INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2026, Vol. 5, NO. 1, 43-53
doi: https://doi.org/10.56612/ijaaeb.v5i2.173
ARK: https://n2t.net/ark:/71142/IJAaEB.v5i2.173
https://eminentscientists.com/

Cytoprotective role of *p*-cymene against alcohol-induced toxicity in human embryonic kidney cells

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Abstract

p-Cymene (p-isopropyl toluene) is a monocyclic monoterpene and is the main compound of many aromatic plant species. *p*-cymene also exhibits various pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antiviral, and antitumor activities. *p*-cymene also acts as a strong anti-inflammatory and antioxidant agent. Alcohol use is widespread in the Western world and is found to be nephrotoxic, and can cause kidney injury, especially during pregnancy and kidney development during intrauterine life. Alcohol can lead to reduced levels of antioxidants and increased synthesis of pro-inflammatory markers. This study aimed to investigate the cytoprotective role of *p*-cymene against alcohol-induced toxicity in human embryonic kidney (HEK293) cells. In this study, we developed four groups: a negative control group, a positive control group, an injury group, and an injury plus treatment group. Injury was induced by ethanol, and then different concentrations of *p*-cymene, i.e., 50 µM, 100 µM, and 500 µM, were added to check the cytoprotective action of p-cymene. The MTT assay and oxidative stress marker (glutathione reductase) were assessed to check cell viability. The KIM-1 and 8-OHdG ELISA were done to evaluate the apoptotic pathways. The results concluded that the 500 µM treatment increased cell viability more than the other levels. According to the MTT Assay, Crystal Violet Assay, and Trypan Blue Assay, *p*-cymene increased cell viability in ethanol-injured cells. KIM-1 ELISA showed lower levels of kidney injury molecule-1 in *p*-cymene-treated cells. While 8-OHdG ELISA showed that 8-hydroxy guanosine levels were also reduced in the cells treated with *p*-cymene, in contrast to cells left untreated. In the Antioxidant assay, a GSH ELISA was performed, and the glutathione value increased in treated cells. In this research, it has been observed that *p*-cymene has a cytoprotective role against alcohol-induced toxicity in human embryonic kidney cells.

ARTICLE TYPE
Research Paper (RP)

SECTION
Human Biology (HB)

HANDLING EDITOR
Fernando, S. (HB)

ARTICLE HISTORY
Received: 25 Jun, 2025
Accepted: 12 Sep, 2025
Online: 15 Oct, 2025
Published: 01 Jan, 2026

KEYWORDS
Antioxidants; Cell viability; Cytoprotective; HEK293: Monocyclic monoterpene

Introduction

Natural products are compounds or substances that originate from living organisms. They are abundant on Earth and are produced by marine and terrestrial plants, bacteria, fungi, insects, animals, and mammals. Human beings have always looked upon natural resources and their products to

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CITATION (APA): Akhtar, S., Afnan, H., Shahnawaz, S., Rafiq, M., Azhar, M.M., Maqbool, T. (2026). Cytoprotective role of p-cymene against alcohol-induced toxicity in human embryonic kidney cells. *International Journal of Applied and Experimental Biology*, 1(1):43-53 https://doi.org/10.56612/ijaaeb.v5i2.173

meet their daily needs, which include food, housing, clothing, communication, and transportation (Rahman et al*.,* 2021). Meanwhile, natural products are vigorously used in different cultures in different treatment of many diseases (Ozkur et al., 2022; Shenvi, 2024). Natural products have been the main source of drugs for many centuries, and almost half of the pharmaceuticals are made from natural products (Chaachouay and Zidane, 2024), and many natural compounds are being used in the treatment of diseases like cancer, autoimmune diseases, infectious diseases, etc. (Shenvi, 2024). Natural products are divided into primary metabolites and secondary metabolites, two different classes (Salam et al., 2023). Primary metabolites have an intrinsic function and are necessary for survival, while secondary metabolites play an external role and are not required for existence, but they increase the quality of life (Salam et al., 2023). Carbohydrates, lipids, amino acids, and nucleic acids are primary metabolites. Secondary metabolites include alkaloids, terpenoids, phenylpropanoids, and polyketides (Kumar et al*.,* 2022; Al-Khyari et al., 2023).

Among all the natural compounds, terpenoids are the most common and diverse natural products, which are found mainly in many plants (Masyita et al*.,* 2022; Li et al., 2023). They can be extracted and isolated from plants and obtained through metabolic engineering, synthetic biology, and biotransformation. Terpenoids are widely used in pharmaceuticals due to their pharmacological properties. They can be grouped into classes according to the number of isoprene units (C5H8) in the molecule. Terpenes are grouped as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes depending on the carbon unit present in the molecule that ranges from C=5,10,15,20….., $n > 40$ (Xavier et al., 2023). Most of the terpenoids are biologically active and are used as anticancer, anti-inflammatory, antibacterial, antiviral, and antimalarial drugs. Terpenoids are widely used in the industry of food, drugs, flavors, fragrances, cosmetics, hormones, vitamins, etc. They also exhibit properties like immunoregulation, antioxidation, antiaging, neuroprotection, and geroprotection. Several terpenes have a protective effect against oxidative stress induced by heavy metals (de Lima et al., 2025).

p-Cymene is a monocyclic monoterpene and is a major compound of many aromatic plant types, including the kinds of *Artemisia* (Asteraceae), *Protium* (Burseraceae), *Origanum*, *Ocimum*, *Thymus*, and *Eucalyptus*. Many foods, such as cinnamon, carrots, orange juice, grapefruit, tangerine, raspberries, nutmeg, butter, and several spices, have *p*-cymene as a naturally occurring ingredient. *p*-cymene is known as an alkylbenzene that is related to the monoterpene class. In its structure, it contains a benzene ring that is attached to an isopropyl group and a methyl group (Balahbib et al*.,* 2021). Contingent on the geometric substitution, *p*-cymene exists in two isomer forms: *m*-cymene and *o*-cymene (Shafodino et al., 2022). *p*-cymene is not soluble in water, but is miscible with organic solvents.

p-Cymene is considered a significant manufacturing compound that is utilized in the production of fungicides, pesticides, perfumes, and fragrances (Balahbib et al., 2021), and the synthesis of precursors of standard antioxidants, such as *p*-cresol. During the third week, there is a beginning of morphogenesis. Endoderm, mesoderm, and ectoderm are the three germ layers (Baginska et al*.,* 2023).

Type 1 transmembrane glycoprotein, also known as kidney injury molecule (KIM-1), that contains mucin-like domains and immunoglobulin in the extracellular part and a small domain having tyrosine phosphorylation signalling motif in its intracellular part. KIM-1 is not present normally in the proximal tubule of the nephron (Karmakova et al*.,* 2021). The extracellular portion of KIM-1 is released into the tubule after an injury to the kidney and is then detected in the urine of the patient. Any pathological state leads to the undifferentiation of epithelial cells, which leads to KIM-1 mRNA synthesis, followed by the synthesis of KIM-1, which accumulates on injured proximal tubule cells' apical membrane. The KIM-1 molecule acts as a receptor on epithelial cells and converts proximal tubule cells into phagocytes. Urinary KIM-1 levels are raised in ARF and CRF and are considered an important biomarker representing kidney injury. KIM-1 is also found in the ATN patients' urine. and acts as an important biomarker in early diagnosis of the disease (Brilland et al., 2023). A sensitive urine biomarker for all renal injury-related disorders, including nephrotoxic injury, kidney injury brought on by heart surgery, kidney injury associated with transplant rejection, and chronic kidney injury, is KIM-1 (Karmakova et al., 2021).

The objective of this study was to find out the cytoprotective role of *p*-cymene in human embryonic kidney cells against the injury caused by alcohol in these cells.

Materials and Methods

Study design

The study was separated into four groups: 1. Negative control; 2. Positive control (silymarin); 3. Injury group; 4. Injury + treatment (*p*-cymene) group.

Study site and setting

The study was done at CRiMM and IMBB, The University of Lahore, Pakistan. HEK293 cells were chosen for this study.

Experimental design

Groups	Treatment
Negative control	No treatment
Positive control	Silymarin
Injury group	Ethanol
Injury + treatment group	*p*-cymene

Culturing of the HEK293 cell line

The cells were thawed before being cultured and sub-cultured. Dulbecco's Modified Eagle's Medium (DMEM) (high glucose), added with streptomycin and penicillin as well as 10% fetal bovine serum (FBS), was grown in T75 flasks at 37 °C in a humidified incubator with 5% CO_2. Every two to three days, the medium was changed. For treatment, DMEM was used, but without FBS. After culturing, when cells reached 70 to 80% confluency, their subculturing was done in a 96-well plate, and after 90% to 100% confluency, treatment was applied as described elsewhere (Maqbool et al., 2019).

Treatment of the cell line with *p*-cymene

The HEK293 cell line was cultured in a 96-well plate for checking cell viability and a 6-well plate for wound healing. We divided the cells into three groups: one group was controlled, the second was injured with ethanol, and the third was the treated one, in which, after injury with ethanol, *p*-cymene was added. After treatment, the cells were incubated for 24 hours. The post-treated cells were then analyzed by the MTT assay, crystal violet assay, and trypan blue assay. The cultured medium that was collected from the post-treated cells, also called secretome, was used for ELISA of KIM-1 and 8-OHdG. The cells treated in the 6-well plate were used for wound healing analysis, and the secretome from the post-treated cells was also used for the GSH assay.

MTT assay

Cell viability was analyzed through the MTT assay technique. It involves the conversion of MTT into formazan crystals by live cells, which measures the activity of mitochondria. Since the activity of mitochondria is generally proportional to the number of live cells, the MTT assay is widely utilized to evaluate the toxic effects of drugs on primary patient cells or cell lines *in vitro*. Each experimental group was tested three times. For assessing the *p*-cymene effect on the HEK293 cells, an MTT assay was conducted in a 96-well plate. The cells were cleaned with phosphate-buffered saline (PBS), then cultured for two hours in 100 µL of serum-free DMEM and 25 µL of MTT solution (5 mg/mL). After the formation of purple formazan crystals, 10% sodium dodecyl sulfate (SDS) or dimethyl sulfoxide (DMSO) was used to dissolve them, and absorbance was measured at 570 nm. The percentage of cell viability was read by using a previously described method (Ahmad et al., 2025; Nayila et al., 2024).

Crystal violet assay

This procedure was carried out in a 96-well plate. The medium from various experimental groups was removed from the wells, and the wells were washed with PBS. After washing, 0.1% crystal violet dye and 1.5% ethanol were added to cover the exposed surfaces of the wells. The plate was incubated for 15 minutes. The wells were then thoroughly cleaned, and the dye was carefully disposed of to prevent the cells from detaching. Next, 100 µL of 1% SDS was added to each well to solubilize the dye for 10 minutes. Lastly, absorbance was measured at 540 nm, using a microtiter plate reader. Crystal violet staining was used to determine cell viability, following the methodology described elsewhere (Kalsoom et al., 2024; Azhar et al., 2025).

Trypan blue assay

Trypan blue was used to measure cell viability and distinguish between living and dead cells. Trypan blue (Invitrogen Inc., USA) was added to the cells from the different experimental groups, and the cells were cultured for 15 minutes after being cleaned three times with PBS. Following incubation, the cells were examined under a microscope and cleaned three more times using PBS. The trypan blue-stained cells were regarded as dead. The trypan blue assay was conducted as reported

elsewhere (Maqbool et al., 2019).

Wound healing

The wound-healing assay was carried out to check and measure whether the wound heals. Using a pipette tip, a straight scratch was produced once the cell confluency reached 80% in each well of a 96-well plate after the cells had been grown in each well for 24 hours at 37 °C. Except for the control, the medium was removed, new medium was added, and then removed once more to remove the detached cells. The cells were photographed using an inverted microscope as soon as the treatment was completed (0 hours). Images were taken and compared to the same wound region after a 72-hour incubation period at 37 °C.

Oxidative stress assay by 8-OHdG ELISA

A competitive multispecies ELISA kit was employed to quantitatively assess the levels of 8-hydroxydeoxyguanosine (8-OHdG). A standard provided with the kit was used to construct a calibration curve, against which all sample readings were evaluated. The HEK 293 cells from the control, ethanol-damaged, and treatment groups were added to a microtiter plate pre-coated with the relevant antibody. A biotinylated detection antibody specific for 8-OHdG was then introduced into each well. After removing unbound components through washing, Avidin conjugated with horseradish peroxidase (HRP) was added and allowed to incubate. Subsequently, a TMB substrate solution was applied to initiate the enzymatic colorimetric reaction, which was terminated using a stop solution. The resulting color intensity was read using a microplate reader, and 8-OHdG concentrations were calculated by comparing optical density (OD) values of the samples to the standard curve.

Injury reversal potential analysis by KIM-1 ELISA

A Sandwich-ELISA was performed to evaluate the KIM-1 expression levels in the control, ethanol-injured, and *p*-cymene-treated HEK 293 cells. The assay began with a micro-ELISA plate pre-coated with a monoclonal antibody specific for human KIM-1. The sample solutions were added, allowing the target antigen to bind to the immobilized capture antibody. A biotinylated detection antibody specific to human KIM-1 was then applied, followed by the introduction of Avidin conjugated to horseradish peroxidase (HRP). The plate was incubated to facilitate antigen-antibody interactions, after which excess reagents were removed via washing steps. A chromogenic substrate solution was subsequently added to initiate a colorimetric reaction. Wells containing the full antigen-antibody-HRP complex developed a blue color, which turned yellow upon addition of the stop solution. Absorbance was measured at 450 ± 2 nm using a microplate reader. The optical density (OD) values corresponded directly to KIM-1 concentrations in the samples, which were quantified by referencing a standard calibration curve.

Evaluation of antioxidant enzymes

Glutathione reductase (GSH) assay

Glutathione reductase (GSH) was performed according to Shamim and Rehman (2015). A 96-well plate contained a reaction combination of 200 µL in each well. A reaction mixture was prepared by mixing 20 mM KH_2PO_4 buffer (PH 7.5), 40 mM EDTA, and 10 mM oxidized glutathione. The secretomes acquired through various experimental groups of post-treatment of the HEK293 cell lines were added to the reaction mixture. Finally, 20 mM NADPH was added, and absorbance was taken using a spectrophotometer at 340 nm.

Statistical analysis

The statistical analysis was carried out using GraphPad Prism. $P < 0.05$ was deemed statistically significant in the one-way analysis of variance (ANOVA) performed on the data, which included a Bonferroni comparison among groups (**Table 1**).

Results

MTT assay

The MTT assay was used to evaluate the cytoprotective effects of *p*-cymene treatment in the HEK293 cells. The assay revealed that *p*-cymene-treated cells after injury showed a decrease in tetrazolium and an increase in formazan dye, indicating that cell viability had improved (**Figure 1**).

Cell adhesion assay by crystal violet staining

Furthermore, cell viability was estimated using crystal violet staining of the HEK 293 cells. According to our demonstration, the treated HEK 293 cells with *p*-cymene showed more live cells as compared to ethanol-injured cells (**Figure 2**).

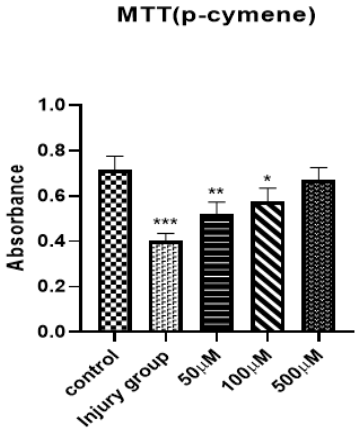

Figure 1: Cell viability assessed by MTT assay in different treatment groups. The level (500 µM) showed a significant difference in cell viability compared to the injury group.

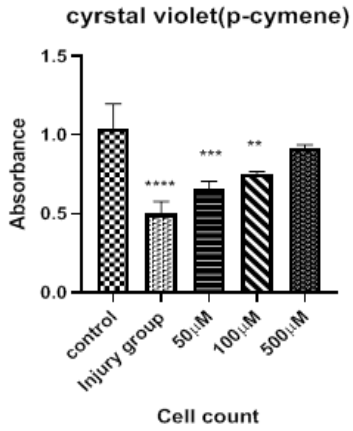

Figure 2: Cell viability assessed by the crystal violet assay in different treatment groups. The level 500 µM showed a significant difference in cell viability compared to the injury group.

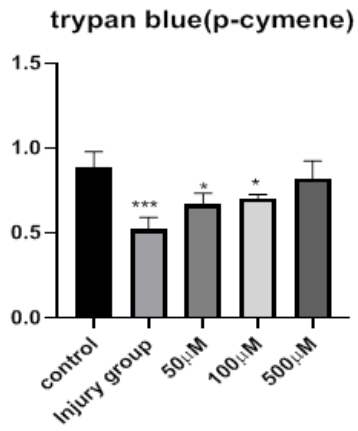

Figure 3: Effects of *p*-cymene on the primary adhesion of the HEK293 cells after 24 hours of treatment

Mean ± SD. (*P* < 0.05, where * shows the significant difference between normal and treated groups at 0.001 percent probability).**

Trypan blue assay

The trypan blue dye selectively binds to floating cells, allowing both living and dead cells to be counted. The treated HEK293 cells were stained with trypan blue. When the HEK293 cells were treated, a disproportionately lower number of blue cells were found, whereas higher blue cells were seen in ethanol-injured cells, indicating fewer dead cells as compared to the injured cells (**Figure 3**).

Determination of KIM-1 molecule expression through ELISA

The treated HEK 293 cells with *p*-cymene showed different means ±SEM. After treating the ethanol-injured HEK 293 cells with *p*-cymene, decreased expression of KIM-1(kidney injury molecule) was observed using ELISA. According to our results, the cell treatment with *p*-cymene reduced the expression of KIM-1 after treatment, as shown in **Figure 4**.

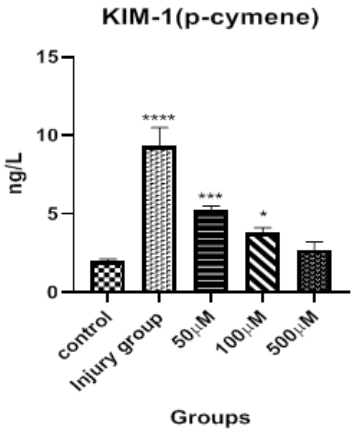

Figure 4: Decreased expression of KIM-1 showing *p*-cymene has cytoprotective potential.

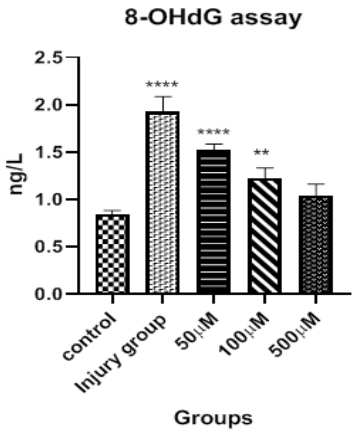

Figure 5: Percentage analysis of 8-OHdG by different groups.

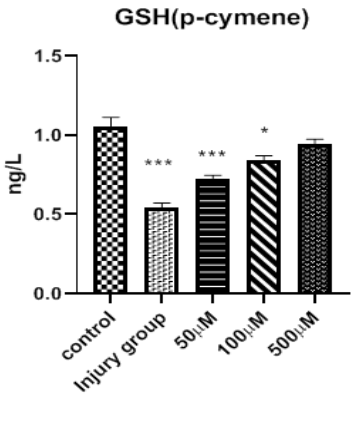

Figure 6: Percentage analysis of GSH Activity by different groups.

Mean ± SD. (*P* < 0.05, where * shows the significant difference between normal and treated groups at 0.001 percent probability).**

Oxidative stress assay via 8-OHdG ELISA assay

After treating the ethanol-injured HEK293 cells with *p*-cymene, the medium collected from all treatments was analyzed for the 8-OHdG activity separately. The results showed decreased oxidative stress after treatment of the HEK293 cells with *p*-cymene (**Figure 5**).

GSH assay through ELISA

The cellular secretome from the HEK293 cells that had been treated and collected was examined independently for the GSH activity, and the results showed that the treated cells at 500 µM concentration expressed a rise in the GSH levels as compared to the untreated group (**Figure 6**).

Scratch assay

The Scratch assay (**Figure 7**) revealed decreased proliferation of the ethanol-injured HEK293 cells as compared to the control cells. The 500 µM-treated group showed better proliferation as compared to the injured group.

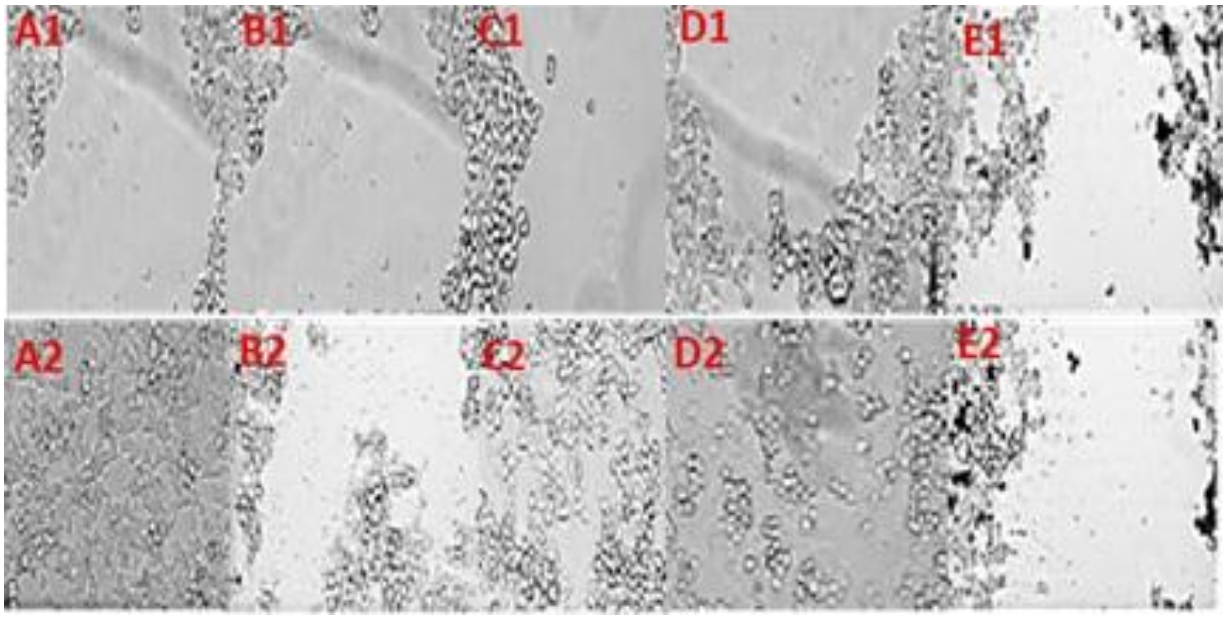

Figure 7: Representative images from the scratch assay demonstrating cell migration across different groups at 0 and 24 hours. The control group shows untreated cells with normal wound closure over time. B1, C1, and D1 represent the treatment groups at 0 hours with low, medium, and high doses, while B2, C2, and D2 show the same treatment groups after 24 hours, respectively; at higher doses, the best migration was observed.

Discussion

Pregnancy-related alcohol consumption is a modifiable health behavior that puts the unborn child at risk for several conditions, such as fetal alcohol spectrum disease, stillbirth, and stunted growth (Reyes et al., 2022; Popova et al., 2023). Various factors contribute to alcohol consumption during pregnancy among women. These include a lack of awareness regarding its harmful effects on the fetus, challenges in coping with stressful life events, reliance on personal instincts or peer influence, misconceptions about alcohol's health benefits, guidance from healthcare professionals, unplanned or unwanted pregnancies, alcohol dependency, and cultural or traditional norms that promote drinking (Ujhelyi Gomez et al., 2022).

According to research, renal function is decreased, and renal tubular reabsorption is also decreased with long-term ethanol use (Das Kumar and Vasudevan, 2008). Cholesterol-induced modifications in membrane composition and lipid peroxidation may be linked to various functional problems of renal tubules (Song et al., 2021). The long-chain polyunsaturated fatty acid concentration in the kidney has been linked in part to the organ's susceptibility to oxidative injury (Duailibe et al., 2024). The origin of functional problems may be influenced by renal ultrastructural abnormalities resulting from exposure to ethanol. A significant component of atherogenesis in chronic renal failure is increased oxidative stress and endothelial dysfunction, both of which have intricate interactions. Polyphenols in particular, being antioxidants, are predicted to lessen the kidneys' susceptibility to

oxidative stressors (Wojtaszek et al*.,* 2021).

In the developmental origin of kidney disease, oxidative stress is a major factor. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), in particular, are pro-oxidant molecules (Jomova et al., 2023). Oxidative stress is characterized as an imbalance in favor of one molecule over antioxidant defenses. Throughout pregnancy, ROS have two roles. While excessive production of ROS harms pregnancy and fetal outcomes, moderate amounts of ROS aid in normal organogenesis and cell differentiation (Grzeszczak et al., 2023). The bioavailability of nitric oxide (NO), a critical modulator of maternal and fetal homeostasis during gestation, is also decreased by excessive ROS generation that causes oxidative stress. Recent research suggests that the developmental origins of kidney disease may be related to the imbalance between ROS and NO (Tirichen et al., 2021).

The primary ingredients in extracts and essential oils of different plants are monoterpenes, which include borneol, bornyl acetate, camphor, carvacrol, 1-methyl-4-(1-methylethyl)-benzene (*p*-cymene), eucalyptol, γ-terpinene, and thymol (Marchese et al., 2017). Thyme (*Thymus vulgaris*) and origanum (*Origanum vulgare*) are only two examples of the over 100 plant species that contain the monoterpene *p*-cymene (Kosakowska et al., 2024), which is utilized in both cooking and medicine. Anxiolytic, anti-inflammatory, antioxidant, anticancer, and antibacterial properties are just a few of the biological actions exhibited by *p*-cymene and its derivatives, carvacrol and thymol (Peter et al., 2024).

The production of linoleyl hydroperoxides is the result of linoleyl peroxyl radicals (LOO•) aggravating linoleic acid peroxidation, a process that is significantly inhibited by γ-terpinene (Valgimigli, 2023). The *p*-cymene is the main organic product of this peroxidation process, which is inhibited by the presence of γ-terpinene. The rapid interaction between lipid peroxyl radicals (LOO•) and hydroperoxyl radicals (HOO•) facilitates swift chain termination, which underlies the observed reduction in oxidative stress (Ayala et al., 2014). The antioxidant potential of *Thymus pubescens* essential oil was evaluated by Nickavar and Malekitabar (2022), revealing it has strong antioxidant activity. The oil's primary constituents, γ-terpinene (7.46%) and *p*-cymene (5.54%), were shown to exhibit dose-dependent antioxidant effects *in vitro*. The oil demonstrated notable free radical scavenging ability, with IC50 values of 0.45 (0.33–0.62) µg/mL in β-carotene bleaching assays and comparable efficacy in ABTS assays, indicating its capability to counteract free radicals and prevent lipid peroxidation.

In our study, we investigated the role of *p*-cymene as a cytoprotective agent against the injury caused by ethanol in the HEK-293 cells. When ethanol was added to the cells, they showed less viability, less proliferation, more oxidative stress, lower antioxidant levels, and more injury markers. In addition to the roles of the flavoenzymes xanthine oxidase and aldehyde oxidase, the study emphasized the involvement of ethanol-inducible cytochrome P450 enzymes within microsomes. It was also shown that ethanol exposure can influence mitochondrial free radical generation and disrupt iron metabolism, highlighting iron's role as a pro-oxidant factor in these processes (Contreras-Zentella et al., 2022). Accordingly, it has been demonstrated that isolated microsomes produce more reactive oxygen species when they consume alcohol over time.

Antioxidant compounds found in the essential oils include monoterpenes like *p*-cymene and carvacrol (Marchese et al., 2017). Carvacrol and the essential oil of oolites both have strong antiradical properties. The antioxidant activity of *p*-cymene was found to be very poor at doses ranging from 5 to 25 µg/mL. In the DPPH experiment, *p*-cymene was employed at a low concentration. These findings showed that *p*-cymene concentrations above a certain level might exhibit free radical scavenging properties (Guo et al., 2021).

To better understand how thyme essential oils protect gene expression *in vivo* from oxidative stress generated by nanoparticles, a study was conducted on their antioxidant properties. The data showed that 17 chemicals in all were found in TEO by GC-MS. Thymol, carvacrol, γ-terpinene, *p*-cymene, terpinolene, (E)-caryophyllene, myrcene, *α*-phellandrene, *α*-phellandrene, and *α*-terpinene comprise 125.9 mg/g of oil. The administration of TiO_2-NPs was found to cause oxidative damage in the liver and kidney, as evidenced by changes in the lipid profile, pro-inflammatory cytokines, oxidant/antioxidant balance, serum biochemistry, and their gene expression, as well as histological changes in the liver tissues (Sallam et al., 2023). These *in vivo* results confirm the findings. Additionally, administering TiO_2-NPs and TEO together offers protection against these changes and functions as a strong antioxidant (Fletes-Vargas et al., 2025). The *p*-cymene also showed good antioxidant activity by reducing KIM-1 and 8-OHdG levels in the HEK-293 cells.

Glutathione (GSH) synthesis and antioxidant defenses may be impacted by the rise in superoxide dismutase (SOD) levels brought on by *p*-cymene. Through lowering superoxide levels, research has demonstrated that SOD is essential for oxidative stress protection. Furthermore, *p*-cymene has

been discovered to possess antioxidant qualities, diminishing lipid peroxidation and nitrite concentration in the mouse hippocampal tissues (de Oliveira et al., 2015). But *p*-cymene exposure has also been associated with the downregulation of antioxidant genes, such as paraoxonase 1 (PON-1) (Wu et al., 2020), which is well-known for its antioxidant properties. Although *p*-cymene possesses antioxidant qualities of its own, its effect on SOD levels may also have an indirect effect on GSH synthesis and general antioxidant defenses, which may have an impact on the equilibrium between antioxidant protection and oxidative stress in biological systems.

Furthermore, *p*-cymene has been shown in a study on colorectal cancer linked to high-fat diets to lower oxidative stress by reducing IL-1 expression and raising IL-6 levels, which in turn stimulates the synthesis of GSH (Jin et al., 2021). These results emphasize the potential of *p*-cymene as a naturally occurring substance with antioxidant qualities by indicating that it may enhance GSH levels in cells through its capacity to influence inflammatory factors and encourage the establishment of advantageous gut microorganisms. Increased GSH levels are associated with reduced oxidative stress in cells. Our study also emphasized these findings that GSH levels are increased and ROS levels are reduced in the cells after treatment with *p*-cymene (Balahbib et al., 2021).

When combined, thymoquinone and curcumin shield the HEK-293 cells against renal damage caused by cisplatin. According to *in vitro* investigations, the group treated with cisplatin had a lower glomerular filtration rate and higher serum levels (Alqahtani, 2024). The expression of KIM-1 was induced by cisplatin. As a result of attenuating KIM-1 and NFκB and upregulating survival signals like Akt and Nrf2/HO-1, Tq + Cur, in summary, demonstrated protective effects against cisplatin-induced nephrotoxicity and renal damage (Jin et al., 2021; Rashid, 2025). This showed that when oxidative stress is induced in HEK-293 cells, the KIM-1 levels are increased, showing increased oxidative stress.

Origanum vulgare is an essential oil whose major component is *p*-cymene. Its anti-inflammatory and antioxidant activity was studied in a human keratinocyte cell model (Avola et al., 2020). *In vitro* keratinocytes are stimulated by histamine and interferon-γ, which results in inflammation. The activation of histamine and interferon-γ results in elevated levels of COX-2, ROS, ICAM-1, and iNOS. The essential oil of *Origanum vulgare* can decrease and/or modify ROS, ICAM-1, iNOS, COX-2, 8-OHdG, MMP-1, and MMP-12 (Pavarino et al., 2023).

Antioxidative enzymes such as APOX, CAT, SOD, and GSH have the effect of increasing cell proliferation in the injured cells. They can also improve therapy efficacy by lowering levels of reactive oxygen species (ROS) (Hatami et al., 2024). Our study has revealed that applying *p*-cymene to the ethanol-injured HEK 293 cells showed the activities of the antioxidants SOD and GSH, which in turn led to a decrease in oxidative stress levels. As part of the ongoing investigation, an examination of the antioxidant and cytoprotective qualities of *p*-cymene has been carried out, yielding encouraging results with regard to increasing the proliferation of treated HEK cells. According to our findings, treating the ethanol-injured HEK cells with *p*-cymene increases the synthesis of antioxidants, reduces oxidative lesions such as 8-OHdG, and also lowers injury markers like Kidney Injury Molecule (KIM-1), as already reported earlier (Rosas et al., 2021).

Conclusion

In conclusion, *p*-cymene at 500 µM has been the most effective in injury reversal in the HEK-293 cells by increasing cell viability, antioxidant levels, cell proliferation, and decreasing the oxidative stress and kidney injury molecules in the cells. There is potential for using *p*-cymene as a cytoprotective agent against the injury caused by alcohol in the HEK-293 cells.

Author(s), Editor(s) and Publisher's declarations

Acknowledgement
None declared.

Source of funding
None declared.

Contribution of authors
Conceptualized and designed the study: HA, SA, MMA, TM. Conducted research and recorded data: HA, SA, MMA, TM. Wrote up the first draft of the manuscript: SA, HA, SS, MR, MMA, TM. Reviewed and edited the manuscript: SA, HA, SS, MR, MMA, TM.

Permissions and ethical compliance
This study is an *in vitro* investigation conducted on cultured cell lines. Therefore, it does not directly involve any human or animal subjects, and an Institutional Ethical Review Board (IERB) approval was not required for this research.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination.

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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