) is widely used as liver toxicant in the laboratory, known as potent hepatotoxicant that induces hepatic damage in human liver and models (Pandey *et al.* 2023). Carbon tetrachloride is metabolized in the liver and causes production of reactive oxygen species by the activity of microsomal cytochrome P450 oxygenase, ROS production leads to the lipid peroxidation, consequently hepatic cells are injured. Abnormal glucose and lipid metabolism also indicate the liver toxicity. Recently, in modern medicinal system, many effortful treatments have been used to recover from the hepatic damage but unfortunately, most of them have limitation in restoration of the liver from damage due to their lower efficacy and adverse effects (Amanat *et al.* 2021). Plant derived bioactive compounds are more potent in restoration from the hepatic injuries due to their mechanism and lower side effects. We have used herbal alternative as *Amomum subulatum* rhizome and their corresponding callus, a perennial herb of the family Zingiberaceae, known as black cardamom or Badi Elaichi and widely grown in moist tropical regions of the world. This plant is traditionally used to treat liver congestion, cough, fever, anorexia, gonorrhoea, hyperacidity, dysentery, dyspepsia, skin diseases, ulcer, wounds and cardiac debility (Sharma *et al.* 2002). The present study was designed to isolate and characterize phytochemicals of rhizome and rhizome callus of *Amomum subulatum* using gas chromatography-mass spectroscopy (GC-MS), total phenolic (TP), total flavonoid (TF), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) analysis and hepatoprotective potential. This finding was further extended to evaluate the protective effect of pre-treatment with less explored rhizome and rhizome callus of *Amomum subulatum*.

Material and methods

Plant material

Rhizome and callus (The callus produced from the rhizome explant of the mother plant (*Amomum subulatum*) using WPM media supplemented with 2.0mg/L 2,4-D + 0.5 mg/L kn) of *Amomum subulatum* were collected in November (during winter season) from the Department of Botany, Chaudhary Charan Singh University Meerut Uttar Pradesh. The plant material was identified by the Botanical Survey of India, Central National Herbarium Howrah (BSI, Howrah), and deposited as specimen no. Bot/Mahendra/01(*Amomum*) Fig. 1A, B.



Fig. 1A *Amomum subulatum*, 1B *Amomum subulatum* rhizome callus

Preparation of methanolic extract from rhizome and rhizome callus

For the preparation of extracts of rhizome and its callus; rhizome was sterilized with Twin-20 and distilled water and both rhizome and callus were dried at room temperature to constant weight and pulverized by an electric grinder to a fine powder. Since, **Vinaykumar et al. (2020)** have reported methanolic extracts to yield higher amount of phytochemicals than other solvents. Hence, the dried rhizome and callus material were separately refluxed in 95% methanolic solution at 60°C temperature using Soxhlet for 48 hours. The extracts were filtered using Whatman no. 1.0 filter paper and lyophilized.

Gas chromatography-mass spectroscopy profiling (GC-MS)

The screening of rhizome and callus was done at Jawaharlal Nehru University, Delhi (Science instrumentation Centre, AIRF) using GC-MS-QP2010 ultra equipment (SHIMADZU, Kyoto, Japan). The obtained data was identified with the help of a database of the National Institute of Standards and Technology (NIST, US). The name of identified compounds, structure, molecular weight and function were listed through NIST and WILEY8 libraries (**Singh and Yerramilli, 2023**).

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) assay

Free radical scavenging activity of rhizome and rhizome callus of *Amomum subulatum* was determined in methanolic extracts according to **Singh and Vimala (2023)** DPPH assay. 10 µl sample concentration was added to 2.9 ml methanolic solution of DPPH (60 µM), the reaction mixture was well shaken and incubated for 12 hours at room temperature in dark, absorbance was recorded at 515nm using UV-2600, SHIMADZU Spectrophotometer. The blank was made against DPPH without extracts. Ascorbic acid (1.0 mg/ml stock solution) was used as standard.

Ferric-reducing anti-oxidant activity (FRAP) assay

The ferric-reducing activity was estimated by using freshly prepared 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) in 10 ml of 40mM HCl, 30mM acetate buffer (pH-3.6) and 20mM FeCl₃.6H₂O, in the ratio 10:1:1, respectively. 3.99 ml FRAP reagent was taken in a test tube and 10µl plant sample extract was added, absorbance was recorded at 593nm against the blank, by using spectrophotometer (UV-2600 SHIMADZU). Ascorbic acid (1.0 mg/ml stock solution) was used as standard (**Polovnikova and Voskresenskaya, 2008**).

Estimation of total phenolic content (TPC)

Total phenolic content was determined by *Folin-Ciocalteu* spectrophotometric method. A 0.5gm dried plant tissue was homogenized in 5ml 80% ethanol, centrifuged at 5000 rpm for 10 minutes; clear supernatant was collected and residue was recentrifuged in 2ml 80% methanol.

The collected supernatant was well shaken in a water bath until it slightly dried, followed by dissolving in 5ml distilled water. The prepared extracts (0.2, 0.4, 0.6, 0.8, 1.0 µl) mixed with 3ml double diluted Folin–Ciocalteu reagent and 3ml 20% Na₂CO₃ were added making a final volume up to 10 ml. Finally obtained blue coloured mixture was shaken well and incubated for 25 min at 45°C in a water bath. The absorbance was measured at 760nm by UV-2600 Shimadzu spectrophotometer against a reference blank. Gallic acid (GAE mg equivalent) was used as a standard for the calibration curve (**Bray and Thorpe, 1954**).

Estimation of total flavonoid content (TFC)

The aluminium chloride spectrophotometric method determined the total flavonoid content (TFC) of *Amomum subulatum* (rhizome and rhizome callus). To 1 ml fresh supernatant, 0.3ml NaNO₂ was added, followed by addition of 10% AlCl₃ after 5 minutes, incubated for 6 minutes and added 2ml 1M NaOH to the mixture, the final volume was made 10 ml using distilled water. The absorbance was measured at 510 nm using a UV-2600 Shimadzu spectrophotometer against blank. Rutin (Rut mg equivalent) was used as a standard for the purpose of calibration (**Zhishen et al. 1999**).

Drugs and chemicals used:

Carbon tetrachloride (CCl₄, Sigma-Aldrich, USA) and silymarin (Sigma-Aldrich, USA) were gifted by PBRI Bhopal, standard Kits for assay of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) were purchased from Span diagnostic Ltd. Bhopal, Madhya Pradesh.

Experimental models (Animals):

All the experimental models (Male albino Wistar rats 200±20g-BW) were acquired from PBRI Bhopal Madhya Pradesh. According to OECD's guidelines, all the experimental models were housed in polypropylene cages in a well-ventilated room maintained at 25±2°C temperature, under 12 hours light/dark cycle in a pathogen free environment. Feed in the form of pellets and water was provided ad libitum for the experimental models (rats). All the experimental study was started after ethical approval from the Institutional Animal Ethical Committee (IAEC). The experimental work was initiated after acclimatization (12 days) of albino Wistar male rats, it is divided into five groups of six rats each.

Toxicity evaluation:

The toxicity of the extracts was evaluated according to the OECD guideline, (2000). Experimental models were used for the evaluation of toxicity. *Amomum* rhizome and rhizome callus extracts were prepared in methanolic (90%) solution. The methanolic extracts of *Amomum subulatum* rhizome and rhizome callus were administered at dose of 180mg/kg bw.

The dose of extracts of *Amomum subulatum* and callus were selected based on the previous standardization studies (**Amanat et al. 2021**), silymarin (100mg/kg bw) was administered to the experimental models orally as standard.

Experimental design:

The hepatoprotective activity of methanolic extracts of amomum rhizome and rhizome callus were evaluated using 30 Albino Wistar male rats, it is divided into five groups, each group had six rats. CCl₄ (1.5ml/kg bw) diluted with olive oil in ratio of 1:1 was orally transmitted for 14 days (Rayhana et al 2022), while plant extracts (*Amomum* rhizome and rhizome callus, 180mg/kg bw) and standard (100mg/kg bw) drug were administered through intraperitoneal injection. Silymarin is a potent natural polyflavonolignan drug that is widely used in the treatment of liver diseases as reported by several scientists (**Adhikari and Arora ,2014, Alfonso and Ruggero 2019**).

Dose design:

The single dosages of treatment for rats (each group) were determined according to **OECD guidelines (2000)**.

Group I (Normal control): No treatment (Saline water)

Group II (CCl₄): 1.5ml/kg-BW/day (1.5ml/kg bw in olive oil)

Group III (CCl₄ + silymarin): 100mg/kg bw/day

Group IV (CCl₄ + AmoR): 180mg/kg bw/day

Group V (CCl₄ + AmoRC): 180mg/kg bw/day

After 12 hours of administration of dose, the experimental models (Albino Wister rats), were euthanized by decapitation and liver was immediately removed from the euthanized rats and washed with cold saline water at 4°C followed by blotting it dry, further it was used for histopathological evaluation and blood samples were obtained by retro-orbital puncture. The collected blood samples were centrifuged at 3000rpm for 10 min. at 4°C to isolate the serum. After centrifugation, serum was stored at -20°C for further biochemical analysis.

Biochemical analysis:

The biochemical or serum markers viz Serum glutamic oxaloacetic transaminase (SGOT; **2007**), Serum glutamic pyruvic transaminase (SGPT; **Ghosh 1984**), Alkaline phosphatase (ALP; **Wolf 1972**), Total protein (TP) and Total bilirubin (TB; **Wilkinson et al 1969**) were analyzed through liver function assays utilizing the standard manual of Span diagnostic kit.

Histopathological studies:

After dissection of the experimental models (rats), blood samples were collected through the retro-orbital puncture, liver was removed immediately and fixed in 10% formalin solution for histopathological examination. The obtained tissues were processed by dehydrating series of alcohol, cleaned with toluene and embedded in molten paraffin wax for a specific time duration, until wax is set. The processed tissues were sectioned and stained by eosin for structural identification of cells. Stained tissues were again processed for dehydration in different series of alcohol, cleared by xylene and mounted in Canada balsam. Finally prepared slides were viewed under microscope using a 10X objective lens for studying histopathological changes.

Statistical analysis:

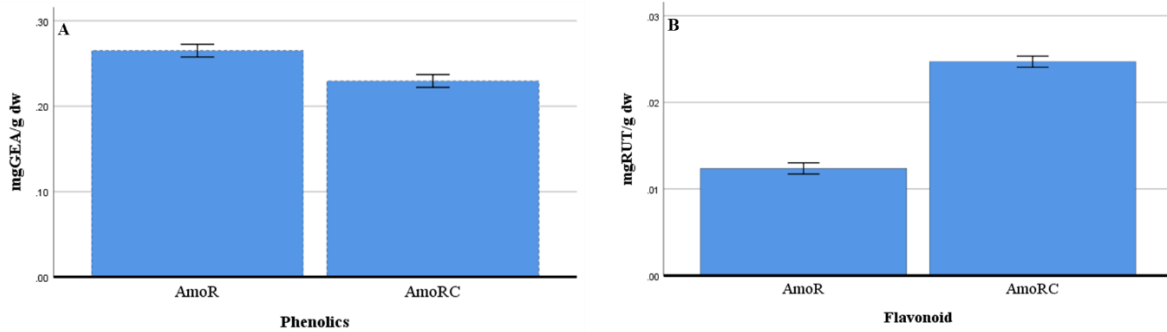
The results were statistically (Mean± SD) analyzed through one-way analysis of variance (ANOVA; P<0.005) and multiple comparison procedures available in the statistical package program version (SPSS 25.0). The significance level is expressed as **P<0.001 for Bonferroni test.

Results

The quantitative and qualitative estimation of *Amomum subulatum* rhizome and rhizome callus extracts (Methanolic) revealed significant yield of secondary metabolites. The screening of methanolic extracts of *Amomum subulatum* rhizome and rhizome callus revealed the presence of phenolics and flavonoid compounds at the concentration of 0.26±0.004 mg GEA should be GAE/gdw and 0.12±0.00004 mg RUT/gdw in AmoR, as against 0.23±0.005 mg GAE/gdw and 0.24±0.0004 mg RUT/gdw in AmoRC,

respectively. Overall, total phenolic content was higher in AmoR and total flavonoid content was starkly higher in AmoRC (Fig.2 A, B).

Fig.2A, Total phenolic and 2B, Total flavonoid content in methanolic extracts of *A. subulatum* rhizome and rhizome callus



Gas chromatography-Mass spectroscopy profiling

The GC-MS fraction of *Amomum subulatum* rhizome and rhizome callus were analysed by National Institute of Standards and Technology (NIST) and WILEY8 libraries. (E)-Labda-8(17),12-diene-15,16-dial (10.02+8.06%); Betulin (8.06%); Cholestan-3,22,26-triol,16-(2-(formylthio) ethyl)-(5.36%); Humulane-1,6-dien-3-ol (3.92%); Spiro (Furan-2(5H),2(1H)-Naphtho (2,1-B) Furan)- (3.11%); 2-(5-(2,2-dimethyl-6-methylene-cyclohexyl)-3 (3.66%); 2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, cis-S (3.99%); (E)-15,16-Dinorlabda-8(17),11-diene-13-one (3.41%) were found in rhizome, while 2-Pyrrolidone (5.05%); 1,3-Propanediol,2-(Hydroxymethyl)-2nitro (14.15%); 11-Octadecenoic acid, methyl ester (5.10%); 1,3-Dioxolane,4-(2-methoxy-4-hexadecenyl (5.62%); 1,1'-bicyclohexyl]-2-ol (5.62%); Gamma-Sitosterol (3.59%) and 4AH-Cycloprop(e)Azulien-4A-ol, Decahydro-1/ Palustrol (2.28) showed maximum area % in rhizome callus.

Antioxidant capacity

The methanolic extracts of *Amomum* rhizome and rhizome callus showed antioxidant potential that could be useful for medicinal application. The free radical scavenging activity of *Amomum* rhizome and rhizome callus against DPPH and FRAP is shown in figure 4A, B. Free radical scavenging activity was slightly higher in *Amomum* rhizome (69 ± 0.3 mg/ml Ascorbic acid) but Fe reducing activity (0.56 ± 0.01 mg equivalent of Ascorbic acid/g dw) was significantly higher in rhizome callus, which can be attributed to higher amount of flavonoids.

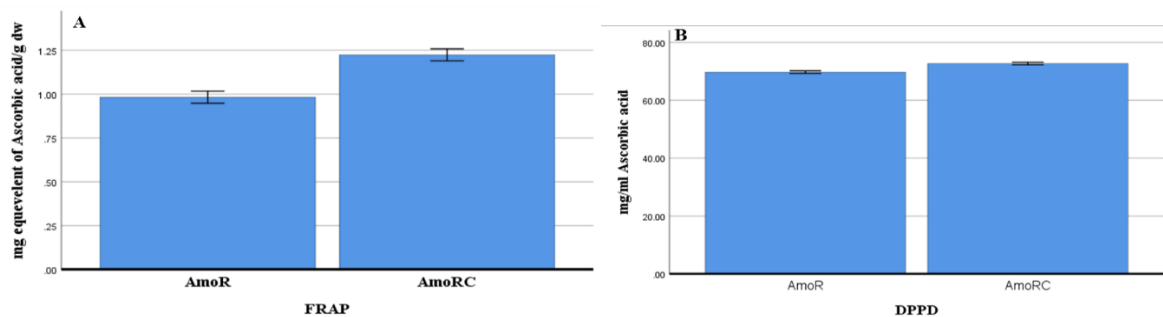


Fig. 4A, DPPH and B, FRAP scavenging capacity in methanolic extracts of *A. subulatum* rhizome and rhizome callus

In vivo hepatoprotective activity evaluation through biochemical markers

The status of established biochemical markers of liver function were evaluated using methanolic extracts of *Amomum* rhizome and rhizome callus to CCl₄-induced rats. The study revealed a significant ($p < 0.001$) increase in the levels of SGOT and SGPT during CCl₄ (1.5ml/kg bw) toxicity as compared to normal control (Fig. 5). After the treatment with methanolic extracts of *Amomum* rhizome and rhizome callus (180mg/kg bw), the levels of SGOT and SGPT could be significantly ($p < 0.001$) lowered compared to normal control groups (Fig.5). The level of ALP could be significantly restored after the treatment with methanolic extracts of *Amomum* rhizome and rhizome callus (180mg/kg bw), compared to normal control groups. During the treatment with CCl₄ (1.5ml/kg bw), TB increased, TP decreased but after treatment with methanolic extracts of *Amomum* rhizome and rhizome callus (180mg/kg bw), TB significantly ($p < 0.001$) decreased and TP significantly increased ($p < 0.001$) as compared to normal control groups (Fig.5).

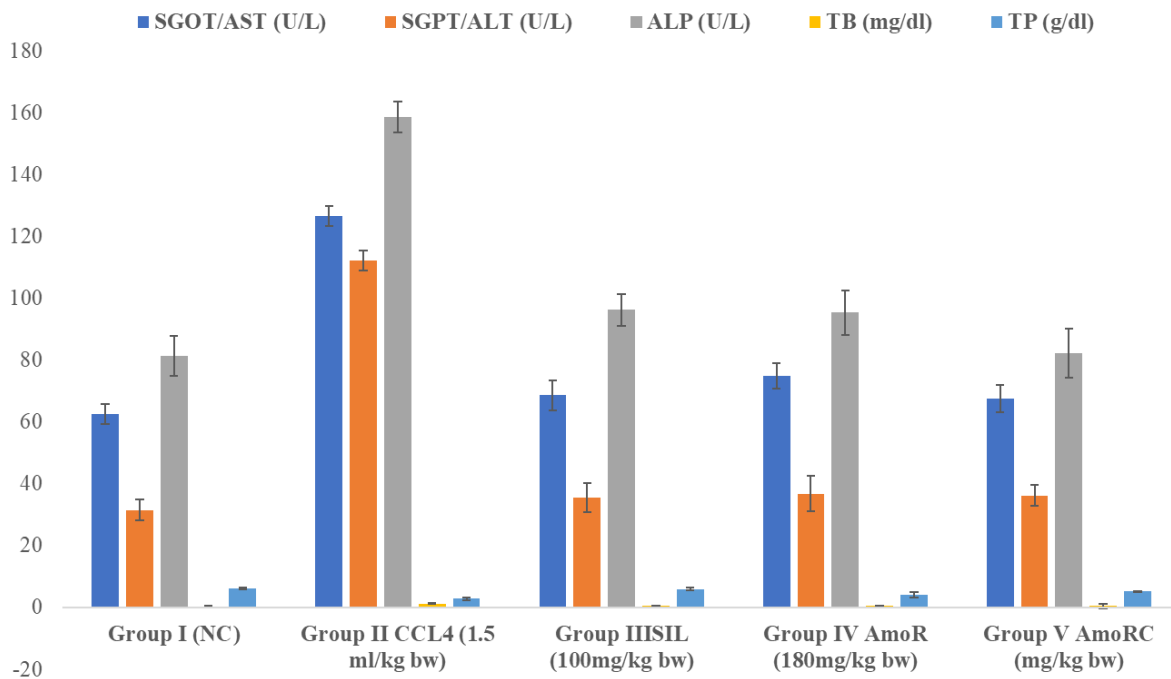


Figure 5: Liver functioning attributes SGOT, SGPT, ALP, TB, and TP of CCl₄ -injured liver of rats, under standard drug treatment and rhizome /rhizome callus extract treatment. Values are expressed as Mean±SD at n=6, One-way ANOVA followed by Bonferroni test, **P<0.001 and ^{NS}P>0.001 compared to the CCl₄ treated rats.

Histopathological examination

In the histopathological examination, after treatment of CCl₄ toxicity, hepatic cells were damaged when compared with normal control groups. However, CCl₄ damaged the hepatic architecture as compared to the normal architecture of normal control groups. The treatment of rhizome and rhizome callus extracts at the single dose of 180mg/kg bw restored normal architecture of liver when compared with normal control groups (Fig.6). The single dose of *Amomum* rhizome and rhizome callus methanolic extracts (180mg/kg bw) offered protection as silymarin (100mg/kg bw). Silymarin is a potent hepatoprotectant drug that showed normal architecture of liver, it reduced the degenerative changes in the liver cells. The administration of rhizome callus methanolic extract at a dose of 180mg/kg bw is more effective when

compared with *Amomum* rhizome (180mg/kg bw). Thus, the results of these experiments indicate the rhizome callus exhibited more potential against CCl₄-induced hepatic-injury in albino wistar rats (**Fig.6**).

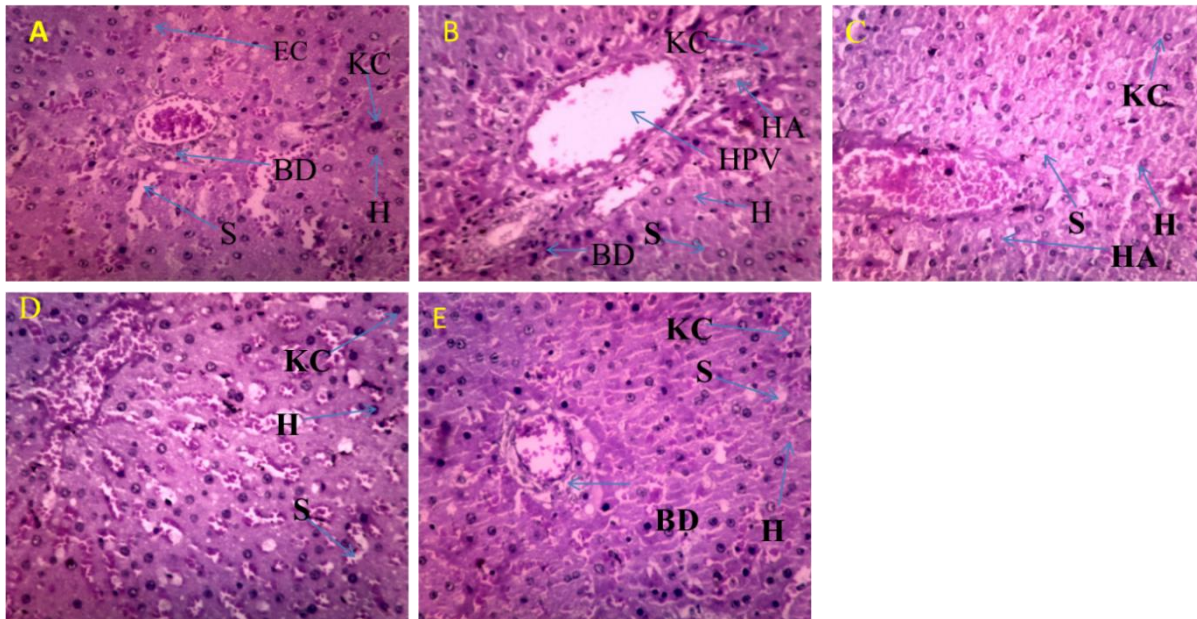


Fig. 6; Effects of *Amomum* rhizome and rhizome callus extracts on structural changes in liver tissue of normal control and treated groups of Albino Wistar rats. (**Fig. 6A**) Normal architecture in normal control groups. (**Fig. 6B**) Representative section of the liver from the CCl₄-treated group showing centrilobular vacuolar degeneration of hepatocytes and parenchyma. (**Fig. 6C**) Treated with silymarin showing normal architecture as normal control. (**Fig. 6D**) (AmoR) & **Fig. 6E**, (AmoRC), showing normal architecture as normal control. EC, Epithelial cell; KC, Kupffer cell; BD, Bile duct; H, Hepatocytes; S, Sinusoids; HA, Hepatic artery; HPV, Hepatic portal venule.

Discussion

Oxidative stress and redox imbalance cause liver damage, however, inflammatory hepatitis, cirrhosis and carcinoma have typical symptoms of liver damage associated with oxidative stress and redox imbalance as reported in alcoholic liver disease by **Wu *et al.* (2009)**. For the intracellular system, oxidant and antioxidant balance is necessary because it plays vital role in cell function and adaptation under diverse growth condition. The excessive generation of reactive oxygen species (ROS) leads to the loss of cell function, oxidative stress and apoptosis/necrosis. Recent research revealed the important pathophysiological role of free radicals and oxidative stress in the development and advancement of liver disease.

CCl₄ used in the study changes into trichloromethyl free radicals and peroxytrichloromethyl with the action of P450 oxygenase enzyme. These free radicals covalently bind with proteins or lipids, thus starting lipid peroxidation of the cell membrane. Trichloromethyl (CCl₃·) and peroxytrichloromethyl, damage the cell membrane by disturbing Ca²⁺ homeostasis. CCl₄- generated free radicals, cause hepatic injury leading to leakage of lipid membrane of liver cells. This results in the release of serum marker enzymes such as SGOT, SGPT and ALP from the cytosol of liver cells to the blood stream. The serum marker enzymes decide the assessment of liver functions (**Vinaykumar *et al.* 2020**).

However, antioxidants, play a significant role in recovery from oxidative liver injury, meaning thereby, the frequent use and consumption of antioxidants could be important in preventing or suppressing the liver

diseases (Zhang et al. 2015). The present study investigated the effect of *Amomum* rhizome and rhizome callus extracts against CCl₄ toxicity in albino wistar rats.

Phytochemicals of *Amomum* rhizome and rhizome callus effectively reduced the excessive production of free radicals and metal chelators causing oxidative stress or other inflammatory response in liver. Similar activity has been reported by Jeyadevi et al. (2019) in *Ipomoea staphylina* Linn. during hepatoprotective and antioxidant activity assays. Presently traditional medicines and their therapeutic potentials are being explored and accepted world over.

Recent reports on medicinal plants revealed about phenolic and flavonoid compounds with potential biological properties (Mounir et al. 2021). The extracts of *Amomum* rhizome and rhizome callus revealed the presence of a richness of phytochemicals viz phenolic, flavonoid, terpenoids and alkaloids through phytochemical profiling using spectrophotometer and GC-MS. GC-MS profiling of *Amomum* rhizome and rhizome callus extracts revealed the screening of 114 phytochemicals in both the samples. Among them (E)-Labda-8(17),12-diene-15,16-dial (10.02+8.06%); Betulin (8.06%) and 2-Pyrrolidone (5.05%); 1,3-Propanediol,2-(Hydroxymethyl)-2nitro (14.15%); 11-Octadecenoic acid, methyl ester (5.10%); 1,3-Dioxolane,4-(2-methoxy-4-hexadecenyl) (5.62%); 1,1'-bicyclohexyl]-2-ol (5.62%) showed the highest area % in *Amomum* rhizome and rhizome callus extracts. These phytochemicals are potentially biologically active. Quantitative analysis revealed high total phenolics and total flavonoids in AmoR (0.26±0.004 mgGAE/gdw), and Amo RC (0.24±0.0004mgRUT/g dw), respectively indicating flavonoids to be potentially active antioxidants. DPPH and FRAP activity of *Amomum* rhizome and rhizome callus extracts were evaluated with ascorbic acid as standard (Fig.4 A, B). Effective antioxidant activity through reduction of metal ion by *Amomum* rhizome callus extracts indicate flavonoids of callus to be potential antioxidants overcoming CCL4 induced toxicity. In CCl₄-treated rats, *Amomum* rhizome and rhizome callus extracts (180mg/kg bw) showed significant (p<0.001) reduction in toxicity- induced increased levels of SGOT, SGPT, ALP and TB, whereas significant increase in total protein levels. (Fig.). The recovery of serum enzymes (SGOT and SGPT) indicated that rhizome and rhizome callus extracts have potential to protect and maintained the structural integrity of the hepatocytes, while increased level of protein also stabilized the endoplasmic reticulum leading to protein synthesis. It proved that rhizome and rhizome callus extracts showed strong potential of overcoming CCl₄-induced toxicity. The histopathological examination revealed the toxicity of liver when treated with CCl₄ (1.5ml/kg bw) as compared to normal control groups. CCl₄ toxin disturbed the normal architecture of liver when compared with normal control group (Fig. 6 A, B). The combination of rhizome and rhizome callus extract+CCl₄ (180mg/kg bw+ 1.5ml/kg bw) exposure revealed the restoration from hepatic injury and retained the arrangement of central vein, hepatocytes and other disappeared cells of liver when compared with normal control group (Fig 6A, D, E) and further regeneration of liver cells was seen when treated with silymarin + CCl₄ (100mg/kg bw + 1.5ml/kg bw, respectively). The histopathological examination provides further support to the potency of methanolic extracts of *Amomum* rhizome and rhizome callus against liver damage.

Conclusion

Based on our findings, it can be concluded that methanolic extracts of *Amomum* rhizome and rhizome callus exhibit hepatoprotective efficacy against CCl₄-induced toxicity to the hepatocytes. *Amomum* rhizome and especially rhizome callus is being reported for the first time in the literature to have potent protective role against hepatic-damage. The methanolic extracts revealed the presence of phenolics and

flavonoids, which show significantly high antioxidant activity in terms of free radical scavenging and metal ion reducing assays (DPPH and FRAP). The hepatoprotective potential of *Amomum* rhizome and rhizome callus extracts might be due to the presence of mainly flavonoids, besides phenolics. GC-MS profiling revealed the several new phytochemicals which have unknown biological activity so far, that may offer antioxidant activity and thus, may prevent excessive generation of ROS. *Amomum* rhizome and rhizome callus extracts may act as curative drugs against hepatic-injury.

Data availability statement: Data are contained within the manuscript.

Conflicts of interest: There is no conflict of interest.

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