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Cytotoxicity Study of *Fagonia arabica* L against Breast (MCF-7), Liver (HepG2), and Normal (MCF 10A)

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ABSTRACT

Cancer continues to be a leading cause of morbidity and mortality despite decades of scientific and clinical research and trials of promising new medicines (Siegel et al., 2023).In this study, two larger types of cancer were selected: which are Breast and Liver cancer. Breast cancer is the most common incidence and prevalent cancer worldwide, as well as the leading cause of death from any cancer in women (Juarez-Vignon Whaley et al., 2023).Although conventional therapy is effective, it gives a serious inclusion of drug resistance (Altalhi et al., 2023). A combination of therapy of anti-tumor drugs has been employed for quite a long time now, as it improves drug efficacy(Gunasekaran et al., 2023a).

In this study, the antioxidant property of the ethanolic extraction of Dhamaso, botanically known as a *Fagonia arabica* L (Prajapati et al., 2020) has been investigated. *F. arabica*genius falls under Zygophyllaceae, flowering dicot (angiosperms), is considered a vital curative plant (Khasim et al., 2020). It has a wide spectrum of medical traditional uses(Iftikhar et al., 2021). We investigated Phytochemical compositions that exist in FAE which has been very attractive for their potential effects on the health of humans. The qualitative biochemical screening by the chemical tests by using a 98%±2 ethanol solvent.The secondary metabolites of the extract were Phenols, Tannin, Flavonoids, Saponins, Alkaloids, Terpenes, and Glycosides using chemical reagents (Rahman et al., 2022).The MTT assay was employed to evaluate the cytotoxicity of FAE ethanolic extract(Oh & Hong, 2022). MCF10A cell line (normal cell line), MCF7 cell line (Breast cancer), and HepG2 cell line (liver cancer) were used in the current study. In 96-well plates, cells were initially plated. The extract has Moderate anticancer properties on HepG2 cells, MFC7, and weak on normal cell lines, IC50 is 88.46 ± 5.16 µg/mL, 78.87649 ±3.60 and 271.40 ± 3.21 µg/mL, respectively for FAE which described being (Moderate), while it was weak against normal cell lines after a 24-hour incubation period.The selectivity index (SI) for the combination between FAE and Cisplatin (FCP) shows that FCP reduced the side effects of Cisplatin when they were combined together. FCP showed synergistic results on the HepG2 cell line while showing antagonistic results on 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: *Fagonia arabica* L extract, phytochemical, Cisplatin, Selectivity index SI, IAI.

INTRODUCTION

It has been demonstrated by many studies that plant secondary metabolites not only enhance the curative benefits of cisplatin but also mitigate its chemotherapy-induced cytotoxicity (Farghadani & Naidu, 2022). Many natural products provide their therapeutic activity by promoting apoptosis by adjustment of “mitogen-activated protein kinase” (MAPK) and p53 signal transduction pathways and enhancing the chemosensitivity of cisplatin (Dasari *et al*., 2022).

Also, phytochemicals defend cisplatin-induced toxicity by facilitating many gene transcription factors and inducing apoptosis/necrosis. In addition, delay drug release, prolong half-life, and reduce systemic toxicity while other ingredients, like nano gels and hydrogels, facilitate cell membrane penetration, target cancerous cells, and inhibit tumors (Zhao *et al*., 2022).(Lubis *et al.,* 2023) Cisplatin is a stronger cytotoxic drug than the extract with

an IC_{50} value of 28.61 ± 0.13 µg/m01L. For normal cell lines (MCF10A), another study showed that cisplatin's cytotoxicity is strong in this type of cell line (Rashidi *et al.*, 2017).

MATERIAL AND METHODS

3.1. Plant Collection and Preparation

The utilized samples of the herb have been collected locally from the territories around Kufa city. The plant is taxonomized at the University of Kufa, department of Sciences.Arial parts of the plant were taken and cleaned up from the dust and any other strange materials, dried in an oven at 40° C and crashed via the mechanical grinder into a fine powder, and kept in a refrigerator at 4C (Shu et al., 2022). Then, the powder has extracted directly by the Soxhlet apparatus. 100 gm of the plant powder and placed into an extraction thimble, extracted with 750 ml ethanol (98%±2 concentrate), and the extraction was left at room temperature for 24 hours. The extract was filtered through gauze and then through filter paper (Wattman No.1), and then evaporated for dryness (45 C°) under depressed pressure in a rotary evaporator.

Chemical identification of phytochemicals

All reagents were prepared according to (Shaikh, 2020). The presence of alkaloids is proved by Dragendorff's test, while hydroxide potassium (KOH) revealed the flavonoids, shaking the test tube reflected the saponins, while Salkowski's test used terpenes. Froth tests & Haemolysis tests for Saponins (Manik et al., 2022). Benedict examines the presence of glycosides positively and ferric chloride solution was positive for phenols and finally lead acetate was positive for tannins. It is the chemotherapy used at different phases of the cell life cycle (Dasari et al., 2022). Assessment of the antioxidant properties of FAE was carried out using DPPH radical scavenging assay. Preparation of the JRSE, CSD, and combine JCP Concentrations.The combination of certain two substances was synergistic with a selectivity index <1 and was a very strong synergy. However, in terms of the mechanism of action of this combination is not yet identified and known clearly, so further investigation is needed (Lubis et al., 2023).1 mg of the FAE was mixed with 1 ml of deionized distilled water, then the concentrations were prepared (1000,500,250,125,62.5,31.25µg/ml). From the cisplatin ampoule 1000µg/ml, prepared (100,50,25,12.5,6.25µg/ml) after diluting them with deionized distilled water, then combined doses of equal volumes from the extract and the drug have been madeas follows: (500,250,125,62.5,31.25,15.625 µg/ml) to be used in MTT assay.

The MTT Assay Cytotoxicity Studies

The (IC_{50}) of extracts has been estimated following the described procedure: firstly 100 µL of DPPH (1 mg/mL) was added to 100 µL of each dose of (1000, 500, 250, 125, 62.5 µg/mL) of the fresh crude extract of Fagonia arabica. After incubation for 30 min at room temperature in the dark, the absorbance was measured at wavelength 517 nm by a spectrophotometer (Teng et al., 2022). The vital dose of the plant extract for 50 % inhibition of DPPH° (IC50) was gained from a plot of percentage inhibition versus extract concentration. Butylated hydroxyl toluene (BHT) is employed as a positive control, which is an antioxidant too (Arjeh *et al.*, 2022).

RESULT AND DISCUSSION

A group of researchers unveiled in a recent study (Walbi et al., 2023) the full agreement with the medical phytochemicals extracted from the same species (F. arabica) and confirmed the antioxidant properties they do have(Wazir *et al*., 2022).

The FA sample safety index toward;

Safety Index (SI) $= \frac{MCF10A\ cell\ line}{HepG2\ cell\ line}$ =343.36 µg/ml÷88.46 µg/ml = **3.88 > 1** (non-selective)

Safety Index (SI) $= \frac{MCF10A\ cell\ line}{MCF7\ cell\ line}$ = 343.36 µg/ml÷78.87 µg/ml = **4.35 > 1** (non-selective)

The CSD sample safety index toward;

Safety Index (SI) $= \frac{\text{MCF10A}}{HepG2}$= 26.10 µg/ml ÷ 28.48µg/ml= **0.91<1** (selective)

Safety Index (SI) $= \frac{\text{MCF10A}}{MCF7}$= 26.10 µg/ml ÷ 12.88 µg/ml= **2.02>1** (non-selective)

The FCP sample safety index toward;

Safety Index (SI) $= \frac{MCF10A\ cell\ line}{HepG2}$= 26.10µg/ml ÷ 214.17µg/ml= **0.12<1** (selective)

The results showed a selective index of the combination FCP against the HepG2 cell line, and CSD showed a selectivity index for the HepG2 cell line, while FAE indicates non-selectivity for each HepG2 and MCF7 like CSD for MCF7.

The combination of certain two substances was synergistic with a selectivity index <1 and was a very strong synergy. However, in terms of the mechanism of action of this combination is not yet identified and known clearly, so further investigation is needed (Lubis et al., 2023).

Phytoconstituents have a better safety index supporting some cancer drugs to increase the safety index from their side effects (Catanzaro, 2018), especially on normal cells mainly through their free radical scavenging properties (Halliwell, 1995). Flavonoids were demonstrated as agents of selective antiproliferative in most applied cancer cell lines, whereas cisplatin, the reference drug, showed non-selectively(Tronina et al., 2023).

Interaction index (IAI) of drug combinations.

The Interaction indices (IAI) of CSD as 'combined' against cancerous cell line HepG2 and normal cell line (MCF10A) were calculated according to the following equation;

IAI=d1/D1+d2/D2 FORMULA

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

(d, 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.

FAE=0.5, CSD=0.5

1.2.1. LIVER CANCER IAI

Combined FCP:

IC50% of FCP = 28.15 µg/ml

d1= d_{CSD} =0.5 x IC50 % 28.15 µg/ml = 14.07 µg/ml

d2= d_{FAE} =0.5 x IC50 % 28.15 µg/ml = 14.07 µg/ml

D1=D_{CSD} =28.48 µg/ml

D2=D_{FAE} =88.46 µg/ml

IAI = 14.07/28.48 + 14.07/88.46 = 0.64

IAI = 0.64 <1, there is **synergism**.

1.2.2. Human normal cell line (MCF10A).

Combined FCP:

IC50% of compounds mixture = 214.17 µg/ml

d1=d_{CSD}=0.5 x IC50 % 214.17 µg/ml = 107.08 µg/ml

d2=d_{FAE}=0.5 x IC50 % 214.17 µg/ml = 107.08 µg/ml

D1= D_{CSD} =26.09 µg/ml

D2=D_{FAE} =343.36 µg/ml

IAI = 107.08 µg/ml /26.09 µg/ml + 107.08 µg/ml /343.36 µg/ml

IAI = 4.41 >1, there is **antagonism**.

Though cisplatin is a really successful drug, it induces a number of toxic side effects caused by an overdose of cisplatin (Gunasekaran et al., 2023b). The major side effects of cisplatin are hepatotoxicity, nephrotoxicity, ototoxicity, and gastrointestinal toxicity (Appel, 2023). Therefore, efforts currently paid to combine cisplatin with natural ingredients, ethanolic extract and cisplatin were recognized by their IC_{50} values as guidance for the combination test through the MTT method (Lubis et al., 2023).

CONCLUSIONS

1. FAE including bioactive phytoconstituents.
2. FCP has a selectivity index toward MCF10A normal cell lines.
3. FCP effectively reduced of CSD side effects.
4. FCP showed synergistic role against HepG2 cancer cell line. while showed antagonistic results against MCF10A normal cell lines.

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