Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

In vivo assessment of imoxide and diluted vitamin C's inhibitory effects on cervical cancer in mice

Toktam Dehghani [1], Seyed Mousalreza Hosseini [2], Janbibi Dorazehi [1], Seyedeh Elnaz Nazari [3], Bahram Memar [4], Mojtaba Meshkat [5], Hoorieh Soleimanjahi [6], Omid Rajabi [7], Saeid Zibaee [8], Majid Khazaei [3]*, Zahra Meshkat [9]*

[1] Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Gastroenterology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Metabolic Syndrome Research Center, Department of Physiology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran
[4] Surgical Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Department of Community Medicine, MMS.C. Islamic Azad University, Mashhad, Iran
[6] Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[7] Medical Chemistry Department, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[8] Department of Research and Development of Biological Products, Agricultural Research, Education and Extension Organization (AREEO), Razi Vaccine and Serum Research Institute, Mashhad, Iran
[9] Antimicrobial Resistance Research Center, Basic Sciences Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Nov 30, 2025
Accepted: May 6, 2026

Keywords:
Cervical cancer
H_2O_2
Hydrogen peroxide
Imoxide
Papillomavirus
Vitamin C

ABSTRACT

Objective(s): Cervical cancer, the fourth most common malignancy among women, is strongly associated with high-risk human papillomavirus (HPV) infection. Emerging evidence suggests that hydrogen peroxide, alongside standard therapies, may enhance treatment. This study investigated the effects of Imoxide (0.5% hydrogen peroxide compound) and diluted vitamin C on tumor growth in the TC-1 papillomavirus mouse model.

Materials and Methods: Cytotoxicity of 3% hydrogen peroxide and 100 mg/ml vitamin C was assessed using the MTT assay on TC-1 cells. Six-week-old female C57BL/6 mice were used to establish TC-1 tumor models in groups by grafting. Mice were divided into seven groups: intratumoral (IT) control, intraperitoneal (IP) control, IP Imoxide, IT Imoxide, cisplatin, IP vitamin C, and IT vitamin C. Tumor size was measured every other day. Histopathological analysis and real-time PCR were conducted to evaluate gene expression (P53, P21, BAX, BCL-2).

Results: Dilutions of 10^{-3} and 10^{-4} of hydrogen peroxide resulted in cell survival rates of 53.7% and 58.8%, respectively. Imoxide markedly inhibited tumor growth *in vivo*, in some groups exceeding cisplatin. Histological findings showed limited apoptotic features. Gene-expression analysis revealed no statistically significant differences after multiple-testing correction.

Conclusion: Imoxide substantially suppressed tumor growth in the murine model; however, modulation of apoptosis-related genes was minimal, suggesting that non-apoptotic pathways may contribute to its antitumor effects.

▶Please cite this article as:
Dehghani T, Hosseini SM, Dorazehi JB, Nazari SE, Memar B, Meshkat M, Soleimanjahi H, Rajabi O, Zibaee S, Khazaei M, Meshkat Z. *In vivo* assessment of imoxide and diluted vitamin C's inhibitory effects on cervical cancer in mice. Iran J Basic Med Sci 2026; 29: 971-978. doi: https://dx.doi.org/10.22038/ijbms.2026.93220.20105

Introduction

Cervical cancer is the fourth most commonly detected cancer and the fourth major cause of cancer death in women. Cervical cancer is the most frequently detected cancer in 23 countries, with an estimated 348,000 global deaths in 2022 (1, 2). Virtually all of these deaths could be prevented through widespread access to all-inclusive cervical cancer prevention and control plans: human papillomavirus (HPV) vaccination for all girls aged 9 to 13; cervical cancer screening and treatment of pre-cancerous lesions for all women, and treatment of invasive tumors (3–5).

HPV is an envelope-free, small, double-stranded DNA virus that prefers human epithelial cells, both in the skin and mucous membranes (6-8). An ORF coding for the capsid protein L1 has been identified as a criterion for categorizing 229 known HPVs (9–11). Cervical cancers are found to have DNA from HPV in 99.7% of cases, with HPV oncogenic types 16 and 18 making up over 70% of cases (12, 13). In this case, E7 binds to and inactivates the cellular tumor suppressor protein Rb, while E6 binds to, and degrades the cellular tumor suppressor protein p53 through the ubiquitin pathway (14). In cervical intraepithelial neoplasia (CIN), changes occur in squamous cells of the

**Corresponding authors:* Majid Khazaei. Metabolic Syndrome Research Center, Department of Physiology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran. Email: khazaeim@mums.ac.ir; Zahra Meshkat. Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran Email: MeshkatZ@mums.ac.ir

cervix, and if left untreated, it can progress to cancer *in situ* or invasive cancer. HPV plays a crucial role in transforming cervical epithelial cells, mainly subtypes 16 and 18 (15, 16). According to a study, the prevalence of HPV increased from 62.64% for patients with CIN1, to 86.91% for those with CIN2/3, and 89.21% for those with cervical cancer (17).

Many physiological processes generate hydrogen peroxide (H_2O_2) a non-radical reactive oxygen species (ROS). This molecule may represent a signaling messenger or may result in oxidative damage depending on its spatiotemporal accumulation profile (18, 19). ROS levels are elevated in cancer cells in part due to mitochondrial dysfunction and increased metabolism, which may provide a unique opportunity to eliminate cancer cells by elevating ROS levels within cells to highly toxic levels (20–22).

Vitamin C (ascorbic acid) is a pro-drug for extracellular hydrogen peroxide generation. According to *in vitro* studies, high-concentration ascorbate produces H_2O_2 that kills cancer cells with or without radiation or chemotherapy. Consequently, high-dose intravenous ascorbate-based cancer treatments were tested in clinical trials (23–26).

Based on the positive report about the effect of H_2O_2 low dose in the treatment of a wide range of diseases especially before 1940, this study aimed to compare H_2O_2 antitumor effect with vitamin C IV as a pro-drug for H_2O_2 generation.

Materials and Methods

Cell culture

TC-1 cells were developed from primary epithelial cells from C57BL/6 mice cotransformed with HPV-16 E6 and E7 oncogenes and activated c-Ha-ras. We obtained the TC-1 cell line from the virology department at Tarbiat ModaresUniversity, Tehran, Iran. We cultured TC-1 cells in high glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin antibiotic and finally kept it at 37 °C under 5% CO_2 during experiments.

Cell viability assay

The cells were plated in 96-well plates at a cell density of 2×10^4 per well. After culturing overnight, vitamin C and Imoxide were serially diluted in a serum-free medium. Two hundred microliters of each dilution were poured into the wells, and four repetitions were done. Imoxide was applied to the cells for 24 hr. Vitamin C was applied for 2 hr. After 2 hr, the cells were washed with phosphate-buffered saline (PBS) and cultured for an additional 24 hr in a growth medium with ascorbic acid. Cell proliferation was estimated using an MTT assay. After 24 hr, 100 microliters of serum-free medium and 10 microliters of MTT solution were added to each well. Wrapped in foil, the plate was incubated for 4 hr. After removing the MTT solution, 200 µl of dimethyl sulfoxide (DMSO) was added to the cells. The optical density was then measured at 540–630 nm. Finally, viability was calculated and graphed using GraphPad Prism version v8.

Animals

Three C57BL/6 mice (20–30 g) were purchased from the Laboratory Animal Center of Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. The Ethical Committee at the Experimental Animal Center of MUMS, approved all protocols for animal experimentation (code: IR.MUMS.MEDICAL.REC.1400.198). During the experiments, the mice were housed in a laboratory environment (with 12 hr of light and 12 hr of darkness), and standard food and water were provided. The mice were injected subcutaneously with cell suspensions (3×10^6/100 µl) of TC1 cells suspended in PBS, and approximately two weeks later, when the tumors reached a size of 80–100 mm^3, the tumor was carefully removed from under the skin of the mice and was divided into one mm parts. After anesthetizing, a small piece of the tumor was placed under the skin of the shaved right flank of the C57BL/6 mice. After four days, 80 mm^3 and above tumors were established in all the study mice.

Table 1. Experimental administration of drug based on routes and timing in C57BL/6 mice

Group	Drug	Time
Control IT	PBS	Every other day
Control IP	PBS	Every other day
cisplatin	5mg/kg	Days 9 &12
Imoxide IP	Imoxide	Every other day
Imoxide IT	Imoxide	Every other day
Vit C IP	20 mg/mice	Every other day
Vit C IT	8 mg/mice	Every two days

Tumor size and drug injection

Mice were divided into groups of six. The studied groups included Control IT (intratumoral), Control IP (intraperitoneal), cisplatin Group (common cervical cancer chemotherapy drug), H_2O_2 IP Group, H_2O_2 IT Group, Vit C IP Group, and Vit C IT. Tumor measurements were conducted every other day on the animals. The tumor volume (V) was calculated according to the formula V = AB2/2, where A is the length of the major axis and B is the length of the minor axis using the digital caliper. The injection of medications for each group is shown in Table 1.

Histology

On day 18, each mouse's tumor, lungs, kidneys, heart, and liver were collected for further investigation using hematoxylin and eosin (H&E) staining methods. The tumor tissue was examined for apoptosis and necrosis, and the side effects of injecting each substance were tested by examining the tissues of the collected organs.

RNA extraction

Total RNA was isolated using an RNJia kit (ROJE Technologies, Iran), and cDNA was synthesized using the Yekta Tajhiz kit (Iran). The correctness of the RNA extraction and cDNA synthesis was determined using the PCR test with a GAPDH primer. All locally manufactured kits used in this study were approved by the Iranian Ministry of Health and were applied according to the manufacturer's instructions with standard quality-control validation.

Primer design

BAX, BCL2, and GAPDH genes were selected from the reference articles (27–29). Gene primers were designed using the NCBI database, P53 (NM_011640.3) and P21(NM_007669.5), and their characteristics analyzed with Gene Runner software (Table 2).

Real-time PCR

Real-time PCR was performed on a Roche light cycler Real-time PCR System (Switzerland) using RealQ Plus 2x Master Mix Green, High ROX™ Ampliqon according to the Manufacturer's specification. The reaction was performed in 25 µl containing 12.5 µl RealQ Plus 2x Master Mix Green, High ROX, 0.5 µl of 100 µM forward primer, 0.5 µl of

Table 2. Oligonucleotide primer sequences designed in present experiments for qRT-PCR

Gene	Amplicon size	Sequence (5′-3′)
GAPDH	123	Forward: 5′-AGGTCGGTGTGAACGGATTTG-3′
		Reverse: 5′-TGTAGACCATGTAGTTGAGGTCA-3′
P53	146	Forward: 5′-CTTCTCCGAAGACTGGATGACT-3′
		Reverse: 5′-GATCGTCCATGCAGTGAGGT-3′
P21	147	Forward: 5′-GACCAGCCTGACAGATTTCTA-3′
		Reverse: 5′-CACACAGAGTGAGGGCTAAGG-3′
BAX	151	Forward: 5′-GCCTTTTTGCTACAGGGTTTCAT-3′
		Reverse: 5′-TATTGCTGTCCAGTTCATCTCCA-3′
BCL-2	166	Forward: 5′-CCAGCGTGTGTGTGCAAGTGTAAAT-3′
		Reverse:5′-ATGTCAATCCGTAGGAATCCCAACC-3′

100 µM reverse primer, 1 µl of cDNA, and 10.5 µl double-distilled water (ddH_2O). The real-time PCR was carried out in a three-step PCR program as follows: 1 cycle of 95 °C for 15 min, followed by 40 cycles of 95 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 30 sec.

Statistical analysis

In the description of the data, appropriate statistical tables and indicators such as mean ± SD have been used. Data normality was assessed using the Shapiro-Wilk test. For non-normal data, the Kruskal-Wallis test with pairwise comparisons was applied. For normally distributed data, parametric methods, including analysis of variance (ANOVA) with Tukey's *post hoc* test, were used. Benferroni correction was applied for pairwise comparisons, where appropriate. To account for multiple testing across the four gene-expression endpoints (P53, P21, BAX, and BCL2) within each administration route, Holm–Bonferroni correction was additionally applied and both unadjusted and adjusted *P*-values were reported. Statistical analyses were performed using SPSS v.26 and GraphPad Prism 8, with significance set at $P<0.05$ (values <0.05 marked with * and <0.01 with **).

Results

For each experiment, serial 2-fold and 10-fold dilutions were prepared. The concentration used for hydrogen peroxide was 3%, and for vitamin C was 100 mg/ml. The lethal effect of hydrogen peroxide is much higher than vitamin C, even at lower concentrations. After 24 hr, only 53% and 58% of TC-1 cells in 10^{-3} and 10^{-4} concentrations of H_2O_2 survived, respectively, as determined by the MTT assay. In the microscopic H_2O_2 examination, in dilutions 1, 1/2 to 1/32, as well as concentrations of 10^{-1}, and 10^{-2}, after 24 hr, the cells were completely dead, the contents of the nucleus and cytoplasm were disintegrated, and empty vacuoles were observed.

The percentage of living cells near vitamin C was higher than hydrogen peroxide in different dilutions. However, due to the acidic nature of the vitamin and its ability to produce hydrogen peroxide after being oxidized in dilutions of 1/4, 1/8, and 1/16, the percentage of cell death observed was high. Cell death is not observed in 10^{-3} and 10^{-4} dilutions of vitamin C 100 mg/ml. In a microscopic view of high doses, deformed cells with vitamin C crystals were observed.

The injection was started with the prescribed doses and schedule once the tumor size obtained from the malignant tissue graft reached 80–100 mm^3, and the tumor size was assessed every other day. The IP Imoxide group showed the highest reduction in tumor size. Although the size in the Imoxide IT group was smaller than that in the IT control group, it exhibited nearly the same trend as that in the cisplatin group. Mortality occurred exclusively in the intraperitoneal (IP) vitamin C group, with up to two animals dying during the early treatment period. No deaths were observed in the Imoxide-treated groups. The Imoxide IP group experienced the most significant tumor weight decrease compared to the cisplatin group, and the tumor weight loss in the Imoxide IT group was less (Figure 1).

The vitamin C IP group in the vitamin C group showed the greatest reduction in tumor size. Although the size reduction in the vitamin C IP group was greater than that of cisplatin, there was no size difference between the vitamin C IT group and the IT control group. Weight loss was seen in the vitamin C IP group when compared to other groups in terms of tumor weight, but in the vitamin C IT group, the average tumor weight was larger than in the cisplatin and IT control groups (Figure 2). There is a size comparison of all groups in Figure 3.

Necrosis and apoptosis were seen during the histological examinations in the tissue sections linked to the cisplatin group, and apoptosis was also seen in some tissue sections

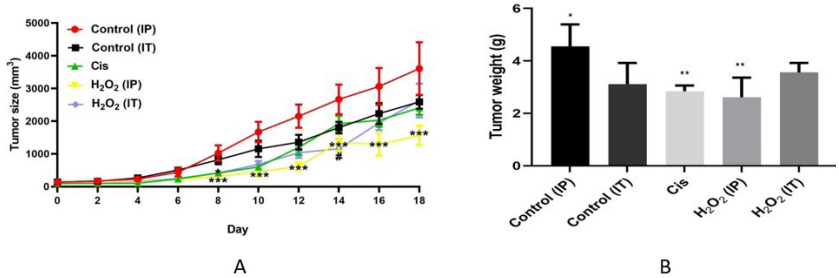

Figure 1. A. Comparison of tumor size reduction in mice treated with Imoxide (H_2O_2) versus the reference drug and control groups. B. Comparison of tumor weight reduction among the Imoxide (H_2O_2), reference drug, and control groups
IT: Intratumoral, IP: Intraperitoneal; Cis: Cisplatin

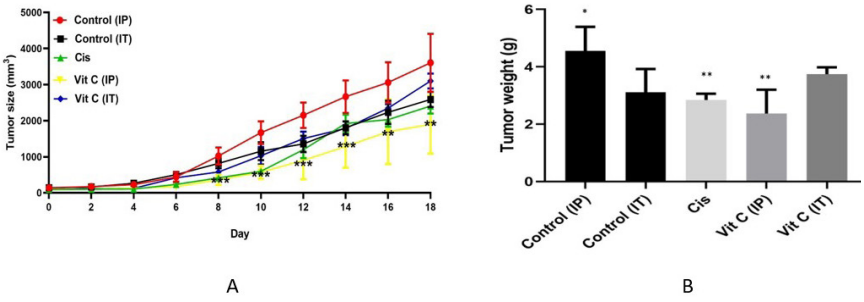

Figure 2. A comparison of tumor size reduction in mice treated with vitamin C versus the reference drug and control groups. B. Comparison of tumor weight reduction among the vitamin C, reference drug, and control groups

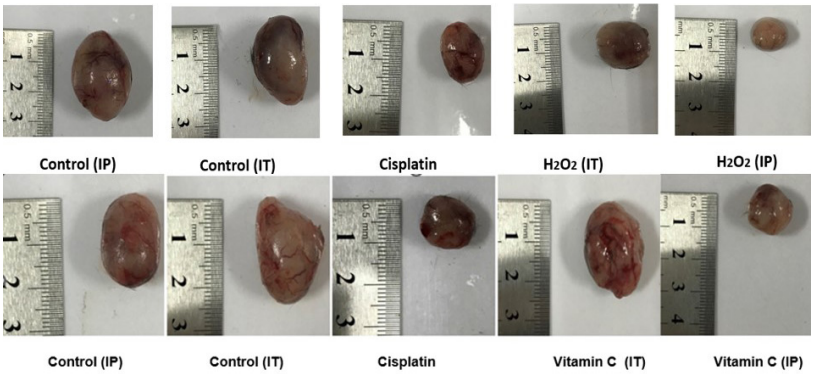

Figure 3. Comparison of tumor size reduction in two mouse Imoxide (H_2O_2) and vitamin C groups with reference drug, and control groups

associated with Imoxide. It should be mentioned that the IP control group's tissue displayed some necrosis (Figure 4). According to a pathologist's analysis of all the slides, apoptosis was only sometimes seen in tissue sections taken from tumors.

Neutrophil infiltration was seen in the liver port spaces and inside the liver lobules and lung interstitium, which was also observed in the control group. In other organs such as the heart and the kidney, it was completely normal and no significant findings were observed (Figure 5).

In the intratumoral (IT) groups, overall group comparisons showed no significant differences in P53 (ANOVA, *P*=0.878), P21 (ANOVA, *P*=0.064), or BCL2 (ANOVA, *P*=0.128). A borderline difference was observed for BAX expression (ANOVA, *P*=0.049). *Post hoc* Tukey analysis indicated a significant difference between the

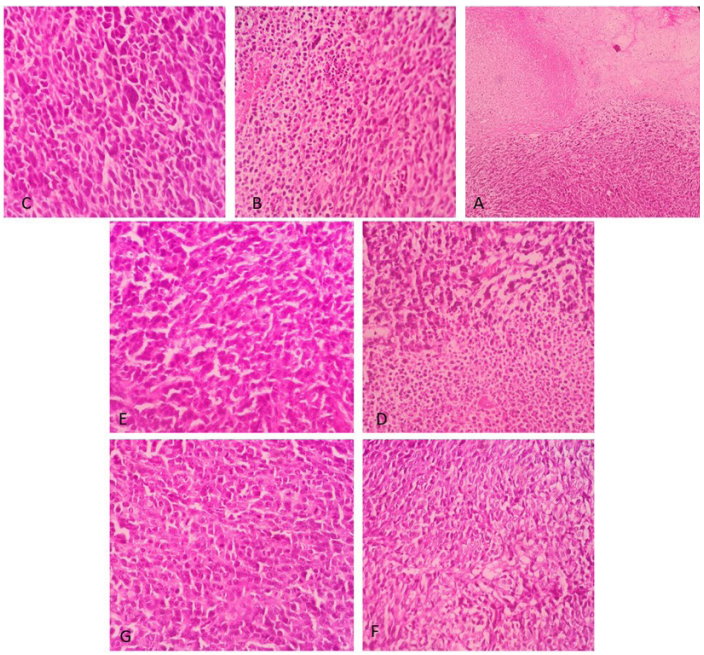

Figure 4. Histological examination of C57BL/6 mice tumor in different groups of investigation by using hematoxylin and eosin (H&E) stain
A. cisplatin Extensive necrosis in the upper half of the field. B. control IP undifferentiated carcinoma with prominent necrosis in the left side of the field. C. control IT undifferentiated carcinoma with large tumoral cells. D. Imoxide IP prominent necrosis and apoptosis in the lower half of the field. E. Imoxide IT undifferentiated carcinoma with numerous mitotic figures. F. Vit C IP undifferentiated carcinoma. G. Vit C IT undifferentiated carcinoma with numerous mitotic figures.

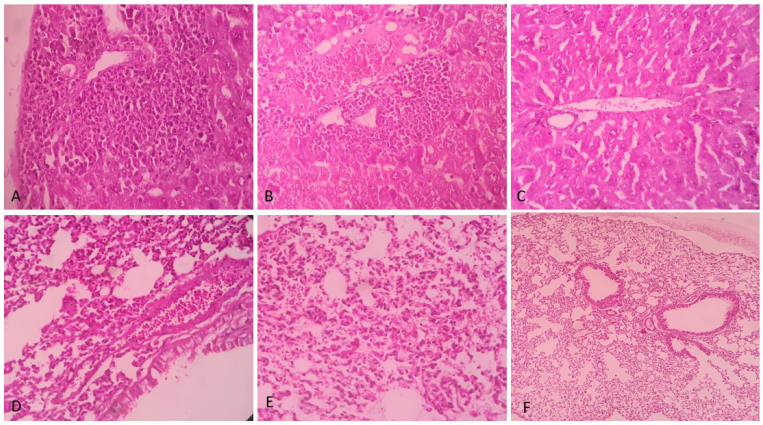

Figure 5. Analysis of side effects in mouse organs using H&E staining
A. Imoxide Ip Cluster of neutrophils in liver portal tract. B. Control IP Cluster of neutrophils in liver lobule. C. Cis IP Normal liver histology and a portal tract at center. D. Imoxide IP Interstitial neutrophilic infiltration in lung a section of bronchiole at (right lower), covered by columnar epithelium. E. Control IP - Interstitial neutrophilic infiltration in lungs. Cis IP - Normal lung histology and two bronchioles near center.

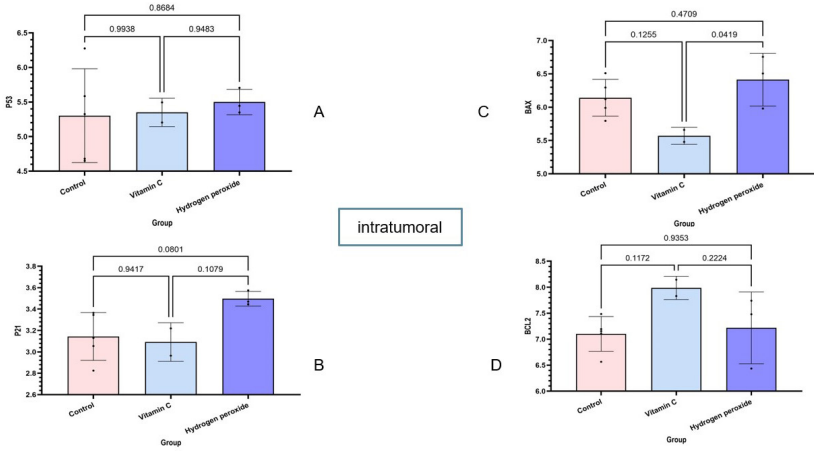

Figure 6. Gene analysis of variance between groups receiving intratumoral injections. A. P53, B. P21, C. BAX, and D. BCL-2

vitamin C and hydrogen peroxide groups (P=0.042). (Figure 6) However, after Holm–Bonferroni correction across the four gene-expression endpoints, none of these differences remained statistically significant.

Among the intraperitoneal (IP) groups, Imoxide treatment showed numerically higher expression of the pro-apoptotic genes P53 and BAX, as well as P21, while the anti-apoptotic gene BCL2 tended to be higher in the Vitamin C group. However, overall group comparisons revealed no statistically significant differences in P53 (Kruskal–Wallis, P=0.232), P21 (Kruskal–Wallis, P=0.934), BAX (Kruskal–Wallis, P=0.255), or BCL2 (Kruskal–Wallis, P=0.249). These findings remained non-significant after multiple-testing correction (Figure 7). Exact P-values for all overall group

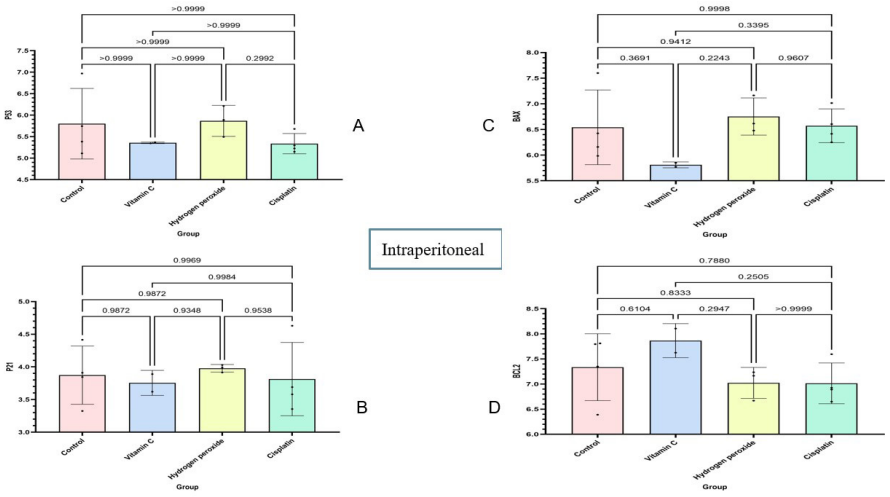

Figure 7. Gene analysis of variance between groups of C57BL/6 mice as a result of receiving intraperitoneal injections
A. P53, B. P21, C. BAX, and D. BCL-2

Table 3. Overall group-comparison *P*-values (unadjusted) and Holm–Bonferroni adjusted *P*-values for gene expression endpoints

Administration route	Gene	Statistical test	Unadjusted *P*-value	Holm–Bonferroni adjusted *P*-value
Intratumoral (IT)	P53	One-way ANOVA	0.878	0.878
Intratumoral (IT)	P21	One-way ANOVA	0.064	0.196
Intratumoral (IT)	BAX	One-way ANOVA	0.049	0.196
Intratumoral (IT)	BCL2	One-way ANOVA	0.128	0.256
Intraperitoneal (IP)	P53	Kruskal–Wallis	0.232	0.928
Intraperitoneal (IP)	P21	Kruskal–Wallis	0.934	0.934
Intraperitoneal (IP)	BAX	Kruskal–Wallis	0.255	0.928
Intraperitoneal (IP)	BCL2	Kruskal–Wallis	0.249	0.928

comparisons are summarized in Table 3.

Discussion

This study evaluated the antitumor effects of Imoxide and vitamin C in a papillomavirus-associated mouse model of cervical cancer. The simultaneous investigation of hydrogen peroxide and vitamin C was based on their shared redox-related mechanisms, whereby pharmacologic ascorbate can generate extracellular H_2O_2 through metal-catalyzed reactions that subsequently enter cells via aquaporin channels (30).

Following TC-1 tumor establishment, both intraperitoneal and intratumoral administration of Imoxide and intraperitoneal high-dose vitamin C resulted in significant tumor size reduction compared with control groups. Notably, intraperitoneal Imoxide demonstrated greater tumor suppression than intratumoral administration and exceeded the therapeutic effect observed with cisplatin. These findings suggest that systemic delivery of hydrogen peroxide–based strategies may be particularly effective in limiting tumor progression, as intraperitoneal administration is generally considered comparable to systemic delivery (31).

Consistent with previous reports, high-dose vitamin C suppressed tumor growth *in vivo*, as previously observed in colorectal cancer models, where ascorbate inhibited proliferation, angiogenesis, and metastasis following intraperitoneal administration (32, 33). Vitamin C treatment was associated with mortality in the intraperitoneal group, suggesting potential toxicity at high systemic doses, in contrast to earlier reports of minimal adverse effects (33). These deaths occurred during the initial phase when injections were prepared from ascorbic acid powder, and were markedly reduced after switching to a commercially prepared formulation, indicating that preparation-related factors rather than intrinsic ascorbate toxicity likely contributed to this limitation.

Vitamin C IP suppressed cancer growth in the current investigation, although this group also had higher expression of the anti-apoptotic gene BCL-2 and lower levels of the apoptotic proteins P53, P21, and BAX than the other groups. Statistical analysis, however, did not reveal any appreciable differences between the groups. Even though none of the groups receiving H_2O_2 injections saw any mouse deaths. These null and partially contradictory molecular findings indicate that tumor regression in this model does not directly correlate with classical apoptosis signaling.

Hydrogen peroxide has been evaluated both historically and in modern clinical contexts for its biological activity, including enhanced tumor reduction when combined with radiotherapy in human studies (34, 35) as well as earlier therapeutic applications in infectious and ischemic conditions (36–40). Together, these findings support the biological activity of systemic H_2O_2, which the present study extends to a cervical cancer mouse model with quantitative evidence of tumor growth inhibition.

Overall, the results demonstrate that Imoxide, particularly via intraperitoneal administration, produces substantial tumor growth suppression with fewer adverse effects compared with high-dose vitamin C. Although apoptosis-related pathways were not significantly modulated, the pronounced antitumor response highlights the need for future studies to elucidate alternative oxidative stress–mediated mechanisms and optimize therapeutic strategies.

Conclusion

In a mouse model of cervical cancer, intraperitoneal and intratumoral administration of Imoxide, as well as high-dose intraperitoneal vitamin C, resulted in marked tumor growth inhibition. Among the treatment groups, intraperitoneal Imoxide demonstrated the greatest reduction in tumor size. However, gene-expression analysis indicated that apoptosis-related pathways were not significantly modulated after multiple-testing correction, suggesting that alternative mechanisms may underlie the observed antitumor effects. Overall, Imoxide administration appeared to be systemically well tolerated, with substantial tumor suppression and no evident adverse effects compared with vitamin C. While these findings highlight the potential of Imoxide as an antitumor agent, further mechanistic and translational studies are required before consideration of clinical application, particularly in treatment-resistant or metastatic tumors.

Acknowledgment

The study was funded by Mashhad University of Medical Sciences' Vice-Chancellor for Research. The results are part of a student's thesis (Grant No. 992064). The funder did not influence study design, data collection, analysis, interpretation, manuscript writing, or publication decision.

Funding

The study received funding from the Vice-Chancellor of Research at Mashhad University of Medical Sciences in

Mashhad, Iran (Grant No. 992064).

Data Availability

Data will be made available upon request.

Authors' Contributions

T D helped with investigation, data curation, writing the original draft, review, and editing. SM H, O R, and S Z provided the concept and methodology. J D, SE N, and B M performed investigation and data curation. M M performed statistical analysis. H S provided methodology and acquired resources. M KH performed project administration, provided methodology and supervision, and acquired resources. Z M performed project administration, provided methodology and supervision, acquired resources, and helped conceive the study.

Conflicts of Interest

There are no conflicts of interest.

Declaration

The author used Grammarly and ChatGPT for grammatical correction and language polishing. The final content was reviewed and edited by the author, who takes full responsibility for the text.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-249. Yuan M, Hong Y, Feng Y, Sun J, Zhao X, Hu S, Zhao F. Cervical cancer incidence and mortality trends in China: The role of screening. Cancer Lett 2026; 27:218286.
2. Oliveira CR, Shapiro ED, Sheth SS, Ellingson MK, Johnson NP, Sullivan EL, Querec TD, Unger ER, Niccolai LM. Clinical effectiveness of HPV vaccine by age at vaccination: A matched case-control study. Lancet Reg Health Am 2025; 51:101225.4. Tin KN, Ngamjarus C, Rattanakanokchai S, Sothornwit J, Aue-Aungkul A, Paing AK, Pattanittum P, Jampathong N, Lumbiganon P. Interventions to increase the uptake of cervical cancer screening in low-and middle-income countries: A systematic review and meta-analysis. BMC Women's Health 2023; 23:120.
5. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem. World Health Organization; 2020.
6. Shen X, Guo W, Yao C, Wu H, Xiang Y, Meng Q. Epidemiological analysis of human papillomavirus and its subtype infections in 36,248 women in Wuhan, China. Front Public Health 2026;14:1745604.
7. Lorenzi AT, Syrjänen KJ, Longatto-Filho A. Human papillomavirus (HPV) screening and cervical cancer burden. A Brazilian perspective. Virology 2015;12:112.
8. Bernard HU, Burk RD, Chen Z, Van Doorslaer K, Zur Hausen H, De Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010;401:70-79.
9. Ljubojevic S, Skerlev M. HPV-associated diseases. Clin Dermatol 2014;32:227-234.
10. Bzhalava D, Eklund C, Dillner J. International standardization and classification of human papillomavirus types. Virology 2015;476:341-344.
11. Abouelkhair MA, Kennedy M. Papillomaviridae and Polyomaviridae. Vet Microbiol 2022:484-888.
12. Mendoza RP, Haidary T, Gabutan E, Zhou YY, Bukhari Z, Connelly C, *et al.* Mixed and nonvaccine high risk HPV types are associated with higher mortality in Black women with cervical cancer. Sci Rep 2021;11:14064.
13. Mittelstadt S, Kelemen O, Admard J, Gschwind A, Koch A, Wörz S, *et al.* Detection of circulating cell-free HPV DNA of 13 HPV types for patients with cervical cancer as potential biomarker to monitor therapy response and to detect relapse. Br J Cancer 2023;128:2097-2103.
14. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, *et al.* Role of a p53 polymorphism in the development of human papilloma-virus-associated cancer. Nature 1998;393:229-234.
15. Chrysostomou AC, Stylianou DC, Constantinidou A, Kostrikis LG. Cervical cancer screening programs in Europe: The transition towards HPV vaccination and population-based HPV testing. Viruses 2018;10:729.
16. Tao L, Han L, Li X, Gao Q, Pan L, Wu L, *et al.* Prevalence and risk factors for cervical neoplasia: A cervical cancer screening program in Beijing. BMC Public Health 2014;14:1185.
17. Chen G, Zheng P, Gao L, Zhao J, Wang Y, Qin W. Prevalence and genotype distribution of human papillomavirus in women with cervical cancer or cervical intraepithelial neoplasia in Henan province, central China. J Med Virol 2020;92:3743-3749.
18. Lismont C, Revenco I, Fransen M. Peroxisomal hydrogen peroxide metabolism and signaling in health and disease. Int J Mol Sci 2019;20:3673.
19. Sinenko SA, Starkova TY, Kuzmin AA, Tomilin AN. Physiological signaling functions of reactive oxygen species in stem cells: from flies to man. Fron Cell Dev Biol 2021;9:714370.
20. Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Reactive oxygen species and cancer paradox: To promote or to suppress?. Free Radic Biol Med 2017; 104:144-164.
21. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, *et al.* ROS in cancer therapy: The bright side of the moon. Exp Mol Med 2020;52:192-203.
22. Kim SJ, Kim HS, Seo YR. Understanding of ROS-inducing strategy in anticancer therapy. Oxid Med Cell Longev 2019;2019:5381692.
23. Mojić M, Pristov JB, Maksimović-Ivanić D, Jones DR, Stanić M, Mijatović S, Spasojević I. Extracellular iron diminishes anticancer effects of vitamin C: An *in vitro* study. Sci Rep 2014;4:5955.24. Di Tano M, Raucci F, Vernieri C, Caffa I, Buono R, Fanti M, *et al.* Synergistic effect of fasting-mimicking diet and vitamin C against KRAS mutated cancers. Nat Commun 2020;11:2332.
25. Schoenfeld JD, Sibenaller ZA, Mapuskar KA, Wagner BA, Cramer-Morales KL, Furqan M, *et al.* $O_2^{\cdot-}$ and H_2O_2-mediated disruption of Fe metabolism causes the differential susceptibility of NSCLC and GBM cancer cells to pharmacological ascorbate. Cancer Cell 2017;31:487-500.
26. Du J, Cieslak III JA, Welsh JL, Sibenaller ZA, Allen BG, Wagner BA, *et al.* Pharmacological ascorbate radiosensitizes pancreatic cancer. Cancer Res 2015;75:3314-3326.
27. Giri A, Sundar IK. Evaluation of stable reference genes for qPCR normalization in circadian studies related to lung inflammation and injury in mouse model. Sci Rep 2022;12:1764.
28. Ma L, Wang R, Dong W, Li Y, Xu B, Zhang J, Zhao Z. Long-term caloric restriction in mice may prevent age-related learning impairment via suppression of apoptosis. Behav Brain Res 2016;315:45-50.
29. Pan XH, Zhang XJ, Yao X, Tian NN, Yang ZL, Wang K, *et al.* Effects and mechanisms of mUCMSCs on ovarian structure and function in naturally ageing C57 mice. J Ovarian Res 2021;14:133.
30. Szarka A, Kapuy O, Lőrincz T, Bánhegyi G. Vitamin C and cell death. Antioxid Redox Signal 2021;34:831-844.
31. Al Shoyaib A, Archie SR, Karamyan VT. Intraperitoneal route of drug administration: Should it be used in experimental animal studies? Pharm Res 2020;37:12.
32. Chen J, Qin F, Li Y, Mo S, Deng K, Huang Y, Liang W. High-dose vitamin C tends to kill colorectal cancer with high MALAT1 expression. J Oncol 2020;2020:2621308.
33. Chen Q, Espey MG, Sun AY, Pooput C, Kirk KL, Krishna MC,

et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci U S A 2008;105:11105-11109.
34. Ogawa Y, Kubota K, Ue H, Kataoka Y, Tadokoro M, Miyatake K, *et al*. Phase I study of a new radiosensitizer containing hydrogen peroxide and sodium hyaluronate for topical tumor injection: A new enzyme-targeting radiosensitization treatment, Kochi Oxydol-Radiation Therapy for Unresectable Carcinomas, Type II (KORTUC II). Int J Oncol 2009;34:609-618.
35. Nimalasena S, Gothard L, Anbalagan S, Allen S, Sinnett V, Mohammed K, *et al*. Intratumoral hydrogen peroxide with radiation therapy in locally advanced breast cancer: results from a phase 1 clinical trial. Int J Radiat Oncol Biol Phys 2020;108:1019-1029.
36. Love IN. Peroxide of hydrogen as a remedial agent.: Read before the St. Louis Medical Society, February 4, 1888. JAMA 1888;10:262-265.
37. Oliver TH, Murphy DV. Influenzal pneumonia: The intravenous injection of hydrogen peroxide. Lancet 1920;195:432-433.
38. Finney JW, Urschel HC, Balla GA, Race GJ, Jay BE, Pingree HP *et al*. Protection of the ischemic heart with DMSO alone or DMSO with hydrogen peroxide. Ann N Y Acad Sci 1967;141:231-241.
39. Urschel Jr HC. Progress in cardiovascular surgery: Cardiovascular effects of hydrogen peroxide: Current status. Dis Chest 1967;51:180-192.
40. Armogida M, Nisticò R, Mercuri NB. Therapeutic potential of targeting hydrogen peroxide metabolism in the treatment of brain ischaemia. Br J Pharmacol 2012;166:1211-1224.