Evaluation of Genotoxic effects of a Hydro-alcoholic extract of flowers of Nargis (*Narcissus Tazetta L.*)

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Abstract: The goal of this study was to determine the genotoxic potential of a hydroalcoholic extract of Nargis flowers (*Narcissus Tazetta L.*). The genotoxicity potential of standardized hydroalcoholic extract of Narcissus Tazetta L at different concentrations was assessed using human breast cancer cells applying the MTT test for cell cytotoxicity. In control cells, Nargis flowers extract did not totally break DNA, however, flowers-treated cells showed high amounts of damage in a dose-dependent way. According to the findings, the crude hydroalcoholic extract of Narcissus Tazetta (Nargis flowers) had a potent inhibitory effect on human breast carcinoma cancer cell proliferation, which was mediated by its pro-apoptotic antiproliferative activity.

Keywords: Hydroalcoholic Extract, *Narcissus Tazetta L.*, Breast Carcinoma, Apoptotic

1. Introduction

Adenocarcinomas of the breast are the most common kind of breast cancer. These tumors grow in glands or ducts that release fluid and are found in a variety of different malignancies. Breast adenocarcinomas develop in the lobules, or milk-producing glands, or in the milk ducts. The overwhelming amount of breast cancers are carcinomas, which are tumors that develop in the epithelial cells that line the body's organs and tissues. The most frequent type of carcinoma that occurs in the breast is adenocarcinoma, which begins in cells in the milk ducts or milk-producing glands. Due to its latent course, difficulties in early identification, metastasis, forceful invasion, and poor prognosis, human breast cancer is very deadly and aggressively malignant. The only curative treatment for breast carcinoma cancer is surgical resection. However, the end result is not always pleasing. Systemic therapies are drugs that can reach cancer cells practically anywhere in the body and are used to treat breast cancer. They can be taken orally or immediately injected into the bloodstream. Different sorts of pharmacological treatments may be employed depending on the type of breast cancer including chemotherapy, hormone therapy, targeted drug therapy and immunotherapy. Paclitaxel is probably the most well-known plant-derived anti-cancer medication.

Both natural compounds collected from plants or animals and synthetic compounds generated from natural prototype structures are currently being used as cancer treatments and chemo preventive agents.

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Recent investigations have shown that thymoquinone has cancer cell specific actions on many targets, implying that Nigella sativa could have a promising function as an anticancer drug (Padhye et al., 2008). The vinca alkaloids and taxanes are the two main groups of antimitotic medicines used to treat cancer. Taxanes are microtubule-targeting medicines that bind to polymerized microtubules, stabilize them, and prevent them from disassembling, resulting in cell death through apoptosis (Risinger et al., 2009; Moris & Fornier, 2008). In the twenty-first century, drug-based therapeutic techniques will predominate. As a result, finding novel medications that are effective against resistant cancers is a critical method for enhancing chemotherapy. Natural medications can be employed as chemical models as well as drug entities (Eddy et al., 2007).

Nargis (*Narcissus tazetta L.)* is a well-known traditional medicine medication from the Amaryllidaceae family. Narcissus is the Greek name for the genus. (Valadon & Mummery, 1968). Its bulb and flower have therapeutic characteristics and are used to treat headaches, colds, central nervous system illnesses, hair and skin ailments, arthralgia, alopecia areata, and gynecological issues etc., because of its deobstruent, detergent, demulcent, (Ying et al., 2018), anti-inflammatory, concoctive, rubefacient, absorbent, siccative, irrigative, and deobstruent characteristics (Kalam & Qayoom, n.d.), antibacterial, antiviral, antifungal, antimalarial, anticancer, antioxidant, immunomodulatory and acetylcholinesterase inhibitory activity.

Narcissus tazetta L. is a bulbous perennial plant that grows to be 38-43 cm tall with fresh, colorful, gracefully perfumed blossoms. Umbels can have up to eight flowers, each of which is white with a golden corona. The flowers feature a cup (trumpet) shaped corona with six petals, and a cup (trumpet) formed corona that is occasionally significantly decreased in size. The leaves range in color from light to dark green. Corona length is usually short and semi-spherical, and perianth segments are flat. The scent is frequently noticeable. Depending on the cultivar, flowers can be seen in clusters ranging from a few to over a dozen per stalk (Valadon & Mummery, 1968) (Hanks, n.d.) ; (Karakoyun et al., 2019).

In the east, the Narcissus flower is seen as a symbol of vanity, whereas in the west, it is regarded as a symbol of vanity. The primary flowering season is spring, but it can also be late fall or early summer. (Zhenzhen & Bin, 2016). Narcissus spp. are a few natural sources of phenethyl alcohol, a strong fragrance essential ingredient (Hanks, n.d.).

The plant, sometimes called Nargis or Daffodil, is a common species. Apart from its decorative appeal, the medical properties of this plant are detailed in the Unani System of Medicine's classical literature. It has anti-inflammatory, detergent, diuretic, emetic, purgative, and other properties. It is used to cure various ailments, including headaches, the common cold, and alopecia and CNS disease etc.

The alkaloids, flavonoids, saponins and tannins are present in the Narcissus bulb. The primary chemical constituents of *Narcissus tazetta L*. are essential oils, lectin, galantamine, haemanthamine, lycorine, pseudolycorine, narciclasine, narcisine, dimethyhomolycorine, pretazattine, tazettine and suisenine. Galantamine has been found helpful for alzehmier's disease. Chemical constituents of Narcissus tazetta L. are essential oils, lectin, galantamine, haemanthamine, lycorins, pseudolycorine, narciclasine etc. Galantamine has been found helpful for Alzheimer's disease. In addition, mucilage, T-glucomannan, and narcissus have also been extracted from bulbs that exhibit significant hypoglycaemic activity in mice (Zhenzhen & Bin, 2016); (Talib & Mahasneh, 2010); (Evans, 2009). Oils have been derived from flowers, bulbs and roots of the plant used as medicine.

There are very few pharmacological investigations on the blooms of this plant. This floral extract has shown some promise in the fight against cancer. Its anticancer properties stimulated substantial research into the structure-activity link. Lycorine, the principal representative alkaloid of Amaryllidaceae plants, has risen to prominence in biological activity, notably cancer (Karakoyun et al., 2019; Kintsurashvili & Kemoklidze, 2019). Regardless of cancer cell sensitivity to apoptotic cell death, haemanthamine showed intense anticancer activity in vitro (Karakoyun & Ünver-Somer, 2019).

2. Material and Methods

Fresh flowers of Nargis (*Narcissus Tazetta L.)* were collected from the Sulaimani province of Iraq, Hawraman area. (Figure 1 A and B). Fresh flowers of Nargis were collected to remove any impurities present and were dried at room temperature. The flowers were powered in a mortar and pestle, followed by the extraction process. 100 gm of powder was soaked in 600 ml of ethanol and kept in a seven-day shaker. After seven days, the extract was filtered and dried on the hot plate. A crude extract of flowers of 5.8 gm was obtained. This residue was stored in aseptic conditions.

A: Intimate view of the flower

B: The flower at harvesting point.

Figure 1: Flowers of Narcissus used in the study

2.1 Chemicals and Reagent

Gibco (USA) provided the DMEM medium, Fetal Bovine Serum (FBS), and antibiotic solution; Sigma (USA) provided DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazoliumbromide) (5 mg/ml); and Himedia provided 1X PBS (India). Tarson provided the 96-well tissue culture plate and the wash beaker (India).

Gibco (USA) provided the DMEM medium, Penicillin/Streptomycin antibiotic solution, and Trypsin-EDTA, and Thermo Scientific provided the Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit (USA). Fluorescence microscope with fluorescence.

2.2 Preparation of Plant Extracts

To prepare the final extract, the extraction mixture was heated to 90 °C on a steam bath and refluxed for 2 hours, then cooled in the refrigerator, sonicated for 5 minutes, and diluted to 50 ml with ddH2O. All of the experiments were done in duplicate. The tetrazolium/formazan (MTT) assay for cell cytotoxicity and Apoptosis Assay were used to determine cell viability.

2.2.1 Cell Culture

The MCF-7 (Human breast carcinoma cells) cell line was bought from NCCS in Pune and cultivated in liquid medium (DMEM) supplemented with 10 % Fetal Bovine Serum (FBS), 100 u/ml penicillin, and 100 g/ml streptomycin, and kept at 37°C in a 5 % CO_2 atmosphere.

2.2.2 MTT Assay for Cell Cytotoxicity

The in vitro cytotoxicity of the Nargis flower extract was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the MCF-7 cell trypsinization was used to collect the cultivated MCF-7cells, which were then pooled in a 15 ml tube.

The cells were then plated at a density of 1×105 cells/ml cells/well (200 L) in a DMEM medium with 10% FBS and 1% antibiotic solution for 24-48 hours at 37°C in a 96-well tissue culture plate. The wells were cleaned with sterile PBS and treated with various doses of the Nargis extract in a serum-free DMEM medium. Each sample was tripled, and the cells were cultivated in a humidified 5 percent CO2 incubator for 24 hours at 37°C. After the incubation period, MTT (20 µL at 5 mg/ml) was added to each well.

Under an inverted microscope, the cells were incubated for another 2-4 hours until purple precipitates were evident. Finally, MTT (220 µL) was used to aspirate the medium out of the wells, and 1X PBS (200 µl) was used to rinse the wells. To dissolve formazan crystals, 100µL DMSO was added, and the plate was shaken for 5 minutes. The absorbance of each well was measured at 570 nm using a microplate reader (Yan et al., 2010). (Thermo Fisher Scientific, USA). The percent cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

2.2.2.1 Apoptosis Assessment

To detect the apoptosis induction in MCF-7 cells for each assay. The cells are treated with ethanolic extract of Nargis at different concentrations required for 50 % inhibition of growth of MCF-7 cells for 72 h. As a negative control, cells were treated with DMSO (0.05 percent v/v) vehicle (Kiran Aithal et al., 2009).

2.3 Statistical Analysis

Graph Pad Prism 6.0 software was used to calculate the % cell viability and the IC50 value. The growth inhibition study's data values are reported as Mean ± SD.

3. Results

3.1 Inhibition of Cell Viability

The effect of a crude hydroalcoholic extract of Nargis on cell viability was investigated using the MTT test. To examine the cytotoxicity of different concentration of Nargis hydroalcoholic extract, MCF-7 cells were treated for 72 hours. Nargis reduced MCF-7 cell viability in a concentration-dependent way, according to the findings (Table 1 and Figure 2). Typical MCF7 cells look healthy, polygonal, and adhere to the well plate under the microscope. However, 24 hours after applying Nargis (NT), MCF-7 cells showed considerable alterations in morphology and density (Figure 3).

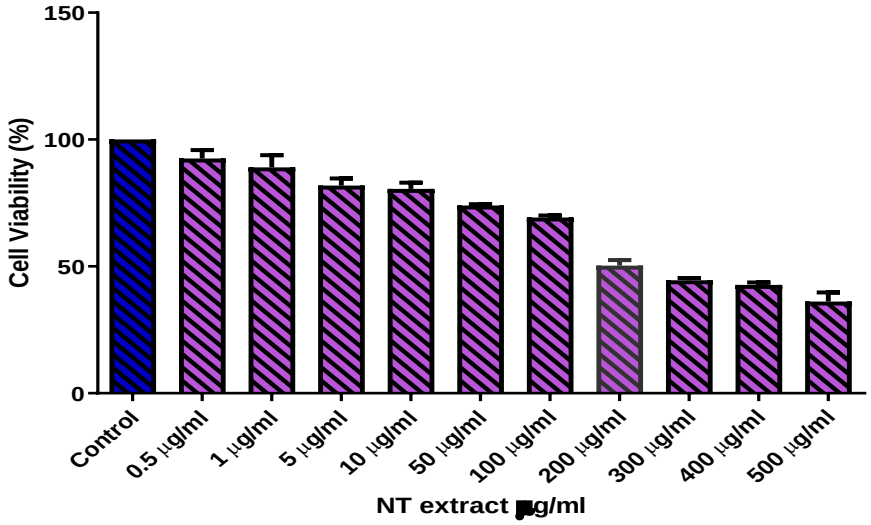
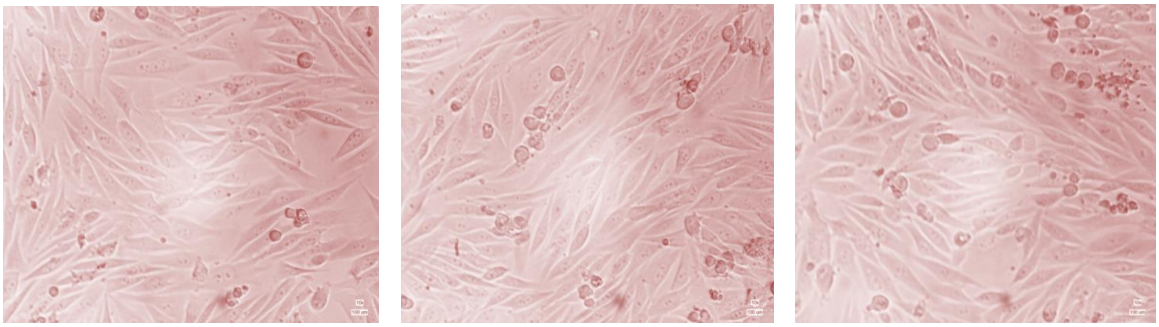


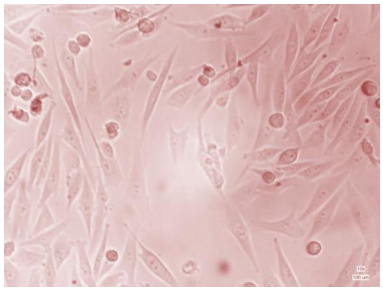
Figure 2: Nargis' (NT) cytotoxicity was determined using the MTT Assay (The results are reported as the mean of three independent measurements± SD.)

3.2 Apoptosis Assessment

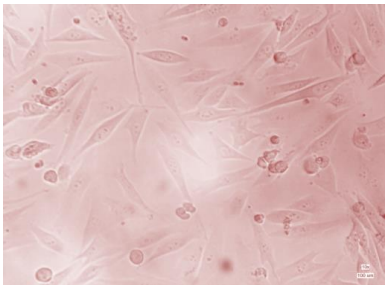
In-vitro cytotoxic evaluation of Nargis exhibited the most cytotoxic effects. Therefore, the crude ethanolic extract of Nargis was selected to investigate apoptosis induction ability. Apoptosis was induced by treating MCF-7 cells with Nargis flower extract at their IC50 values approximately 81.37 after 72 h, respectively (Cetintas et al., 2012) (Eidi Nita et al., 1998). (Table 1 and Fig 3 (A to G)). As a negative control, cells were treated with DMSO (0.05 percent v/v) vehicle.



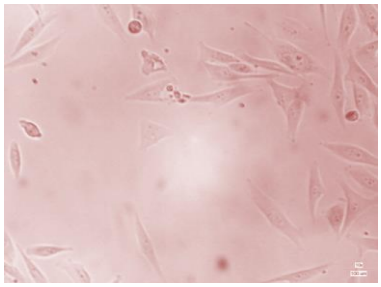
A: Control B: NT (Nargis) extract 1 µg/ml C: NT (Nargis) extract 10 µg/ml



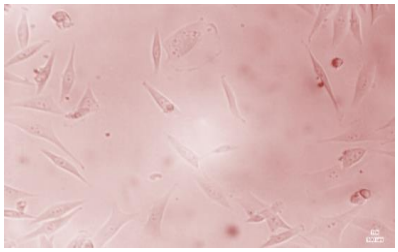
D: NT (Nargis) extracts 50 µg/ml



E: NT (Nargis) extracts 100 µg/ml



F: NT (Nargis) extracts 300 µg/ml



G: NT (Nargis) exracts 500 µg/ml

Figure 3: Nargis (NT) causes cytotoxicity in human breast cancer MCF-7 cells, as shown in figure (A). Figures B to G show cells that have been treated with Nargis (conc. 500-10 µg/ml respectively).

Table 1: Inhibitory activity of Nargis flower extract at their IC_{50} values

IC50 values of tested sample (Nargis extracts)

Log (inhibitor) vs. normalized response -- Variable slope	NT (Nargis extract(µg/ml)
Best-fit values	
LogIC50	1.910
HillSlope	-1.095
IC50	81.37
Std. Error	
LogIC50	0.05230
HillSlope	0.1327
95% Confidence Intervals	
LogIC50	1.803 to 2.018
HillSlope	-1.367 to -0.8237
IC50	63.58 to 104.1
Goodness of Fit	
Degrees of Freedom	28
R square	0.9274
Absolute Sum of Squares	2710
Sy.x	9.839
Number of points	
Analyzed	30

4. Discussion

Apoptosis is a highly controlled and programmed physiological mechanism for destroying injured or defective cells (Aigner, 2002). Many clinically utilized and successful anticancer drugs can cause it. Furthermore, it is easy things that are screening for apoptosis will rule out drugs that are harmful using non-specific methods (El-Menshawi et al., 2010). A wide range of secondary metabolites can cause cell proliferation in various human cancer cells (Mathur et al., 2009; Shiezadeh et al., 2013).

Many of these chemical compounds can be found in medicinal plants that have pharmacological value. Humans consume a variety of vegetables, flowers, and fruits. As a result, assessing apoptotic inducers from plants, whether as crude extracts or chemical entities isolated from herbal plants, is necessary (Taraphdar et al., 2001). In phytochemical analysis, Narcissus Tazetta (Nargis flowers) is high in phenolic compounds, saponins, flavonoids, and glycosides. Narcissus Tazetta's secondary metabolites contain various biological and pharmacological properties, including anti-inflammatory, anticancer, and antioxidant properties. The aerial parts and flowers were utilized as an anticancer, anti-inflammatory, memorigenic, and sedative in Jordan (Uthirapathy, 2021). Narcissus tazetta bulbs were utilized in the treatment of breast cancer (Tomoda et al., 1980). The extracts of Narcissus tazetta bulbs

were found to contain flavonoids (rutin, quercetin, and kaempferol) and phenolic acids (Rameshk et al., 2018).

The IC50 values of the ethanolic extract of Narcissus Tazetta (Nargis) obtained from the MTT experiment in this investigation were shown in Table 1 and Figure 3 (A to G). Active chemical components presumably cause the observed cytotoxicity. Based on the chemo-physical properties of various substances and the polarity of solvents, it is plausible to estimate the cytotoxic effect that may be present in Nargis extract, which was investigated in the MCF-7 cell line to determine its apoptotic activity. In vitro and in vivo, there are a variety of methods for detecting apoptotic cell death. The use of a fluorescent microscope in the detection of apoptosis has some significant benefits. First of all, fluorescence can ensure a better signal-to-noise ratio compared to the color development method, thereby increasing sensitivity. The second option is to use fluorescent dyes and fluorescent fusion proteins. Finally, because the detection approach does not rely on an enzyme reaction, its effectiveness may be influenced by various parameters such as buffer solution, pH, and temperature (Uthirapathy et al., n.d.; Baskić et al., 2006). As a response, distinguish clearly sub populations of apoptotic cells, such as early or late apoptotic cells. The extract of Nargis flowers was proven to increase early and late apoptosis more than as whole apoptosis in this investigation (Necrosis of cells).

5. Conclusion

This study established for the first time that a crude ethanolic extract of Narcissus Tazetta (Nargis flowers) had a potent inhibitory effect on the human breast carcinoma cancer cell. Proliferation, which was mediated by its pro-apoptotic antiproliferative activity. Furthermore, ethnobotany data on this species is utilized for cytotoxicity, Alzheimer's illness, and CNS agent. Apoptosis' molecular mechanism and other signal pathways will need to be investigated further. Nevertheless, these findings place a solid scientific foundation for developing new anticancer drugs based on the bioactive chemicals detected in the most effective extract. Narcissus Tazetta (Nargis flowers) had a potent inhibitory effect on human breast carcinoma cancer cell proliferation. The findings place a solid scientific foundation for developing new anticancer drugs based on the bioactive chemicals detected in the most effective extract.

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