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Enhanced cytotoxicity of sorafenib in hepatocellular carcinoma through synergistic combination with naringenin: a molecular and cellular perspective.

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Abstract: The most prevalent kind of liver tumor is hepatocellular carcinoma (HCC). Sorafenib is an efficient multikinase inhibitor used for treatment of HCC. However, the expensive cost and severe side effects of sorafenib limit its therapeutic potential. Therefore, the use of combination therapies to increase survival is advised. Naringenin is a naturally occurring flavonoid that has anti-inflammation properties and has been utilized for many years as a powerful antioxidant. Hence, this research objective is to study the ability of naringenin to increase the sensitivity of HepG2 cells towards sorafenib. Using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test, the damaging effects of naringenin and sorafenib on HepG2 cells were assessed. In the present research, HepG2 cells were separated into 4 groups; control group, group given sorafenib treatment in its IC50 conc (4.3 µM), group treated with naringenin in sub-lethal dose ¼ IC50 (7.25 µM) and combination group. The co-administration of naringenin and sorafenib had more deleterious effects on cell viability than does either drug alone. Expression variations of genes associated with angiogenesis and apoptosis such as vascular endothelial growth factor-A (VEGF-a), tumor suppressor protein (TP53), caspase-8 and mitogen activated protein kinase 5 (MAP3K5) were examined using quantitative real-time PCR. Compared with single drug therapy, sorafenib/naringenin combination therapy showed higher inhibitory effects on VEGF-A and enhancing effects on TP53, Capase-8 and MAP3K5. Together, the present study results suggest that low concentration of naringenin enhances the HepG2 cells sensitivity towards sorafenib alone. For naringenin to be used therapeutically in different types of cancer, more in vivo and in vitro studies on the dosage and duration of naringenin use are needed.

Keywords: Hepatocellular carcinoma (HCC); sorafenib, naringenin; sensitivity; VEGF-a; Tp53; caspase-8, MAP3K5.

1. INTRODUCTION

The sixth most frequent cancer in the world is HCC. As it's one of the most common malignancies1, Sorafenib is an oral multikinase inhibitor which has been demonstrated to have antiproliferative properties in HCC cell lines. Additionally, it reduced tumor-cell signaling and tumor angiogenesis, even though it raised the apoptosis 2. However, the expensive cost, poor tumor response especially in prolonged use, and severe adverse reactions of sorafenib restrict its therapeutic potential 3, 4. Therefore, it's necessary to find a useful natural neo-adjuvant product to improve the tumor response and to lessen sorafenib's adverse effects.

The flavonoid naringenin is a natural product found in citrus fruits, particularly grapefruit, having the ability to treat many cancer forms, either when administered alone or in combination with other agents5, 6. Flavonoids like naringenin are potent angiogenesis inhibitors, they are considered to be a promising treatment for cancer7. Since angiogenesis contributes to the development of liver fibrosis into cirrhosis and HCC, anti-angiogenesis therapy can ameliorate hepatic fibrosis 8. Vascular endothelial

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growth factor-A is a crucial signaling component that promotes angiogenesis and hence facilitates HCC growth and metastasis 9. Apoptosis is the natural physiological reaction of cells to a variety of stimuli, infections, or damage, such as radiation therapy protocols or cytotoxic drug treatments that cause irreversible damage to deoxyribonucleic acid (DNA), fail to induce cell death and the resulting gathering of damaged cells within the body can cause a variety of cancers 10.

From the most important genes involved in apoptosis are: Caspase-8, TP53 and MAP3K5. Caspase-8 is an essential component of the extrinsic apoptotic pathway, which triggers fast cell death upon external stimulation of death receptors 11. Tumor suppressor protein is participating in cell death, DNA repairing, senescence, and cell cycle arrest 12.

It's been proven that several proteins are mutated in HCC; TP53 is one such protein that is altered in about 25% of HCC cases 13. Apoptosis signal-regulating kinase 1 (ASK1) belongs to MAP3K family that is also recognized as MAP3K5 14. Research has demonstrated that the ASK1 signaling pathway is induced by oxidative damage or inflammation, ASK1 deregulation relates to a variety of human illnesses, comprising neurological disorders, cancer, and inflammation 15.

This work aims to explore the synergistic beneficial impact of sorafenib and naringenin therapy on angiogenesis, cell death, and cell cycle stages in HepG2 cells. The target is to propose a novel approach for treating HCC enhancing the responsiveness of HepG2 cells to sorafenib by augmenting its anticancer properties through combination with naringenin. To reject the null hypothesis, we need to analyze the changes in gene expressions of angiogenesis and apoptosis-related genes (VEGF-A, TP53, caspase-8, and MAP3K5) when treated with a combination of sorafenib and naringenin. This helps us understand the possible clinical use of naringenin as a supplementary treatment to sorafenib for HCC.

2. METHODS

2.1. Study design and Materials

This work involved categorizing HepG2 liver cancer cells into four different groups. Group 1: Untreated HepG2 cells incubated with DMSO, Group 2: HepG2 cells treated with 4.3 µm Sorafenib, Group 3: HepG2 cells treated with 7.25 µm Naringenin, Group 4: HepG2 cells treated with 7.25 µm Naringenin + 4.3 µm Sorafenib.

HepG2 cells were acquired through Nawah Scientific Inc., (Mokatam, Cairo, Egypt). They were cultivated with Dulbecco's Modified Eagle's Medium (DMEM) accompanied with high glucose with stable glutamine, fetal bovine serum (FBS) and Penicillin-Streptomycin were purchased from Serana Europe GmbH, Germany. GeneJET RNA Purification Kit (Cat. #: K0731), TaqMan Reverse Transcription Kit (Cat. #: 4387406), TaqMan™ Gene Expression Master Mix (Cat. #: 4370048) and TaqMan Gene Expression Assay for both " MAP3K5 (Ask1), TP53", "Caspase8" and "VEGF-A" were purchased from Applied Biosystems by Thermo Fisher Scientific, Graiciuno, Vilnius, Lithuania.

For cellular metabolic activity and as an indicator of cell viability MTT was obtained through Sigma Aldrich, St. Louis, MO, USA.

Sorafenib (Nexavar®) Free base, >99% was purchased from Genuine LC Laboratories brand PMA, U.S.A. Naringenin (Natural (US) 98%) was acquired through Sigma Aldrich, St. Louis, MO, USA.

2.2. Cell Culture and Viability Assay

The Cells were grown in DMEM enhanced with 1% of 100 mg/ml of streptomycin,100 units/ml of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C in the cell culture laboratory, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt. At 80–90% confluency, cells were passage through the assays.

In this study, HepG2 cells were cultured at a density of 1 × 10^5 cells/well in 96 well plates accordance with the MTT cell viability assay protocol. Sorafenib and naringenin were prepared as a stock solution of 100 mM and serial diluted at 5 differing concentrations (0.01, 0.1, 1, 10 and 100 µM). Distinct sorafenib and naringenin amounts were administered following 24hour incubation. Cells were fed with 100 µl of growth media comprising 0, 0.001, 0.01, 0.1, 1, 10, 100, and 1000 µM of the desired drug in triplicate after a 24-hour incubation period.

Sorafenib and naringenin were applied to cells at the appropriate concentration for 48h. Concentrations of sorafenib and naringenin that reduced the survival of the cells to its half (IC50) were established and outcomes were spectrophotometrically assessed at 450 nm through a microplate reader (BioTek, USA).

IC50 of sorafenib and naringenin was found to be 4.3 µM and 29 µM for 48h respectively. There were observed time- and dose-dependent declines in the HepG2 cell viability. In this study, HepG2 cells received treatment with sorafenib in its IC50 conc (4.3 µM), and naringenin in sub-lethal dose (¼ IC50; 7.25 µM) and their combination to recognize if naringenin will increase the sensitivity of HepG2

cells towards sorafenib. The choice of sublethal dose is to lessen the action of naringenin and only examine its ability to sensitize the HepG2 cells towards sorafenib treatment This is predicated on earlier research like Ahmed *et al*. [16] who used sublethal doses of FTY720 (Fingolimod) to improve the viability decline brought on by treating HCC cell lines (HepG2 and HuH7) with escalating sorafenib dosages.

2.3. Real-Time PCR Analysis

On collagenated six-well plates, 3 × 10^5 cells/well were used to seed HepG2 cells. 24 hours after the incubation process with the exception of the control well, all wells received the previously stated quantities of sorafenib, naringenin, and their amalgamation. The wells were then incubated for 48 hours.

Total RNA was taken out by means of the Gene JET RNA Purification Kit protocol. Liquifying the extracted RNA in 40 µl of RNase free water, it was stored at -80 ◦C till the following procedure. RNA that had been extracted was measured with a NanoDrop® 1000. The extracted RNA was split, moved to RNAse free Eppendorf tubes, and kept at -80°C until needed.

The TaqMan Reverse Transcription Kit was used for reversing transcribe 100 ng of total RNA in accordance with the manufacturer's instructions.

Analysis of gene expression of (VEGF-A, TP53, caspase-8, and MAP3K5) was done by means of quantitative real-time PCR system (Applied-Biosystems, USA) regarding TaqMan™ Gene Expression (Thermo Fisher Scientific, Graiciuno, Vilnius, Lithuania) technique. For the real-time PCR study, certain primers were used for every gene. The GAPDH housekeeping gene was used to standardize the results of the expression of the chosen gene.

2.4. Cell-Cycle Analysis

After treating HepG2 cells with sorafenib and naringenin at the specified dosages, they were incubated for 48 hours. The impact of sorafenib and naringenin on cell cycle distribution was detected by flow cytometry. Trypsinization was used to gather the cells, and they were then double washed in ice-cold phosphate buffered saline. After being suspended again in 2 ml of 60% ice-cold ethanol, the cells are fixed for one hour at 4ºC. The fixed cells were washed double once more with PBS (pH 7.4) and suspended again in one milliliter of PBS comprising 50 µg/mL RNAase A and 10 µg/mL propidium iodide. Following a twenty-minute darkened incubation period at 37 ºC, cells were subjected to flow cytometry investigation utilizing the FL2 (λex/em 535/617 nm) signal detector (ACEA Novocyte™ flow cytometer) to determine the DNA content. There were 12,000 occurrences for every sample. The distribution of the cell cycle has been ascertained using ACEA NovoExpress™ software.

2.5. Statistical analysis

Every experiment was individually performed in triplicate. GraphPad Prism® version 8.01 (GraphPad Software, San Diego, USA) were utilized to analyze the entire data and create the charts. Fold change of the relative expression of qRT-PCR was calculated using the relative cycle threshold (2-ΔΔCt) technique. Data was normalized against GAPDH. Findings were provided as mean ± standard deviation (SD). The gathered information was exposed to Shapiro Wilk normality test to examine the parametric distribution pattern. The entire data passes the normality test, consequently, group variances were calculated using the student's t-test or ANOVA. The acceptable level for statistical significance was set at P value < 0.05.

3. RESULTS

3.1. Cytotoxic activity

Demise of HepG2 cells following administration of sorafenib, naringenin, and a combination therapy was evaluated by using the MTT test. The viability of HepG2 cells was observed to exhibit time- and dose-dependent decline patterns. In this work, IC50 of sorafenib and naringenin was determined to be 4.3 µM and 29 µM for 48h respectively. While the combination treatment (sorafenib 4.3 µM + naringenin 7.25 µM) reduced the IC50 0f sorafenib to 2.29 µM as shown in Figure (1).

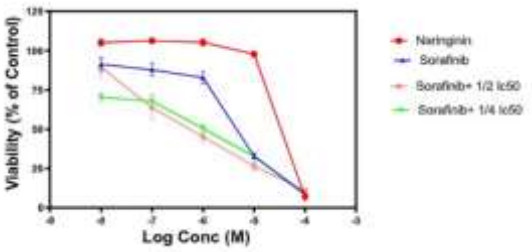

Figure 1. Dose response curve for determination of IC50 of sorafenib and naringenin and the effect of their combination on HepG2 hepatocellular carcinoma cell line.

3.2. Real-time PCR analysis:

The quantity of mRNA for some genes varied significantly, according to real-time PCR analysis results. As shown in Figure (2), in the group

administered with 4.3 μM sorafenib, in comparison with the control group, the degree of VEGF-A expression was significantly lesser. (0.2343±0.0360 vs 1.003±0.0921) whereas there was a significant rise in the expression level of TP53, caspase-8, and MAP3K5. (24.32±1.165 vs 1.003±0.0981, 3.797±1.225 vs 1.005±0.1204 and 4.631±0.6385 vs 1.006±0.1356) respectively compared to control group. These finding display that single treatment of sorafenib causes suppression of angiogenesis and induction of apoptosis in HepG2 cells. Alternatively, in 7.25 μM naringenin treated group, VEGF-A expression level was significantly decreased in comparison to control group (0.4077±0.0313 vs 1.003±0.0921). while TP53, caspase-8 and MAP3K5 expression level was non-significantly increased respectively (7.652±2.132 vs 1.003±0.0981, 1.757±0.0136 vs 1.005±0.1204 and 2.504±0.8373 vs 1.006±0.1356) compared to control group. In 4.3 μM sorafenib + 7.25 μM naringenin combination group, the degree of VEGF-A expression was significantly decreased (0.06413±0.0181), while TP53, caspase-8 and MAP3K5 expression level was significantly increased respectively (64.19±13.90, 6.404±0.1551 and 10.34±0.7999) compared with the control and the single treatment groups. In this regard, treating HepG2 cells with both sorafenib and naringenin together can exhibit both angiogenesis suppression and apoptotic induction.

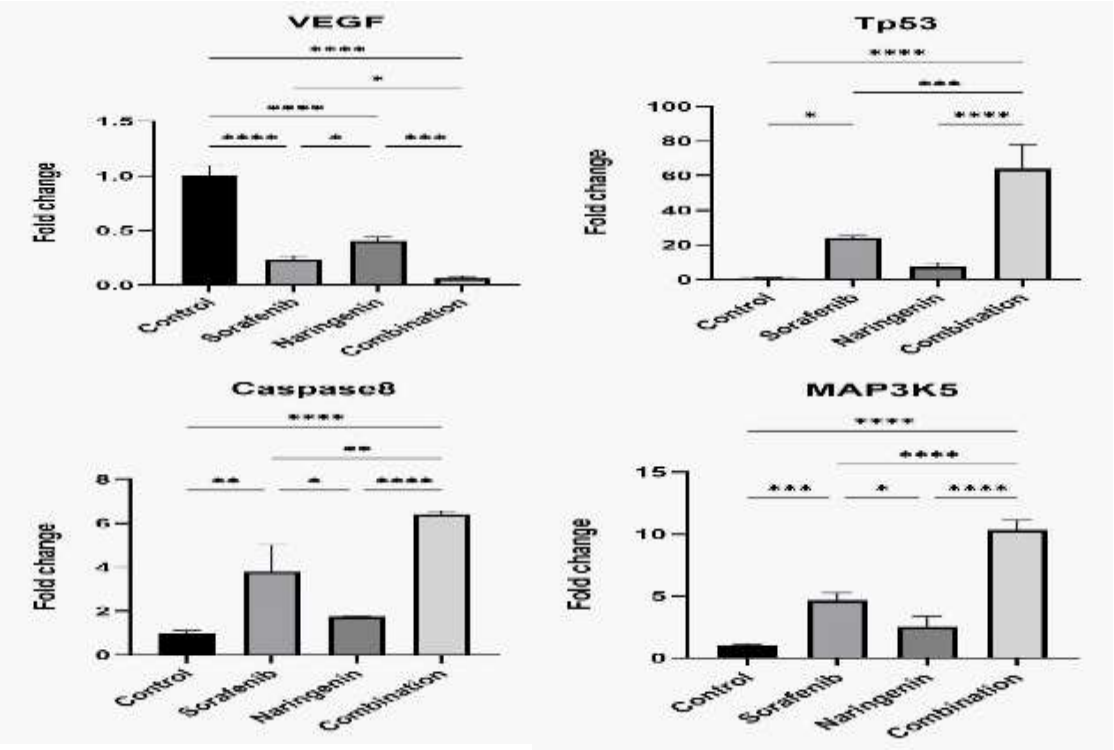

Figure 2. Fold change of VEGF-A, TP53, caspase-8 and MAP3K5 in HepG2 cells in the studied groups (mean ± SD). Statistical analyses were performed using repeated-measurers ANOVA followed by Tukey's post hoc test and the criterion for statistical significance was set to be significant at $p < 0.05$.

3.3 Sorafenib and Naringenin Regulated Cell Cycle Progression

It has been confirmed that disruption of the cell cycle has a role in abnormal cell growth and the occurrence of cancer[17]. As exposed in Figures (3, 4, 5, 6) HepG2 cells treated with sorafenib (4.3 μM), the cell growth arrest in G0/G1 phase was enhanced compared with the control group (64.67 ± 2.897 vs. 48.183 ± 2.550). As for naringenin, the cell growth arrest in G0/G1 phase was non-significantly increased compared to control group (54.69 ± 2.875 vs. 48.183 ± 2.550).

Dual treatment of sorafenib and naringenin 4.3 μM and 7.25 μM respectively caused cell growth arrest in G0/G1which was significant compared with the control group (76.86 ± 3.88 vs. 48.183 ± 2.550) but non-significant in comparison with sorafenib treated group.

4. DISCUSSION

Hepatocellular carcinoma, which accounts for around 75% of liver cancer cases, is the predominant histological form of primary liver cancer[18]. The present study included the use of two drugs for the treatment of HCC, the first one is sorafenib which is a multikinase inhibitor chemotherapeutic agent.

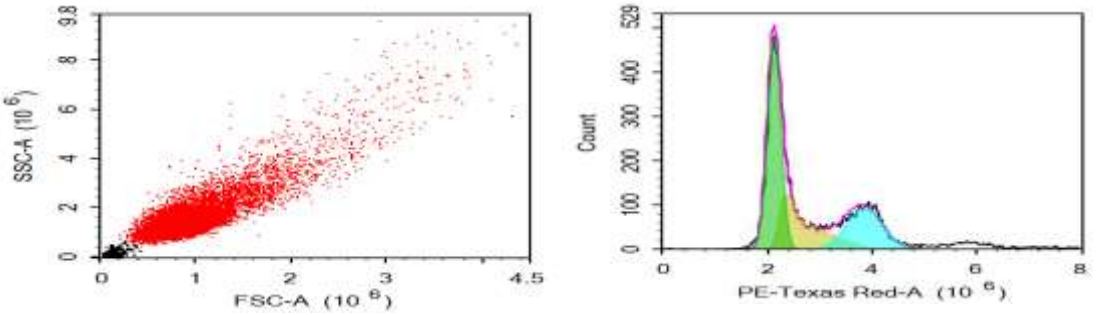

Figure 3. Cell cycle analysis of HepG2 cells in control group.

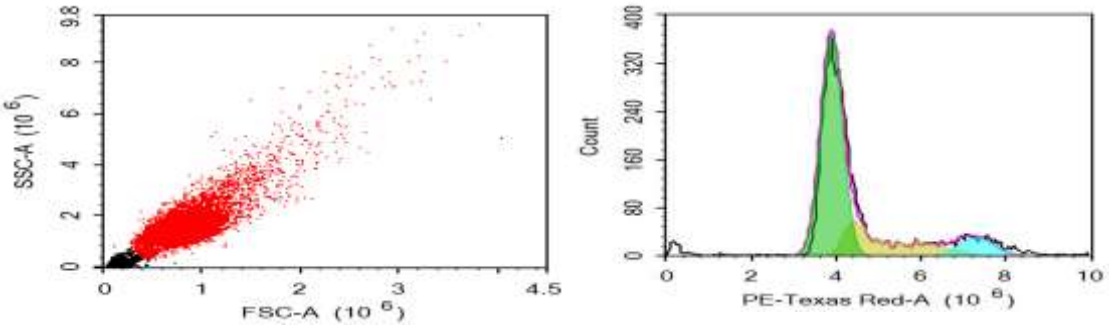

Figure 4. Analysis of the cell cycle in the HepG2 cells treated with sorafenib.

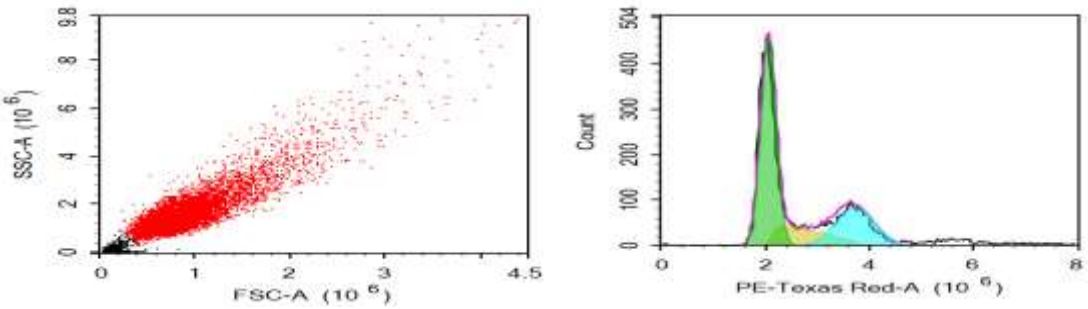

Figure 5. Analysis of the cell cycle in the HepG2 cells treated with naringenin.

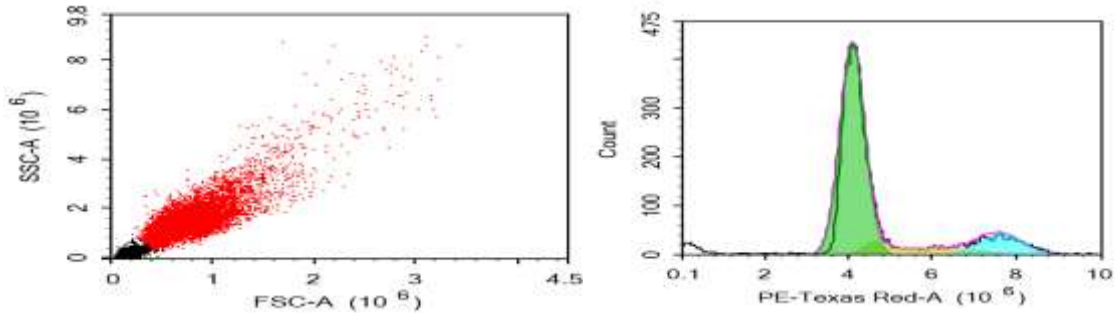

Figure 6. Cell cycle analysis of HepG2 cells in the dual treated group.

Sorafenib causes reduction of angiogenesis, promotion of apoptosis, and prevention of the growth of malignant cells [19]. Unfortunately, there are numerous side effects associated with sorafenib therapy, which forces many patients to lower their dosage or terminate treatment [20]. This is the reason why studies on the use of natural products or active ingredients in conjunction with chemotherapy drugs for the therapy of HCC have recently been conducted. Naringenin is a natural compound, which regarded as one of the primary bioactive flavanones. In a previous study, it exhibited cytotoxicity against HepG2 cells and minimized cytotoxicity against LO2

normal liver cells, demonstrating the selective cytotoxicity of naringenin [21].

In the current study, sorafenib (0.01, 0.1, 1, 10, 100 μM) dramatically repressed the propagation of HCC cells in a dose dependent way when compared to the control group, through an estimated IC50 value of 4.3 μM for 48 h using MTT analysis. This outcome was in line with Wang *et al.* [22] who stated that sorafenib significantly suppressed the propagation of HepG2 cells in dose dependent way, with IC_{50} 4.79 μM. Şirin *et al.* [23] also stated that sorafenib reduced the propagation of HepG2 cells with IC_{50} 7.5 μM for 48 h by means of XTT assay. The IC50 dose variances between the present findings and those found in previous literature are assumed to be the result of various cell viability analysis techniques.

In the present study, the proliferation of HCC cells was dramatically decreased in a dose-dependent way after treatment with naringenin (0.01, 0.1, 1, 10, and 100 μM) compared to the control group, with an approximate IC50 value of 29 μM for 48 h using MTT analysis. This outcome was consistent with a study conducted by Zhang *et al.* [21] which indicated that naringenin induces apoptosis in HepG2 cells by lowering mitochondrial membrane potential.

Li *et al.*[26] also reported that Treatment with Naringenin inhibited the development of Human Mammary Epithelial Cells (HMECs) and human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner; the IC50 values were 26.17 ± 3.62 μM and 29.97 ± 4.18 μM, respectively.

Zhao *et al.*[37] stated that naringenin was able to inhibit the growth of human breast cancer cells (MCF-10A and MDA-MB-231) by causing cell death, which was associated with changes in the cell cycle and the encouragement of apoptosis. In an animal model of breast cancer induced by 12-dimethylbenz[a] anthracene (DMBA), it also showed anti-inflammatory activity and was implicated in controlling the mitochondrial-mediated apoptotic cell signaling pathway.

In the present study, to determine the drugs concentrations that would be used, naringenin with different sublethal doses was used to choose the least possible concentration that would result in sensitization of HepG2 cells to sorafenib and in the meantime does not exhibit significant cytotoxic effect. The selected concentrations were the IC50 of sorafenib and ¼ IC50 of naringenin (4.3 μM and 7.25 μM) respectively. The viability of HepG2 cells using these concentrations was reduced showing that the addition of naringenin even in ¼ IC50 dose has decreased the IC50 of sorafenib which reflects that naringenin significantly increased sensitization of HepG2 cells to sorafenib.

Based on the above, this research intends to provide a new treatment strategy for HCC and sensitize HepG2 cells to sorafenib by enhancing its anticancer activity when combined with naringenin. It also aims to explore the combination therapeutic impact of sorafenib & naringenin over angiogenesis, cell death, and cell cycle on HepG2 cells.

In the present work, sorafenib significantly decreased the gene expression of VEGF-A. This result is consistent with Xu *et al.* [24] Who stated that following sorafenib administration, a decrease in VEGFA mRNA expression was seen in human hepatoma cells (SMMC 7721) and (MHCC 97H). According to their findings, sorafenib's antiangiogenic properties, induction of apoptosis, and reduction of cell proliferation could all be caused by blocking tumor signaling pathways, specifically the HIF-1a/VEGFA pathways.

Wang *et al.* [25] also stated that in subcutaneous and orthotopic H22 hepatic carcinoma models, sorafenib reduces tumor proliferation by dramatically downregulating VEGF-A expression and mitigating the VEGF-mediated angiogenesis process.

In the present study, there was a significant downregulation of VEGF-A level after naringenin treatment. This was consistent with another research project conducted by Li, Q *et al.*, [26] who found naringenin not just prevented Akt, focal adhesion kinase (FAK), and paxillin phosphorylation, which in turn prevented VEGF-induced kinase insert domain-containing receptor (KDR) signaling cascades in HUVECs, but it also reduced KDR's tyrosine kinase activity, which is necessary for VEGF's mitogenic effects on endothelial cells.

In the current study, the TP53 expression was significantly increased after sorafenib treatment. This was in accordance with Wei *et al.* [27] who showed that following sorafenib treatment, Tp53 levels had significantly increased.

Kerdput *et al.* [28] also indicated that sorafenib promoted apoptosis in rats carrying HCC, which in turn caused the production of TP53 and Bcl-2-associated X protein (Bax) mRNA.

In the present study, the low concentration of naringenin slightly increased TP53 level but was not statistically significant in comparison with control group. Xu *et al.* [29] stated that the mRNA transcription of the p53 genes was somewhat reduced by naringenin at a low dose (10 μM).

In the present work, the level of caspase-8 expression was significantly upregulated after the treatment of HepG2 cells with sorafenib. This result was in accordance with a research done by Gao *et al.* [30] who explained that following a 48-hour sorafenib

treatment of HepG2 cells and Huh7, the amount of cleaved caspase-8 protein was significantly up-regulated. They explained that by the drug's ability to inhibit he development of HCC cells and encourage cell death via the PKA/AMPK/eEF2K signal pathway.

In the current research, after treatment of HepG2 cells with low concentration of naringenin, there was no significant alteration in the expression of caspase-8 when related to the control group. The outcomes were consistent with Filho *et al.* [31] and Noori *et al.* [32] who stated that in human breast cancer cells (MDA-MB-231) administrated with naringenin, there was no apparent change in the expression of caspase-8.

In the current study, the expression of MAP3K5 (ASK1) was significantly upregulated when HepG2 cells treated with sorafenib. This was in line with El Dika *et al.* [33] who stated that the observed effect was attributed to sorafenib ability to inhibit rapidly Accelerated Fibrosarcoma (Raf-1) and increase ASK1. They explained that the cytotoxic effect of chemotherapy and the induction of apoptosis in endothelial cells are dependent on ASK1, which is bound to and neutralized by Raf-1[28].

In the current study, after treatment of HepG2 cells with naringenin, there was no significant change in the expression of MAP3K5 (ASK1) when compared to control group.

In the existing study, VEGFA expression level was significantly decreased in the combination group treated with (7.25 μM naringenin + 4.3 μM sorafenib) in comparison with control and sorafenib-treated groups.

There was also statistically significant rise in each of (TP53, caspase-8 and MAP3K5) expression level in comparison to control and sorafenib treated groups.

Based on the mentioned above, co-treatment of naringenin 7.25 mM offered improved sensitivity of HepG2 cells towards sorafenib at 4.3 μM ($P < 0.001$). Moreover, several molecular changes took place as compared to sorafenib alone, suggesting an increased sensitivity of sorafenib to HepG2 cells.

The present findings provide evidence that Naringenin possess pro-apoptotic and chemo-sensitizing effects on HepG2 cells to sorafenib through down regulation of angiogenic gene (VEGF-A) and upregulation of pro-apoptotic genes (Tp53, caspase-8 and MAP3K5).

The dysregulation of the cell cycle system was demonstrated previously to enhance the development of different kinds of cancer cells [34, 35]. Cell cycle arrest is a significant feature of anticancer drugs and is usually used in the design of sequential chemotherapy[35].

In the present study, sorafenib significantly inhibited cell development and caused the arrest of cell cycle at G0/G1. These findings were in line with Zhang *et al.* [36] who showed that sorafenib had promoted apoptosis dose-dependently, caused cell cycle arrest in the G0/G1 stage, and lowered cell proliferation in the acute promyelocytic leukemia cell line (NB4). Such effects could be brought on by the overexpression of caspase-3 and caspase-8, and by the downregulation of Myeloid cell leukemia-1 (MCL1), an antiapoptotic protein, and cyclin D1, which is a protein linked to the cell cycle.

In the present study, there was an alteration in the cell growth of naringenin treated group although this alteration was non significantly different compared to control. Xu *et al.* [29] showed that higher doses of naringenin caused significant cell cycle arrest in the G0/G1 stage.

In the existing research, the combined therapy of HepG2 cell with 4.3 μM sorafenib and 7.25 μM naringenin caused an improvement in G0/G1 cellular arrest, compared to the sorafenib-treated group although this improvement was not statistically significant ($p > 0.05$).

5. CONCLUSIONS

The gene expression of VEGF-A was downregulated while that of (Tp53, caspase-8 and MAP3K5) were raised in regard to the dual administration of ¼ IC50 of naringenin and IC50 of sorafenib. The combined treatment resulted in enhanced cell arrest in G0/G1 phase although it was non-significant compared to sorafenib treated group.

The current study's findings suggest a sensitizing action of naringenin on HepG2 cells towards sorafenib treatment as demonstrated by enhanced apoptosis and reduced angiogenesis.

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Conflicts of Interest: The authors declare that they have no competing interest.

Ethical Statement: The research was conducted in agreement with the ethical guidelines and standards established by the Ethics committee of faculty of

pharmacy, (Girls), Al-Azhar University (REC. No. 150).

Author Contribution: This work was carried out in collaboration between all authors. **Doha E. Gouda**: Investigation, Formal analysis, Resources and Writing Original Draft. **Ahmed I. Abulsoud**: Conceptualization, Validation, Software, Editing manuscript, Supervision, Management and Coordination. **Noha A. Eldesoky**: Visualization, Writing Review, Editing, Supervision and gaining ethical approval.

List of Abbreviations: HCC: Hepatocellular carcinoma, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, VEGF-A: tumor suppressor protein ascular endothelial growth factor-A, TP53: Tumor suppressor protein, MAP3K5: Mitogen activated protein kinase 5, DNA: deoxyribonucleic acid, Ask1: Apoptosis signal-regulating kinase 1, DMEM: Dulbecco's Modified Eagle's Medium, FBS: fetal bovine serum, FTY720: Fingolimod, HepG2 & HuH7: Human hepatoma cell lines, HMECs: Human Mammary Epithelial Cells, HUVECs: human umbilical vein endothelial cells, MCF-10A and MDA-MB-231: Human breast cancer cells, DMBA: 12-dimethylbenz[a] anthracene, FAK: focal adhesion kinase, KDR: kinase insert domain-containing receptor, Bax: Bcl-2-associated X protein, Raf-1: rapidly Accelerated Fibrosarcoma, NB4: acute promyelocytic leukemia cell line, MCL1: Myeloid cell leukemia-1.

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