Hepatoprotective Effects of Opuntia dillenii Haw Fruit in a Mice Model of Lung Cancer

Reza Shirazinia 1, Goudarz Sadeghi Hashjin1*, Ahad Muhammadnejad2

1Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Introduction: Lung cancer is an important burden, causing a massive rate of deaths every year. Using a specialized treatment regimen can improve treatment outcomes and reduce treatment-related adverse events. This study aimed to evaluate the protective effects of the Opuntia dillenii fruit hydroalcoholic extract (ODHAE) in a mouse model of lung cancer.

Methods: Eight groups of lung cancer-induced BALB/c mice (each containing twelve animals) were included in this study. They were the control group, groups receiving the ODHAE extract at doses of 50, 100, and 200 mg/kg by oral gavage, the cisplatin group receiving cisplatin intraperitoneal injection, and groups receiving cisplatin and different doses of ODHAE (50, 100, and 200 mg/kg). Weight and tumor changes were evaluated in the short and long phases of the study, including 30 and 90 days, respectively. Liver transaminase and survival time were evaluated in different groups. Malondialdehyde (MDA) was evaluated in different groups as an indicator of lipid peroxidation. Statistical tests were performed using GraphPad Prism 8. Graphs were designed using GraphPad Prism 8 as well.

Results: The observed weight changes were not statistically significant across all groups, except for group 8. However, in groups that did not receive cisplatin treatment, there was a significant increase in tumor volume. Conversely, tumor volume change did not reach statistical significance in all groups receiving cisplatin. The administration of ODHAE, along with cisplatin, demonstrated a dose-dependent increase in the survival time among the relevant groups. Furthermore, oral administration of ODHAE exhibited a significant reduction in transaminase levels, serving as a reliable biomarker for assessing the hepatoprotective effect of the ODHAE extract. Additionally, ODHAE displayed a dose-dependent reduction in MDA levels, indicating its potential as a therapeutic agent for mitigating oxidative stress.

Conclusion: The administration of ODHAE was not effective in reducing tumor growth but significantly increased survival time and healed the liver injury induced by cisplatin.

Keywords: Lung cancer, Mouse, Opuntia dillenii, Hydroalcoholic extract, Liver

Introduction

Cancer is a pathological condition characterized by the uncontrolled proliferation of cells in the body, which may also exhibit metastatic behavior. Failure to initiate prompt treatment may result in severe and irreversible consequences. Extant research indicates that virtually all malignant neoplasms possess the capacity to manifest unregulated and disseminated growth patterns.1,2 In 2019, the global prevalence and incidence rates of lung cancer were recorded at 38.84 per 100,000 individuals and 27.66 per 100,000 individuals, respectively.3 Lung cancer remains the primary cause of cancer-related fatalities on a global scale. Nevertheless, the incidence and mortality rates of lung cancer exhibit significant variations across different regions, representing diverse patterns of tobacco smoking, exposure to environmental risk factors, and genetic factors. Notably, tobacco smoking stands as the foremost risk factor associated with the development of lung cancer.4 It is important to highlight that lung cancer continues to be the leading cause of cancer-related mortality, accounting for 1.8 million deaths annually worldwide.5

Opuntia dillenii, belonging to the Cactaceae family, is widely recognized for its medicinal properties and is cultivated in various countries, particularly in desert, semi-desert, tropical, and subtropical regions. This fruit


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possesses a wide range of pharmacological attributes, including antioxidant, anti-inflammatory, anti-tumor, neuroprotective, liver-protective, and hypotensive effects. The therapeutic potential of *O. dillenii* is attributed to its rich composition of essential components, such as betaines, ascorbic acid, total phenol, protein, and essential elements. These constituents are believed to contribute significantly to the plant’s pharmacological properties.6

Scientific investigations have underscored the plant’s capacity for synthesizing valuable compounds with potential commercial applications in herbal medicine. Additionally, numerous studies have extensively examined the pharmacological properties of this plant family, encompassing anti-inflammatory, antioxidant, neuroprotective, antidiabetic, analgesic, hypotensive, antibacterial, and hepatoprotective effects. A study was conducted to investigate the potential anticancer properties of *O. dillenii*, specifically its fruit extract, by examining its ability to induce apoptosis in a lung carcinoma cell line. The findings revealed that the isolated polysaccharides from this plant demonstrated inhibitory effects on cell division and growth within the tested cell line. In addition, treatment with this plant extract led to cell cycle arrest in the S-phase, effectively impeding the proliferation of cancer cells.

The extract induced apoptosis, as evidenced by Annexin V evaluation, indicating promising anticancer effects on specific cell lines, although in an in vitro setting. Another study also demonstrated the hepatoprotective effects of the hydroalcoholic extract of *O. dillenii* on lead acetate-induced liver toxicity. Given the beneficial pharmacological properties of *O. dillenii*, the present study sought to evaluate the protective effects of *O. dillenii* fruit hydroalcoholic extract (ODHAE) in a mouse model of lung cancer.

Materials and Methods

**Experiment Design**

This study was performed in two distinct phases, namely, a short phase spanning the first month of the study and a subsequent long phase lasting 90 days. Each experimental group consisted of 12 mice, with 6 mice being sacrificed after the short phase, while the remaining 6 mice were included in the long phase to assess their survival time. At the end of the initial 30-day period (short phase), 6 mice from each study group were euthanized, and blood samples were collected for subsequent analysis. These collected blood samples were utilized to evaluate the levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). The remaining animals were subjected to a long-term phase as part of the study.

**Preparation of the Extract**

The method of extraction was based on the previous studies with slight modifications. The fruit of the *O. dillenii* was collected from a local market in May 2021 in Zahedan, Sistan and Baluchestan province, Iran. The plant and fruits were evaluated, and the samples were stored in the School of Pharmacy Herbarium, Mashhad University of Medical Sciences, Iran (No. 13161).

To prepare the extract, the seeds were carefully extracted, followed by a drying process in a controlled environment where fresh air and shade were provided. The powdered fruits underwent maceration in a mixture of ethanol and water (50% V/V) at room temperature for 48 hours with intermittent shaking. Subsequently, the extracts were subjected to filtration using Whatman® No. 4 filter paper, resulting in the removal of solid particulates. The resulting liquid extract was concentrated at 40 °C using a rotary evaporator and subjected to incubation at the same temperature to remove any residual organic solvents. A yield of 100 mL of the pure extract was obtained from every 500 g of fruit powder.

**Cell Culture**

The LL/2 (LLc1) cells were cultured in Dulbecco’s Modified Eagle medium containing 10% fetal bovine serum, 100 units per mL of penicillin, and 100 μg/mL of streptomycin in a controlled environment containing 5% carbon dioxide at 37 °C.

**Tumor Induction**

The LL/2 (LLc1) cell line was used for cancer induction. The cells were cultured in the cell culture medium until the number reached 10 × 10⁶ cells/mL, then 1/10 mL of the culture solution was suspended in ice-cold phosphate-buffered saline and inoculated in mice using subcutaneous injection. Seven to fifteen days were considered for tumor inoculation (Figure 1).

**Animals**

Overall, 96 male BALB/c mice (22 ± 1.9 g) were used in this study. Mice were kept in standard cages under controlled lighting conditions of 12 hours light and 12 hours dark and a constant temperature (23 ± 2 °C) with

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Figure 1. Tumor-Bearing Mice
ad libitum access to pellets (Javaneh-Khorasan, Iran) and fresh water.

Cisplatin was employed as a standard drug as it is a highly prescribed chemotherapeutic medication and is extensively utilized in the treatment of various malignancies affecting both pediatric and adult populations. It proves effective in a diverse range of cancers, including the ovaries, testicles, bladder, head, neck, breasts, and lungs.\textsuperscript{15}

Tumor-bearing mice were classified into 8 groups, each containing 12 animals:

- **G1** (control group): This group received daily distilled water orally for the first month (short phase) but received physiological saline (0.9% w/v NaCl, i.p.) at the same volume as other groups during cisplatin administration.
- **G2** (ODHAE 50): It received ODHAE (50 mg/kg/d, p.o.) during the short phase of the study.
- **G3** (ODHAE 100): This group received ODHAE (100 mg/kg/d, p.o.) during the short phase of the study.
- **G4** (ODHAE 200): It received ODHAE (200 mg/kg/d, p.o.) during the short phase of the study.
- **G5** (Cisplatin): This group received cisplatin (5 mg/kg/d, i.p.) during the 6\textsuperscript{th}, 11\textsuperscript{th}, 16\textsuperscript{th}, and 21\textsuperscript{st} days of the short phase.
- **G6** (ODHAE 50 + Cisplatin): It received ODHAE (50 mg/kg/d, p.o.) and cisplatin (5 mg/kg/d, i.p.) during the 6\textsuperscript{th}, 11\textsuperscript{th}, 16\textsuperscript{th}, and 21\textsuperscript{st} days during the short phase.
- **G7** (ODHAE 100 + Cisplatin): This group received ODHAE (100 mg/kg/d, p.o.) and cisplatin (5 mg/kg/d, i.p.) during the 6\textsuperscript{th}, 11\textsuperscript{th}, 16\textsuperscript{th}, and 21\textsuperscript{st} days during the short phase.
- **G8** (ODHAE 200 + Cisplatin): It received ODHAE (200 mg/kg/d, p.o.) and cisplatin (5 mg/kg/d, i.p.) during the 6\textsuperscript{th}, 11\textsuperscript{th}, 16\textsuperscript{th}, and 21\textsuperscript{st} days during the short phase.

### Evaluation of Biochemical Parameters

In each study group, 6 mice were euthanized at the end of 30 days (short phase), and blood samples were collected to evaluate AST, ALT, and ALP using relevant commercial kits and the Selectra Pro, M autoanalyzer (Vital Scientific, SpanNeren, Netherlands).

### Evaluation of Lipid Peroxidation

In each study group, 6 mice were euthanized at the end of 30 days (short phase), and malondialdehyde (MDA) levels were measured using the relevant commercial biochemistry kits (TEB PAZHOUHAN RAZI) according to the manufacturer’s instructions.

### Tumor Growth Kinetics and Weight Change

To investigate the tumor growth kinetics and weight change in the short- and long-term phases of the study, the mice were weighed on days 0, 2, 5, 8, 11, 14, 18, 21, 24, and 28 of each month. The size of the tumors was measured using a ruler, and the tumor volume was calculated using the following formula:\textsuperscript{9}

\[
\text{Tumor volume} = \frac{\text{length} \times \text{width}^2}{2}
\]

### Survival Rate

The mice used in the long-term phase were kept for 90 days to evaluate their survival time. The survival rate and Kaplan-Meier curve were provided using GraphPad Prism 8.

### Statistical Tests

GraphPad Prism 8 for Windows (San Diego, CA) was utilized for statistical analysis. The data on oxidative stress and transaminases were expressed as means ± standard deviations (SD) and analyzed by a one-way analysis of variance (ANOVA) using Tukey’s posttest. The tumor growth and weight changes were evaluated using repeated measure ANOVA in GraphPad Prism 8 as well.

### Results

#### Weight Change

The analysis of weight changes during the short phase of the study revealed that significant weight changes were only observed in the G8 from day zero to day 28 (\(P<0.05\)). No significant weight changes were observed in any other study groups, although it is noteworthy that all groups, except for the control group (G1) group, experienced weight increases during the study (Figure 2). Moreover, monthly weight changes were recorded in the mice during the long phase of the study. The results showed that weight changes in the long phase were not significant in all groups (Figure 3).

#### Tumor Growth

The results revealed a statistically significant rise in the
tumor volume between the initial and final stages of the short phase within the groups (G1-G4) that did not receive cisplatin ($P<0.05$ to $0.01$ for all groups, Figure 4). Furthermore, at the end of the short phase (day 28), tumor growth in G1 was significantly higher than that observed in the cisplatin-receiving groups ($P<0.05$ to $0.01$ for all groups). During the first, second, and third months of the long phase, the groups who received no cisplatin (G1-G4) experienced a more dramatic increase in the volume of the tumor. Moreover, this was significant in the 1st and 3rd months of the long phase and in the 2nd month of the long phase, except for G1 ($P<0.05$ to $0.001$ for all groups (Figure 5).
Effects of Opuntia Dillenii Fruit Hydroalcoholic Extract on the Serum Levels of Liver Function Enzymes

The administration of cisplatin significantly increased the level of transaminases in comparison with other groups. Additionally, the administration of ODHAE significantly reduced the level of transaminases in a dose-dependent manner ($P<0.05$ to $0.001$, Table 1).

Effects of Opuntia Dillenii Fruit Hydroalcoholic Extract on the Serum Levels of Lipid Peroxidation

The administration of cisplatin increased the levels of MDA as an indicator of lipid peroxidation when compared to other groups, and ODHAE administration significantly reduced the level of MDA in comparison with the G5 group in a dose-dependent manner ($P<0.05$ to $0.01$, Table 1).

Survival Rate

The administration of ODHAE significantly increased the survival rate in the cisplatin-receiving groups in comparison to the groups that received no cisplatin (G1-G4; $P<0.01$ to $0.05$ for all groups, Figure 6). Moreover, the cisplatin-alone group (G5) had a significantly higher rate of survival compared to the G3 group ($P<0.05$).

Discussion

The objective of this study was to assess the impact of O. dillenii on LL/2 (LLc1) tumor growth and weight change in a mouse model of lung cancer. The results showed that the extract had no inhibitory effects on tumor growth at the examined doses. Additionally, the weight gain induced by the extract was not significant in the different treatment groups.

ODHAE administration resulted in a significant reduction of the cisplatin-induced hepatic side effects and improved survival rates. These effects were accompanied by a significant decrease in liver enzymes. The findings suggest that ODHAE may have a beneficial effect when used in combination with cisplatin chemotherapy. In a study for the evaluation of the effects of the O. dillenii extract on skin melanoma cells, the results indicated that the extract could stimulate cell growth, as confirmed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay results. However, these findings may align with the results of the current study.16

In another study conducted by Li et al to investigate the anti-cancer effects of O. dillenii, the results indicated that the polysaccharides isolated from this plant exhibited anti-cancer effects in the SK-MES-1 cell line, as confirmed by MTT assay results. However, the findings of this study may differ from those of the present study due to the differences in cell lines and applied experimental methods.17

A study for the evaluation of the anticancer effects of the O. dillenii indica revealed that glycosides isolated from this plan have anti-tumor effects in the HT-29 cell line. It also discussed the molecular mechanism underlying cytotoxicity, which involves a complex series of events, including cell cycle arrest, the formation of apoptotic bodies, nuclear condensation, exposure to phosphatidylserine, and mitochondrial dysfunction.17 Another study investigating the protective effects of cactus seed oil on gentamicin-induced renal toxicity demonstrated that the cactus seed oil extract had no significant effect on weight changes in study groups,11 which conforms to the results of the present study.

The present study elucidates the protective effects of ODHAE against cisplatin-induced hepatic toxicity. Extensive research has consistently demonstrated the hepatotoxic effects induced by cisplatin, whereby the generation of free radicals plays a pivotal role in...
triggering oxidative stress, inflammation, and apoptosis within hepatic cells. Consequently, the utilization of natural compounds possessing potent antioxidant properties assumes paramount importance in effectively countering and ameliorating cisplatin-induced hepatotoxicity.\textsuperscript{18} The antioxidant effects of ODHAE are evaluated in the present study, revealing that ODHAE significantly reduced cisplatin-induced oxidative stress, as indicated by a significant reduction in MDA.

A review study of the pharmacological effects of \textit{O. dillenii} highlighted its prominent antioxidant and anti-inflammatory properties as its key pharmacological effects. The active compounds present in the fruit extract interact directly with free radicals, effectively modulating their activity and thereby providing protection against oxidative stress.\textsuperscript{6} In another study conducted by the same researcher, the protective effects of the \textit{O. dillenii} hydroalcoholic extract against lead acetate-induced hepatic toxicity were investigated, and the results revealed its dose-dependent hepatoprotective effects.\textsuperscript{8} These findings are consistent with those of the present study.

Furthermore, this study showed that ODHAE can increase survival rates in a dose-dependent manner. This effect may be partly due to the hepatoprotective and antioxidant effects of the ODHAE, as there was a significant difference in survival rates between the groups receiving doses of 100 and 200 mg/kg of the extract compared to the control group. In another study conducted on alloxan-induced diabetes in mice, the consumption of \textit{O. dillenii-indica} fruit extract was found to significantly increase their survival rates due to antioxidant mechanisms and prevent diabetes-induced mortality. These results conform to the findings of the present study. Inflammation is a natural response for protection against different injured tissues and is an important part of the homeostatic responses of the immune system, but prolonged and uncontrolled inflammation definitely can cause unwanted consequences, even leading to a reduced survival rate.\textsuperscript{19,20} The increased survival rate in the present study may also be partly due to the anti-inflammatory effects of \textit{O. dillenii}; however, more investigations are needed to elucidate this hypothesis.\textsuperscript{21}

\textbf{Conclusion}

This study evaluated the beneficial effects of ODHAE as a concomitant treatment with cisplatin to reduce the relevant side effects and increase efficacy in vivo. However, it did not investigate the in vitro effects of the ODHAE on LL/2 (LLc1) and different cell lines, and the in vitro evaluation of the ODHAE on LL/2 (LLc1) may be a promising option for future evaluations.

\textbf{Authors’ Contribution}

\textit{Conceptualization:} Goudarz Sadeghi Hashjin.  
\textit{Data curation:} Goudarz Sadeghi Hashjin, Reza Shirazinia.  
\textit{Formal analysis:} Reza Shirazinia.  
\textit{Funding acquisition:} Goudarz Sadeghi Hashjin.  
\textit{Investigation:} Goudarz Sadeghi Hashjin.  
\textit{Methodology:} Goudarz Sadeghi Hashjin, Reza Shirazinia.  
\textit{Project administration:} Goudarz Sadeghi Hashjin.  
\textit{Resources:} Reza Shirazinia.  
\textit{Software:} Reza Shirazinia.  
\textit{Supervision:} Goudarz Sadeghi Hashjin, Ahad Muhammandnejad.  
\textit{Validation:} Goudarz Sadeghi Hashjin, Ahad Muhammandnejad.  
\textit{Visualization:} Goudarz Sadeghi Hashjin.  
\textit{Writing–original draft:} Reza Shirazinia.  
\textit{Writing–review & editing:} Goudarz Sadeghi Hashjin, reza Shirazinia.

\textbf{Competing Interests}

The authors have no conflict of interest to declare.

\textbf{Ethical Approval}

The Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, approved the procedure (IR.UT.VETMED.REC.1402.002).

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