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# *In vitro* apoptotic, anti-proliferative and antioxidant activities of therapeutic plant *Datura metel*

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## Abstract

Natural bioactive compounds with apoptotic action might be a promising new anti-cancer drug source. The purpose of the present study was to assess the apoptotic, anti-proliferative, antioxidative activities of a therapeutic plant *Datura metel* in liver hepatocellular carcinoma, HepG2 cell lines, as well as in normal baby hamster kidney (BHK) cell lines as controls. Ethanol and n-hexane solvents were used to extract *Datura metel* leaves extract. Standard techniques for identifying components were used to conduct phytochemical analysis. Cell death and viability in all sets of the cells were assessed using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), trypan blue and crystal violet tests. For the estimate of apoptosis and cell death in all groups ELISA of Annexin-V was used. In addition, antioxidant enzyme activity assays were also conducted to estimate $H_2O_2$, nitric oxide, superoxide and DPPH radical scavenging activities. The outcomes revealed that when cancer cells from the HepG2 cell lines were treated with *Datura metel* extracts, they demonstrated decreased viability, proliferation, and enhanced apoptosis as compared to normal BHK cells and untreated control cells. Anti-oxidative scavenging activities were higher in cancer cells treated with *Datura metel* extract than in untreated ones. It was concluded that the *Datura metel* leaves extract induces apoptosis, enhance antioxidant status, decrease proliferation in HepG2 cells.

## Introduction

Herbal plants are widely utilized in traditional medicine due to their various medicinal qualities, piquing researchers' interest in learning more about them [1]. According to the WHO, different versions of these herbal medicines have become the most frequent source of basic health care for around 75-80 percent of the world's population [2]. Medicinal plants are a valuable source of essential phytochemicals with diverse pharmacological actions. Furthermore, as compared to medications made synthetically, therapeutic compounds derived from medicinal plants are generally safe and cost-effective. As a result, there is a pressing need to concentrate on medications made from natural plants, which are affordable, safe, and effective [3].

Plants generate various secondary metabolites, which are a major source of many pharmacological medicines with hepatoprotective, antibacterial, antiviral, nephroprotective, anti-asthmatic, antipyretic, analgesic, and anticancer effects [4]. Cancer is a new illness with a rapidly increasing global prevalence, characterized by unregulated cell proliferation and poor apoptotic activity. Apoptosis is a type of cell death that occurs when a cell is designed to die. Apoptosis is important in cancer because it inhibits the growth of cancerous cells [5]. Many chemotherapeutic drugs are now accessible for the cure and control of cancer, thanks to significant breakthroughs in cancer treatment. These compounds are extremely effective, although they have several drawbacks, such as multiple drug resistance and non-specificity [6]. More than three thousand plant species had been reported to cure cancer, and approximately thirty plant-derived chemicals have been identified and evaluated in clinical trials of cancer [7]. Because of the low toxicity and simple availability compared to chemotherapy, these plant-derived chemicals have been at the forefront as essential components of anticancer treatments for a long time and played a dynamic role in the treatment of cancer [8]. Plant chemicals are easily given orally as patient's food intake part. Plant-derived chemicals are typically non-toxic and more tolerated to normal human cells [9]. *Datura metel* is a medicinal plant with a long history in medicine and is considered to work as an antioxidant, apoptotic, and anti-proliferative agent [10]. *Datura metel* L. is an important therapeutically active genus of family Solanaceae. Datura metel, also known as Indian Thornapple, Hindu Datura,[3] or Metel in Europe and Devil's Trumpet or Angel's Trumpet in the United States, is a shrub-like annual (zones 5-7) or short-lived, shrubby perennial (zones 8-10). *Datura metel* has been naturalized in all the world's warmer climates, particularly in India, where it is known by the old Sanskrit-derived Hindi name (Dhatra), from which the genus name Datura is derived. The plant is grown all over the world as a decorative and for its therapeutic benefits, which are attributable to tropane alkaloid concentration (like in all Datura species). This plant was shown to possess varied array of pharmacological activity, including hyperlipidemia, hypoglycemia, and prevention of stomach ulcers, neuroprotective, and emollient, benefits via anti-inflammatory and anti-oxidant and antiviral and antibacterial properties. It also has a therapeutic and preventive impact in the case of diabetes [11].

Several animal model-based results relating to the *Datura metel* plant have proven pharmacological efficacy and safety which opened a new window for new treatments related to the human health. Because of its various pharmacological and traditional *D. metel* has been identified as a pharmacologically significant species [7, 12]. However, more sophisticated in vivo plus in vitro investigations are needed to determine the precise pharmacological processes and the foundation of therapeutic usefulness.

The present study was intended to evaluate apoptotic, anti-proliferative, and antioxidative effects of *Datura metel* plant extract on HepG2 cell lines. Because no such properties of this plant have previously been reported, our findings here might be a source of knowledge for future research. The current work was aimed to demonstrate targeted therapy using *Datura metel* plant extract for the treatment of cancer cell lines by inhibiting proliferation in HepG2 cells, as well as inducing oxidative stress and apoptosis.

## Materials and Methods

### *Extract Preparation*

Leaves of the understudied medicinal plant *Datura metel* were collected from the Biotechnology Lab of the Institute of Molecular Biology and Biotechnology, The University of Lahore Pakistan. Leaves were crushed and turned into a fine powder using dried samples. For the extraction of plant extract, the cold maceration method was employed, in which 400g of plant leaves material was employed in a stoppered container with 1000ml of ethanol and n-hexane solvent maintained in a shaker for 7 days set at RM (room temperature). These extracts were lyophilized, and stock solutions were made by dissolving 20 mg of

plant extracts in 1ml methanol. The percentage yield of the extract was calculated as follows
% Extraction yield = (W1x100)/W2
W1 = weight after lyophilization of solvent from the extract
W2 = Plant extract weight in powder form

***Phytochemical analysis***

Standard techniques for identifying components were used to conduct phytochemical analysis including percentage yield, retention time, and area sum percent of bioactive compounds of plant extracts.

***Estimation of tannins***

The total tannins in plants were estimated using with Folin-Ciocalteu assay used by Kavitha et al [13].

***Estimation of phenolic compound***

The phenolic compounds in the extract were assessed by the method of Khatoon et al [14].

***Estimation of total flavonoids***

The total flavonoid contents were estimated by using the colorimetric method involving aluminum chloride reported by Pękal and Pyrzynska [15].

***Cell lines***

HepG2 and BHK cell lines were acquired from The University of Lahore's cell culture facility. Cryovials containing liquid nitrogen were used to maintain these cell lines. Cryovials were resurrected and processed for the next step of culturing.

***Cell line culturing***

The cryovials (acquired from liquid nitrogen) were defrosted. In a culture flask, the HepG2 cancer cell line and BHK normal cell line was grown in DMEM-HG media with 10% FBS (fetal bovine serum), streptomycin, and penicillin added. Sub-culture was added to the cultivated HepG2 and BHK cells after they reached 70-80% confluence. The cells adhering to the culturing flask were then rinsed with 1X PBS (phosphate buffer saline) and treated with trypsin-EDTA till their removal from the surface of the flask. Observing the flask under the inverted microscope confirmed the cells' separation. FBS (few drops) were added to the flask with continuous stirring. The mix was then centrifuged for 5 minutes at 2000 rpm. After centrifugation, the supernatant was removed followed by the resuspension of the pellet.

***Treatment with plant extracts***

HepG2 and BHK cells were cultured in 96 well plates for IC50 values, cell viability, and muse analysis on 6 well plates. Three groups were formed from each cell line. One group of each was not given any treatment and was designated as UT-HG for HepG2 untreated cell line and, UT-BHK for BHK untreated cell line. The remaining two groups of each cell line were designated as T-HG-DE (HepG2 treated with *Datura metel* ethanol extract) and T-HG-DH (HepG2 treated with *Datura metel* n-hexane extract). Similarly, T-BHK-DE (BHK cell line treated with *Datura metel* ethanol extract) and T-BHK-DH (BHK cell line treated with *Datura metel* n-hexane extract). Each cell line was treated with plant extract at concentrations of (**10,25, 50,100,200ug/mL**) in DMEM. Treatment was given to cultured cells for 24 hours. The IC50 was calculated using cells (post-treated) in plates of 96 wells after 24 hours. The standard anti-cancer medication was cisplatin. Similarly, trypan blue and crystal violet assays were executed on cells (post-treated) from plates of 96 wells. These distinctive viability assays were assessed by utilizing Graph-Pad Prism 6 to determine the half-maximal inhibitory (IC50) values.

***Cell viability assays***

MTT assay was accomplished by following an already established protocol Maqbool et al [16]. In brief, Datura *metel* plant ethanol and n-hexane extracts were applied in increasing concentrations (10, 25, 50, 100, 200ug/mL) on HepG2 liver cell line and BHK cell lines *via* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay on plates of 96 wells for 72 h. A PBS (phosphate buffer saline) was used to wash the cell's monolayer. Samples without extracts served as control. Besides, it was incubated in a 100 µL complete medium having 25 µL MTT solution for 2 h. In living cells, Tetrazolium was converted to formazan (purple color) which was later solubilized with DMSO (dimethyl sulphoxide), the absorbance of which was taken at 570 nm.

***Trypan blue assay***

Trypan blue was used as an inhibitor of live and dead cells to assess cell viability. The cells of different

experimental groups were washed three times by using PBS, thereby incubating in trypan blue (Invitrogen Inc., USA) for fifteen minutes. Then the washing of the cells was performed 3 times by using PBS and were detected under the microscope. The cells which were stained with trypan blue were taken as dead. Viable cells percentage was intended as follows

Viable cells (%) = 1.00 – (Number of blue cells ÷ Number of total cells) × 100

Viable cells/mL of culture were calculated as follows by employing a dilution factor.

Number of viable cells × $10^4$ × 1.1 = cells/mL culture

***Crystal violet assay***

Cell viability was assessed also by employing crystal violet staining in BHK and HepG2 cells. The method was led in 96-well plates. The secretome was removed from the wells of the plate, and the cells were rinsed with PBS. Following cleaning, the wells were painted with 0.1 percent crystal violet mixed with 2 percent ethanol in such a way that the whole surface was coated. It was then incubated for 15 minutes at room temperature. The dye was properly removed, and wells were thoroughly cleaned to ensure that cells did not rise away from the well. Then, to solubilize the stain, 100l of 1% SDS was applied to each well and allowed for 5-10 minutes. Finally, absorbance was measured on a microtiter plate at 595nm.

***Muse analysis***

The $IC_{50}$ value of plant extracts was applied on HepG2 cells which were cultured in 6 well plates through Annexin V kit (Cat. No MCH100105) of Thermo Fisher offering fluorescent annexin V conjugates as standalone reagents by utilizing the Muse™ cell automated analyzer (Merck-Millipore). After treatment, the cells were centrifuged for five minutes at 2000 rpm. The supernatant was disposed of followed by pellet desolation in Annexin v reagent for 20 minutes and then cells were counted.

***Evaluation of anti-oxidative activity***

The antioxidant potential of the extracts of the plant was used to determine the DPPH scavenging capacity by using the method reported by Martins et al [17]. A DPPH solution (1 mM) was primed in 99.5% methanol. Butyl hydroxyl anisole standards with 50 ppm concentration or 1ml samples of 10 mg/mL were mixed with DPPH solution of 250 µL which was then incubated in the dark for thirty minutes at RM (room temperature). Absorbance was taken then at 517 nm in a SpectraMax (Molecular Devices, USA) 190 Microplate-reader. For blank methanol was used. The DPPH free radical inhibition percentage was computed as:

% Inhibition = (Absblank – Abssample)/(Absblank) × 100

***Nitric Oxide scavenging assay***

Nitric oxide scavenging potential of selected medicinal plant extracts was using sodium nitroprusside according to Vishwakarma et al [18].

***Hydrogen Per-oxide scavenging assay***

The $H_2O_2$ scavenging properties of plant extracts were estimated by a method reported by Wu et al [19].

***Superoxide Dismutase (SOD) activity***

SOD activity was performed based on the NBT (Nitroblue tetrazolium test) method reported by Abid et al [5].

***Statistical Analysis***

All the data of photochemistry and trial groups were presented as mean ± SEM. One-way ANOVA and t-test were employed to compare group means at a 0.05 level of significance. Graph pad prism 9.1.2 software was used for the analysis.

## Results

The outcomes of the current study were analyzed in two steps. In the first step different bioactive compounds of *Datura metel* were analyzed in ethanol and n-hexane solvent with the determination of percentage yield and mean retention time and area sum percentage of different phytochemicals extracted from *Datura metel*. In the second step overall apoptotic, anti-proliferative, and cytotoxic effects with antioxidative activities of *Datura metel* ethanol and n-hexane extracts with these bioactive compounds were evaluated in vitro by using HepG2 and BHK cell lines.

***Evaluation of bioactive compounds of Datura metel extracts***

The extraction results revealed that ethanol and n-hexane solvents extracted *Datura metel* plant bioactive compounds in varying amounts. Because

each compound of the plant has a different chemical nature, its solubility in a particular solvent varies.

***Percentage yield***

The percentage yield of the plant under investigation demonstrated that the n-hexane solvent has a high-potential source of phytochemicals as compared to the ethanol solvent. The percentage yield in n-hexane extract was 4.657±0.02963 and in ethanol extract, it was 4.250±0.02887 (Table 1). Significant difference ($t = 9.831$, $p = 0.0006 < 0.05$) in percentage yield of plant extract was observed between ethanol (Brown colored) and n-hexane (Yellow colored) extracts (**Table 1, Fig. 1**).

***Evaluation of phytochemicals obtained in ethanol and n-hexane extract of the plant***

The mean concentration of seven classes of phytochemicals, flavonoids, tannins, phenol, hydrocarbons, fatty acids, lipids, and aldehyde in ethanol and n-hexane extracts are shown in **Table 2**. The mean concentration of flavonoids, tannins, phenol, fatty acids, and lipids was high in n-hexane extract as compared to the ethanol extract. The difference was significant statistically, ($p<0.05$) for these classes of phytochemicals while the mean concentration difference of hydrocarbon and aldehyde class of phytochemicals measured in ethanol and n-hexane extract was statistically insignificant ($p>0.05$).

***Mean retention time and area sum % of phytochemicals***

The mean retention time (RT) and area sum % (ASP) of seven classes of phytochemicals are presented in **Table 3a**. The mean RT and ASP of the tannins class were greater than any other class of phytochemicals. ANOVA was applied to check the equality of means for retention time and area sum % for the class of phytochemicals. The differences were statistically significant in mean retention time ($F=19.28$, $p=0.000<0.05$), but the differences between means of area sum % for the class of phytochemicals were statistically insignificant, ($F=2.55$, $p=0.061 > 0.05$) (**Table 3b**).

**Table 1:** Percentage Yield of *Datura Metel* Ethanol and n-Hexane Extract

| Extract | Color | Recovery (g) W1 | Recovery (g) W2 | Percentage yield (%) ±SEM | t- Value | p- value |
|---|---|---|---|---|---|---|
| Ethanol | Brown | 20 | 450 | 4.250±0.02887 | 9.831, | 0.0006* |
| n-hexane | Yellow | 22 | 478 | 4.657±0.02963 | | |

*Significant value p<0.05

**Table 2:** Comparative Evaluation of Concentration of Different Bioactive Compounds in Ethanol and n-hexane Extracts

| Bioactive compounds | Ethanol Extract | n-hexane Extract | p- value |
|---|---|---|---|
| Flavonoids | 3.45±0.36 | 4.31±0.05 | 0.004* |
| Tannins | 601.00±213.62 | 323.50±152.99 | .000* |
| Phenol | 12.71±4.72 | 14.55±5.13 | .004* |
| Hydrocarbons | 118.12±65.98 | 116.21±62.21 | 0.238 |
| Aromatic hydrocarbons | 102.90±45.24 | 103.91±46.43 | 0.760 |
| Fatty Acids | 189.45±72.91 | 300.61±142.82 | .000* |
| Fatty Acid Esters | 188.75±72.71 | 302.27±145.45 | .000* |
| Derived Lipids | 152.77±55.61 | 275.23±136.44 | .000* |
| Terpenoids | 282.21±68.72 | 356.46±176.55 | .002* |
| Aldehyde | 130.76±56.12 | 122.41±66 | 0.079 |

*Significant value p<0.05

**Table 3a:** Mean retention time and area sum % of different classes of phytochemicals

| Class of Phytochemicals | Retention Time (RT) (Mean±SEM) | Area Sum % (ASP) (Mean±SEM) |
|---|---|---|
| Flavonoids | 5.21±1.23 | .12±0.14 |
| Tannins | 79.24±9.88 | 9.72±6.11 |
| Phenol | 9.29±1.82 | 0.21±.006 |
| Hydrocarbons | 11.91±6.95 | 0.12±0.07 |
| Aromatic hydrocarbons | 7.11±1.90 | 0.11±0.13 |
| Fatty Acids | 49.26±2.58 | 9.64±9.54 |
| Fatty Acid Esters | 49.15±4.95 | 2.63±3.56 |
| Derived Lipids | 40.45±42.12 | 1.01±1.08 |
| Terpenoids | 74.58±9.76 | 9.42±6.95 |
| Aldehyde | 18.36±0.58 | 0.19±0.03 |

***Evaluation of cytotoxicity level of Datura metel***

The MTT test was used as a reliable and easy technique for measuring cell cytotoxicity. Figure 2 depicts a plot of cytotoxicity as a percentage (%) viability of cells vs different concentrations (10, 25, 50, 100, and 200ug/ml) of *Datura metel* ethanol and n-hexane extracts. It was observed that both the extracts, for the most concentrations demonstrated a dose-dependent mode of cytotoxicity in the HepG2 cell line. It was revealed from the outcomes that ethanol extract has significantly high cytotoxicity on 50, 100, and 200ug/ml concentrations while n-hexane extract showed significant cytotoxicity started from a low concentration level of 25ug/ml to the maximum concentration level of 200ug/ml.

The IC50 values obtained for both the extracts are presented in Table 4. The IC50 value obtained for ethanol extract was 98.78±5.77 while the obtained IC50 value for n-hexane extract was 97.26±5.34. A significant difference in obtained IC50 values of ethanol and n-hexane extracts was observed ($F=65.22$, $p=0.0001<0.05$). Less IC50 value reveals more cytotoxicity as the extract has maximum inhibitory potential at even less concentration. The outcomes revealed a high cytotoxicity level of n-hexane extract (less IC50 value) as compared to ethanol extract for which a high IC50 value was obtained. These IC50 values were utilized for further experimentation.

***Evaluation of Datura metel plant extract on cell viability***

HepG2 cancer cell line and BHK normal cell line was treated with an IC50 value of each extract determined by MTT test to assess the effect of *Datura metel* extracts on cell survival of HepG2, and BHK cell lines. Trypan blue and crystal violet assays were used to determine cell viability after incubation.

***Evaluation of cell viability via trypan blue assay***

In treated HepG2 cells with *Datura metel* extracts, a significantly higher number ($F=1084$, $p=0.000<0.05$) of dead cells and a significantly lower number of viable cells were found compared to untreated HepG2 cells (**Fig. 3A**). Mean Percentage of dead cells in UT-HG: untreated HepG2 cell line was 11±0.6667 while in treated groups T-HG-DE: HepG2with *D. metel* ethanol extract and T-HG-DH: HepG2 with *D. metel* N-hexane extract mean percentage of dead cells observed was 50±0.8819 and 53±0.5774 respectively (**Table 5**).

In normal cell lines BHK, there were fewer dead cells than HepG2 treated cells (**Fig. 3B**), showing that *Datura metel* extracts had no adverse effects on normal cells. In an untreated group of BHK cell line (UT-BHK) mean percentage of dead cells was 15±0.5774 while in the treated group it was 16±0.3333 and 19±0.6667 recorded for T-BHK-DE: BHK with *D. metel* ethanol extract and T-BHK-DH: BHK with *D. metel* N-hexane extract groups respectively. A statistically insignificant difference in the mean dead cells % was observed in treated and untreated groups of BHK cell line ($F=4.200$, $p=0.07>0.05$) (**Table 5, Fig. 3B**).

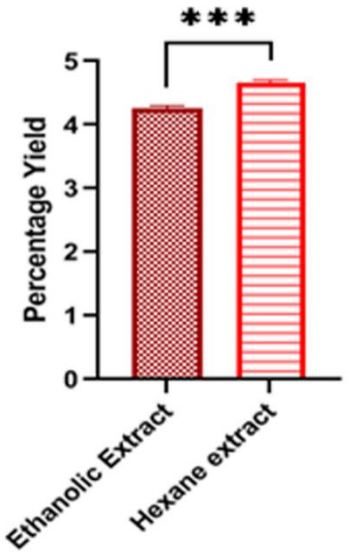



**Fig. 1:** Percentage yield of ethanol and n-hexane extract of *Datura metel*. The comparison revealed a significant difference in percentage yield of plant extract in ethanol and n-hexane.

**Table 3b:** Analysis of Variance for RT and ASP for Different Classes of Phytochemicals

| Variable | Variation | Sum of square | D.F | Mean Square | F | p-value |
|---|---|---|---|---|---|---|
| Retention time | Between Groups | 13299.27 | 6 | 2216.55 | 19.28 | 0.000* |
| | Within Groups | 2413.74 | 21 | 114.94 | | |
| | Total | 15713.01 | 27 | | | |
| Area sum % | Between Groups | 486.12 | 6 | 81.02 | 2.55 | 0.061 |
| | Within Groups | 665.22 | 21 | 31.67 | | |
| | Total | 1151.34 | 27 | | | |

*Significant value $p<0.05$

***Evaluation of cell viability via crystal violet staining***

Cell viability estimation via crystal violet staining revealed a significantly higher number (F=9293, p=0.0004<0.05) of dead cells in treated HepG2 cells with *Datura metel* extracts (Figure 4A). Mean Percentage of dead cells in UT-HG: untreated HepG2 cell line was 0.8±0.05774 while in treated groups T-HG-DE: HepG2 with *D. metel* ethanol extract and T-HG-DH: HepG2 with *D. metel* N-hexane extract mean percentage of dead cells observed was 0.4±0.02887 and 0.37±0.01202 respectively (**Table 6**).

In normal cell lines BHK, fewer dead cells than HepG2 treated cells were obtained (**Fig. 4B**), which depicts that *Datura metel* extracts had no adverse effects on normal cells. In the untreated group of BHK cell line (UT-BHK) mean percentage of dead cells was 0.83±0.08819 while in the treated group it was 0.76±0.03333 and 0.77±0.01528 recorded for T-BHK-DE: BHK with *D. metel* ethanol extract and T-BHK-DH: BHK with *D. metel* extract groups respectively (Table 5). A statistically insignificant difference in mean %age of dead cells was observed in treated and untreated groups of BHK cell line (F=0.4641, p=0.65>0.05) (**Table 6, Fig. 4B**).

**Table 4:** Cytotoxicity level of *Datura metel* ethanol and n-hexane extracts

| Cell Line Groups | Samples | IC50 Values (ug/ml) (±SEM) | F | p- value |
|---|---|---|---|---|
| T-HG-DE | Treated HepG2 with ethanol extract | 98.78±5.77 | | |
| T-HG-DH | Treated HepG2 with n-hexane extract | 97.26±5.34 | 65.22 | 0.0001* |

*Significant value p<0.05

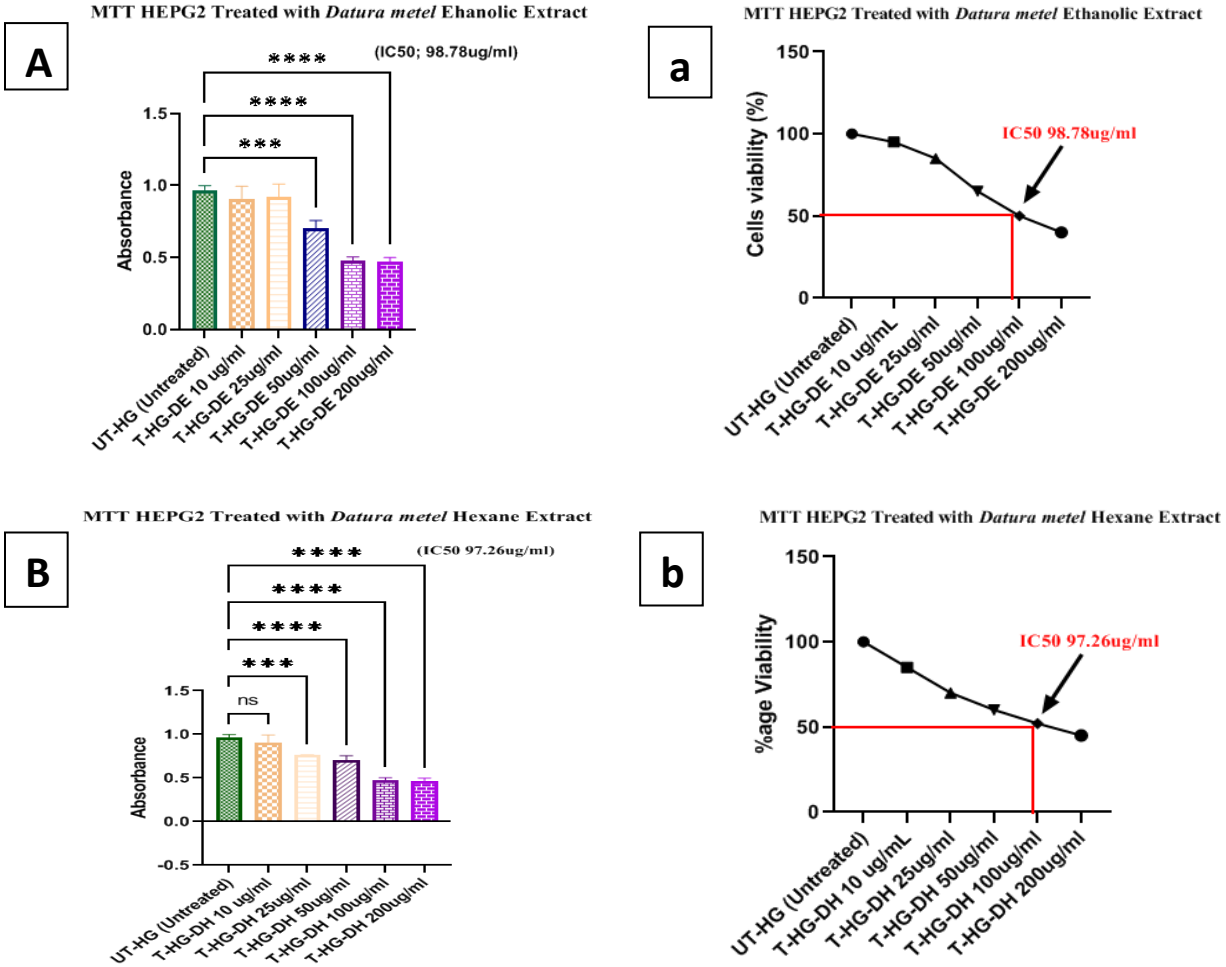



**Fig. 2:** MTT test for cytotoxicity and IC50 values of *Datura metel* ethanol extracts on HepG2 cell line. UT-HG (untreated HepG2 cell lines), T-HG-DE (HepG2 cell line treated with ethanol extract of *Datura metel.* T-HG-DH (HepG2 cell line treated with n-hexane extract of *Datura metel.* (A) Evaluation of cytotoxicity induced in HepG2 cells treated with different concentrations of *Datura metel.* ethanol extract (10ug/ml, 25ug/ml, 50ug/ml, 100ug/ml and 200ug/ml). (a) IC50 values of different concentrations of *Datura metel.* ethanol extract. (B) Evaluation of cytotoxicity induced in HepG2 cells treated with different concentrations of *Datura metel.* extract in n-hexane (10ug/ml, 25ug/ml, 50ug/ml, 100ug/ml and 200ug/ml). (b) IC50 values of different concentrations of *Datura Metel* extract in n-n-hexane.

**Table 5:** Evaluation of cell viability via trypan blue assay

| Groups (HepG2 cell lines) | Dead cell (%) values (±SEM) | F | p-Value | Groups (BHK cell lines) | Dead cell (%) values (±SEM) | F | p-Value |
|---|---|---|---|---|---|---|---|
| UT-HG | 11±0.6667 | | | UT-BHK | 15±0.5774 | | |
| T-HG-DE | 50±0.8819 | | | T-BHK-DE | 16±0.3333 | | |
| T-HG-DH | 53±0.5774 | 1084 | 0.000* | T-BHK-DH | 19±0.6667 | 4.200 | 0.07 |

UT-HG: untreated HepG2 cell lines; T-BHK: untreated BHK cell lines; T-HG-DE: HepG2 with *D. metel* ethanol extract T-BHK-DE: BHK with *D. metel* ethanol extract; T-HG-DH: HepG2 with *D. metel* N-hexane extract T-BHK-DH: BHK with *D. metel* n-hexane extract
*Significant value p<0.05

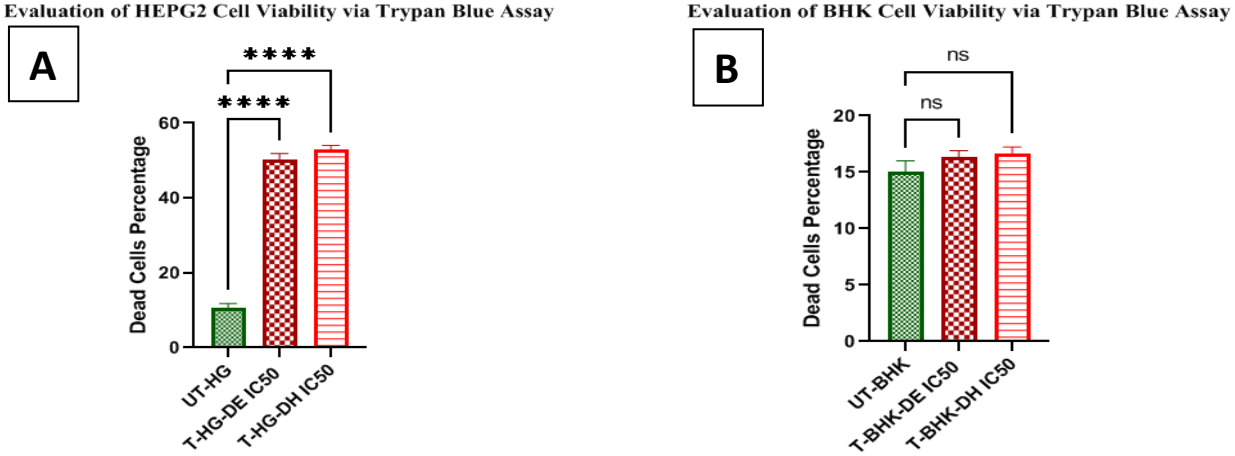



**Fig. 3:** Evaluation of cell viability via trypan blue assay for the estimation of dead cells. UT-HG (untreated HepG2 cell lines), T-HG-DE (HepG2 cell line treated with ethanol extract of *Datura metel).* T-HG-DH (HepG2 cell line treated with n-hexane extract of *Datura metel).* UT-BHK (untreated BHK normal cell lines), T-BHK-DE (BHK normal cell line treated with ethanol extract of *Datura metel).* T-BHK-DH (BHK normal cell line treated with n-hexane extract of *Datura metel).* (A) Representation of HepG2 cell viability (B) Representation of BHK cell viability.

**Table 6:** Evaluation of Cell Viability via Crystal Violet Staining

| Groups (HepG2 cell lines) | Abs values (±SEM) | F | p-Value | Groups (BHK cell lines) | Abs values (±SEM) | F | p-Value |
|---|---|---|---|---|---|---|---|
| UT-HG | 0.8±0.05774 | | | UT-BHK | 0.83±0.08819 | | |
| T-HG-DE | 0.4±0.02887 | | | T-BHK-DE | 0.76±0.03333 | | |
| T-HG-DH | 0.37±0.01202 | 39.40 | 0.0004* | T-BHK-DH | 0.77±0.01528 | 0.4641 | 0.65 |

UT-HG: untreated HepG2cell lines; UT-HG: untreated BHK cell lines; T-HG-DE: HepG2 with *D. metel* ethanol extract; T-BHK-DE: BHK with *D. metel* ethanol extract; T-HG-DH: HepG2 with *D. metel* n-hexane extract; T-BHK-DH: BHK with *D. metel* n-n-hexane extract
*Significant value p<0.05

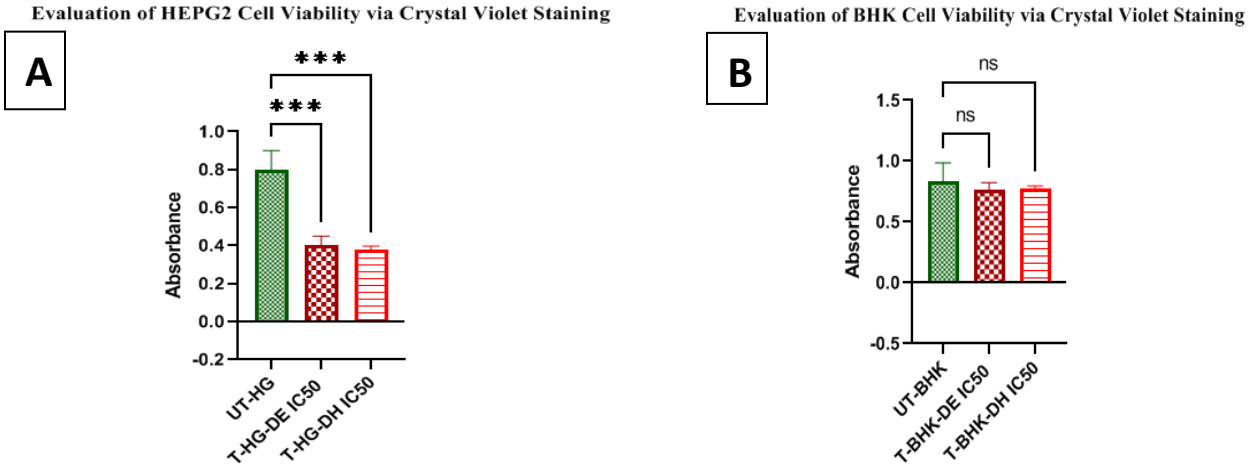



**Fig. 4:** Evaluation of cell viability via crystal violet assay. UT-HG (untreated HepG2 cell lines), T-HG-DE (HepG2 cell line treated with ethanol extract of *Datura metel).* T-HG-DH (HepG2 cell line treated with n-hexane extract of *Datura metel),* UT-BHK (untreated BHK normal cell lines), T-BHK-DE (BHK normal cell line treated with ethanol extract of *Datura metel),* T-BHK-DH (BHK normal cell line treated with n-hexane extract of *Datura metel).* (A) Representation of HepG2 cell viability (B) Representation of BHK cell viability.

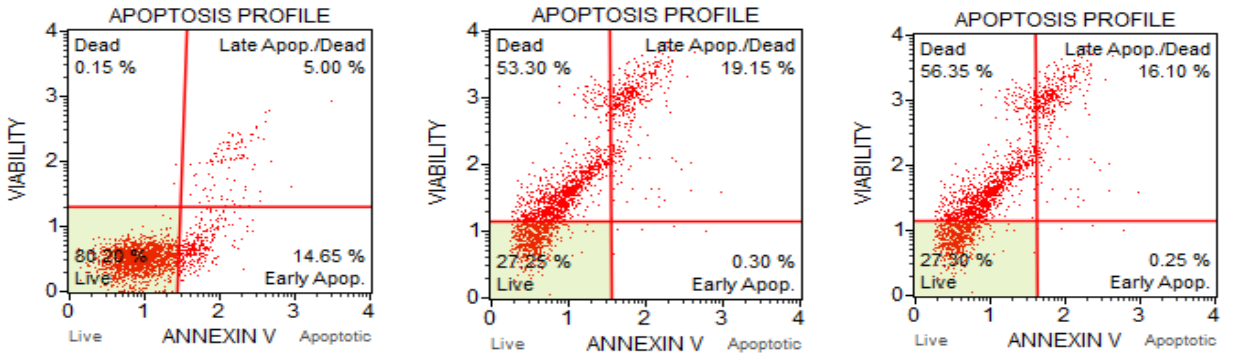
### *Evaluation of apoptotic effect of Datura metel plant extracts*

Using an annexin-V reagent of the kit the degree of apoptosis was assessed. The results demonstrated that *Datura metel Plant* Extracts (ethanol and n-n-hexane) treated groups, T-HG-DE and T-HG-DH displayed a significantly high percentage of late apoptosis and subsequently more dead cells as compared to control untreated group UT-HG. The percentage of initial live cells in the untreated group UT-HG was 80.20%. Early apoptosis was 14.65%, the percentage of late apoptosis was 5.00% and the dead cell percentage recorded for this group was 0.15% (Figure 5A) after incubation time. Percentage viability results of the T-HG-DE group treated with ethanol extract of the plant showed 27.25% live cells with early and late apoptosis of 0.30% and 19.15% respectively (**Fig. 5B**). A significantly high percentage of dead cells, 53.30% was observed in this group. Live cells percentage recorded for the T-HG-DH group treated with n-hexane extract of the plant was 27.50% with early and late apoptosis of 0.25% and 16.10% respectively. The percentage of dead cells recorded for this group was 56.35% (**Fig. 5C**). Significantly high ($F=1068$, $p=0.0001<0.05$) percentage of late apoptosis and dead cells was obtained in both the treated groups as compared to the untreated group. It was also revealed from the outcomes that the number of dead cells and percentage of late apoptosis was higher under n-hexane extract treatment as compared to the treatment with ethane extract but the difference in the percentage of late apoptosis and dead cells was insignificant among these two treated groups ($F=4.4221$, $p=0.721>0.05$) (**Table 7**).

**Table 7:** Evaluation of apoptotic activity of ethanol and n-hexane extract for HepG2 cell line.

| Groups (HepG2 cell lines) | % Viability | | | | F | p-value |
|---|---|---|---|---|---|---|
| | Live cells %±SEM | Early Apoptosis %±SEM | Late apoptosis %±SEM | Dead cells %±SEM | | |
| UT-HG | 80.20±0.011 | 14.65±0.056 | 5.00±0.022 | 0.15±0.006 | 1068 | 0.0001* |
| T-HG-DE | 27.25±0.021 | 0.30±0.043 | 19.15±0.038 | 53.30±0.028 | | |
| T-HG-DH | 27.50±0.030 | 0.25±0.052 | 16.10±0.0332 | 56.35±0.044 | 4.4221 | 0.721 |

UT-HG: untreated HepG2 cell lines; T-HG-DE: HepG2 with *D.metel* ethanol extract; T-HG-DH: HepG2 with *D.metel* n-n-hexaneextract
*Significant value $p<0.05$




(**A**) UT-HG (**B**) T-HG-DE (**C**) T-HG-DH

**Fig. 5:** Evaluation of Apoptotic effect of *Datura metel plant* extracts. UT-HG (untreated HepG2 cell lines), T-HG-DE (HepG2 cell line treated with ethanol extract of *Datura metel).* T-HG-DH (HepG2 cell line treated with n-hexane extract of *Datura metel).* (**A**) Level of apoptosis in untreated control HepG2 cell line (**B**) Level of apoptosis in HepG2 cell line treated with ethanol extract of *Datura metel*. (**C**) Level of apoptosis in HepG2 cell line treated with n-hexane extract of *Datura metel.*

### *Evaluation of in vitro antioxidant activity of ethanol and n-n-hexane extract of Datura metel*

$H_2O_2$, Nitric oxide, Superoxide, and DPPH scavenging activity tests were performed to assess the antioxidative potential of *Datura metel* plant extracts. The results (**Table 8, Fig. 6**) showed significantly high $H_2O_2$ scavenging activity ($F=434.6$, $p=0.0001<0.05$) (**Fig. 6A**), Nitric oxide scavenging activity ($F=344.9$, $p=0.0001<0.05$) (**Fig. 6B**), Superoxide scavenging activity ($F=991.2$, $p=0.0001<0.05$) (**Fig. 6C**) and DPPH radical scavenging activities ($F=161.7$, $p=0.0001<0.05$) (**Fig 6D**) in treated T-HG-DE and T-HG-DH groups as compared to untreated UT-HG group. A significant difference in antioxidative activities was observed between treated and untreated groups of HepG2 cell lines. For BHK cell lines insignificant difference in

$H_2O_2$ scavenging activity (F=3.000, p=0.1250>0.05) (Fig. 6A), Nitric oxide scavenging activity (F=4.000, p=0.0787>0.05) (**Fig. 6b**), Superoxide scavenging activity (F=1.474, p=0.3016>0.05) (**Fig. 6c**), and DPPH radical scavenging activities (F= 0.2418, p=0.7925>0.05) (**Fig. 6d**), was observed between treated T-BHK-DE, T-BHK-DH and untreated control group UT-BHK. Antioxidant levels were nearly the same in untreated and treated groups of BHK normal cell lines.

**Table 8:** Evaluation of antioxidant activity of ethanol and n-hexane extract *in vitro*

| Antioxidant activity | Groups (HepG2 cell lines) | Abs values (±SEM) | F | p-Value | Groups (BHK cell lines) | Abs values (±SEM) | F | p-Value |
|---|---|---|---|---|---|---|---|---|
| $H_2O_2$ scavenging activity | UT-HG | 0.5000± 0.05774 | 434.6 | 0.0001* | UT-BHK | 0.6267± 0.04410 | 3.000 | 0.1250 |
| | T-HG-DE | 1.690± 0.02082 | | | T-BHK-DE | 0.6700± 0.01155 | | |
| | T-HG-DH | 1.770± 0.02517 | | | T-BHK-DH | 0.6867±0.00522 | | |
| Nitric oxide scavenging activity | UT-HG | .5000± 0.05774 | 344.9 | 0.0001* | UT-BHK | 0.6233± 0.008819 | 4.000 | 0.0787 |
| | T-HG-DE | 1.690± 0.02082 | | | T-BHK-DE | 0.6300± 0.005774 | | |
| | T-HG-DH | 1.770± 0.02517 | | | T-BHK-DH | 0.6500± 0.005782 | | |
| Super oxide scavenging activity | UT-HG | 0.6633± 0.01202 | 991.2 | 0.0001* | UT-BHK | 0.6800± 0.005774 | 1.474 | 0.3016 |
| | T-HG-DE | 2.723± 0.06227 | | | T-BHK-DE | 0.6933± 0.01202 | | |
| | T-HG-DH | 2.867± 0.02404 | | | T-BHK-DH | 0.7000± 0.005774 | | |
| DPPH radical scavenging activity | UT-HG | 0.5000±0.05774 | 161.7 | 0.0001* | UT-BHK | 0.5000± 0.05774 | 0.2418 | 0.7925 |
| | T-HG-DE | 2.300± 0.1155 | | | T-BHK-DE | 0.5433± 0.06119 | | |
| | T-HG-DH | 2.367± 0.03333 | | | T-BHK-DH | 0.5567± 0.06173 | | |

UT-HG: untreated HepG2 cell lines; UT-HG: untreated BHK cell lines; T-HG-DE: HepG2with *D.metel* ethanol extract; T-BHK-DE: BHK with *D.metel* ethanol extract; T-HG-DH: HepG2 with *D.metel* n-hexane extract; T-BHK-DH: BHK with *D.metel* n- hexane extract;
*Significant value p<0.05

## Discussion

Natural medicines are thought to be safer than synthetic medicines, attracting human attention to natural medicines like phytotherapeutic agents and phytopharmaceuticals products [20]. This has sparked a renewed interest in the biological impacts of natural goods among scientists. Work on novel anti-tumorigenic chemicals, which have a strong effect on cancer cell growth, is expanding to address the problem of treatment resistance in many cancers. Although some medicines have little toxicity, they nonetheless impede the cancer cell's angiogenic capacity [21]. Currently, naturally occurring compounds, particularly those derived from plants, are seen as essential actors in the creation of new medicines for life-threatening illnesses. Furthermore, studies have shown that phytotherapeutic products rich in antioxidants might reduce the risk of cancer. As a result, there is a pressing need to identify novel anticancer drugs derived from plants with significant antioxidant activity [22]. The main goal of this investigation was to discover if *Datura metel* extracts had any apoptotic, anti-proliferative, and antioxidative activities against the HepG2 cell line in comparison to the normal BHK cell line. Plant leaves extracts were extracted using ethanol and n-hexane as solvents. From the leaves of this plant, seven types of bioactive compounds were extracted among them flavonoids, tannins, and phenols are considered the most powerful anti-cancer agents. Many studies have shown that these chemicals have anticancer characteristics, as they can suppress tumor cell development, anti-proliferative, and cause apoptosis [23-26]. The present study also revealed the percentage yield of the plant under investigation, and it was revealed that n-hexaneextract yielded more percentage as compared to ethanol extract of the plant. The polarity of the solvents determines the yield, which is an indicator of the plants' medicinal significance. This outcome of the study was novel as

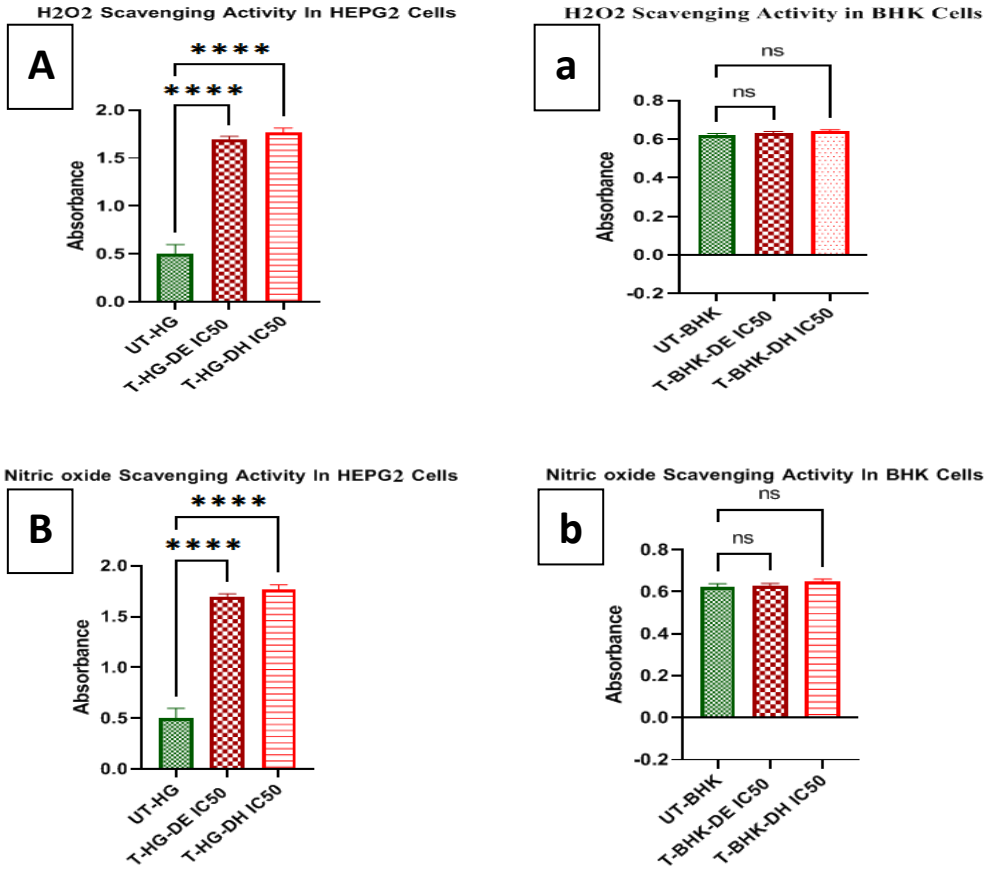



**Fig. 6:** Evaluation of the antioxidative activity of *Datura metel* extract in ethanol and n-n-hexane. UT-HG (untreated HepG2 cell lines), T-HG-DE (HepG2 cell line treated with ethanol extract of *Datura metel).* T-HG-DH (HepG2 cell line treated with n-hexane extract of *Datura metel).* (A) $H_2O_2$ scavenging activity in HepG2 cell line. (a) $H_2O_2$ scavenging activity in BHK cell line. (B) Nitric oxide scavenging activity in HepG2 cell line. (b) Nitric oxide scavenging activity in BHK cell line. (C) Superoxide scavenging activity in HepG2 cell line. (c) Superoxide scavenging activity in BHK cell line. (D) DPPH radical scavenging activity in HepG2 cell line. (d) DPPH radical scavenging activity in BHK cell line.

many other studies previously demonstrated that ethanol extracts of medicinal plants possessed more percentage yield as compared to any other solvent [27-29].

The current study also evaluated apoptotic, and cytotoxic effects with antioxidative activities of *Datura metel* ethanol and extracts in vitro by using HepG2 and BHK cell lines. For this purpose, cytotoxicity level of plant extracts was measured by MTT assay. MTT is a positively charged tetrazolium salt that is reduced by living cells with an active metabolism, where it penetrates easily and produces formazan product (purple-colored). The MTT test evaluates cell viability because of its reductive activity, which allows it to enzymatically convert the tetrazolium molecule to water [16]. Our findings revealed that when HepG2 cells were treated with *Datura metel* extracts, their proliferation reduced considerably, but when BHK cells were treated with the extracts, their proliferation remained unaffected.

Additionally, additional cell viability tests, such as trypan blue and crystal violet, yielded comparable results. The proportion of dead cells in HepG2 cells following plant extract treatment was higher than the %age of dead cells in BHK cells and untreated HepG2 and BHK cells after the same treatment in a trypan blue test. Similarly, when BHK cells treatment was compared to HepG2 cell treatment, the amount of crystal violet absorbed by living cells was higher in the BHK group. This might be related to a decrease in angiogenesis, which limits cancer cell development.

Anticancer medicines, as well as those derived from plants, typically work by triggering apoptosis in tumor cells. Plant-derived anticancer medicines have a variety of modes of action, but the majority of them cause apoptotic kind of cell death, which can be intrinsic or extrinsic, and caspase and/or p53-dependent or independent processes [30, 31]. After treatment with plant extracts, annexin V staining indicates a significant proportion of apoptosis in

several studies. When HepG2 cells were treated with ethanol and n-hexane extract of *Datura metel* it promoted apoptosis in HepG2 cells via annexin V-dependent mechanisms, resulting in an escalating apoptotic pattern. In the present study, it was observed that the n-hexane extract of the plants was more efficient in inducing apoptosis and cell cytotoxicity as compared to the ethane extract. BHK cell line, yielded non-significant findings in this experiment, indicating that these extracts do not increase apoptosis in normal cells, suggesting them as the ideal choice for anticancer treatment.

Oxidative stress causes a rise in reactive oxygen species (ROS), yet cancer cells can use this metabolic shift to protect themselves from apoptosis caused by oxidative damage, allowing for fast growth. Antioxidant enzymes have a favorable influence on cell proliferation, but when they are administered in conjunction with anti-proliferative treatment, they improve the efficacy of the therapy by lowering ROS levels [32]. When the HepG2 cells were treated with *Datura metel* extracts, $H_2O_2$, Nitric oxide, Superoxide, and DPPH scavenging activities were raised which reduced oxidative stress with the increase in anti-proliferative efficacy of the *Datura metel* extract as compared to the untreated HepG2 cells and normal BHK cells where no significant raised in scavenging activity was observed potent. Overall results of the current study revealed that both the ethanol and n-hexane leaves extract of the therapeutic *Datura metel plant* potentially have apoptotic, anti-proliferative, and antioxidative activities against the cancer cell line. The n-hexane extract however was proved to be more efficient in generating apoptotic, anti-proliferative, and antioxidative activities than ethanol extract of the plant.

## Conclusion

It was concluded from the current investigation that the *Datura metel* leaves extracts can induce apoptosis and reduce cancer cell growth while also increasing their antioxidant ability. Furthermore, the efficiency of leaves extract produced in n-hexane was considerably more efficient in terms of apoptotic, anti-proliferative, and antioxidative activities against cancer HepG2 cell line as compared to the extract of plant in ethanol.

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### Conflict of interest

The authors declare no conflict of interest.

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