Cytotoxicity of the Methanol Extract of *Datura innoxia* Petals on MCF-7 and HEK-293 Cell Lines

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Abstract

Background: Medicinal plants are traditionally used to prevent and treat various diseases, including cancer. Despite the development of advanced methods of treatment, cancer mortality is still increasing every year. Medicinal plants are one of the most important sources of anticancer agents.

Objectives: The present study was conducted to evaluate the cytotoxic effects of the methanolic extracts of *Datura innoxia* petals, native to central and South America and distributed in Africa, Asia, Australia, and Europe, on the breast cancer cell line (MCF-7).

Materials and Methods: The petals were collected, cleaned, and powdered. The extraction was conducted using the maceration method and then filtered, centrifuged, freeze-dried, and kept at 4°C. The MCF-7 and HEK-293 cell lines were treated with the methanolic extract of *D. innoxia* petals at various dilutions. Cell viability was quantitated using the MTT assay after 48 and 72 hours.

Conclusions: These findings indicate that the methanolic extracts of *D. innoxia* petals can act as a possible food supplement with anticancer effects and can be used after complementary tests.

Keywords: Cytotoxicity, MCF-7, HEK-293, Methanolic Extract, Datura innoxia

1. Background

Cancer is the second leading cause of death in the world. Although great advances have been made in the treatment and control of this disease, significant shortcomings remain to prevent further development. Some unwanted side effects are often observed during chemotherapy. Hence, natural treatments, such as the use of plant-derived products, may reduce these harmful side effects. At present, there are few herbal products, which have shown anticancer effects in vitro, but are still being evaluated in humans. Therefore, it is essential that future studies indicate the beneficial effects of these plants for the treatment of cancer in humans.

*Datura innoxia* is a type of wild grass that belongs to the Solanaceae family. Its name is derived from the word Dhutra meaning divine; therefore, it is named after its healing properties. Many varieties of Datura are known and are widely used for their medicinal properties and toxicity that is associated with the presence of more than 30 different alkaloids. The species such as *D. innoxia*, *D.stramonium*, and *D.wrightii* are cultivated as ornamental plants due to the funnel-shaped forms and fragrant blossoms at night; however, *D.metel* is known as a wild variety. Its chopped dried roots are used to reduce fever. The poultice from which it is made is also used for the treatment of inflammation and bruising (1). Furthermore, the plant in China known as Yangjinhuais known and used for the treatment of asthma, epilepsy, pain, and rheumatism (2), while *D. stramonium* is mainly used due to its toxic effects. Toxication with datura is very common in India. Unintentional poisoning is observed suddenly or when an extraction is completed (3). Datura poisoning symptoms, therefore, include delirium, excitement, seizures, dilated pupils, dry mouth, nausea and vomiting, blood pressure, and coma (4-6). Alkaloids inspecies such as *D. stramonium* belongs to the tropane alkaloid groups (7-9). *D. innoxia* alkaloids show a similar pattern (10).

2. Objectives

The main purpose of this study was to investigate the cytotoxic effects of plant extracts on the MCF-7 breast cancer cell line and the non-malignant cell line, HEK-293, in vitro.
3. Materials and Methods

3.1. Plant Materials

*D. innoxia* petals were collected from Sabzevar city in September 2014. In addition, the breast cancer cell line MCF-7 and non-malignant cell line HEK-293 were used in this study and were purchased from the cell bank of the Pasteur institute of Iran.

3.2. Extraction

Following the drying of the petals, samples were powdered in the absence of light. The extraction was completed using the maceration method. For this purpose, the petals were dried (4g) and then incubated for 12 days in the presence of methanol (50 \times 2 \text{ mL}) at room temperature.

The materials were then filtered and the clear supernatant was then concentrated under a reduced pressure with a vacuum rotary evaporator at 40°C. Finally, the residue (1.1 g) was used in the following experiments, whereby the extract was kept at 4°C.

3.3. Determination of Total Phenolic Content (TPC)

For determining TPC of the extract, Folin-Ciocalteu's reagent method was applied. In brief, a 0.5 mL of FCR solution (10% in distilled water) was added to a test tube containing 0.5mL of extract (800 µg/mL in methanol) and 1.5 mL of distilled water. This mixture was thoroughly shaken. After 5 minutes, 2 mL of 10% sodium carbonate solution was added and the mixture was shaken for a second time. The mixture was then incubated in the dark for 2 hours at room temperature. The absorbance was measured at 760 nm with a UV-VIS spectrophotometer. The analyses were conducted in triplicate. Moreover, a gallic acid standard curve was completed (5 - 100 µg/mL). TPC was determined in µg of gallic acid equivalents (GAE)/g (11, 12).

3.4. MTT Assay

One of the methods used to evaluate the cytotoxic effects of different materials was the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) diagnostic test. The basis of this method regards specific metabolic reactions in the mitochondria of living cells. On mitochondrial metabolism, MTT in the mitochondria changed into solid crystals and formed a purple formazan. After the dissolution, different values of crystals in solvents, such as DMSO, formed varying degrees of a purple color. Measuring the absorbance of the solution at a wave length of 545 nm it can be attributed to the optical density of each well to a number of metabolizing MTT viable cells (13).

In this study, the cytotoxic effects of the methanol extract of the petals of *D. innoxia* on the breast cancer cell line MCF-7 and non-malignant cell line HEK-293 was evaluated by MTT assay. Initially, to study the cytotoxic effects of the extract on the cell lines, extracts at concentrations between 5 µg/mL and 100 µg/mL of this material were prepared at 48 and 72 hours after treatment, where the viability percentage of the cells was evaluated by the MTT assay.

The cytotoxic effects of different extract concentrations for each time range was performed for the cell line and then used to determine the most effective concentrations and times for these effects. Following this, curves for the extract concentration and cell viability percentage were drawn, and finally, the IC$_{50}$ concentration was calculated.

4. Results

4.1. TPC

The TPC of the methanolic extract of *D. innoxia* petals was determined by the Folin-Ciocalteau assay. Therefore, the calibration curve of gallic acid was drawn (Figure 1). The amount of phenol in the methanolic extract was 105.684 ± 0.513 mg GAE/g extract.

![Figure 1. Standard Graph of Gallic Acid for TPC Determination](image)

4.2. Cytotoxic Effect

Figures 2 and 3 show the results of the MTT assay by measuring the optical density (OD) based on concentrations compared with the rate of cellular viability. The percentage of living cells that had received the extract were calculated using the following formula (14).

Equation 1.

\[
\text{Cell Viability, \%} = \left( \frac{\text{Sample optical density}}{\text{Control optical density}} \right) \times 100
\]

The illustrations showed the effects of the different extract concentrations on cells after 48 and 72 hours, respectively. These findings suggest that the extract will have little effect on non-malignant cells after 48 hours; however, at concentrations of 50 - 100 µg/mL the extract exhibits a high inhibitory effect on tumor cells. Comparing these illustrations, 50% of the cell growth inhibitory concentration...
A study was performed on the plant extracts of different organs and it became clear that *D. innoxia* contains compounds such as alkaloids, saponins, flavonoids, essential oils, and phenolic compounds (16). In a study completed on the leaves and stems of the *D. metel* extracts, it was confirmed that there was anticancer activity against the cancer cell line MCF-7. The leaf extract also had more anti-cancer ability and contained plant compounds such as alkaloids, sterols, saponins, phenols, tannins, and flavonoids (17). In another study, the phytochemical screening of *D. innoxia* petals revealed the presence of alkaloids, saponins, flavonoids, coumarins, and tannins. In addition, the results showed that the methanolic extracts of petals of the *D. innoxia* plant have the potential to be an antioxidant (18). As aforementioned, *D. innoxia*, containing a combination of several compounds, is reported to have anticancer effects for these compounds. Therefore, it can be concluded that the anticancer effects observed in the extract are associated with the presence of these compounds.

Furthermore, the steroids in this genus have anticancer activity against the colon cancer cell line, HCT-116 (19, 20). Many species of the solanaceae family are rich in calystegin, which has glycosidic inhibitory properties against cancer (4). In another study, the anticancer effects of methanol extracts on the leaves of the *D. innoxia* in vitro through the induction of apoptosis in cancer cell lines, such as Hep-2 and adenocarcinoma of the colon (HCT-15) was studied. The anticancer effects after 48 hours of incubation, regarding HCT-15 and Hep-2 cell lines with methanolic extract leaves of *D. innoxia*, have been found (21). In another study, which was conducted on the seeds of the *D. innoxia*, the anticancer effects of this plant on the Hela cell line was established.

5. Discussion

The results of this study showed that the *D. innoxia* extract has cytotoxic effects on the breast cancer cell line MCF-7. The methanolic extract of this plant has a severe impact after 72 hours of cell treatment compared to that at 48 hours of incubation. However, the results indicate that the extract at 48 hours has less cytotoxic effects on normal cells. The effect on the cells was dose-dependent and therefore, significantly increased with a rising concentration. With an increasing time from 48 hours to 72 hours, there was significant change in IC\textsubscript{50} values. However, with this time change, the toxicity effect on normal cells greatly increased and was derived from the toxicity of the plant. The *D. stramonium* plant has anticancer effects on nasopharyngeal carcinoma at the treatment doses (15).

5.1. Conclusion

According to the results of this study and also in comparison with similar studies, it could be deduced that the *d. innoxia* extract having anticancer effect, is to be used in treatment of the breast cancer. However, further research is warranted to find effective compounds.

References


