RESEARCH PAPER

Phytochemical profiling and in vitro, in silico hepatoprotective investigation of sprouted Panicum glaucum L. grains intended for navigating the liver disorders

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ABSTRACT

Rising interest in natural biocatalytic compounds for liver diseases reflects the limitations of symptom-based conventional therapies and the significant global burden of these conditions. The sprouting process of pearl millet (*Pennisetum glaucum* L.), a chief cereal offers a variety of nutritional and human health benefits, for augmentation in navigating the liver diseases. The ethanol extract of twelve days sprouted and unsprouted *P. glaucum* grains (EESPG & EEUSPG) was investigated for hepatoprotective and anti-oxidant effects followed by different *in vitro* model and EESPG was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) for phytochemical analysis. The major compounds observed by GC-MS *viz* 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF), Maltol, and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP), were reported in literature to have antioxidant and hepatoprotective effects. The EESPG showed significant (p<0.01) anti-oxidant and hepatoprotective effects among the *in vitro* cell viability, thiobarbituric acid reactive substances assay (TBARS) to measure lipid peroxidation, and restored the altered levels of hepatic biomarkers including aspartate aminotransferase (AST); alanine aminotransferase (ALT); glutathione (GSH) content; and super oxide dismutase (SOD) activity assay whereas EEUSPG exhibits nonsignificant effects. DMHF interacted with the antioxidant enzyme ascorbate peroxidase (APX; PDB ID: 1OAF), maltol bound to the Keap1 Kelch domain regulating Nrf2, and DDMP showed interaction with the target protein (PDB ID: 6TYM). The combined phytochemical, *in vitro,* and *in silico* findings support the potential application of sprouted *P. glaucum* grains as a natural therapeutic agent for the management and prevention of liver disorders.

Key words: Panicum glaucum, Sprouted grains, Hydroxydihydromaltol, Hepatoprotective effects, in vitro and in silico study.
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1. INTRODUCTION

Plants have been the foundation of nourishment and medicine, playing an essential role in human survival since the beginning of civilization. Health and disease have constantly co-existed. The remedial properties of plants would have been recognized through human predisposition. This information might have been complemented over time based on additional annotations and usefulness in retrieval. Primeval civilizations show that the use of plants for healing purposes dates to the earliest part of recorded history [1]. Traditional healthcare systems like Ayurveda, Unani, and Siddha have been in practice for eras. Nowadays, the perception of Traditional Systems of Medicine, which practice plants to treat ailments, has a worldwide reputation. Millets belong to the family Poaceae and are regarded as one of the primogenital cultivated crops. Bulrush millet, generally recognized as Pearl millet (*Pennisetum glaucum* L.), and classified as *P. typhoides* and *P. americanum,* is propagated as minor grain in tropical and sub-tropical cereal grass for animal forage. The scientific validation of the therapeutic effects of plants gained prominence [2]. Pearl millet is a chief cereal, widely grown as the sixth most vital cereal in the world after wheat, rice, barley, maize, and sorghum, and is used in West African and Asian countries. It is commonly known as 'Bajra' in India, 'Gero' in Nigeria, 'Hegni' in Niger, 'Dukhon' in Sudan, Arabic, and 'Mahangu' in Namibia. Sub-humid uplands of East Africa and sub-Sahara African are thought to be the regions of origin of Bulrush millet [3].

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The major chemical constituents and main nutrient contents of the grains are carbohydrate, protein, fat, vitamins, minerals, and antinutrient phytochemicals. The percentage of amylose and soluble sugars in *P. glaucum* grain starch is relatively lower than other species of *Pennisetum* (nonwaxy) starches. The prolamin (aqueous alcohol-soluble) proteins, constitute 31–41% of the total protein, form the major part in this millet. The amino acid content of this plant grain is marked by a relatively high content of vital amino acids such as leucine, isoleucine, lysine, tyrosine, phenylalanine, threonine, tryptophan, valine, cystine, and methionine [4]. Bulrush millet grain is a good source of essential dietary minerals such as calcium, phosphorus, and potassium. It also serves as an important source of B-vitamins, concentrated in the aleurone layer, with niacin occurring partially bound to carbohydrate. Compared with other cereal grains, its fat content is typically high, with oleic acid, linoleic acid, and palmitic acid as the key components [5].

Three C-glycosyl flavones- glucosylvitexin, glycosylorientin, and vitexin are present in millet and can exert goitrogenic effects when pearl millet is consumed without dehulling. *P. glaucum* grains contain efficient levels of bound hydroxycinnamic acid and ferulic acid, while their very low soluble content and bound hydroxybenzoic acids. The presented data on phytochemicals in *P. glaucum* grains remain very limited [6]. The de-iodination of thyroxine hormone to tri-iodothyronine, is inhibited by thyroid peroxidase activity, which is suppressed by the C-glycosylflavones present in millet. These compounds are responsible for imparting the brownish-grey coloration of pearl millet and responsible for mousy flavour in moist millet flour, which are possibly responsible for goitrogenic effects [7].

Growing interest has emerged in natural bioactive components for their possible therapeutic aids, since traditional treatments for liver diseases often focus mainly on symptom management [8]. Liver diseases such as liver inflammation, cirrhosis, fatty liver disease, and hepatocellular carcinoma characterize important global health tasks. Natural bioactive compounds, found in herbs and plants, are increasingly recognized for their potential in managing liver disorders [9]. These compounds, often with antioxidant, anti-inflammatory, and liver protective properties, can help recover liver function, lessen liver enzyme levels, and alleviate symptoms. They offer a promising avenue for complementing traditional treatments and addressing the underlying causes of liver damage [10].

Millet grains contain antinutrients that may adversely affect nutrient bioavailability. On the other hand, *P. glaucum* grains are gluten-free and are regarded as one of the most suitable grains for individuals with celiac disease and liver disorders [11]. Many people consume sprouted grains due to their numerous health benefits like to regulate blood sugar, weight management and for strengthening the immune system [12]. Sprouting of grains offers a variety of nutritional and health supports, producing them a valued addition to the diet. It enhances levels of vitamins (especially B vitamins, vitamin C, and vitamin E), increases antioxidants, which help fight free radicals in the body, improved digestibility. The sprouting process of *P. glaucum* grains wash out the goitrogenic C-glycosylflavones and breaks down complex starches, into oligosaccharides and different other compounds making it potent antioxidant intended for augmentation in navigating the liver diseases [13]. After sprouting there were enhancements in mineral and vitamin contents of *P. glaucum* that could be useful in the formulation and production of human food supplements in respect of minerals and vitamins to improve health, combat covid-19 and optimize cereal potentiality [14]. This research work was designed to evaluate the *in vitro* antioxidant and hepatoprotective activities of sprouted and unsprouted *Panicum glaucum* grains (EESPG and EEUSPG). Additionally, the phytochemical constituents of the sprouted grains were characterized by GC–MS analysis, and hepatoprotective mechanisms of active constituents were explored through *in silico* molecular docking studies.

2. MATERIALS AND METHODS

2.1 Procurement and certification of plant material

Panicum glaucum (L.) variety HHB 67 were purchased in October 2022 from Dharmshala market of district Gorakhpur, UP., (India) and validated by the All-India Institute of Ayurveda (AIIA), New Delhi. The reference number is (RRDR/AIIA/phg. /133).

2.2 Chemicals and drugs

Human liver cancer cell line (hepatoblastoma) $HepG_2$ cell lines were attained from the National Centre for Cell Science (NCCS), Pune, India. Trolox (Otto Kemi - Cat no.–T7723), Cupric Chloride (SD fine – Cat no. 37834K05), Ammonium acetate (Fischer scientific – Cat no.- 11145), Neocuproine (SRL chem – Cat no. 93007), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution (SRL Chem Cat no. SR-29128), Acetic acid, Methanol (SD fine- Cat no. 10930lC250), Ascorbic Acid (SRL, Cat no 23006), Para-nitrophenol, Tris HCl/NaCl, 5,5'-Dithiobis (2-Nitro benzoic acid), Phenazonium methosulphate (HiMedia-GRM1677), Nitro blue tetrazolium (SRL-Chem-11207), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (SRL-Chem-11326), Potassium pyro-phosphate buffer (Sigma -Adrich-322431), Riboflavin (SRL-Chem – 34392), Sodium Lactate Lactic acid (HI media Cat. No. GRM243), Potassium phosphate buffer, Gelatin (Fisher Scientific, Cat. No-900-70-8), NAD^+ Enzyme- 400U/mL, TBARS assay kit (Cat No. OPKA003), Ecoline diagnostic kits, Glutathione (GSH) assay kit, Super oxide dismutase (SOD) action examine kit, Alkaline phosphatase (ALP) assay kit (Cat No. KEA017).

2.3 Instruments

UV spectra were recorded using a Lambda Bio 20 Spectrophotometer (Shimadzu-U, Singapore), and absorbance measurements were obtained with a

microplate reader (iMark, Bio-Rad). GC–MS study was accomplished on a Shimadzu QP-2010 Ultra system prepared with a standard nonpolar TRX 5-MS capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness).

2.4 Sprouting of plant materials and extract preparation

For the optimal sprouting, 250 g of *P. glaucum* grains were divided in to four parts and each parts were thoroughly washed with distilled water to remove dust, debris, and broken particles. To reduce microbial contamination, the grains were surface sterilized by soaking in 0.1% sodium hypochlorite solution for 5 minutes. The grains were then rinsed 3–4 times with sterile distilled water to remove any residual disinfectant. The sterilized grains were soaked in sterile distilled water for 10–12 hours at room temperature (32–40°C) to facilitate hydration and initiate germination. After soaking, excess water was drained and the grains were spread evenly on sterile germination trays lined with sterile moist muslin cloth. The trays were covered with sterile cloth and incubated under controlled conditions at 32–40°C. To maintain adequate moisture and prevent microbial growth, the grains were rinsed with sterile distilled water every second day and excess water was removed to avoid water stagnation. The grains were allowed to germinate for 12 days, and the germination process was monitored regularly. Any grains showing visible fungal or bacterial contamination were immediately discarded. After completion of sprouting, the germinated grains were collected and dried in a hot air oven at 40–45°C until constant weight to stop further metabolic activity. The dried sprouted grains were ground into coarse powder using a mechanical grinder [15]. Following a 72-hour cold maceration process, 100 g of the powdered sprouted grains and 25g fresh unsprouted grains were extracted with 250 and 100 mL of 90% v/v ethanol. The extract was passed through muslin cloth for filtration, after which the filtrate was concerted under reduced pressure and subsequently vacuum-dried [16]. The yield of ethanol extract of sprouted and unsprouted of *P. glaucum* grains (EESPG) and (EEUSPG) was 9.4% and 6.8% w/w. EESPG was subjected to preliminary phytochemical investigation and GC–MS analysis to identify various phytoconstituents. Both EESPG and EEUSPG was *in vitro* evaluated for hepatoprotective and antioxidant activities **(Fig.1).**

Figure 1. Days 12 sprouted seed of *Pennisetum glaucum* grains

2.5 Determination of *in vitro* hepatoprotective effect of EESPG and EEUSPG

2.5.1 In vitro cytotoxic activity of EESPG and EEUSPG

Mitochondrial synthesis was estimated using the tetrazolium assay to determine the 50% cytotoxic concentration (CTC_{50}). $HepG_2$ cells (5.0×10^3 cells/well) were preserved in 96 well-culture plates for 72 h in the existence of 100 µL of EESPG and EEUSPG at the concentrations of 50, 100, 150, 200, 250 and 300 µg/mL. After the incubation time, the test solutions were withdrawn from the wells, and 60 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution made in modified Eagle's Medium was poured to all well. The plates were slightly agitated and incubated up to the 2.5 h at 35 ± 3 °C in a 5% CO_2 environment. During incubation, the supernatant was rejected, and 50 µL of propanol was introduced to dissolve the formazan crystals, with constant shaking up to the 40 min at room temperature. Absorbance of the sample was taken at the wavelength of 540 nm by microplate finder from BioTek Instruments, Inc., (Winooski, VT) USA [17]. The growth inhibition was measured by using the following formula:

(%) Growth inhibition = 100 − (Mean of OD for specific test /Mean of OD for control) × 100

A dose response curve was plotted with % growth inhibition on the Y-axis and concentrations of extract (µg/mL) on the X-axis, from which the CTC_{50} value was derived.

2.5.2 Measurement of cell viability, ALT, and AST activities

The cell viability activity of EESPG and EEUSPG was assessed in HepG2 cells, with ethanol (80 mM) as the hepatotoxic agent and silymarin as the positive control. The experimental study was divided into four groups, and each group was conducted five times. Group I cultured cells considered as normal control (NC) and 100 µL of silymarin, EESPG, and EEUSPG as per se controls. The group II toxic group (TC) cells were exposed to 100 µL of 80 mM ethanol for 24 h. The group III cells were treated with 100 µL Silymarin and EEUSPG comprising 80 mM ethanol up to 24 h. The group IV cells were conserved with EESPG and EEUSPG (50, 100, and 200 µg/mL) holding 80 mM ethanol for 24 h. Further, cell viability, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) assays were conducted following standard protocols with Ecoline diagnostic kits [18].

2.5.3 Measurement of LDH release in HepG2 cells

When cells are damaged, they release LDH into the extracellular space; under normal conditions, LDH remains a stable enzyme within the cytoplasm of most cells. The LDH assay is a widely used procedure for assessing cell viability and cytotoxicity. For the evaluation of cytoprotective activity of EESPG and EEUSPG four sets of well selected, in which 50 µL stock solution (1M Acetic acid), 10 µL buffer solution Tris. HCl-NaCl at (p*H* 8.0) and 80 µL enzyme reaction mixture containing Solution A (5% Gelatin), Solution B (10mM Phenazine methosulfate), and Solution C, (100 mM Nitro blue Tetrazolium) was added. HepG2 cells were pre-treated with 100 µL of 80 mM ethanol for 24 h to induce hepatotoxicity. Cells were then treated with tested sample EESPG, EEUSPG and silymarin in Solution D (NAD^+ and Enzyme 400U/mL) and incubated at 37±4 °C for 24 h. Finally, 10µL substrate mixture was added to each well of all four groups and the absorbance was taken at 570 nm [19-20].

LDH release activity = (A_{Sample} - $A_{Control}$ /$A_{Control}$) ×100
(A_{test} = Absorbance of test sample)
($A_{Control}$ = Absorbance of control)

2.5.4 Estimation of MDA content (TBARS Assay)

The TBARS assay is a common technique for estimating MDA content, a product of lipid peroxidation, in plant extracts. In this method, lipid peroxidation is induced in a 0.5 mL of linoleic acid solution (0.2%) prepared in ethanol and the inhibitory effect of the EESPG and EEUSPG are evaluated. The assay involves reacting MDA with thiobarbituric acid (TBA) in acidic environments and at high temperature, forming a pink-colored adduct that can be measured spectrophotometrically. In a clean test tube 100 µL of EESPG and EEUSPG at (50, 100, and 200 µ/mL) concentrations were mixed thoroughly to 500 µL of Solution A (TCA-Trichloroacetic acid). The content was centrifuged at 2500 rpm for 5 mins, and the supernatant was collected for further experiment. The 100 µL of tested sample was employed in a tube and assorted with 1800 µL of TBA reagent and the solution was incubated for 15 min at 80 °C. Then the absorbance was taken at 532 nm with spectrophotometer [21]. Malondialdehyde levels were determined by means of an extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$.

MDA concentration (M) = (Absorbance $_{532\ nm}$ / 1.56×10^5) × dilution factor

2.5.5 Alkaline phosphatase assay

Alkaline phosphatase (ALP) is an isoenzyme found throughout the body, with higher concentrations in the liver, bile ducts, and bones. It plays a role in various biological processes, like bone formation, liver function, and kidney function. The liver is the primary source in most patients with elevated enzyme levels. The ALP activity assay was performed with alkaline phosphate assay kit (Cat no. KEA017). 10µL of sample (cell lysate) and standard was taken in the defined wells of 96 well plate. 50 µL of AMP buffer containing 50 µL PNP substrate is added to the each well of the defined set. Then, it was incubated at 37°C for 15 minutes. Then, 10 µL of NaOH was added to stop the reaction. Then, the absorbance was taken at 405 nm in a microplate reader [22].

ALP Activity (U/mg of protein) = (A/B)

A [ALP activity in test (mol/min.)] = (Test absorbance × standard amount)/Standard absorbance ×15
B [Protein quantity (mg)] = {Test absorbance × Standard amount (g) × 210}/ (Standard absorbance × 10 × 1000)

2.5.6 Estimation of reduced glutathione

A 140 µL enzyme solution (Glutathione reductase + Potassium phosphate buffer) was added in reaction mixture (Potassium phosphate buffer and enzyme) to each well of 96 well plate in all the four sets and after that 10 µL of each sample was added to all well of set two and three, and 10 µL standard solution was added to all well of set four and extra buffer was added to make up the volume 100 µL in each well of set 1-4. Test sample EESPG and EEUSPG at (50, 100, and 200 µ/mL) concentrations, blank, standard, and control tubes were prepared then pipetted into 96 well plate. After that the plate was incubated for 5 minutes at room temperature and then 50 µL substrate solution 5,5'-Dithiobis (2-Nitro benzoic acid), was added to each well of the set 1-4 and the absorbance was collected immediately at 415 nm in microplate reader (iMark Biorad USA). Total GSH concentration was estimated in µM with the help of standard curve of Glutathione [23].

2.5.7 Super oxide dismutase (SOD) activity assay

The SOD assay relies on the ability of superoxide dismutase to suppress the production of blue-colored formazan crystals generated from the reaction of GSH,

phenazine methosulfate, and nitro blue tetrazolium. The absorbance of color product is noted at 560 nm using a spectrophotometer. During bacterial killing, white blood cells employ enzymes like NADPH oxidase to yield superoxide and other reactive oxygen species (ROS). Under stressful conditions, the cellular concentration of SOD generally rises, as these enzymes function as antioxidants that safeguard cellular components from ROS-induced oxidation. All reagents and homogenate were brought to 37°C. Test sample EESPG and EEUSPG at (50, 100, and 200 µ/mL) concentrations, blank, standard, and control tubes were prepared then pipetted into 96 well plate. SOD enzyme was diluted at different concentrations (6.25 to 100 U/mL) to prepare the standard curve. In a glass cuvette, 1.2 mL potassium pyrophosphate buffer (pH 8.3 0.025M) was taken then 1mL to Phenazonium methosulphate (PBS) (186µM) and 0.3mL Nitro blue tetrazolium (300µM) was added and mixed thoroughly. After this 05mL PBS (1X) and 0.05mL enzyme crude extract (10 times diluted) was added in blank as well as sample cuvette and it was mixed properly. To make the volume of reaction up to 8mL, 1.15 mL deionized water was added and then, the reaction was initiated by adding 0.2mL GSH (780µM) and the reading was recorded at absorbance of 560 nm [24].

2.6 *In vitro* antioxidant activity

2.6.1. CUPRAC assay

Mixed (10Mm) Cupric chloride solution, 1.0M Ammonium acetate in buffer at pH 7.0 and Neocuproine (Nc) in equal proportion to make the desired quantity of reagent mixture. 10 µL of different concentration of the EESPG, EEUSPG and Trolox (50, 100, 150, 200, 250, & 300 µg/mL) were poured in defined wells of 96 well plate. Then, 200 µL of reagent mixture was added in a 96 well plate. Reaction mixture in quadruplicate form and blank in duplicate form were prepared in methanol and 10 µL of compound of different concentrations for sample and standard incubated for 30 minutes in dark. Wells without any treatment served as the control group. Following incubation, the decrease in absorbance was restrained at 490 nm with a microplate reader. For the control reaction, 20 µL of deionized water was added in the place of the sample or standard. The scavenging effect was stated as percentage inhibition relative to the control [25]. The IC_{50} rate was determined and graph was plotted with sample concentration on the X-axis and percentage inhibition (compared to control) on the Y-axis.

2.6.2. DPPH scavenging assay

10 µL of various stock of the test sample EESPG, EEUSPG and Ascorbic acid (50, 100, 150, 200, 250, & 300 µg/mL) were poured to 0.2 mL of 0.1mM DPPH in methanol in a 96 well plate. The wells without sample were considered as controlled and wells without DPPH were measured as blank. The response was established in quadruplicate and all plats were incubated for 30 min in dark. The absorbance was measured at 540 nm by means of a microplate reader (iMark, BioRad) after incubation. The reaction mixture with 20 µL double distilled water served as the control. The scavenging capacity was articulated as % inhibition relative to control, sample concentration (X-axis) against % inhibition (Y-axis) [26].

2.7 GC-MS analysis of EESPG

GC–MS investigation of EESPG was carried out by means of a Shimadzu GCMS-QP2010 Ultra system equipped with a TR 5-MS nonpolar capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium served as the transporter gas at a flow rate of 1.21 mL/min. The oven temperature was automated from 100 °C to 260 °C at 10 °C/min, with an injection volume of 5 µL. Samples dissolved in ethanol were scanned up to the mass range of 10–650 m/z, and compounds were identified through the Wiley spectral library. Detected components (0–25 min) were characterized by name, molecular weight, molecular formula, and structure, while their relative abundance was determined from peak area normalization [27-28].

2.8 Molecular docking study

Based on literature, maltol, DMHF, and DDMP are reported as potential antioxidant as well as hepatoprotective phytoconstituents. So molecular docking studies were conducted to predicts the binding affinity of ligands to receptor proteins using the LibDock protocol of discovery studio. For antioxidant activity, DMHF were docked against the antioxidant-related protein 1OAF**,** with ascorbic acid serving as the standard compound. To assess the cytoprotective and anti-apoptotic potential, Maltol and DDMP were docked against the human Keap1 Kelch domain (PDB ID: 6TYM)**,** which regulates antioxidant defines. Prior to docking, proteins were prepared by removing co-crystallized ligands and water molecules, adding hydrogen atoms, and defining active binding sites, while ligands were energy minimized for optimal fitting [29]. This approach was aimed at predicting molecular interactions that could support the proposed antioxidant and hepatoprotective mechanisms of these compounds.

3. STATISTICAL ANALYSIS

Results were articulated as mean ± standard deviation (S.D) and mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way ANOVA following Dunnett's t-test. in GraphPad Prism 5.0 (San Diego, CA, USA), with $P < 0.05$ and < 0.01 considered as significant. IC_{50} values were obtained through linear regression analysis.

4. RESULTS AND DISCUSSION

The sprouting process of *P. glaucum* grains wash out the C-glycosylflavones glycosides and breaks down complex starches, making the grains easier to digest and allows better absorption of minerals like iron, calcium, magnesium, and zinc. The different amino acids present in EESPG have antioxidant, anti-inflammatory, and regenerative properties, offer promising therapeutic potential for liver diseases. Preliminary phytochemical test of the EESPG discovered the existence of carbohydrates, reducing sugar, phenolic compounds, and amino acids as major phytoconstituents.

4.1 *In vitro* hepatoprotective activity of the EESPG and EEUSPG

4.1.1 In vitro cytotoxic activity of EESPG and EEUSPG

This is a colorimetric assay for measuring the cell metabolic activity and measures mitochondrial dehydrogenase activity. In which viable cells reduce yellow MTT into purple formazan crystals. The results of cytotoxicity reveal that EESPG and EEUSPG exhibited low level of toxicity to HepG2 cells **(Figure 2).** However, at the highest dosage (300 µg/mL) EESPG and EEUSPG exhibited 13.1 and 28.4% cell death whereas standard drug Paclitaxel showed 96.4%. However, at the highest dosage (300 µg/mL) both tested EESPG and EEUSPG, showed less than 50% cell death.

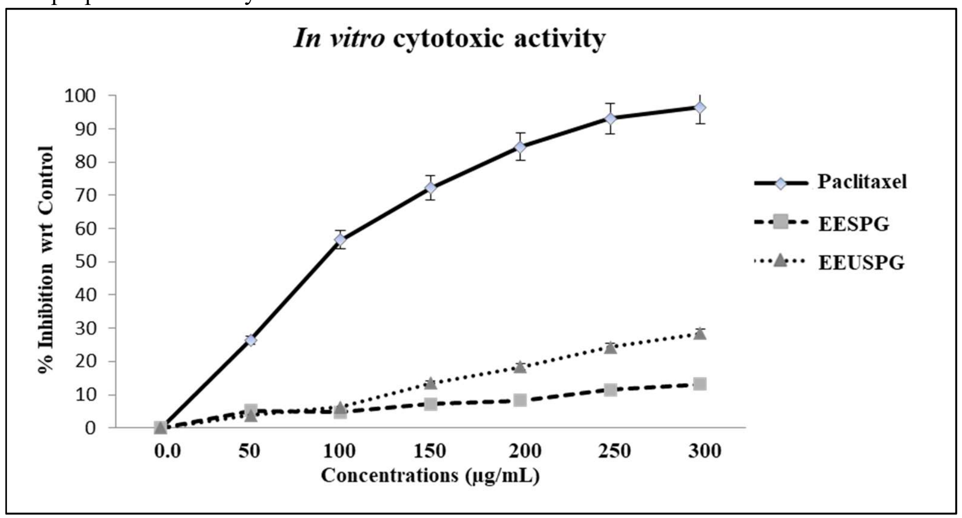

Fig. 2: MTT cytotoxicity effect of the ethanol extract of sprouted and unsprouted *Panicum glaucum* grains (EESPG & EEUSPG) in HepG2 liver cells. Data are expressed as percentage of mean ± SD (n = 3).

4.1.2 Measurement of cell viability, ALT, and AST activities

The outcomes of the study cell viability**,** AST and ALT levels are represented in **(Table 1).** The group-I NC HepG2 cells showed 94.61 ± 1.20% whereas per se Silymarin and EESPG treated HepG2 cells showed 93.14 ± 1.12% and 91.16 ± 1.18 %. Silymarin (100 µg/mL) and EESPG (200 µg/mL) treated with 80 mM ethanol on HepG2 cells showed 188.31 ± 2.63% and 77.53 ± 2.63% cell viability. There was a significant ($^{##}p < 0.01$) reduction in cell viability and significant ($^{##}p<0.01$) rise in the level of AST and ALT in the group-II, treated with 80 mM ethanol as related with normal control group-I. The HepG2 cells treated with EESPG (50, 100, and 200 µg/mL) exhibited a significant renovation of HepG2 cell viability and demolish of hepatic enzyme levels (ALT and AST), comparable to that observed with the reference drug silymarin and EEUSPG did not show significant effects. All the tested doses (50, 100 and 200 µg/mL) of EESPG exhibited the cytoprotective activity and dose 200 µg/mL showed more significant effect. AST is an enzyme found in various tissues, but high levels in the blood often indicate liver damage. When cells are damaged, AST leaks into the bloodstream, causing elevated levels in a blood test. The liver is a primary source of AST, so elevated levels are often a sign of liver damage or disease [30].

Table 1: *In vitro* hepatoprotective activity of ethanolic extract of sprouted and unsprouted *Panicum glaucum* grains against damage induced by 80 mM ethanol in HepG2 cells towards AST, ALT and LDH production.

Groups	Cell viability (%)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Group I Normal control (NC)	94.61 ± 1.20	12.24 ± 2.21	9.23 ± 3.11	137.26 ± 1.41
EESPG 100 µg/mL (Per se control)	93.14 ± 1.12	13.26 ± 3.14	10.36 ± 4.16	140.42 ± 1.48
EEUSPG 100 µg/mL (Per se control)	91.16 ± 1.18	13.44 ± 2.16	11.41 ± 4.26	138.65 ± 1.61
Silymarin 100 µg/mL (Per se control)	93.86 ± 1.24	12.76 ± 2.64	10.86 ± 3.12	139.06 ± 1.94
Group II 80 mM ethanol (Toxin)	$36.77 \pm 1.42^{##}$	$58.6 \pm 3.32^{##}$	$46.58 \pm 4.42^{##}$	$242.56 \pm 2.11^{##}$
Group III (Standard)				
80 mM ethanol + silymarin (100 µg/mL)	$82.32 \pm 1.45^{**}$	$16.28 \pm 3.42^{**}$	$13.33 \pm 4.62^{**}$	$144.68 \pm 1.88^{**}$
Group IV (Test)				
80 mM ethanol + EESPG (50 µg/mL)	$52.55 \pm 1.51^{*}$	$34.4 \pm 3.18^{*}$	$24.3 \pm 2.26^{*}$	$197.40 \pm 1.22^{*}$

80 mM ethanol + EESPG (100 µg/mL)	68.16 ± 2.72*	27.62 ± 3.46*	17.21 ± 4.14*	172.38 ± 1.64*
80 mM ethanol + EESPG (200 µg/mL)	77.53 ± 2.63**	18.64 ± 2.83**	14.24 ± 3.43**	158.96 ± 2.31**
80 mM ethanol + EEUSPG (50 µg/mL)	38.97 ± 1.86	51.56 ± 3.39	42.18 ± 3.32	239.55 ± 1.73
80 mM ethanol + EEUSPG (100 µg/mL	42.57 ± 2.24	48.94 ± 2.62	39.72 ± 2.48	237.45 ± 1.89
80 mM ethanol + EEUSPG (200 µg/mL	48.82 ± 1.82	46.71 ± 3.45	38.67 ± 2.42	236.52 ± 1.84

Data represent mean ± SEM (n = 5), one-way (ANOVA) analysis of variance was performed followed by Dunnett's t-test. $^{##}p<0.01$ Group-I (Normal control) versus Group-II (Toxin treatment 80 mM ethanol). $^{*}p<0.05$ & $^{**}p<0.01$ Group-II (Toxin treatment 80 mM ethanol) versus test Group-III & Group-IV (Different concentrations of EESPG and EEUSPG).

ALT is primarily found in the liver, and elevated levels can indicate liver damage or disease. ALT levels are a key indicator of liver health, with elevated levels often suggesting liver damage from various causes, including hepatitis, alcohol abuse, or other liver diseases. The assay can be utilized to screen the progression of liver disease or the efficacy of treatment [31]. Levels of the AST and ALT enzyme activity looks to be precise by the quantity of EESPG. Interestingly, EESPG showed comparatively higher cytoprotective effects than EEUSPG, indicating that the sprouting process might enhance the beneficial phytochemicals such as phenolic compounds, flavonoids, and antioxidants, which may contribute to improved cellular tolerance and protective effects.

4.1.3 In vitro cytotoxicity evaluation by LDH release assay in $HepG_2$ cells

LDH is a stable cytosolic enzyme that is released into the culture medium when the plasma membrane is damaged; therefore, its extracellular activity serves as a reliable marker of cell membrane integrity and cytotoxicity. Treatment of HepG2 cells with the toxic control 80 mM ethanol caused a marked elevation in LDH release compared with the normal control group. In contrast, EESPG and EEUSPG treated groups showed a concentration-dependent reduction in LDH leakage and significantly lower LDH release when compared with the toxic control. At concentrations of 50, 100 and 200 µg/mL, EESPG confirmed protective effects on HepG2 cells by maintaining membrane integrity. The highest concentration (200 µg/mL) showed the significant (**p<0.01) reduction in LDH levels, which was comparable to the standard hepatoprotective drug Silymarin whereas EEUSPG showed non-significant effect (Table 1). The reduction in LDH release indicates stabilization of the cellular membrane and prevention of enzyme leakage. These results indicate that the extracts were non-cytotoxic at the tested concentrations and were able to protect the hepatocytes against toxicant-induced cellular damage.

4.1.4 Estimation of MDA content (TBARS assay)

Lipid peroxidation is one of the major mechanisms involved in oxidative stress-induced cellular injury. In the present study, the toxic control group exhibited a significant increase in MDA levels compared with the normal control group, indicating enhanced lipid peroxidation and oxidative stress in the hepatic cells. Conversely, the dose-dependent treatment with EESPG significantly (**p < 0.01) reduced MDA levels in the treated groups compared to the toxic control, with the higher dose (200 µg/mL) showing greater reduction, suggesting inhibition of lipid peroxidation and potential antioxidant activity. These actions collectively reduce ROS-mediated damage and stabilize cellular membranes. EESPG showed a comparatively greater reduction in MDA formation than EEUSPG, suggesting a stronger protective effect against lipid peroxidation. The reduction in MDA levels at the highest concentration (200 µg/mL) was comparable to that of the standard hepatoprotective drug Silymarin. The TBARS assay can be susceptible to interference from other compounds in plant extracts that also react with TBA, potentially leading to overestimation of MDA. While the TBARS assay is widely used, it's important to acknowledge that TBA can react with other aldehydes, present in the tested sample not just MDA **(Table 2).** The TBARS assay results demonstrate that EESPG effectively attenuate oxidative stress by inhibiting lipid peroxidation in HepG2 cells, thereby supporting their potential role as natural hepatoprotective agents.

4.1.5 Alkaline phosphatase assay

The activity of Alkaline Phosphatase was estimated in HepG2 cell lysates to evaluate hepatocellular damage and the protective effect of the EESPG. The level of the ALP enzyme activity seems to be controlled by the quantity of EESPG **(Table 2).** In the present study, the toxic control group exhibited a significant elevation ($^{##}$p<0.01) in ALP activity compared with the normal control group. Treatment with EESPG shows a dose-dependent significant (**p<0.01) restored in enzyme activity in the given concentrations (200 µg/mL) as compare to toxic group. The standard Silymarin also significantly (**p<0.01) restored ALP levels toward normal values, whereas EEUSPG showed nonsignificant restoration of ALP levels. Liver diseases cause a large upsurge in the activity of liver ALP that takes place entirely in the plasma membrane [32]. The activity of the membrane phosphatase may be elevated in the livers by permeating the toxic drugs. The results indicate that the EESPG exhibits notable hepatoprotective potential by mitigating toxin-induced elevation of ALP in HepG2 cells, likely due to its antioxidant phytochemicals found in the EESPG.

4.1.6 Estimation of reduced glutathione

GSH in plant extracts can be valuable for understanding a plant's antioxidant capacity and potential health benefits. GSH is a crucial antioxidant that helps protect cells from damage caused by free radicals. The toxic control group showed a significant depletion in the

intracellular GSH level compared with the normal control group, indicating the induction of oxidative stress in HepG2 cells. Similarly, treatment with EESPG at higher concentrations (200 µg/mL) resulted in a significant (**p<0.01) increase in GSH levels compared to the toxic control group. Among the tested samples, EESPG showed a more pronounced elevation of GSH levels than EEUSPG, suggesting enhanced antioxidant activity due to sprouting (Table 2). Estimating Analysing GSH levels in plant extracts can reveal their potential as natural antioxidants neutralizing harmful ROS [33]. Plant extracts with high GSH content can be incorporated into functional foods and supplements to promote human health.

4.1.7 Super oxide dismutase (SOD) activity assay

Measuring SOD activity in plant extracts provides valuable insights into their antioxidant potential and potential health benefits. In contrast, the toxic control group showed a significant reduction in SOD activity compared to the normal control group, suggesting the induction of oxidative stress and excessive generation of reactive oxygen species (ROS) in HepG2 cells. Treatment with the standard drug silymarin and EESPG significantly restored SOD activity toward normal levels, and EESPG demonstrated a greater enhancement of SOD activity than EEUSPG **(Table 2).**

Table 2: *In vitro* effects of ethanolic extract of sprouted and unsprouted *Panicum glaucum* grains against damage induced by 80 mM ethanol towards MDA, ALP, GSH and SOD.

Groups	MDA content (nmoles/mL)	ALP (U/mg Protein)	GSH (U/mg Protein)	SOD (U/mg Protein)
Group I Normal control (NC)	1.51 ± 0.26	51.33 ± 2.24	8.71 ± 3.14	28.248 ± 1.28
EESPG 100 µg/mL (Per se control)	1.71 ± 0.32	53.26 ± 3.14	9.26 ± 2.16	30.42 ± 1.48
EEUSPG 100 µg/mL (Per se control)	1.61 ± 0.38	53.44 ± 2.16	9.41 ± 2.26	29.65 ± 1.61
Silymarin 100 µg/mL (Per se control)	1.76 ± 0.24	54.76 ± 2.64	9.86 ± 2.12	32.26 ± 1.94
Group II 80 mM ethanol (Toxin)	8.74 ± 0.39$^{##}$	182.65 ± 2.56$^{##}$	2.71 ± 2.14$^{##}$	12.26 ± 1.32$^{##}$
Group III (Standard) 80 mM ethanol + silymarin (100 µg/mL)	1.98 ± 0.32**	82.15 ± 2.92**	6.71 ± 2.14**	26.41 ± 2.11**
Group IV (Test)				
EESPG (50µg/mL)	6.53 ± 0.23	138.69 ± 2.21	3.74 ± 2.11	16.26 ± 1.21
EESPG (100 µg/mL)	4.78 ± 0.28*	116.41 ± 2.34*	5.32 ± 2.16*	20.98 ± 1.24*
EESPG (200 µg/mL)	2.64 ± 0.25**	98.22 ± 2.26**	6.50 ± 1.86**	24.46 ± 1.31**
EEUSPG (50 µg/mL)	7.46 ± 0.22	166.14 ± 2.31	3.14 ± 1.94	13.83 ± 1.22
EEUSPG (100 µg/mL)	7.323 ± 0.38	152.68 ± 2.38	3.32 ± 2.16	14.13 ± 1.28
EEUSPG (200 µg/mL)	7.177 ± 0.35	147.23 ± 2.78	3.48 ± 2.12	15.99 ± 1.34

Data represent mean ± SEM (n = 5), one-way (ANOVA) analysis of variance was performed followed by Dunnett's t-test. $^{##}p<0.01$ Group-I (Normal control) versus Group-II (Toxin treatment 80 mM ethanol). $^{*}p<0.05$ & $^{**}p<0.01$ Group-II (Toxin treatment 80 mM ethanol) versus test Group-III & Group-IV (Different concentrations of EESPG and EEUSPG).

The restoration of SOD activity in HepG2 cells treated with EESPG suggests that these extracts possess considerable antioxidant and hepatoprotective properties. The improvement in SOD activity may be attributed to the presence of phenolic compounds, flavonoids and other antioxidant phytoconstituents in *P. glaucum*, which can neutralize reactive oxygen species and enhance the cellular antioxidant defines mechanism. Measuring SOD activity helps determine the plant's overall antioxidant capacity and its potential for protecting against oxidative stress-related damage [34].

4.2 *In vitro* antioxidant activity

The cupric ion reducing antioxidant capacity (CUPRAC) and DPPH radical sequencing assay was performed to evaluate the total antioxidant potential of EESPG and EEUSPG. As shown in **figure 3** and 4 the EESPG sample, showed a dose-dependent antioxidant activity, by using the CUPRAC and DPPH radical methods. Based on the results obtained from the experimental work, antioxidant property (CUPRAC) assay was observed in samples and 50% inhibitory concentration of EESPG was 194.8 ± 0.54 µg/mL and equivalent to 107.6 ± 0.82 µg/mL of Trolox. The results obtained from the experimental work, antioxidant activity (DPPH assay) estimated in EESPG sample, IC_{50} of tested sample was found to be 230.3 ± 0.24 µg/mL and equivalent to 77.3 ± 0.42 µg/mL Ascorbic acid. The CUPRAC assay is a method used to estimate the antioxidant capacity of a sample by measuring the reduction of Cu (II) to Cu (I) in the presence of antioxidants compounds.

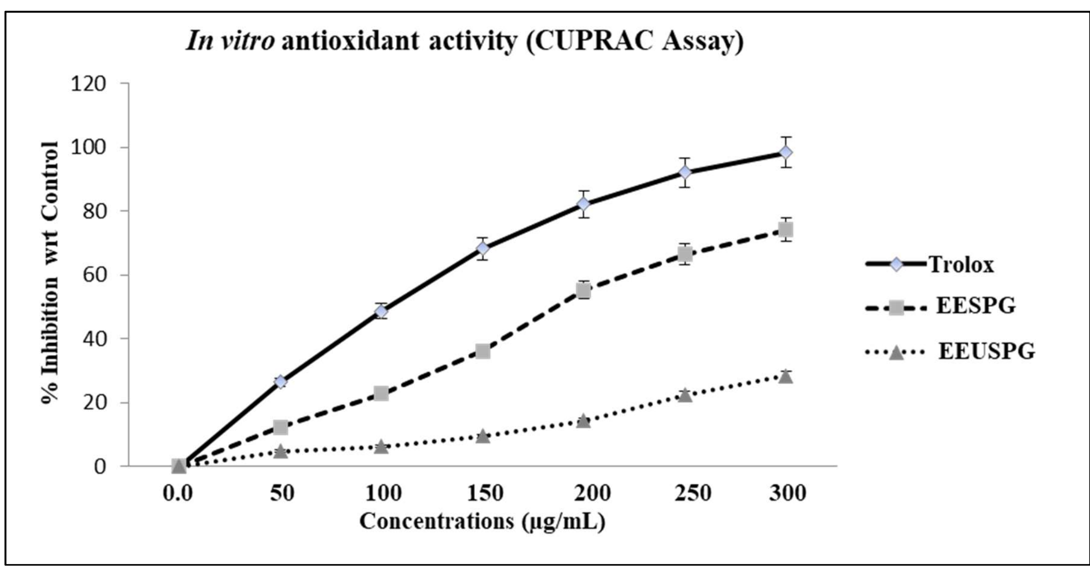

Fig. 3: Dose-dependent antioxidant activity, by the ethanol extract of sprouted and unsprouted *Panicum glaucum* grains (EESPG & EEUSPG) using the CUPRAC. The data represent the percentage reduction of Cu (II) to Cu (I) by the tested sample. Each value represents mean ± S.D., (n = 4). IC_{50} of tested sample EESPG and Trolox was found to be 194.8 ± 0.54 and 107.6 ± 0.82 µg/mL. EEUSPG was found to be very low active as compared to standard – Trolox and 50% inhibitory concentration was observed above dose limit.

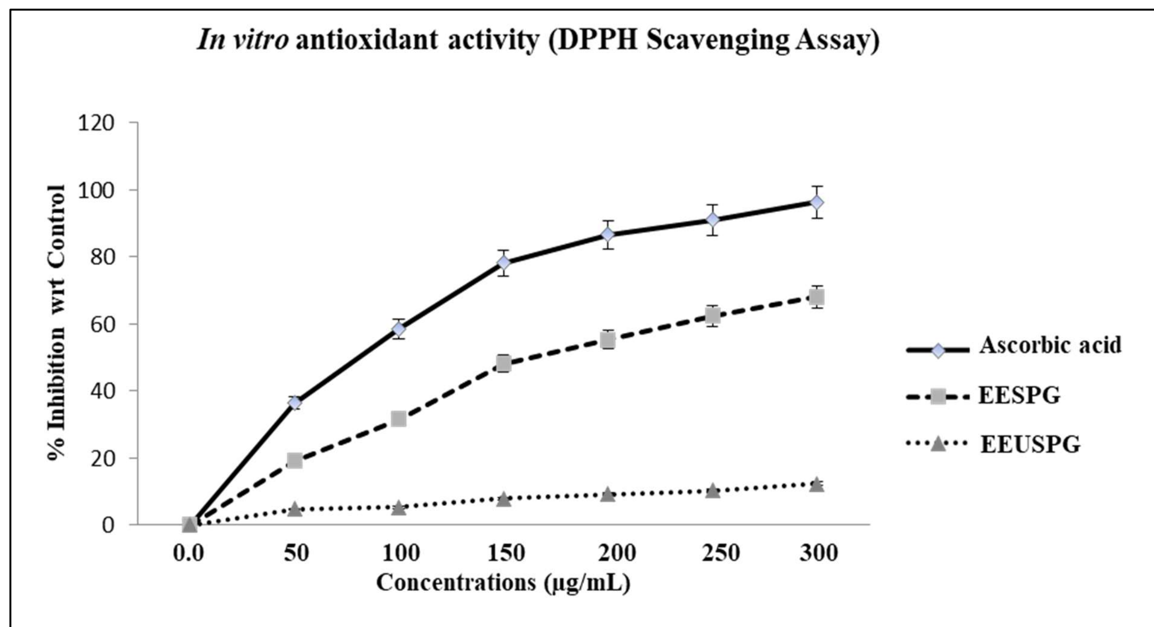

Fig. 4: Dose-dependent scavenging of DPPH radicals by the ethanol extract of sprouted and unsprouted *Panicum glaucum* grains (EESPG and EEUSPG). The data represent the percentage inhibition of DPPH radicals. Each value represents mean ± S.D., (n = 4). IC_{50} of tested sample EESPG and Ascorbic acid was found to be 230.3 ± 0.24 and 77.3 ± 0.42 µg/mL. EEUSPG was found to be very low active as compared to standard – Ascorbic acid and 50% inhibitory concentration was observed above dose limit.

This method is based on the formation of a coloured Cu (I) neocuproine chelate, which is then measured spectrophotometrically. The antioxidant activity of the EESPG was found to be 68.11% by CUPRAC assay and 74.62% DPPH assay at 300 µg/mL). The percentage antioxidant activity increased with increasing concentration, at 300 µg/mL, EESPG showed the maximum cupric ion reducing activity, whereas EEUSPG exhibited comparatively lower activity.

4.3 GC-MS data of EESPG

The retention times, peak areas, chemical names, molecular weights, and molecular formulas of the compounds are presented in **table 3** and **figure 5** while

figure 6 shows a typical GC chromatogram of EESPG. These compounds were identified through GC-MS analysis, where gas chromatography separated the bioactive compounds and mass spectrometry confirmed their structures. By linking each average peak range to the entire peak range, the relative percentage quantity of all the components was calculated. A total of 24 phytoconstituents were observed in EESPG whose molecular structures are given in figure 5. The observed phytocomponents found in EESPG are α-Furfuryl alcohol (1.39%), Cyclopent-4-ene-1,3-dione (0.47%), 3-(Methylthio)-Propanal (0.43%), α-Butyrolactone (0.46%), 2-Hydroxy-2-cyclopenten-1-one (0.48%), α-Methyl maleic anhydride (0.29%), n-Butyl 2-butenoate (0.43%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone (1.85%), Pentyl phenylacetate (1.86%), 2,5-Dimethyl-4-hydroxy- 3(2H)-furanone (0.64%), Maltol (0.87%), Pentanedioic acid, dimethyl ester (0.75%), 2-Acetyl-2-Hydroxy-γ-butyrolactone (1.92%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (21.46%), Isoamyl trimethylacetate (0.47%), Ethyl cyclopro3panecarboxylate (0.89%), 6-Ethyl-3,4-dimethylphenol (0.68%), p-Hydroxybenzaldehyde (2.68%), 4-Ethyl-2,6-dimethyl-4-heptanol (2.60%), Anhydro-D-mannosan (0.95%), 6-O-Methyl-α-D-glucopyranoside (47.71%), 2-(Hydroxymethyl)cyclohexanone (0.71%), 2-Methylenecyclopropanecarboxylic acid (0.73%), and 2,4-Dimethyl-3-heptanol (0.77%).

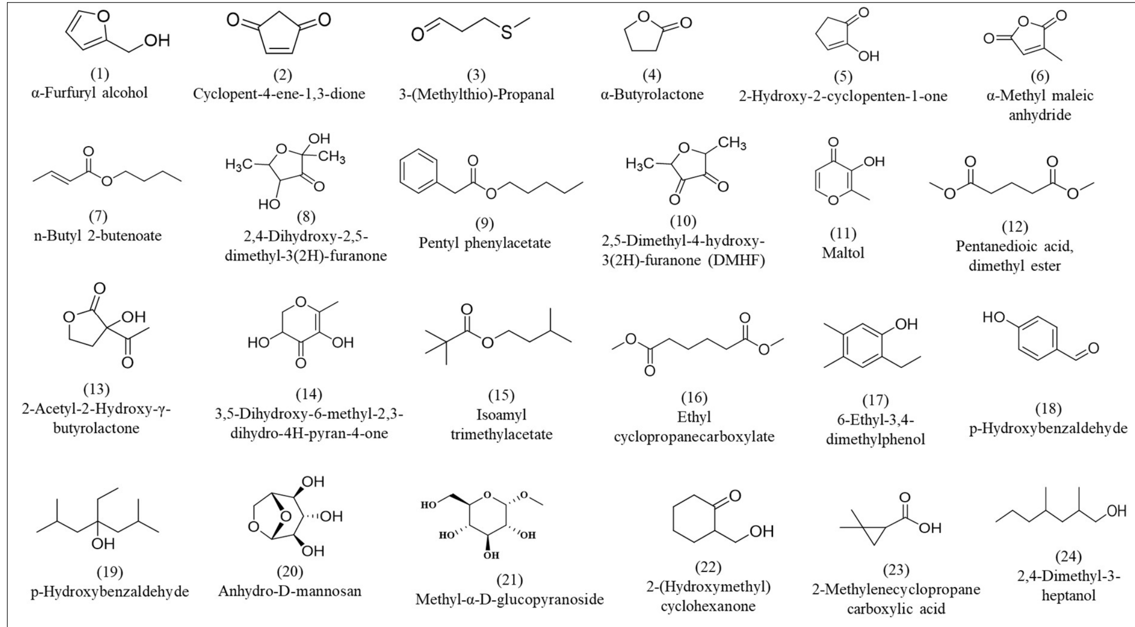

Fig. 5: Chemical compounds hit upon the GC-MS chromatogram of the ethanol extract of sprouted *Panicum glaucum* seeds.

Table 3. Bioactive compounds present in the ethanol extract of sprouted *Panicum glaucum* seeds using GC-MS analysis

Peak No.	R. Time	Area%	Molecular Formula	m/z (g/mol)	Compounds Name
1	4.489	1.39	$C_5H_6O_2$	98	α-Furfuryl alcohol
2	4.983	0.47	$C_5H_4O_2$	96	Cyclopent-4-ene-1,3-dione
3	5.272	0.43	C_4H_8OS	104	3-(Methylthio)-Propanal
4	5.475	0.46	$C_4H_6O_2$	86	α-Butyrolactone
5	5.705	0.48	$C_5H_6O_2$	98	2-Hydroxy-2-cyclopenten-1-one
6	6.014	0.29	$C_5H_4O_3$	112	α-Methyl maleic anhydride
7	6.523	0.43	$C_8H_{14}O_2$	142	n-Butyl 2-butenoate
8	6.686	1.85	$C_6H_8O_4$	144	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone
9	7.833	1.86	$C_{13}H_{18}O_2$	206	Pentyl phenylacetate
10	8.096	0.64	$C_6H_8O_3$	128	2,5-Dimethyl-4-hydroxy- 3(2H)-furanone (DMHF)
11	8.990	0.87	$C_6H_6O_3$	126	Maltol
12	9.237	0.75	$C_7H_{12}O_4$	160	Pentanedioic acid, dimethyl ester
13	9.435	1.92	$C_6H_8O_4$	144	2-Acetyl-2-Hydroxy-γ-butyrolactone
14	9.575	21.46	$C_6H_8O_4$	144	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one

					(DDMP)
15	10.423	0.47	C_5H_{12}	72	Isoamyl trimethylacetate
16	10.672	0.89	$C_6H_{10}O_2$	114	Ethyl cyclopropanecarboxylate
17	11.955	0.68	$C_{10}H_{14}O$	150	6-Ethyl-3,4-dimethylphenol
18	13.092	2.68	$C_7H_6O_2$	122	p-Hydroxybenzaldehyde
19	14.132	2.60	$C_{11}H_{24}O$	172	4-Ethyl-2,6-dimethyl-4-heptanol
20	14.663	0.95	$C_6H_{10}O_5$	162	Anhydro-D-mannosan
21	15.644	47.71	$C_7H_{14}O_6$	194	Methyl-α-D-Glucopyranoside
22	18.304	0.71	$C_7H_{12}O_2$	128	2-(Hydroxymethyl)cyclohexanone
23	18.750	0.73	$C_5H_6O_2$	98	2-Methylenecyclopropanecarboxylic acid
24	19.652	0.77	$C_9H_{20}O$	144	2,4-Dimethyl-3-heptanol

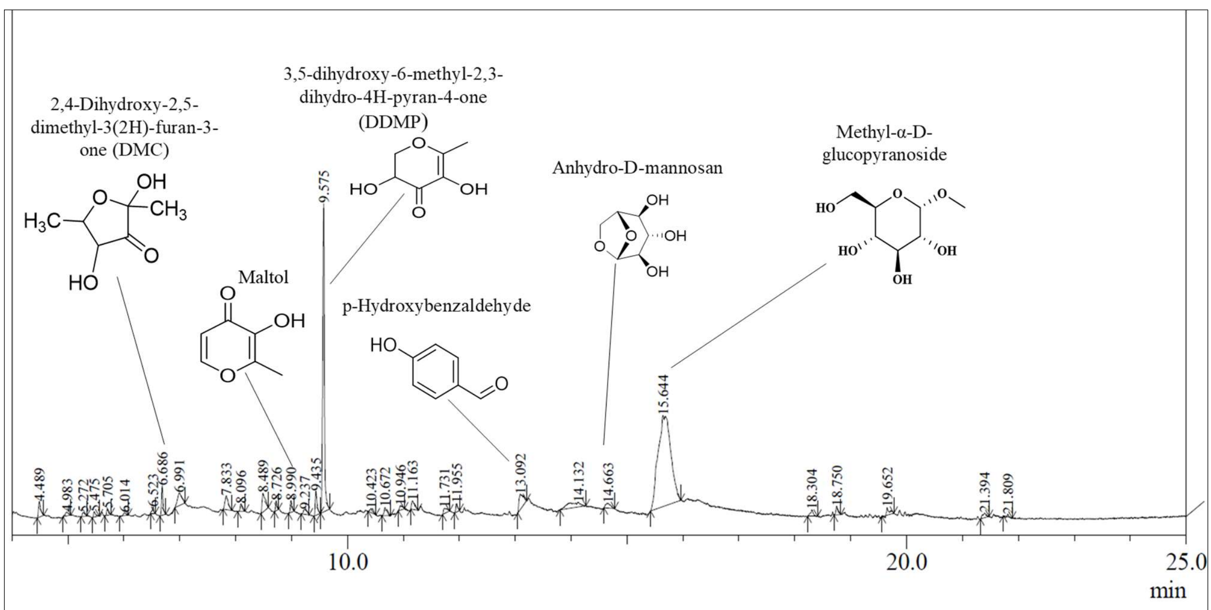

Fig. 6: GC-MS chromatogram of ethanol extract of sprouted *Panicum glaucum* seeds with probable marker compounds for hepatoprotective activity.

The hepatoprotective and antioxidant effects of the sprouted seeds consequence the presence of DMHF, Methyl-α-D-Glucopyranoside, Maltol, and DDMP in EESPG. DMHF also known as furaneol, is a naturally occurring aroma compound found in many fruits and foods. It has been shown to possess various biological properties, including antimicrobial and antioxidant activities, and may also have a hepatoprotective effect, though this requires further investigation. While, DMHF is primarily known for its flavor and aroma properties, studies suggest it may also have a protective effect on the liver. A study on mice supplemented with DMHF showed a slight decrease in TBARS in the liver, which are indicators of lipid peroxidation. Lipid peroxidation is a process that can damage liver cells, and a reduction in TBARS suggests a potential protective effect against this damage. Furthermore, DMHF has antioxidant properties, which can help neutralize harmful free radicals that can contribute to liver damage [35]. Maltol and DDMP found in some foods and maillard reaction products, have been shown to have hepatoprotective effects in studies. It exhibits protective activity against liver damage induced by substances like carbon tetrachloride and alcohol by reducing inflammation, suppressing apoptosis, and enhancing antioxidant enzyme activity. This protection is likely due to its potent antioxidant properties, which help combat oxidative stress and other damaging processes in the liver. Maltol's hepatoprotective effects are thought to stem from its ability to scavenge free radicals and reduce oxidative stress, a key contributor to liver damage. It also helps to suppress inflammation and prevent cell death (apoptosis). Research has shown maltol to be effective in animal models of liver injury, such as those induced by carbon tetrachloride or alcohol. In these models, maltol has been observed to reduce levels of liver enzymes like ALT and AST, indicators of liver damage, and to protect against histopathological changes like fatty degeneration and apoptosis. Maltol can help alleviate the harmful effects of oxidative stress on the liver by increasing the movement of antioxidant enzymes. Maltol may also play a role in mitigating liver fibrosis, a scarring process, and other types of liver injury. Primarily DDMP protects the liver by its strong antioxidant activity. It neutralizes free radicals, which is key contributor to liver damage. In

animal studies, DDMP has been shown to improve liver architecture and reduce signs of liver damage, such as necrosis and inflammation, according to a study. DDMP is a common product of the maillard reaction, a chemical reaction between amino acids and reducing sugars, and it is known to contribute to the antioxidant properties of maillard reaction products [36]. Methyl α-D-glucopyranoside, also known as methyl α-D-glucoside or α-Methyl-glucoside, is a derivative of glucose. Methyl α-D-Glucopyranoside is a compound used in the synthesis of DMHF, which is formed through the Maillard reaction by enolisation of sugars and subsequent cyclisation and dehydration steps [37]. Methyl α-D-glucopyranoside is like Methyl 6-O-cinnamoyl-α-D-glucopyranoside (MCGP) demonstrates hepatoprotective effects by alleviating liver injury and oxidative stress in various models. It reduces liver damage, including intrahepatic haemorrhage and nuclear pyknosis, and decrease liver index. Additionally, MCGP inhibits the increase in serum ALT/AST levels and reduces necrotic areas in the liver, suggesting its liver protective role [38]. The GC–MS analysis confirms that EESPG contains several biologically active compounds which may collectively contribute to its antioxidant and hepatoprotective effects.

4.4 Molecular docking study

In the literature it was found that molecular docking study was carried out to predict the binding interactions between the phytoconstituents like 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF), Maltol, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP), and Methyl α-D-glucopyranoside identified from the EESPG targeted protein associated with antioxidant and hepatoprotective activity **(Table 4).**

Table 4: Predictive molecular docking studies identified conceivable hepatoprotective compounds in the ethanol extract of twelve-day-sprouted *P. glaucum* grains (EESPG).

Conceivable hepatoprotective compounds	Mechanisms of action and predictive molecular docking
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	A study on mice supplemented with 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone and DMHF showed decrease in TBARS in the liver, which are indicators of lipid peroxidation. Lipid peroxidation is a process that can damage liver cells, and a reduction in TBARS suggests a potential protective effect against this damage. DMHF has antioxidant properties, which can help neutralize harmful free radicals that can contribute to liver damage [35]. Both 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone and DMHF exhibited strong binding affinity through hydrogen bonding and hydrophobic interactions with critical amino acid residues located within the active sites of antioxidant and hepatoprotective targets. The presence of hydroxyl functional groups in the compound may further enhance its free radical-scavenging capability, contributing to reduced lipid peroxidation and protection against oxidative damage. Therefore, the predictive docking results support that 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone may exert hepatoprotective effects by improving antioxidant defense systems and mitigating oxidative stress-induced liver injury.
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF)	
Maltol	Maltol and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP) showed hepatoprotective effects by scavenging the free radicals and reduce oxidative stress, and helps to suppress inflammation and prevent cell death [36]. The docking analysis of both compounds exhibited favourable binding affinities with key proteins involved in oxidative stress regulation and liver protection. Maltol showed stable interactions within the active site through hydrogen bonding and hydrophobic contacts, which may contribute to its ability to scavenge reactive oxygen species and reduce oxidative damage. The presence of hydroxyl and carbonyl functional groups in Maltol is likely to enhance its antioxidant capability and support the stabilization of target proteins associated with cellular defences mechanisms. Similarly, DDMP displayed strong binding interactions with critical amino acid residues at the active sites of antioxidant-related enzymes. The multiple hydroxyl groups in DDMP may facilitate hydrogen bond formation, improving ligand–protein stability and enhancing its free radical scavenging potentials.

3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP)	
Methyl α-D-glucopyranoside	Methyl α-D-glucopyranoside like Methyl 6-O-cinnamoyl-α-D-glucopyranoside (MCGP) demonstrates hepatoprotective effects by alleviating liver injury and oxidative stress in various models. It reduces liver damage, including intrahepatic hemorrhage and nuclear pyknosis, and decrease liver suggesting its liver protective role [38].

The tested compound DMHF exhibited a LibDock score of 66 and 60, in comparison to the reference standard ascorbic acid, which achieved the score of 77. Ascorbic acid formed strong stabilizing interactions, including conventional hydrogen bonds with ILE165 and PRO34 and a carbon–hydrogen bond with HIS163, along with van der Waals contacts involving GLY162, GLY166, ALA167, TRP179, LEU37, LEU159, ARG38, and TRP41, indicating a highly stable binding orientation **(Figure 7).** Although the binding energies of the phytoconstituents were slightly lower than that of the standard, the ligands established multiple hydrogen bonds, van der Waals, and hydrophobic interactions with the active site residues of 1OAF. Maltol showed stabilization mainly through van der Waals and a Pi–Alkyl contact with ALA556, whereas DDMP established a conventional hydrogen bond with VAL463 along with multiple hydrophobic and alkyl contacts (VAL604, VAL606, ILE559).

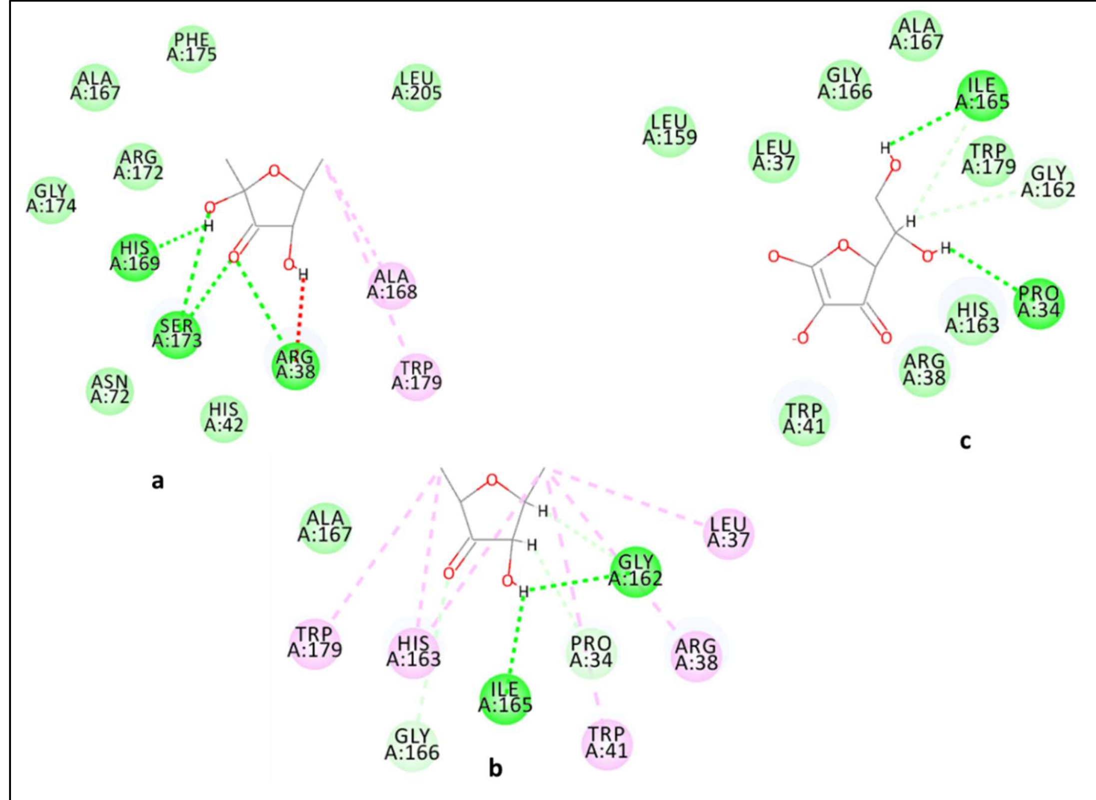

Fig. 7: 2D interaction diagrams of selected ligands with the antioxidant target protein 1OAF: **(a)** 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone, **(b)** 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF), and **(c)** Ascorbic acid as standard. The interactions illustrate hydrogen bonding, van der Waals forces, and hydrophobic contacts contributing to ligand stabilization within the active site.

These findings suggest that DDMP may exhibit a higher binding affinity and stronger potential to modulate Keap1–Nrf2 signalling compared to Maltol, thereby contributing more effectively to antioxidant defence. In addition, DDMP was docked with the anti-apoptotic protein Bcl-xL (PDB ID: 2YXJ, bound to an inhibitor), where it formed a conventional hydrogen bond with GLU124 and several van der Waals contacts with residues including TYR120, THR172, HIS177, ASP176, and TRP169, supported by alkyl and Pi–Alkyl interactions with VAL127, PHE123, and TYR173 **(Figure 7).**

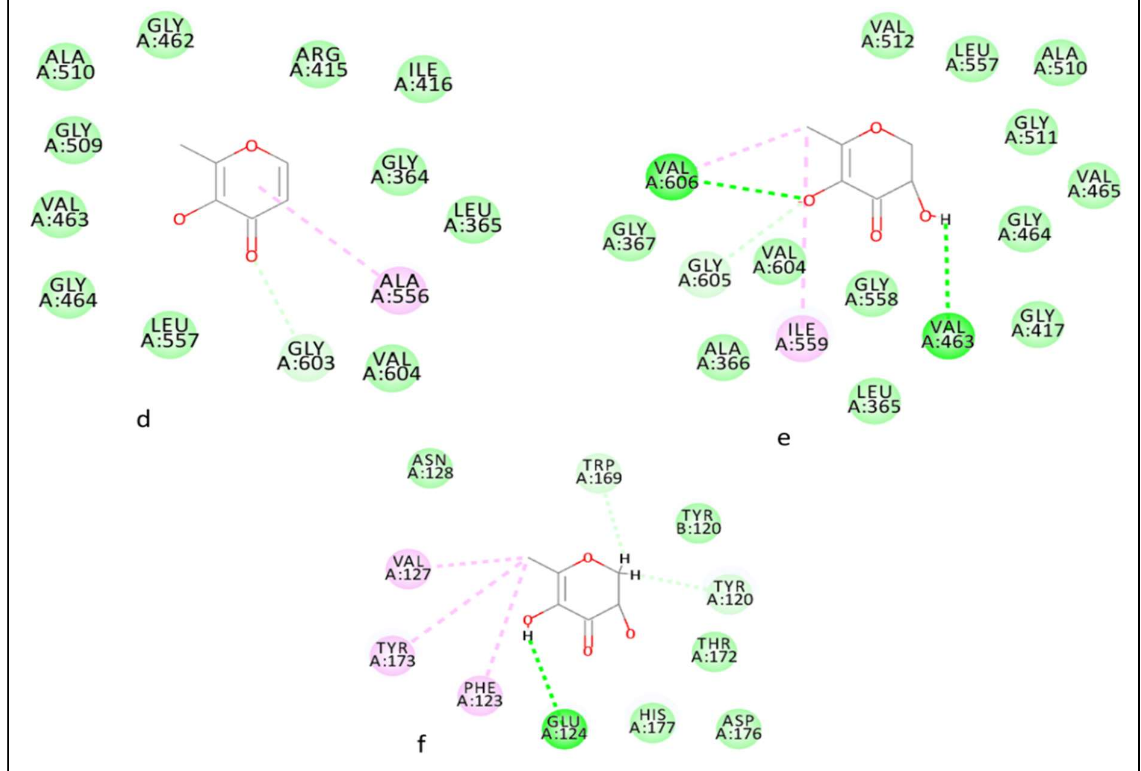

Fig.8: 2D interaction diagrams of the docked ligands: **(d)** Maltol with the Keap1 Kelch domain (PDB ID: 6TYM), **(e)** DDMP with the Keap1 Kelch domain (PDB ID: 6TYM), and **(f)** DDMP with the anti-apoptotic protein Bcl-xL (PDB ID: 2YXJ). The interactions highlight hydrogen bonding, hydrophobic, and van der Waals contacts that contribute to ligand stabilization within the respective protein binding pockets.

The selected ligands exhibited promising binding affinities toward 1OAF, while docking with the human Keap1 Kelch domain (PDB ID: 6TYM) showed that maltol and DDMP fit well within the active binding pocket.

5. CONCLUSION

The findings of this study indicate that sprouted *P. glaucum* grains possess promising antioxidant and hepatoprotective activities, which may be attributed to their enriched phytochemical composition developed during the sprouting process. The compounds maltol, DMHF, and DDMP identified by GC-MS in the EESPG have been reported to possess antioxidant and hepatoprotective effects. These compounds are like the standard compound (Trolox) which is the analog of vitamin E. The *in vitro* antioxidant assays confirmed the strong free-radical scavenging capacity of the EESPG, by improved the levels of endogenous antioxidant markers such as GSH and SOD, along with reduced lipid peroxidation indicated by lowering of MDA levels. The hepatoprotective potential was supported by biochemical parameters, including the restoration of ALP levels and reduced cytotoxicity in HepG2 cells as assessed through the LDH release assay. In addition, *in silico* molecular docking studies demonstrated favourable binding interactions between the identified phytocompounds and target proteins, suggesting their potential involvement in the modulation of key biological pathways. Collectively, the integrated *in vitro* and *in silico* findings indicate that sprouted *P. glaucum* grains may serve as a promising natural therapeutic candidate for the management of liver disorders. However, further *in vivo* studies and clinical investigations are required to validate these findings and to explore the precise mechanisms of hepatoprotective effects of EESPG for social protection of people with jaundice and other liver diseases.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception methodology design, editing, visualization, validation, and original draft preparation. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

9. ETHICS STATEMENT

The study did not involve animal treatment.

10. DATA AVAILABILITY STATEMENT

The data included in this article are not stored in public databases. However, the corresponding author can be contacted to provide access upon reasonable request.

11. PUBLISHER'S NOTE

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12. DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The study did not involve generative AI and AI-assisted technologies

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