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Chemical Analysis and Determination of the Cytotoxicity *Juglans Regia* L. Shell Extract with Cisplatin Drug

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Abstract

The history of the walnut tree dates back to 7000 B.C. in Asia, making it one of the earliest trees from which people have ever harvested items (Vahdati, 2019) [38]. Different portions of walnuts offer curative or preventative qualities (Mutha, 2015) [25]. Medicine, many underestimate the value of walnuts as an important medicinal plant (Delaviz, 2017) [11]. A qualitative analytical study was conducted to detect the phytochemicals in the ethanolic walnut shell extract using chemical reagents (Meyer, Dragendorf, phenols, flavonoids, glycosides, tannins, terpenes and saponins), where the results showed the presence of more than one active compound in this extract such as: alkaloids, phenols, flavonoids, tannins, terpenes and glycosides and the absence of saponins. Chemotherapy with cisplatin has been a staple of cancer care (Kelland, 2007) [18]. Cisplatin is a substance that kills cancer cells by creating intra- and inter-strand DNA crosslinks (Wang D. &., 2005) [40]. Cisplatin (CSD) was used as a standard drug in order to comparison with the JRSE. In addition, equal parts of chemotherapy were mixed with the JRSE to find out its toxic activity against certain normal and cancer cell lines and to know the index of interaction. The results showed that cisplatin chemotherapy has a strong to moderate inhibition capacity toward (PC3, A549 and MCF10A cell lines) compared with JRSE, which was characterized by being weakly inhibition after a 24-hour incubation period. Also, the results showed a higher ability of cisplatin to inhibit cell lines (A549 and MCF10A) than (PC3) cell lines. The ability of cisplatin chemotherapy stems from the ability of the treatments that contain platinum in its compounds and their ability to restrict DNA division in cells and thus contribute to cell death (Wang D. &., 2005) [40]. Selectivity index (SI); *Juglans regia* plus Cisplatin combine (JCP) shows safety index against MCF10 normal cell lines by reduced cisplatin toxicity toward normal cells, which means that JCP reduced the side effects of Cisplatin when they were combined together. JCP showed synergistic results towards PC3 cell line, while showed antagonistic results towards MCF10A cell lines, which means that it increased the inhibition activity against cancer cells and decreased the ability of cisplatin inhibition on normal cells.

Keywords: Juglans Regia Shell Extract, Phytochemical, Cisplatin, Selectivity Index SI, IAI

Introduction

Currently, cancer ranks first or second among the causes of early death in the majority of the world's nations, from east to west. Due to demographic shifts, population expansion, and aging populations around the world over the next 50 years (Soerjomataram, 2021) [34]. The most prevalent cancers include prostate, breast, lung, colon, and rectum cancers (Bray, 2018) [6]. Chemotherapy is a crucial part of the fight against cancer. It has helped increase cancer patient survival rates. The most significant adverse effect of chemotherapy is its psychological impact on the patient. Other side effects include nausea, vomiting, exhaustion, anemia, hair loss, and changes in taste and smell (Wagland R, 2016) [39]. Cancer is one of the most expensive diseases in most countries of the world, especially countries that do not have health insurance, especially poor countries. Therefore, the cost of chemotherapy to treat tumors may push people to sell their savings and everything they own for treatment (Kircher, 2019) [20]. Chemotherapy continues to be the cornerstone of cancer treatment techniques despite significant advancements in the field. They have been demonstrated to have considerable toxic effects on the heart, kidneys, marrow, neurons, liver, gastrointestinal toxicity, mucositis, and negatively impact the quality of life of cancer patients, among other adverse effects (Liu, 2021) [21].

Material and Methods

Walnut Shell Collection and Preparation

With a nut cracker, the walnut shells were manually removed. They were then carefully cleaned, rinsed, dried, and ground into

minute pieces using an electric grinder. Then it was sifted through a sieve the powder was then collected in airtight nylon bags and maintained in the laboratory at room temperature until it was needed.

Walnut Shells Extraction

The sample putted in a thimble in the Soxhlet extraction's conventional approach. Gradually filled with brand-new condensed extract (a name for the extraction solvent) from a distillation flask. The extracted analytics are transferred to the bulk liquid by the siphon, which suctions it from the thimble when the questioner reaches the overflow level and dumps it out again into a distillation flask.Until the extraction is finished completely (López-Bascón, 2020) [22]. From a plant's shell, 100 grams of fine powder are removed and put in an extraction thimble. Extract with 750 ml of 98±2% concentrated ethanol and let sit in the soxhelt for 24 hours at 45 C°. The final extraction product was filtered through Whatman No. 1 filter paper before the supernatant was evaporated in oven it out (at 45 °C) to remove solvent.

Chemical Identification of Phytochemicals in J. Regia Shell Extracts (JRSE)

All reagents were prepared according to (Shaikh, 2020) [32]. Alkaloids are detected in JRSE by used Meyers-Dringendorf's reagent, in addition, phenols by ferric chloride, flavonoids by potassium hydroxide, terpenes by sulfuric acid, glycosides by Benedict's reagent, tannins by lead acetate, and the saponins by shaking to form foam in the tube that contains the extract.

Preparation of the JRSE, CSD and Combine JCP Concentrations

1 mg of the extract was weighed and dissolved in 1 ml of deionized distilled water, then the rest of the concentrations were prepared after dilution to obtain (***1000***, 500, 250, 125, 62.5, 31.25 µg/ml). From the cisplatin ampoule containing the chemotherapy at a concentration of 1000µg/ml, the following dilutions were prepared (100, 50, 25, 12.5, 6.25µg/ml) after diluting them with deionized distilled water, then the treatment collection concentrations were prepared with the extract.

Finally, the concentrations consisting of an equal volume of the drug CSD and the extract JRSE were prepared and were as follows (500, 250, 125, 62.5, 31.25, 15.625 µg/ml) to use in order to MTT assay.

The MTT Assay Cytotoxicity Studies

One of the most useful and well-liked experiments is the MTT assay. During the MTT test, the water-soluble yellow dye MTT is transformed into an insoluble purple formazan by mitochondrial reductase. The optical density is then used to quantify the solubilized formazan. The end result is a sensitive test with remarkable consistency, with a linear range in the cells in each well. The MTT test is used to assess the viability of cells at a reasonably high throughput (96-well plates) (Xuemei Liu, 2018) [42]. Create the growth curve by calculating the cell density using the absorbance signal from the previously completed calibration. To evaluate cytotoxic potential, determine the percentage difference between the signal intensities of the sample and the control culture. The proportion of cell viability or the following formula was used to determine the degree of inhibition: Viability % = (optical density of sample⁄optical density of control) ×100

IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line (Rahman, 2011) [28].

Result and Discussion

In this study the results showed the presence of alkaloids in the extract used by Meyers-Dringendorf's reagent, in addition, phenols by ferric chloride, flavonoids by potassium hydroxide, terpenes by sulfuric acid, glycosides by Benedict's reagent, tannins by lead acetate, and the absence of saponins by shaking to form foam in the tube that contains the extract. The results agreed with Herrera *et al.* (2020) for the presence of chemical compounds flavonoids, tannins, phenols, terpenes, and glycosides in walnut shell extract, while (Sultanova, *et al*, 2022) they mentioned in their results that walnut shell contain large concentrations of phenols, flavanoids, and tannins, and these biochemicals are a great importance as antioxidants oxidative stress. Further, when studying the constituents of the portions of the walnut fruit, (Akbari, *et al.*, 2012) [3] noticed the presence of phenols and flavonoids.

The CSD Sample Safety Index Toward:

SI=MCF10A IC50% 26.0955 µg/ml /PC3 IC50% 49.1637 µg/ml
= 0.53 <2 SI, absent.

The JCP Sample Safety Index Toward:

SI=MCF10A IC50% 203.495 µg/ml /PC3 IC50% 49.14 µg/ml = 4.14>2SI, present.

The results showed a selective index of combination JCP for the MCF10A cell line, while the JRSE and CSD did not have a selectivity index, and this indicates the possibility of using them on the cell line (MCF10A) safely.

The biologically active molecules contained in the walnut tree make it one of the plants that help some cancer drugs to increase the safety index from their side effects, especially on normal cells, because of the effective antioxidants that inhibit free radicals and their accumulation (Catanzaro, 2018) [7].

The Interaction indices (IAI) of JCP as 'combined' against cancerous cell line (PC3) and normal cell line (MCF10A) were calculated according to the following equation;

IAI=d1/D1+d2/D2

IAI <1, Synergistic; >1, Antagonistic; =1, Addtive (Tong, 2015).

(d1, d2= drug partition; D1, D2 dose inhibit cells)

The stock solution of mixture combined plant extract + cisplatin prepared according to (0.5:0.5) partition.

JRSE=0.5
CSD=0.5

Human Prostate Cancer Cell Line (PC3)

Combined JCP;
IC50% of compounds mixture = 49.14µg/ml
d1= d_{CSD} =0.5 x IC50 % 49.14µg/ml = 24.57 µg/ml

d2= d_{JRSE} =0.5 x IC50 % 49.14µg/ml = 24.57 µg/ml
D1=D_{CSD} =49.1637µg/ml
D2=D_{JRSE} =360.64µg/ml
IAI=24.57 µg/ml/49.1637µg/ml + 24.57 µg/ml/360.64µg/ml
IAI = 0.568 <1, there is synergism.

Human Normal Cell Line (MCF10A)

Combined JCP;
IC50% of compounds mixture = 203.495µg/ml
d1=d_{CSD}=0.5 x IC50 % 203.495µg/ml = 101.75 µg/ml
d2=d_{JRSE}=0.5 x IC50 % 203.495µg/ml = 101.75 µg/ml
D1= D_{CSD} =26.0955µg/ml
D2=D_{JRSE} =430.03µg/ml
IAI = 101.75 µg/ml /26.0955µg/ml + 101.75 µg/ml /430.03µg/ml
IAI = 4.24 >1, there is antagonism.

Most of the walnut plant's components, including the fruit's shell, contain useful chemicals. It can suppress the activation of the signaling pathways, which inhibit the growth of cancer cells and stop the process of angiogenesis, which results in the formation of new blood vessels for malignant tissues. This molecule also acts as a brake material for the accumulation of free radicals in normal cells, which enhances the action of chemical treatments for cancer or to be an alternative to them (Karki, 2020) [17].

Conclusion

1. JRSE contains active compounds with biological activity.
2. The JCP has selectivity index toward MCF10A normal cell lines.
3. The JRSE has decreased the side effect of CSD when mixed together.
4. The combined JRSE and CSD showed synergistic activity with CPD against PC3 cancer cell line. While showed antagonistic results towards toward CSD against MCF10A normal cell lines.

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