

(RESEARCH ARTICLE)



THE CYTOPROTECTIVE AND ANTIOXIDANT EFFECTS OF ATRIPLEX HALIMUS EXTRACT AGAINST H₂O₂-INDUCED OXIDATIVE STRESS IN HEPG2 CELLS

Maher Rasheed Salman *

Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences- College of Pharmacy, Iraq.

* Corresponding Author

ORCID Details

Maher Rasheed Salman: <https://orcid.org/000-0002-4216-3639>

GSC Biological and Pharmaceutical Sciences, 2026, 36(03), 098–105

Publication history: Received on 02 August 2026; revised on 10 September 2026; accepted on 13 September 2026

Article DOI: <https://doi.org/10.30574/gscbps.2026.36.3.0318>

Copyright © 2026 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0

ABSTRACT

Oxidative stress is one of the major causes of cell damage due to an increase in oxidative stress, loss of antioxidants in the cells, and activation of lipid peroxidation. *Atriplex halimus* L. is a type of salty medicinal plant that is rich in bioactive substances, which may contain antioxidant properties. In this study, the effect of the alcoholic extract against hydrogen peroxide (H₂O₂)-induced oxidative stress in HepG2 cells was investigated. HepG2 cells were treated with hydrogen peroxide and subsequently exposed to various concentrations of the extract (25, 50, 100, and 200 µg/mL). Cell viability was assessed using the MTT assay, while markers of oxidative stress and cellular damage were analyzed by measuring the levels of reactive oxygen species (ROS), reduced glutathione (GSH), and malondialdehyde (MDA) within the cells. H₂O₂ exposure caused a decline in cell viability measured at $56.8 \pm 3.4\%$, and an increase in ROS and MDA levels registered at $100.0 \pm 5.4\%$ and 6.8 ± 0.5 nmol/mg protein, respectively. The content of GSH decreased to a level of 4.2 ± 0.4 µmol/g protein. The use of *A. halimus* extract promoted gradual restoration of cell viability and reached the highest percentage of $94.3 \pm 2.6\%$ at 200 µg/mL. In addition, a downtrend in the level of ROS and MDA was noted, which amounted to $44.1 \pm 3.2\%$ and 2.3 ± 0.2 nmol/mg protein correspondingly. GSH content increased to the level of 9.1 ± 0.7 µmol/g protein. The ethanolic extracts of *A. halimus* showed cytoprotective properties in terms of concentration against oxidative stress caused by H₂O₂ in HepG2 cells as shown by the reduction in oxidative stress and lipid peroxidation and the preservation of intracellular GSH levels. The current study shows the potential of *A. Halimus* to serve as a source of antioxidant compounds.

Keywords: Atriplex halimus, Oxidative stress, HepG2 cells, ROS, Lipid peroxidation.

1 INTRODUCTION

Oxidative stress is a fundamental cellular mechanism associated with an imbalance between the generation of reactive oxygen species (ROS) and the capacity of antioxidant systems to neutralize them. When ROS production exceeds the cell's defensive capacity, oxidative damage to lipids, proteins, and nucleic acids can occur, leading to cellular dysfunction and the activation of pathways associated with cell death (An *et al.*, 2024). Hydrogen peroxide (H₂O₂) is being used

extensively as an experimental model to produce oxidative stress in cultured cells due to its ability to change the cellular redox state and cause the resulting effects of high levels of oxidants and depletion of endogenous antioxidants. Research on human liver cells has shown that exposure to H_2O_2 can disturb redox homeostasis and result in processes associated with cell death (Li *et al.*, 2000).

HepG2 cells represent a frequently used cell line for studying hepatic reactions to oxidative substances as well as for evaluating the effect of natural ingredients in preventing such reactions. Evidence suggests that when HepG2 cells are exposed to oxidizing agents, an increase in the antioxidant defense mechanism is seen whereby levels of glutathione and other indicators of oxidative stress change (Alfá *et al.*, 2005).

Reactive oxygen species (ROSs) are very important indicators when evaluating the changes in intracellular redox state. The fluorescent probe 2',7'-dichlorofluorescein diacetate (DCFH-DA) is frequently used to determine changes in cellular oxidative activity. However, this probe is not selective for H_2O_2 or other ROS species, and DCF signal can be altered by different oxidizing agents or cellular pathways. Therefore, the probe is regarded merely as an indicator of the changes in redox state (Winterbourn, 2014; Karges, 2026).

Glutathione, also termed reduced glutathione (GSH), is an integral part of the cellular antioxidant defense system. It is responsible for maintaining a reducing environment and preventing cellular macromolecules from undergoing oxidation (Georgiou-Siafis and Tsiftoglou, 2023). Oxidative stress leads to the depletion of GSH and changes in its amount are widely used as an indicator of antioxidant status and oxidative stress resistance of the cell. Sulphydryl groups, among which is GSH, can be quantified by means of DTNB reagent according to Ellman (1959).

Malondialdehyde is used extensively as a measure of lipid peroxidation because of the fact that its activity is associated with the oxidation of polyunsaturated fatty acids found in cell membranes (Mas-Bargues *et al.*, 2021). Ohkawa *et al.* created a spectrophotometric method of measuring products of lipid peroxidation from the reaction of thiobarbituric acid with the products of lipid peroxidation; a method that became widely accepted for testing oxidative injury to lipids (Ohkawa *et al.*, 1979).

The increased interest in natural sources of antioxidants has brought attention to medicinal plants because they possess bioactive secondary metabolites such as phenolic compounds and flavonoids. Naturally, *Atriplex halimus* L., a halophyte that grows in arid and saline regions, contains a variety of beneficial bioactive compounds - phenolic acids, flavonoids, and other phytochemicals that exhibit a wide range of biological activities like antioxidant activity (Salman *et al.*, 2026; Al-Burki *et al.*, 2025).

Studies in chemistry and pharmacology confirm that *A. halimus* extracts contain antioxidant activity, which can be attributed to the presence of phenolic and flavonoid compounds in the extracts (Guedri *et al.*, 2024). In addition, the composition and bioactivity of these herbal extracts depend on the source of the plant (the plant part being used), solvent, extraction conditions, etc. Therefore, an assessment of antioxidant activity using cell models is also important because chemical assay methods have limitations (Roubi *et al.*, 2025).

As a result, this study sought to evaluate the efficacy of the ethanolic extract derived from the leaves of *Atriplex halimus* to reverse oxidative stress in HepG2 cells as induced by hydrogen peroxide, as well as to keep redox homeostasis.

2 MATERIAL AND METHODS

2.1 Preparation of Plant Extract

The leaves of *Atriplex halimus* plant were collected from Al-Shabaka area (165 km southwest of Najaf city, Iraq) in February 2022. The plant material was cleaned with distilled water and kept in darkness at room temperature until dried. After having dried the leaves, they were ground using an electric grinder and put in a closed container for future extraction. The extraction of plant powder was accomplished with the use of 70% ethanol and a 1:10 solvent-to-plant ratio by using the maceration/continuous extraction procedure over a period of 72 hours. The filtrate was filtered with the aid of Whatmann filter paper, and the solvent was stripped away in a rotary evaporator. The crude extract obtained after evaporation was stored at -20°C . Finally, sterile DMSO was used to prepare a stock solution containing 100 mg/mL and the extract was diluted to a concentration of 0.1% v/v.

2.2 Cell Culture and Experiment Treatment

In the present study, HepG2 cell lines were used, and the cells were cultured in complemented DMEM/RPMI-1640 medium, including 10% FBS and 1% penicillin-streptomycin at 37°C in the humidified 5% CO_2 atmosphere (American Type Culture Collection [ATCC], n.d.). When the confluency reached the required degree of 70% - 80%, the cells were harvested and plated into 96-well plates at a density of 1×10^4 cells/well and cultured for 24 hours. Afterwards, cells

were exposed to *Atriplex halimus* extract in doses of 25, 50, 100, and 200 µg/mL for an incubation period of 24 hours. Following treatment with *Atriplex halimus* extract, the cells were exposed to H₂O₂ at the selected dose for the selected period, as determined in the previous experiments (Deferme *et al.*, 2013). The experiment was conducted with the control group without any treatment, H₂O₂ group, and the H₂O₂ plus extract groups at selected doses. There was also a group where extract only was given without H₂O₂ treatment. Each treatment underwent a triplicate process (technical replicates), while the experiment itself was independently repeated three times. In this case, an untreated control, an H₂O₂ group, and H₂O₂ + *A. halimus* extract group formed the core of this experiment. Each treatment was done in five independent replicates.

2.3 Evaluation of cell viability based on MTT test

The assay used to evaluate cell viability was conducted based on a MTT assay. The technique for this assay was described by Mosmann (1983), and the underlying principle of using this assay is based on the ability of viable cells to reduce MTT compound to the formazan crystals. After the completion of the experimental treatment, MTT solution was added to the respective wells resulting in the final concentration of 0.5 mg/mL. Thereafter, the cells were left in the incubator at 37°C in dark for 4 hours.

Once incubation was finished, the liquid was discarded, and the formazan crystals that were created were dissolved after the addition of 100 µL of DMSO into each well. The absorbance reading was then performed at 570 nm. Cell viability was expressed as a percentage relative to the control group with no treatment, as calculated using the following formula: Cell viability (%) = (absorbance of treated group / absorbance of control group) × 100. The results were obtained in order to determine the effect of H₂O₂ on the viability of HepG2 cells as well as the ability of *A. halimus* extract to offer protection.

2.4 Intracellular Reactive Oxygen Species (ROS) Measurement

Presence of intracellular ROS in HepG2 cells was determined using DCFH-DA probe. Cells were initially treated with experimental treatment, and then washed twice with PBS. The probe was then introduced at a concentration of 10 µM DCFH-DA with incubation of 30 minutes in dark conditions at 37°C. Following incubation step, unreacted probe layers were washed out using PBS. Fluorescence readings were collected using fluorescence plate reader with 485 nm and 530 nm as excitation and emission wavelengths, respectively.

The observations were then represented as a percentage compared to the fluorescence signal of the H₂O₂ group (which was considered as 100% signal) and the background was adjusted with results obtained from cell-free wells wherever applicable.

2.5 Evaluation of reduced glutathione (GSH)

The amount of reduced glutathione (GSH) in HepG2 cells was determined by means of a colorimetric method involving reaction with the sulfhydryl containing reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) according to Ellman (1959) method. The harvested cells were subsequently washed with cold PBS followed by preparation of cell lysate using 0.1M phosphate buffer (pH 7.4).

Cellular waste was centrifuged at 10,000 ×g for 10 minutes at 4°C, and then the supernatant formed was used to assess the amount of GSH. A reagent known as DTNB was added to the samples which were kept at room temperature for 15 minutes in complete darkness before measuring the absorbance at a wavelength of 412 nm. The GSH concentration was derived from the glutathione standard curve, and the results were given as the ratio to the total protein amount in the sample.

2.6 Measurement of lipid peroxides (MDA)

Measurement of lipid peroxidation was done by the concentration of the lipid peroxidation product malondialdehyde (MDA) using the thiobarbituric acid (TBA) method proposed by Ohkawa *et al.* (1979). Cell extracts were treated with the reagents including TBA, trichloroacetic acid (TCA), and hydrochloric acid (HCl), followed by heating up to 95°C for 60 minutes. Following cooling and centrifugation of the mixture, the absorbance of the product was measured at 532 nm. The concentration of MDA was obtained using the standard curve of 1,1,3,3-Tetramethoxypropane (TMP) and reported in nmol/mg of protein.

2.7 Assessment of total protein content

The total protein content in cell extracts was assessed using the Bradford technique, where BSA was used for reference. Total protein values served as a baseline for the measurements of GSH and MDA, with final results being presented as a ratio of total protein to sample concentrations (Bradford, 1976).

2.8 Statistical Analysis

Data analysis was carried out using GraphPad Prism software. Two-way ANOVA was carried out to compare different groups with a sample size of 5 in each group. p-value of <0.05 was taken as significant. Further, Tukey's test was employed for running multiple comparisons.

3 RESULTS

3.1 Effects of *A. halimus* extract on cell viability under oxidative stress

Data from the MTT assay presented in Table 1 showed a significant reduction in HepG2 cell viability after the H₂O₂ treatment: it decreased from 100.0 ± 2.5% in the control group to 56.8 ± 3.4% after treatment. The addition of the *A. halimus* extract was associated with an increase in cell viability with increasing concentrations of extract, which resulted in 65.9 ± 3.6%, 76.4 ± 3.2%, 87.5 ± 2.9%, and 94.3 ± 2.6% of cell viability for 25, 50, 100, and 200 µg/mL of the extract, respectively (Table 1, Figure 1). The results suggest a concentration-dependent cytoprotective effect of the extract against the cytotoxic effect of H₂O₂.

Table 1: Effect of *Atriplex halimus* Extract on Cell Viability under Oxidative Stress

Group	Cell viability (%)
Control	100.0 ± 2.5
H ₂ O ₂	56.8 ± 3.4
H ₂ O ₂ + AH 25 µg/mL	65.9 ± 3.6
H ₂ O ₂ + AH 50 µg/mL	76.4 ± 3.2
H ₂ O ₂ + AH 100 µg/mL	87.5 ± 2.9
H ₂ O ₂ + AH 200 µg/mL	94.3 ± 2.6

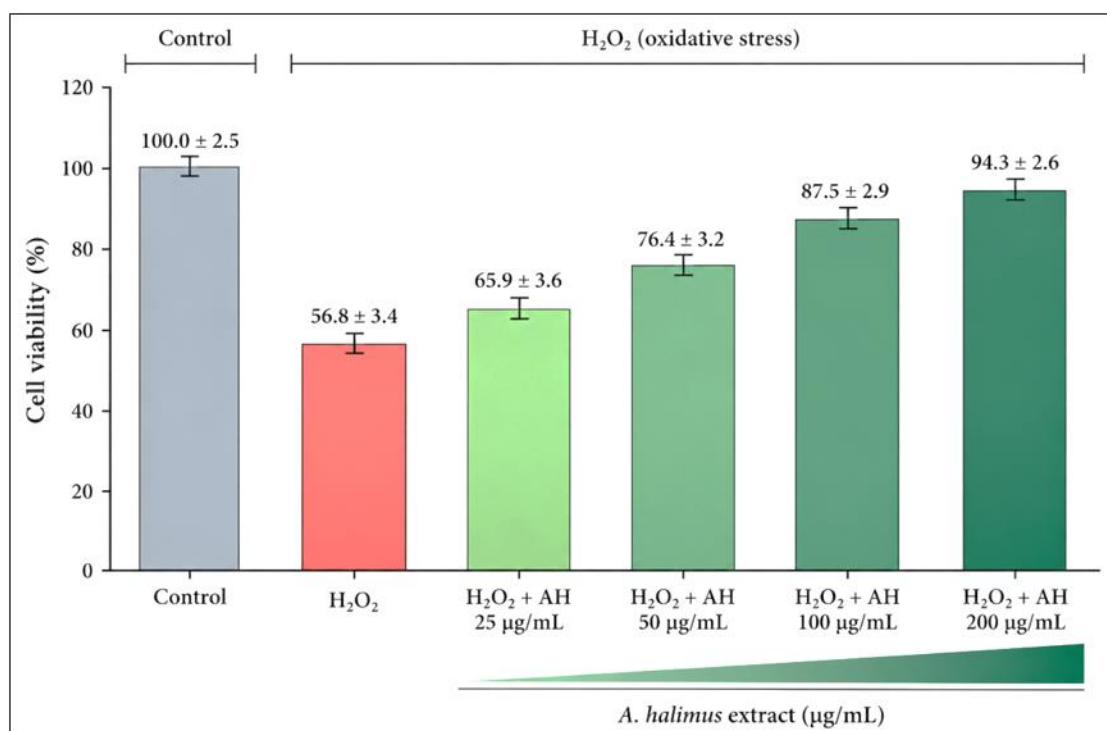


Figure 1: Influence of the alcoholic extract on the HepG2 cell viability

3.2 Influence of the extract on the generation of intracellular reactive oxygen species

The data provided in Table (2) demonstrated a notable rise in the ROS level in HepG2 cells treated with H₂O₂ (100.0 ± 5.4%) compared to the control group (41.8 ± 3.7%). The treatment with the *A. halimus* extract induced a decrease in

ROS levels in a dose-dependent manner and the obtained results were as follows: $84.6 \pm 4.8\%$, $68.2 \pm 4.1\%$, $53.9 \pm 3.6\%$, and $44.1 \pm 3.2\%$ when utilizing extract concentrations of 25, 50, 100, and 200 $\mu\text{g/mL}$ respectively which suggests that the extract is responsible for decreasing the accumulation of ROS in a concentration-dependent manner.

Table 2: Influence of *A. halimus* Extract on Intracellular ROS Generation

Group	ROS (% of H_2O_2 group)
Control	41.8 ± 3.7
H_2O_2	100.0 ± 5.4
$\text{H}_2\text{O}_2 + \text{AH } 25 \mu\text{g/mL}$	84.6 ± 4.8
$\text{H}_2\text{O}_2 + \text{AH } 50 \mu\text{g/mL}$	68.2 ± 4.1
$\text{H}_2\text{O}_2 + \text{AH } 100 \mu\text{g/mL}$	53.9 ± 3.6
$\text{H}_2\text{O}_2 + \text{AH } 200 \mu\text{g/mL}$	44.1 ± 3.2

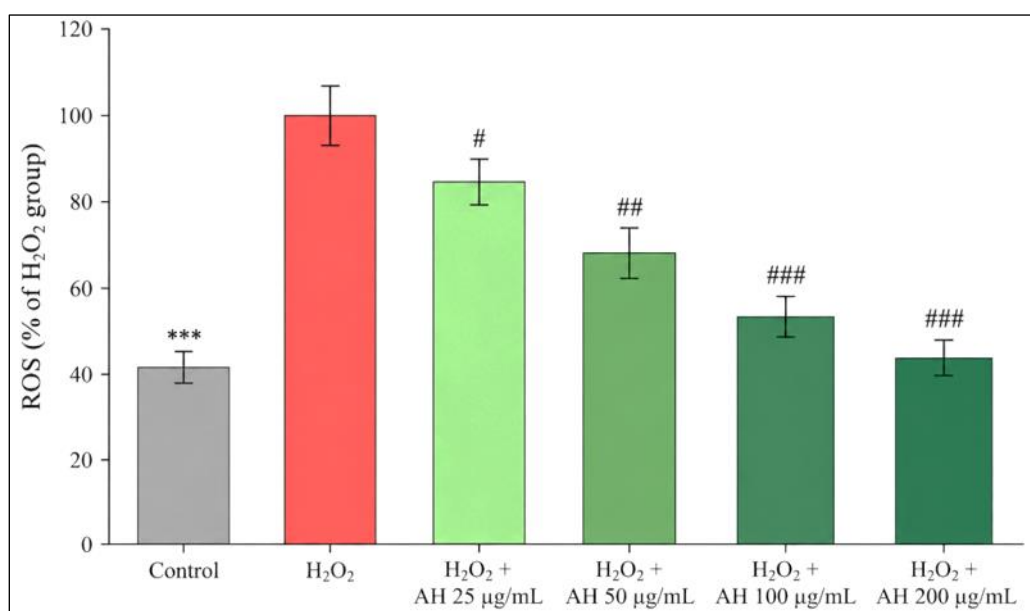


Figure 2: Influence of *A. halimus* extract on ROS generation in HepG2 cells

3.3 Influence of the Extract on GSH Levels

According to the data shown in the table (3), the value of the glutathione (GSH) level declines notably in HepG2 cells treated with H_2O_2 : during the experiment, the degradation level dropped from $9.8 \pm 0.6 \mu\text{mol/g}$ protein in the control group to $4.2 \pm 0.4 \mu\text{mol/g}$ protein. The introduction of the *A. halimus* extract caused the increase of the GSH values that matches the higher concentration of the extract. The values reached 5.6 ± 0.5 , 6.9 ± 0.5 , 8.1 ± 0.6 , and $9.1 \pm 0.7 \mu\text{mol/g}$ protein with the concentration of 25, 50, 100, and 200 $\mu\text{g/mL}$, respectively. This means that the extract is able to recover GSH which is lost due to the effects of H_2O_2 .

Table 3: Influence of *A. halimus* extract on reduced glutathione (GSH)

Group	GSH ($\mu\text{mol/g}$ protein)
Control	9.8 ± 0.6
H_2O_2	4.2 ± 0.4
$\text{H}_2\text{O}_2 + \text{AH } 25 \mu\text{g/mL}$	5.6 ± 0.5
$\text{H}_2\text{O}_2 + \text{AH } 50 \mu\text{g/mL}$	6.9 ± 0.5
$\text{H}_2\text{O}_2 + \text{AH } 100 \mu\text{g/mL}$	8.1 ± 0.6
$\text{H}_2\text{O}_2 + \text{AH } 200 \mu\text{g/mL}$	9.1 ± 0.7

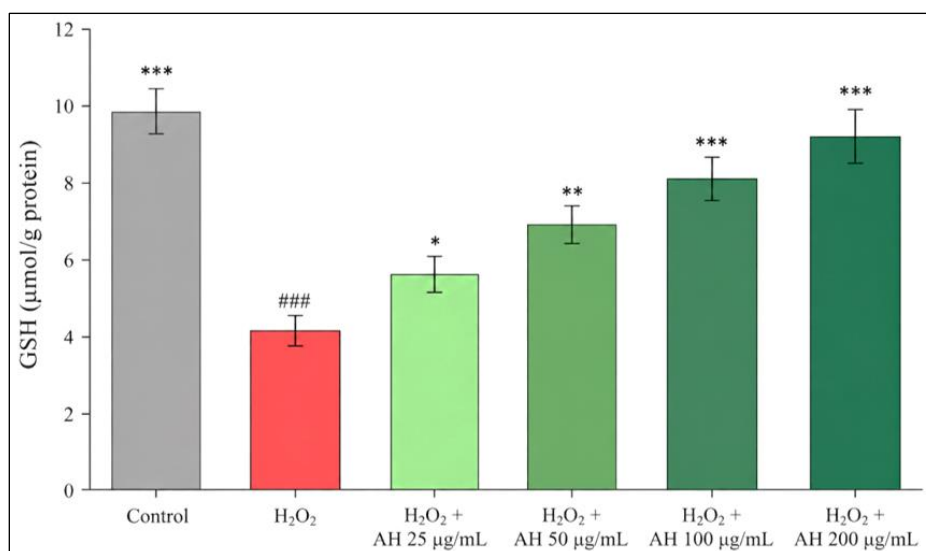


Figure 3: Influence of *A. halimus* extract on GSH levels in HepG2 cells

3.4 Influence of *A. halimus* extract on lipid peroxidation

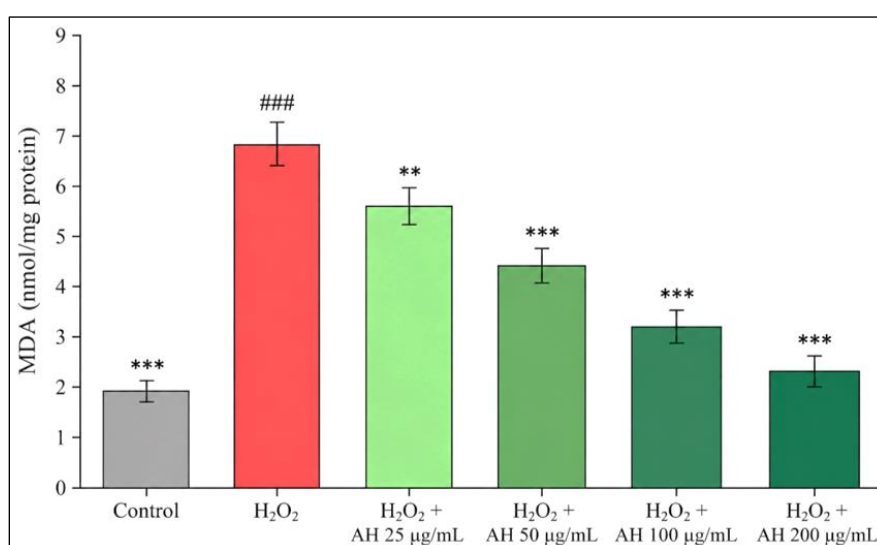


Figure 4: Effect of *A. halimus* extract on lipid peroxidation in HepG2 cells

Table 4: Influence of *Atriplex halimus* Extract on Lipid Peroxidation (MDA)

Group	MDA (nmol/mg protein)
Control	1.9 ± 0.2
H ₂ O ₂	6.8 ± 0.5
H ₂ O ₂ + AH 25 μg/mL	5.6 ± 0.4
H ₂ O ₂ + AH 50 μg/mL	4.4 ± 0.3
H ₂ O ₂ + AH 100 μg/mL	3.2 ± 0.3
H ₂ O ₂ + AH 200 μg/mL	2.3 ± 0.2

The results of the experiments presented in Table (4) indicated that there was an observed increase of MDA levels in HepG2 cell line treated with H₂O₂ up from 1.9 ± 0.2 nmol/mg protein in the control group to 6.8 ± 0.5 nmol/mg protein. The administration of the alcoholic extract of *A. halimus* led to the decrease of MDA levels in the HepG2 cells with the increasing of the extraction concentration from 5.6 ± 0.4 nmol/mg protein, 4.4 ± 0.3 nmol/mg protein, 3.2 ± 0.3 nmol/mg

protein at 25, 50, 100, and 200 µg/mL concentrations, respectively. From the above results, it can be concluded that the extract reduces the lipid peroxidation levels in a concentration-dependent manner.

4 DISCUSSION

The findings showed that the treatment of H₂O₂ resulted in huge oxidative damage in HepG2 cells manifested mainly by a drop in cell viability percentage down to 56.88 ± 3.47. This drop confirms that the oxidative stress model was successful in causing measurable cellular injury. Our findings are totally consistent with the previous data from the literature confirming that H₂O₂ indeed has an ability to disturb the redox balance and induce cellular injury in hepatocytes (Li *et al.*, 2000; Alía *et al.*, 2005). Conversely, treatment with *A. halimus* extract showed a distinct change where the cell viability improved from 65.9% at 25 µg/mL to 94.3% at 200 µg/mL, indicating a concentration-dependent cytoprotective action rather than random increase in cell survival. The increase in cell survival must correlate with the improvement in other oxidative stress markers at the same time. The increase can be attributed, at least partly, to the presence of phenolic and flavonoid compounds having antioxidant activities as described for *A. halimus* previously (Guedri *et al.*, 2024; Roubi *et al.*, 2025). These results obtained from testing GSH confirmed the interpretation, i.e., exposure to hydrogen peroxide leads to significant decline in reduced glutathione levels, whereas the extract of *A. halimus* promotes restoration of reduced glutathione levels in a concentration-dependent manner, thus indicating a possible ability to restore cellular redox balance and mitigate GSH depletion occurring due to oxidative stress (Ellman, 1959; Alía *et al.*, 2005).

The corroborated observation was followed by decreases in MDA levels corresponding with higher extract concentrations suggesting that the extract is capable of inhibiting lipid peroxidation induced by H₂O₂. The simultaneous reductions in ROS and MDA along with GSH recovery and cell viability show the combined protective role of the extract against oxidative stress and cell damage (Mas-Bargues *et al.*, 2021).

In summary, the results suggest that the *A. halimus* alcoholic extract possesses significant cytoprotective properties in HepG2 cells subjected to oxidative stress induced by H₂O₂. These properties may include its ability to reduce oxidative effects and lipid peroxidation while maintaining GSH levels, which contributes to the increased viability of the cells. The findings are consistent with the current literature concerning the antioxidant properties of extracts of *A. halimus* and their link to the components of bioactive phytochemicals. Further studies are needed to identify the mechanisms and compounds responsible for the described effect.

5 CONCLUSION

Results showed that the ethanolic extract of the leaves of *Atriplex halimus* L. has protective action against oxidative stress induced by H₂O₂ in HepG2 cells. It was shown to increase cell viability, reduce the levels of ROS and lipid peroxidation (MDA), and restore GSH levels in a concentration-dependent manner. The results suggest that *A. halimus* may be a reliable source of natural materials with antioxidant and cytoprotective properties, but further studies are necessary in order to identify the active substances and the molecular mechanisms responsible for this activity.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

No conflict of interest to be disclosed.

REFERENCES

- [1] Al-Burki, F. R., Zarka, M. A., Hasan, H. F., Salman, H. K., Hadi, R. S., & Al-Alnkooshi, A. A. (2026). Evaluation of the Therapeutic Effect of A Nanocomposite of Desert Germander Extract and Gold Nanoparticles on Breast Cancer. *Egyptian Journal of Medical Microbiology*.
- [2] Alía, M., Ramos, S., Mateos, R., Bravo, L., & Goya, L. (2005). Response of the antioxidant defense system to tert-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). *Journal of Biochemical and Molecular Toxicology*, 19(2), 119–128. <https://doi.org/10.1002/jbt.20061>
- [3] American Type Culture Collection. (n.d.). Hep G2 [HEPG2] (ATCC® HB-8065™). ATCC. <https://www.atcc.org/products/hb-8065>
- [4] An, X., Yu, W., Liu, J., Tang, D., Yang, L., & Chen, X. (2024). Oxidative cell death in cancer: mechanisms and therapeutic opportunities. *Cell death & disease*, 15(8), 556.

- [5] Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- [6] Deferme, L., Briede, J. J., Claessen, S. M. H., Jennen, D. G. J., Cavill, R., & Kleinjans, J. C. S. (2013). Time series analysis of oxidative stress response patterns in HepG2: A toxicogenomics approach. *Toxicology*, 306, 24–34. <https://doi.org/10.1016/j.tox.2013.02.001>
- [7] Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1), 70–77. [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6)
- [8] Georgiou-Siafis, S. K., & Tsiftoglou, A. S. (2023). The key role of GSH in keeping the redox balance in mammalian cells: mechanisms and significance of GSH in detoxification via formation of conjugates. *Antioxidants*, 12(11), 1953.
- [9] Guedri, M. M., Krir, N., Terol, C. C., Romdhane, M., Boulila, A., & Guetat, A. (2024). Phytochemical analysis, acetylcholinesterase inhibition, antidiabetic and antioxidant activities of *Atriplex halimus* L.(Amaranthaceae Juss.). *Chemistry & biodiversity*, 21(7), e202301941.
- [10] Karges, J. (2026). Reactive oxygen species detection with fluorescent probes: Limitations and recommendations beyond DCFH-DA. *Journal of Medicinal Chemistry*, 69(3), 1970–1981. <https://doi.org/10.1021/acs.jmedchem.5c03494>
- [11] Li, J., Huang, C. Y., Zheng, R. L., Cui, K. R., & Li, J. F. (2000). Hydrogen peroxide induces apoptosis in human hepatoma cells and alters cell redox status. *Cell Biology International*, 24(1), 9–23.
- [12] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275. DOI: 10.1016/S0021-9258(19)52451-6.
- [13] Mas-Bargues, C., Escriva, C., Dromant, M., Borrás, C., & Vina, J. (2021). Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. *Archives of biochemistry and biophysics*, 709, 108941.
- [14] Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63. DOI: 10.1016/0022-1759(83)90303-4.
- [15] Ohkawa H, Ohishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. DOI: 10.1016/0003-2697(79)90738-3.
- [16] Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- [17] Roubi, M., Dalli, M., Azizi, S.-E., & Gseyra, N. (2025). *Atriplex halimus*: Phytochemical insights, traditional applications, and pharmacological promises. *Chemistry & Biodiversity*, 22(4), e202402171. <https://doi.org/10.1002/cbdv.202402171>
- [18] Salman, M. R., Abbas, A. A., Al-Burki, F. R., & Al-Ethari, Y. A. Enhancing Phenolic Content and Antioxidant Activity during Fermentation using a Selected Lactic Acid Bacteria Isolate from a Local Milk.
- [19] Winterbourn, C. C. (2014). The challenges of using fluorescent probes to detect and quantify specific reactive oxygen species in living cells. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1840(2), 730–738. <https://doi.org/10.1016/j.bbagen.2013.05.004>