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Evaluation of Antiproliferative activity of Taxol, Proanthocyanidin and Atorvastatin against human hepatocellular carcinoma cell line (HepG2).

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ABSTRACT

Liver cancer is the sixth most frequent cancer in the world, with a significant morbidity and mortality rate, is liver cancer. Discovery of a suitable therapeutic alternative for this disease is of greatest importance because various types of cancer annually result in thousands of fatalities worldwide. Atorvastatin is a competitive inhibitor of hydroxy methylglutaryl CoA reductase, which plays a role in anticancer activities in a range of malignancies, including human liver cancer. Proanthocynadin is a phytochemical that has a variety of pharmacological effects and chemotherapeutic properties. Taxol, an anti-microtubule chemotherapeutic medication, has been shown to be effective in the treatment of several HepG2 (liver carcinoma cell line). In this study, the antiproliferative and proapoptotic properties of Atorvastatin, Proanthocynadin and Taxol against HepG2 liver cancer cell line was evaluated *in vitro.* Cell survival and apoptosis in liver cancer cell lines were analyzed using MTT assay. The results showed that Taxol, Atorvastatin and Proanthcyanidin inhibited the viability of HepG2 cells in a time- and dose-dependent manner, induced a significant enhancement of treated cancer cells compared to untreated cells.

1. INTRODUCTION

Liver cancer has been placing a huge burden on global health as the third most prevalent etiology of cancer mortality in 2020 and the sixth most frequently diagnosed cancer (Sung et al., 2021). With an Age-standardized incidence and mortality rates (ASR) of 32.5 cases per 100,000 people and a death rate of 31.1 per 100,000 people, liver cancer ranks second among both men and women in Egypt (Sung et al., 2021). Hepatocellular carcinoma (HCC), the most common primary hepatic cancer, is also well known for its poor prognosis and tendency to develop. Most HCC patients receive a diagnosis at an advanced stage, when the bulk of treatment options, including transplant, surgery, or ablation, are not practicable (Johnston and Khakoo, 2019).

Paclitaxel (Taxol) is a chemotherapeutic medication used to treat a variety of tumors. Paclitaxel's molecular target has been identified as tubulin, and it modifies the dynamics of and stabilizes microtubule filaments. Paclitaxel, anti-cancer mechanism, interferes with the activity of microtubules in mitosis, resulting in mitotic arrest followed by death. Recent research shows that non-mitotic processes targeting microtubules may be more significant than suppressing mitosis for paclitaxel's efficacy as an anti-cancer therapy (Elizabeth et al., 2022).

Paclitaxel is reported to trigger drug resistance and aneuploidy or polyploidy because of aberrant mitosis if its cell death mechanism is dependent only on apoptosis (Watanabe et al., 2013; Yasuhira et al., 2016). The paclitaxel-induced cell death mechanism involved not only apoptosis but also the autophagy pathway. Although the paclitaxel-induced apoptotic mechanism in AGS cell line involves the cleavage of poly (ADP-ribose) polymerase (PARP) and caspase-3 by the activation of cleaved caspase-9, it is not related to the release of cytochrome c from mitochondria, and caspase-8 is not involved in the apoptotic mechanism (Khing et al., 2021).

Statins are the most often used drugs for hypercholesterolemia management and therapy. Statins are classified into two types based on their polarity: lipophilic statins and hydrophilic statins. Atorvastatin, lovastatin, simvastatin, pitavastatin, and fluvastatin are lipophilic statins, but pravastatin and rosuvastatin are hydrophilic. (Althanoon et al., 2020; Miller and Thyfault et al., 2020). Atorvastatin lowers circulating lipid levels and is used to treat and prevent coronary heart disease and stroke. (Zhang et al., 2019). Furthermore, atorvastatin is also considered as an anticancer therapeutic candidate since earlier research has shown that it possesses antiproliferative, pro-apoptotic, and immuno-regulatory properties. (Kong et al., 2018). Statin use is associated with lowering the cancer risk, involving HCC, as demonstrated by several meta-analyses (Yi et al., 1017), and their immunomodulatory effect is one of the reasons for its anticancer effect (Sarrabayrouse et al., 2017).

Grape seeds are the most prevalent source of Proanthcyanidins in meals and drinks. Grape seed Proanthcyanidins (GSPs) are a polymerization of catechins

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and/or epicatechins that results in a variety of dimers, trimers, tetramers, and oligomers/polymers (Lan et al., 2015). In vitro and in vivo studies have shown that proanthocyanidins are safe within a specified dosage range (Yamakoshi et al., 2002). Proanthocyanidins have been shown in studies to have anticancer effects in humans (Nandakumar et al., 2008), including head and neck squamous cancer (Prasad et al., 2012), non-small cell lung cancer (Singh et al., 2011), colorectal cancer (Kaur et al., 2008), HCC (Upanan et al., 2019)

Here, an *in vitro* evaluation of the antitumor activity of Atorvastatin, Proanthocynadin and Taxol is presented.

2. MATERIAL AND METHODS

2.1. Chemicals:

Taxol chemotherapeutic drug manufactured by Hikma specialized pharmaceutical, Badr city. Cairo A.R.E was dissolved in RPMI at different concentrations. Atorvastatin (Ator 10mg coated tablets) obtained from EGYPTION INTERNATIONAL PHARMACEUTICA INDUSTRIES CO. E.I.P.I.C. O 10^{th} OF RAMADAN was dissolved in DMSO for preparing stock solution 1000 µg/ml. Proanthocynadin natural compound extracted from grape seed (purity.95%). Proanthocynadin was obtained from Al-Gomhoriya Co., Cairo- Egypt and dissolved in RPMI at different concentrations.

2.2. Cell line and Cell culture:

The hepatocellular carcinoma cell line, (HepG2), was used to evaluate the antitumor activity of Taxol, Atorvastatin and Proanthocynadin. The human liver cancer HepG2 cells was obtained from The Holding Company for Biological Products & Vaccines (Vacsera), Dokki-Giza, Egypt. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS), (100U) 20 g/ml penicillin, and (100 g/ml streptomycin) was used to cultivate the cells. HepG2 cells were cultured in monolayers in Roswell Park Memorial Institute (RPMI) culture medium with glucose and L-glutamine (LSP, UK); in a humidified 5% CO_2 atmosphere at 37 °C. The cellular viability was checked using trypan blue (Strober, 2015). HepG2 cells were treated with different concentrations of Taxol at 0, 0.006, 0.06, 0.6, 6 and 60 µg/ml, Atorvastatin at concentrations of 0, 5, 10, 20, 40, 60 µg/ml and Proanthcyanidin at concentrations0, 20, 40, 60, 80, 100 µg/ml, then incubated for 24 hours. Wells containing HepG2 cells without drugs were considered as the control wells.

2.3. Cytotoxicity assay:

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric test (Cell Titer 96 Aqueous One Solution Cell Proliferation test, Promega, USA) was used to determine cytotoxicity. Briefly, 200 µL/well of the Taxol, Atorvastatin and Proanthcyanidin working solution produced in RPMI were treated with triplicates of 1×10^4 of HepG2 cells. Taxol was added to the medium at concentrations of 0, 0.006, 0.06, 0.6, 6 and 60 µg/ml, Atorvastatin at concentrations of 0, 5, 10, 20, 40, 60 µg/ml and Proanthcyanidin at concentrations0, 20, 40, 60, 80, 100 µg/ml. For 24 or 48 hours, all plates were incubated at 37 °C in a humidified 5% CO2 incubator.

The Mosmann method was used to perform the MTT assay, with a few changes (Mosmann, 1998). Using a microplate reader (LMR-9602, U.S.A.), the cell growth in each plate after 24 h was measured at 570 nm, and the IC_{50} concentrations were determined.

2.4. DNA Fragmentation analysis:

After 24 hours of taxol treatment, cells were lysed in a lysis solution (10 mM Tris-HCl, pH 7.4, 10 mM EDTA, and 0.2% Triton X-100) and dissolved in TE buffer. The DNA content was determined using spectrophotometry. Samples (10-20 g DNA) were run in a 1% agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0) for 3 hours at 110 mV. The gels and running buffer (0.1 g/ml) were treated with ethidium bromide (0.5 g/ml). The FUJIFILM Thermal Imaging System FTI-500 was utilized to image DNA fragments, and Image Master VDS was employed to visualize them.

2.5. Statistical Analysis:

All tests were carried out in triplicate, and the results are reported as means standard deviation. Excel was used for statistical analysis and data graphing. $P< 0.05$was regarded as statistically significant.

3. RESULTS

The Cellular Toxicity and Viability Data by MTT Assay:
Toxicity of HepG2Cells Treated with Taxol for 24hrs.

Figure (1) represents the cell viability percentages of HepG2 cells after 24 hours of incubation with serially increasing Taxol concentrations. According to the findings, the percentages of surviving cells decrease as Taxol concentrations increase in a dose-dependent way. After 24 hours, the inhibitory concentration (IC50) estimated from the treatments that caused toxicity in HepG2 cells was 3.320g/mL.after 24 hrs.

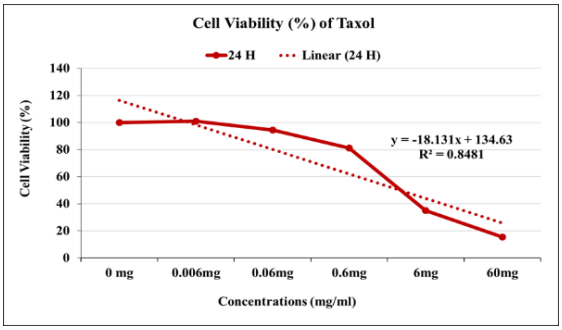

Figure 1 Histogram for the cell viability results of Taxol treatment on HepG2 cells after incubation for 24 hrs. The percentage of dead cells was measured by MTT cytotoxicity assay.

The CellularToxicity (IC50) of HepG2Cells After Treatment with Atorvastatinfor 24 hrs.

Figure (2) displays the percentage of dead cells after 24hrs of incubation of HepG2 cells with different concentrations of Atorvastatin. The results demonstratedthat survival cellspercentage is decreased with increased Atorvastatinconcentrations in a dose-dependent manner. the IC50 of Atorvastatin-treated cells was calculated to be 88.56µg/ml after 24hrs.

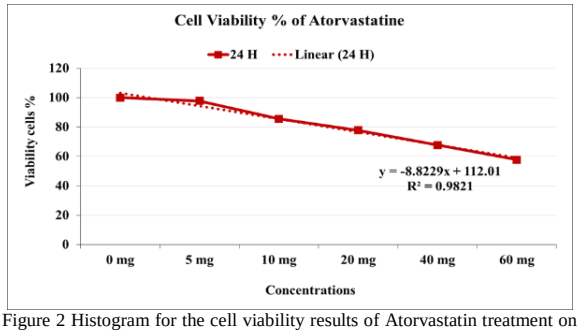

Figure 2 Histogram for the cell viability results of Atorvastatin treatment on HepG2 cells after incubation for 24 hrs. The percentage of dead cells was measured by MTT cytotoxicity assay.

The CellularToxicity(IC50) of HPG2Cells After Treatment with Proanthocynadin for 24hrs.

Figure 3 shows the percentage of dead cells after 24 hrs of incubation of HepG2 cells with different concentrations of Proanthocynadin. The results show that the survival cells percentage is decreased with increased Proanthocynadin concentrations in a dose-dependent manner. the IC50 of Proanthocynadin-treated cells was calculated to be 32.99µg/ml after 24hrs.

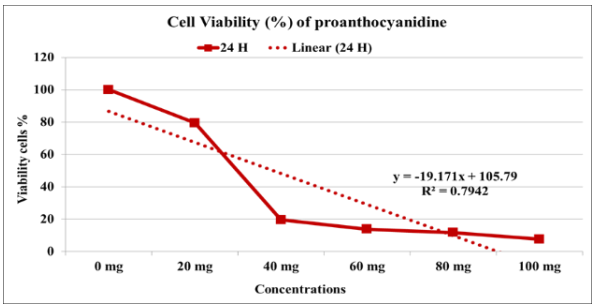

Figure 3 Histograms for the cell viability results of Proanthocynadin treatment on HPG2 cells after incubation for 24 hrs. The percentage of dead cells was measured by MTT cytotoxicity assay showing almost constant cell survival.

Morphology in untreated and treatedHepG2 cells:

When compared to untreated control cells, the morphology of HPG2 cells following treatment with Taxol, Atorvastatin, and Proanthocynadin was abnormal. Dosages of treatment altered the shape of treated cells. In general, morphological changes became apparent when treated cells began to exhibit gradual cell shrinkage, rounding, and separation from the tissue culture surface. Flasks and floating in tissue culture media, eventually leading to cell enlargement and rupture, (these morphological features give an indication of cell apoptosis). Treatment with Taxol does not show distinct changes in contrast to more prominent morphological changes seen in cells treated with other compounds compared with untreated control HPG2 cells (Figure 4).

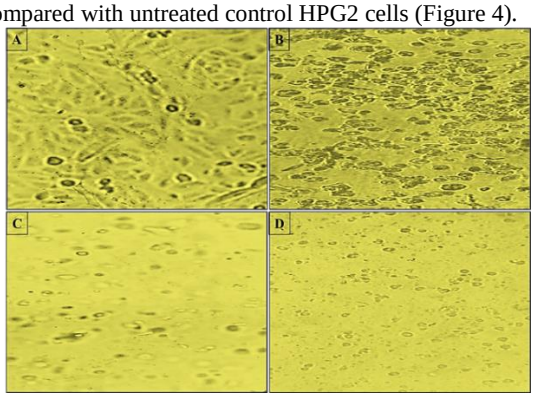

Figure 4 Morphological changes of experimental groups. A; control untreated HPG2 cells, B: Taxol; C: Atorvastatin; D: Proanthocynadin.

DNA fragmentation percent:

The DNA fragmentation test was used to determine the mechanism of action of Taxol, Atorvastatin, and Proanthocynadin against the HPG2 cell line (Figure 5). The different lanes profile the genomic DNA on agarose gel. Lane M: DNA ladder marker, lane 1: untreated cells with (Band 18), lane 2: Taxol-treated cells (Band 21), lane 3Atorvastatin-treated cells (Band 20), lane 4: Proanthocynadin-treated cells (Band 16). Obtained data showed that there was a slightly fragmented DNA in both Taxol or Proanthcyanidin treated groups while treatment with Ator caused a significant increase in DNA fragmentation.

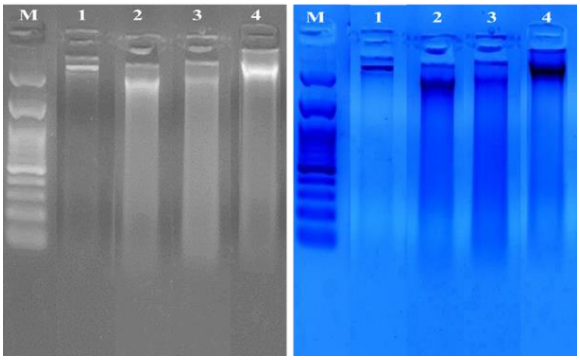

Figure 5 Agarose Gel Electrophoretic Analysis of DNA Isolated from HPG2 Cells untreated and treated with Different Concentrations of different compounds incubated for 48 hrs. M: DNA ladder; Lane 1: Control; Lane 2: Taxol; Lane 3: Atorvastatin; Lane 4: Proanthocynadin.

4. DISCUSSION

Chemotherapy is an important technique in clinical oncology today. The potential for systemic treatment of cancer patients with cytotoxic drugs was identified in the mid-twentieth century. To solve the resistance problem, drug development efforts were more focused on targeted treatments and combinations of anti-cancer medications to minimize off-target harmful effects. (Gilad et al., 2021).

The current study looked at the mechanism of Proanthocyanin's interactions with Taxol and Atorvastatin. To begin, the obtained results revealed that atorvastatin decreased the viability of liver cancer cells in a dose-dependent way. Second, using an MTT test, the current study indicated sub-cytotoxic levels of Proanthcyanidin with Taxol and Atorvastatin on HepG2 cells at various doses. This approach included inducing apoptosis in HepG2 cells that were exposed to DNA fragmentation analysis after being treated with Atorvastatin, Proanthcyanidin, or Taxol.

Taxol (Paclitaxel)is a powerful chemotherapeutic drug used to treat cancer patients. Paclitaxel inhibited HCC carcinogenesis by reducing cell proliferation and increasing apoptosis. CircRNA baculoviral IAP repeat-containing 6 (circ-BIRC6), were increased in HCC tissues and cells, whereas microRNA-877-5p (miR-877-5p) was downregulated. (Liu et al., 2020).

Taxol causes internucleosomal DNA breakage in human myeloid leukemia cells, which is related with programmed cell death. (Bhalla et al., 1993).

Previous research has shown that statins can trigger apoptosis by a variety of methods, including altering the mitochondrial membrane potential and releasing the second mitochondria-derived activator of caspases (Cafforio et al., 2005). The effects of statin concentrations on BCl-2(B-cell

lymphoma 2) have been widely investigated. Indeed, statins may trigger apoptosis by decreasing BCl-2 expression at high doses, but at low concentrations, they tend to prevent apoptosis and cell death by raising Bcl-2 expression (Ahmadi et al., 2023). The decrease of chromosomal DNA damage and nuclear division index NDI values with statin treatment could be indicated by the association between statin use and reduced risk of cancer (Donmez-Altuntas, H et al., 2019).

Shimura et al. (2021) supports the potential therapeutic benefit of combining andrographis with oligomeric proanthocyanidins (OPC), maybe as an additional treatment to standard medications, for patients with colorectal cancer. OPC is a novel allosteric serine-threonine protein kinase AKT inhibitor with significant anti-tumor efficiency, in addition to its antioxidant and anti-inflammatory properties, according to (Liu et al., 2020). Suganya et al., 2019 showed that Proanthcyanidin prevented cell growth, and the CI50 value for 24 hours of exposure was found to be 6.25 M when compared to the normal cell line, which does not exhibit toxicity.

Taxol increases the level of reactive oxygen species (ROS) in yeast oxidative stress response mutant strains. Further, 4',6-Diamidino-2'-phenylindole (DAPI) and acridine orange/ethidium bromide (AO/EB) staining show that Taxol induces apoptotic features such as nuclear fragmentation and chromatin condensation in DNA repair mutants. (Veerabhadrappa et al., 2021). Atorvastatin can modulate the DNA damage repair response in primary human endothelial cells exposed to MMC in a cell line- and incubation scheme-dependent manner (Sinitsky et al., 2023).

5. CONCLUSION

The present study showed a good cancer activity of Taxol, Atorvastatin and Proanthcyanidin against human hepatocellular carcinoma cell line (HepG2) and caused apoptosis for cancer cells *in vitro*.

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