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Fig Seed Oil Induces Mitochondrial-Mediated Apoptosis in HepG2 Cells

Adem YAVAŞ[1]

[1] Aydın Adnan Menderes University, Çine Vocational School, Food Quality Control and Analysis Programme, Aydın, Türkiye

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Abstract

Objective

Hepatocellular carcinoma (HCC) remains a significant global health challenge with limited effective therapies, necessitating the identification of novel therapeutic agents. Fig seed oil (FSO), rich in bioactive compounds, is being explored for its health benefits, including potential anti-cancer effects. This study investigated the cytotoxic, pro-apoptotic (via the mitochondrial pathway), and oxidative stress-modulating effects of FSO on HepG2 cells.

Material and Method

HepG2 cells were treated with FSO (0.1-1% v/v). Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay over 24, 48, and 72 hours. Oxidative stress markers, including Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI), were measured by ELISA. Key apoptotic proteins (Bax, Bcl-2, cytochrome c, cleaved caspase-9, and cleaved caspase-3) were evaluated using Western blot analysis.

Results

FSO demonstrated dose-dependent cytotoxicity against HepG2 cells, with an IC50 of 0.45% v/v at 72 hours. Treatment with 0.5% v/v FSO significantly improved cellular oxidative status by increasing TAS (1.8±0.2 vs 1.2±0.1 mmol Trolox eq/L, $p<0.01$), decreasing TOS (7.8±0.9 vs 12.4±1.3 µmol H_2O_2 eq/L, $p<0.001$), and consequently reducing the OSI by 58%. Furthermore, FSO induced mitochondrial apoptosis, evidenced by a 2.5-fold upregulation of Bax, a 60% decrease in Bcl-2, a 3.2-fold increase in cytosolic cytochrome c, a 2.8-fold increase in cleaved caspase-9, and a 3.1-fold increase in cleaved caspase-3 ($p<0.05$ for all significant changes).

Conclusion

This study shows that fig seed oil effectively induces mitochondrial-mediated apoptosis in HepG2 cells through caspase activation and simultaneously mitigates cellular oxidative stress. These findings suggest that FSO holds significant therapeutic potential for the treatment of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma; Fig seed oil; Apoptosis; Mitochondrial pathway; Oxidative stress.

Correspondence: A.D. / adem.yavas@adu.edu.tr
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ORCID IDs of the Authors: A.D: 0000-0003-3506-4306

Introduction

HCC represents a major global health challenge, ranking as the sixth most common cancer and the third leading cause of cancer-related mortality worldwide (1). The increasing prevalence of risk factors such as chronic viral hepatitis, alcohol-induced liver damage, non-alcoholic fatty liver disease, and metabolic syndrome contributes to its growing incidence (2). Despite progress in diagnosis and treatment, the prognosis for HCC patients remains poor, with five-year survival rates typically below 20%, highlighting an urgent need for more effective therapeutic interventions targeting the underlying molecular mechanisms of the disease (3).

Apoptosis, or programmed cell death, is a fundamental biological process crucial for maintaining tissue homeostasis (4). Evasion of apoptosis is a recognized hallmark of cancer, enabling malignant cells to survive and proliferate uncontrollably (5). In the context of HCC, resistance to apoptosis plays a significant role in tumor initiation, progression, and resistance to conventional therapies like chemotherapy (6). Therefore, strategies aimed at restoring or inducing apoptosis in HCC cells hold considerable promise as a therapeutic approach for this aggressive malignancy.

The intrinsic apoptotic pathway, commonly referred to as the mitochondrial pathway, is fundamental in orchestrating programmed cell death (7). Triggered by various cellular insults such as DNA damage or oxidative stress, this pathway converges on the mitochondria, which function as critical regulatory centers (8). A key event is mitochondrial outer membrane permeabilization (MOMP), leading to the release of pro-apoptotic molecules like cytochrome c (cyt-c) into the cytoplasm. Cyt-c initiates the activation of a caspase cascade, a sequence of proteolytic enzymes responsible for dismantling the cell, resulting in characteristic apoptotic features like DNA fragmentation and membrane blebbing (9). The Bcl-2 family of proteins precisely regulates MOMP, maintaining a balance between pro-apoptotic members (e.g., Bax, Bak) that promote permeabilization and anti-apoptotic members (e.g., Bcl-2, Bcl-xL) that inhibit it, ultimately dictating cell survival or death (10, 11).

In the quest for improved cancer therapies, natural compounds from plants are gaining significant interest. These compounds often possess the ability to modulate apoptotic pathways, potentially offering greater selectivity towards cancer cells and reduced toxicity to normal tissues compared to conventional treatments (12). Plant-derived oils, particularly those rich in bioactive lipids, antioxidants, and phytosterols, have shown considerable promise. Studies on flaxseed and olive oils, for example, have highlighted their anti-inflammatory, antioxidant, and apoptosis-inducing capabilities (13). FSO, extracted from Ficus carica seeds, is emerging as another valuable natural product, with accumulating evidence supporting its diverse pharmacological actions, including antioxidant, anti-inflammatory, and potential anticancer effects, warranting further exploration of its role in modulating apoptosis (14).

Ficus carica, a tree valued for its fruit, also yields seeds rich in bioactive compounds, though these remain relatively underexplored (15). FSO is notably abundant in polyunsaturated fatty acids (PUFAs) (omega-3, omega-6), phytosterols, and phenolic compounds, known as contributors to health benefits like reduced inflammation and oxidative stress protection (16). While FSO's potential anticancer properties are increasingly recognized, the underlying mechanisms require further elucidation. Preliminary studies indicate FSO exhibits cytotoxicity against breast cancer cells via caspase activation and inhibits colorectal cancer cell proliferation by modulating cell cycle and apoptotic pathways (17, 18). However, its specific effects on mitochondrial-mediated apoptosis within the context of HCC have not yet been investigated. This gap highlights the need for research into FSO's potential therapeutic role against HCC through mitochondrial modulation.

Mitochondrial dysfunction is a crucial factor in inducing programmed cell death, positioning it as a significant therapeutic target for HCC. Overcoming apoptosis resistance in HCC cells by targeting mitochondrial pathways is a recognized strategy, supported by numerous studies (19). For example, natural compounds like resveratrol and curcumin have demonstrated efficacy in promoting apoptosis in HCC cell lines (e.g., HepG2) by disrupting mitochondrial membrane potential, activating caspases, modulating Bcl-2 family protein expression, and enhancing cyt-c release (20-22). These findings highlight the therapeutic potential of leveraging natural compounds to trigger mitochondrial-mediated apoptosis in HCC.

Despite the growing interest in plant-derived natural products as anticancer agents, the specific mechanisms through which FSO exerts its effects, particularly on cancer cells, require further investigation. Understanding FSO's influence on the mitochondrial apoptotic pathway in HCC is crucial for evaluating its therapeutic promise. Therefore, this study is designed to meticulously examine the impact of FSO

on apoptosis signaling within the HepG2, focusing specifically on mitochondrial function and associated molecular pathways. By elucidating the mechanisms underpinning FSO-induced apoptosis, this research aims to provide valuable insights that could contribute to the development of novel therapeutic strategies for HCC.

Material and Method

Source and Extraction of Fig Seed Oil

FSO was obtained from İncir Dede Company (Kuyucak, Aydın, Türkiye). The seeds were sourced from dried figs cultivated in the Aydın region, recognized for their quality produce. Following mechanical separation and cleaning, FSO was extracted using cold-pressing techniques. The extracted oil underwent further filtration to remove particulate matter and ensure purity. This method was specifically chosen to preserve the oil's natural composition and prevent the degradation of heat-sensitive bioactive compounds, including PUFAs, phytosterols, and phenolics. After extraction, the oil was immediately packaged in dark, airtight glass bottles and stored under refrigerated conditions (4°C) by the supplier to minimize oxidation and maintain quality. Upon receipt in the laboratory, the FSO was stored at -20°C in the dark until the experiments were conducted. All experiments utilized FSO from the same batch (Batch Number: K150125), and care was taken to minimize exposure to light and air during handling and preparation of working dilutions.

Cell Culture

HepG2 (ATCC #HB-8065) cells were maintained in Dulbecco′s Modified Eagle′s Medium (DMEM, D6171, Sigma-Aldrich, Germany) with 10% Fetal Bovine Serum (FBS, F7524, Sigma-Aldrich, Germany) and 1% penicillin-streptomycin (P4458, Sigma-Aldrich, Germany) at 37°C, 5% CO_2, and 95% humidity. Cells were passaged using trypsin-EDTA (C-41020, Sigma-Aldrich, Germany) upon reaching 80–90% confluence. Before experimentation, cells were seeded into appropriate culture vessels (96-well plates for cytotoxicity assays or 6-well plates for protein extraction) at a density of 1×10^5 cells/mL. All experiments were conducted during the logarithmic growth phase to ensure consistent cellular responses. Dose Determination and MTT Test

The cytotoxic potential of FSO against HepG2 cells was determined using the MTT assay. Briefly, HepG2 cells were seeded into 96-well plates (5×10^3 cells/well) and allowed to adhere for 24 hours. Cells were then exposed to varying concentrations of FSO (0.1% to 1% v/v), prepared by serial dilution of a dimethyl sulfoxide (DMSO, D8418 Sigma-Aldrich, Germany) stock solution in a culture medium. Control groups included untreated cells and cells treated with 0.4% DMSO (solvent control).

Following treatment periods of 24, 48, and 72 hours, MTT reagent (5 mg/mL) was added, and plates were incubated for 4 hours at 37°C. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as the percentage of absorbance relative to untreated controls[(Absorbance treated / Absorbance untreated) × 100]. Half-maximal inhibitory concentrations (IC_{50}) were calculated from the dose-response curves via nonlinear regression analysis using GraphPad Prism software to guide subsequent experiments.

Cell Lysate Preparation and Oxidative Stress Assessment

Following treatment, HepG2 cells were harvested, washed twice with ice-cold Phosphate-Buffered Saline (PBS, pH 7.4), and lysed using a buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM Ethylenediaminetetraacetic acid (EDTA), 1 mM Ethylene glycol tetraacetic acid (EGTA), 1 mM sodium orthovanadate (Na3VO4), 1 mM Phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (diluted 1:100). After incubation on ice for 30 minutes and centrifugation at 12,000 × g for 15 minutes at 4°C, the supernatant containing the total protein extract was collected. Protein concentration was determined using the Bradford assay (Bio-Rad Protein Assay Dye Reagent), and lysates were stored at -80°C until further analysis.

Oxidative stress was evaluated using commercial ELISA kits (Rel Assay Diagnostics, Gaziantep, Türkiye) to measure total antioxidant status (TAS) and total oxidant status (TOS) in the prepared cell lysates. TAS was quantified by the inhibition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) oxidation, while TOS was measured by the oxidation of ferrous to ferric ions. Absorbance values read at 660 nm for TAS were converted to mmol Trolox equivalent/L (TAS), and absorbance values read at 590 nm for TOS were converted to µmol H_2O_2 equivalent/L (TOS). Oxidative stress index (OSI), reflecting the overall oxidative balance, was calculated as the ratio of TOS to TAS (OSI = TOS/TAS). (23).

Apoptotic Protein Determination by Western Blot

As in our previous study, apoptotic protein expression was evaluated using Western blotting (24). Equal

amounts of protein (30 µg) from HepG2 cell lysates were resolved by sodium dodecyl sulfate (SDS–PAGE, 10%) and transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5% skim milk in Tris-buffered saline with Tween-20 (TBST), membranes were incubated overnight (4°C) with primary antibodies targeting Bax, Bcl-2, cyt-c, cleaved caspase-9, cleaved caspase-3 (1:1000), and β-actin (1:5000). β-actin was blotted for all samples as a loading control to ensure equal protein loading across lanes and for subsequent normalization. All antibodies used were obtained from Cell Signaling Technology USA, and the manufacturer's recommended dilution ratios were used. Following TBST washes, HRP-conjugated secondary antibodies (1:5000) were applied for 1 hour. Protein bands were detected using an enhanced chemiluminescence (ECL) system (Bio-Rad, Hercules, CA, USA), and relative band intensities were measured using ImageJ software (NIH, Bethesda, MD, USA) with quantification normalized to the corresponding β-actin band intensity for each sample.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical significance between experimental groups was assessed using GraphPad Prism software (version 9.0). One-way analysis of variance (ANOVA) was first performed to detect overall differences. When significant differences were shown by ANOVA, Tukey's post hoc test was applied for pairwise comparisons between specific group means. Statistical significance was set at p-value<0.05. Dose-response curves were generated for MTT assay data, and IC_{50} values were determined by non-linear regression analysis. The relationships between oxidative stress markers (TAS, TOS, OSI) were evaluated using Pearson correlation coefficient analysis.

Results

Dose Determination and MTT Test

FSO cytotoxicity on HepG2 cells was assessed by MTT assays. A dose-dependent decrease in cell viability was observed at all time points following treatment with FSO (24, 48, and 72 hours). At 24 hours, cell viability decreased significantly from 95.6 ± 2.3% at 0.1% v/v FSO to 45.3 ± 3.1% at 1% v/v FSO (p<0.001). Similar trends were observed at 48 and 72 hours. The IC_{50} values at 24, 48, and 72 h were 0.52%, 0.48% and 0.45% v/v, respectively (Figure 1). Importantly, treatment with the solvent control (0.4% DMSO) alone showed no significant effect on HepG2 cell viability compared to untreated control cells at any time point (data not shown), confirming that the observed cytotoxicity is attributable to FSO. Statistical analysis revealed significant differences between untreated controls and all treatment groups (p<0.01), indicating that FSO exerts potent cytotoxic effects on HepG2 cells. According to the MTT results, a 0.5% v/v dose was selected as the treatment dose, with a 48-hour IC50 close to that. The levels of TAS, TOS, and apoptotic markers were measured in cells incubated with 0.5% FSO for 24 hours.

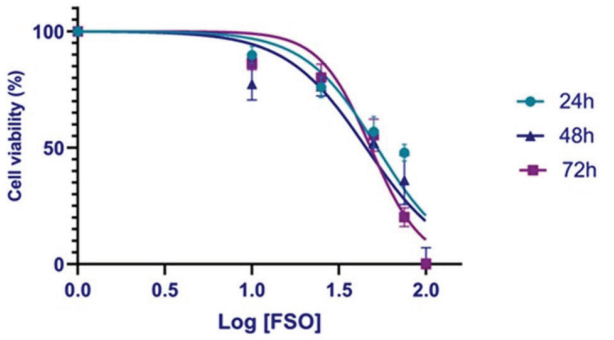

Figure 1

Dose-dependent cytotoxicity of FSO on HepG2 cells. Figure 1 illustrates the dose-dependent cytotoxic effects of FSO on HepG2 hepatocellular carcinoma (HCC) cells over 24, 48, and 72 hours, as assessed by the MTT assay. The x-axis represents FSO concentrations (ranging from 0.1% to 1.0% v/v, with 0.0 representing the untreated control), while the y-axis shows cell viability (% of untreated control). The graph demonstrates a significant reduction in cell viability with increasing FSO concentrations and prolonged treatment durations. The IC50 values (concentration required to inhibit 50% of cell viability) are 0.52%, 0.48%, and 0.45% v/v at 24, 48, and 72 hours, respectively.

Protein Yield Analysis: Cell lysates prepared from FSO-treated HepG2 cells showed no visible signs of contamination or degradation, as confirmed by protein quantification using the Bradford assay. Protein concentrations ranged from 2.5 ± 0.2 mg/mL in untreated controls to 1.8 ± 0.3 mg/mL in cells treated with 0.5% v/v FSO (p<0.05). This reduction in total protein content suggests cellular damage or apoptosis induced by FSO treatment.

Oxidative Stress Assessment

The oxidative stress status of HepG2 cells was assessed by measuring total antioxidant status (TAS) and total oxidant status (TOS). At 0.5% v/v FSO, TAS

levels rose from 1.2 ± 0.1 mmol Trolox equivalent/L in untreated controls to 1.8 ± 0.2 mmol Trolox equivalent/L (Figure 2A, p<0.01). Conversely, TOS levels decreased significantly, dropping from 12.4 ± 1.3 µmol H_2O_2 equivalent/L in untreated controls to 7.8 ± 0.9 µmol H_2O_2 equivalent/L at 0.5% v/v FSO (Figure 2B, p<0.001). OSI, calculated as the ratio of TOS to TAS, decreased from 10.3 ± 1.1 in untreated controls to 4.3 ± 0.5 in FSO-treated cells (Figure 2C, p<0.01), indicating a marked reduction in oxidative stress.

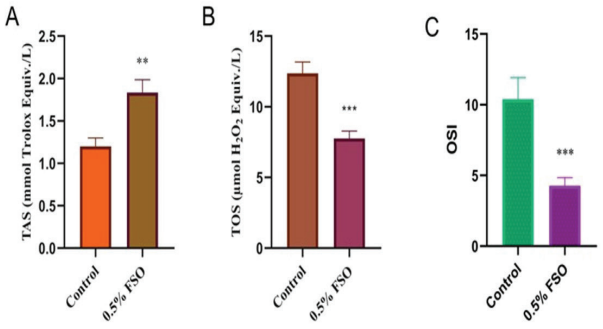

Figure 2

Oxidative stress modulation by FSO.

(A) Total Antioxidant Status (TAS) levels rose significantly in FSO-treated cells compared to untreated controls. (B) Total Oxidant Status (TOS) levels decreased markedly in FSO-treated cells versus controls. (C) The oxidative stress index (OSI) significantly decreased in FSO-treated cells compared to controls. Data are presented as mean ± SD. Statistical significance versus Control is indicated by asterisks: ** p<0.01, *** p<0.001.

Determination of Apoptotic Proteins by Western Blot Method

Western blot analysis provided compelling evidence for the modulation of key apoptotic regulatory proteins in HepG2 cells following treatment with FSO (Figure 3). Figure 3A presents representative Western blots illustrating the expression levels of Bax, Bcl-2, cyt-c, cleaved caspase-9, and cleaved caspase-3, alongside the loading control β-actin, in untreated and 0.5% FSO-treated cells. These blots visually support the quantitative analysis shown in Figures 3B-F. A significant shift towards a pro-apoptotic

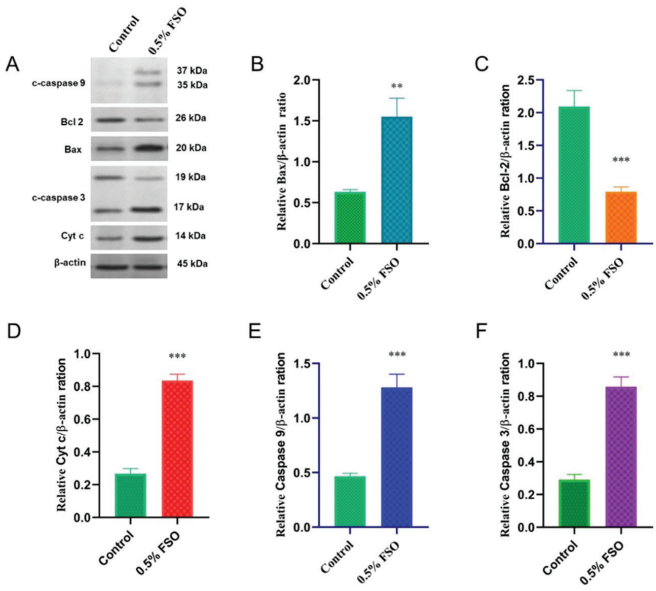

Figure 3

Western blot analysis of apoptotic proteins.

(A) Representative Western blots showing the expression levels of Bax, Bcl-2, cyt-c, cleaved caspase-9, cleaved caspase-3, and the loading control ß-actin in HepG2 cells treated with 0.5% v/v FSO or untreated control. (B-F) Quantification of protein expression relative to the ß-actin loading control. Bax: Pro-apoptotic Bax expression increases by 2.5-fold in FSO-treated cells. Bcl-2: Anti-apoptotic Bcl-2 expression decreases by 60%. Cyt-c. Cytoplasmic cyt-c levels rise by 3.2-fold, indicating mitochondrial outer membrane permeabilization (MOMP). Caspase Activation: Cleaved caspase-9 (2.8-fold) and caspase-3 (3.1-fold) are significantly upregulated. Statistical significance versus Control is indicated by asterisks: ** p<0.01, *** p<0.001.

state was observed with FSO treatment. Specifically, treatment with 0.5% v/v FSO resulted in a statistically significant 2.5-fold upregulation in the expression of the pro-apoptotic protein Bax ($p<0.01$), coupled with a substantial 60% downregulation of the anti-apoptotic protein Bcl-2 ($p<0.001$). This altered Bax/Bcl-2 ratio likely contributes to increased mitochondrial outer membrane permeabilization. Consistent with this, cytosolic levels of cyt-c, released from the mitochondria, were significantly elevated by 3.2-fold ($p<0.001$). This release triggers downstream events, notably the activation of caspases. Indeed, the levels of cleaved (active) initiator caspase-9 increased by 2.8-fold ($p<0.001$), and the key executioner caspase, cleaved caspase-3, showed a 3.1-fold increase ($p<0.001$).

Discussion

In the present study, we investigated the effects of cold-pressed fig seed oil (FSO), obtained from Sarı Lop figs (Aydın, Türkiye), on HepG2 human hepatocellular carcinoma cells. We focused on evaluating its cytotoxicity, ability to modulate cellular oxidative stress, and capacity to induce apoptosis via the intrinsic mitochondrial pathway. Our key findings demonstrate that FSO exhibits significant dose-dependent cytotoxicity against HepG2 cells, notably enhancing cellular antioxidant defenses while reducing the overall oxidative burden. Crucially, FSO effectively induces apoptosis by activating the intrinsic mitochondrial pathway, as evidenced by specific changes in Bcl-2 family protein expression, cytochrome c release, and subsequent caspase cascade initiation. The cold pressing method used for FSO extraction has been shown in studies by Rehman et al. and Irchad et al. to effectively preserve thermally labile bioactive compounds such as PUFAs, phytosterols, and phenolic acids, which are critical for their biological activity and antioxidant capacities compared to solvent-extracted oils (25) (26). This is consistent with our findings that the antioxidant effects observed with the Sarı Lop FSO are likely due to its preserved bioactive profiles.

The dose-dependent cytotoxic effects of FSO on HepG2 cells were evident from the MTT assay results, with an IC_{50} value of approximately 0.5% v/v at 48 hours. This finding is consistent with previous studies demonstrating the cytotoxic effects of plant-derived oils rich in PUFAs on cancer cell lines (27, 28). For example, flaxseed oil, another oil rich in PUFAs, has been demonstrated to suppress proliferation and induce apoptosis in cancer cells through similar mechanisms (29). The observed cytotoxicity of FSO is likely attributed to its ability to disrupt essential cellular processes, including mitochondrial function, which ultimately triggers apoptosis. The relatively low IC50 value underscores FSO's potential as a therapeutic agent effective at accessible concentrations.

The reduction in total protein content observed in FSO-treated cells reflects cellular damage or apoptosis induced by the treatment. Protein quantification serves as an indirect measure of cell viability and structural integrity. The significant decrease in protein concentration at higher FSO concentrations (e.g., 0.5% v/v) corroborates the cytotoxic effects observed in the MTT assay. Similar findings have been reported in studies investigating the effects of other natural oils on cancer cells, where reduced protein levels were associated with apoptosis and cell death (30, 31). These results underscore the potential of FSO to disrupt cellular homeostasis and induce apoptotic signaling pathways. A key finding of our study is the significant improvement in the cellular oxidative balance of HepG2 cells following FSO treatment. We observed a statistically significant increase in TAS and a concomitant decrease in TOS, resulting in a substantial reduction in the OSI. This demonstrates FSO's capacity to enhance cellular antioxidant defenses and reduce oxidative burden. This result stands in contrast to some studies where pro-apoptotic agents exert their effects by inducing oxidative stress. Our findings clearly show that FSO achieves its cytotoxic and pro-apoptotic effects on HepG2 cells alongside a reduction in overall oxidative stress.

This differential effect on oxidative status compared to some other Ficus carica studies warrants specific discussion. A recent study by Kalefa and Al-Shawi (2023) investigated the effects of Ficus carica (specifically, essential oils isolated from Iraqi figs using water and hexane extraction) on HepG2 cells (32). Their work reported that Ficus carica essential oils induced ROS and apoptosis in HepG2 cells. The difference in the observed effect on oxidative status may be attributable to several factors. Firstly, the source material is different: our study uses cold-pressed seed oil from Sarı Lop figs, whereas Kalefa and Al-Shawi used essential oils from the fruit of Iraqi figs. The extraction methods also differ (cold-pressing vs. water/hexane extraction), which significantly impacts the profile and concentration of extracted bioactive compounds, particularly volatile components enriched in essential oils. Furthermore, their study reported inducing oxidative damage at a very high concentration (40%), whereas our effective pro-apoptotic and antioxidant dose was significantly lower (0.5%). It is plausible that high concentrations of certain essential oil components might overwhelm

cellular antioxidant systems, leading to ROS induction, while the balanced composition of cold-pressed seed oil at lower concentrations primarily acts as an antioxidant while simultaneously triggering apoptosis through other pathways.

Our finding that FSO induces mitochondrial apoptosis despite reducing oxidative stress is supported by other literature. Bin Liu et al. (2014) demonstrated that Dihydromyricetin (DHM), a flavonoid, induced mitochondrial apoptosis in HepG2 cells while simultaneously reducing intracellular ROS and GSH levels (33). Their work, like ours, suggests that apoptosis can be triggered by mechanisms that do not rely on the induction of oxidative stress and can even occur in conjunction with enhanced antioxidant capacity. This complex interplay underscores that targeting the apoptotic machinery directly can be an effective strategy independent of (or synergistic with) modulating cellular redox state in a pro-oxidant direction.

Western blot analysis revealed significant modulation of key apoptotic proteins, providing mechanistic insights into the pro-apoptotic effects of FSO. The specific bioactive components within FSO responsible for triggering these apoptotic events warrant further investigation; however, based on the known composition of FSO, components such as PUFAs and specific phenolics are strong candidates (36). Studies have shown that certain PUFAs can directly interact with mitochondrial membranes, affecting permeability and facilitating the release of pro-apoptotic factors. Similarly, various phenolic compounds are known to modulate intracellular signaling pathways that converge on the mitochondria and regulate the activity or expression of Bcl-2 family proteins and caspases (41). The observed marked upregulation of the pro-apoptotic protein Bax, concomitant with the marked downregulation of the anti-apoptotic protein Bcl-2, indicates a significant shift in the cellular balance towards favoring mitochondrial outer MOMP. (19, 42). This altered Bax/Bcl-2 ratio is a well-known feature of the intrinsic mitochondrial apoptotic pathway and indicates that mitochondrial membrane integrity, regulated by proteins involved in this pathway, is disrupted. The functional consequence of this change is confirmed by the significant increase in cyt-c detected in the cytosol. The release of cyt-c from the mitochondrial intermembrane space into the cytoplasm is a key event initiating the downstream caspase cascade. Consistent with this, the data showed a significant increase in the levels of cleaved (active) caspase-9, the initiator caspase specifically activated following apoptosome formation mediated by cytosolic cyt-c. Previous studies by Rahman et al. (2021) and Yadav et al. (2021) have consistently demonstrated that various phytochemicals, including polyphenols, flavonoids, and specific fatty acids, exert their pro-apoptotic effects by directly affecting the expression and activity of Bcl-2 family proteins (43, 44).

In vitro studies utilizing cancer cell lines have provided evidence of Ficus carica's potential for inducing apoptosis. For example, extracts of Ficus carica have demonstrated antiproliferative and apoptosis-inducing effects on colorectal cancer cell lines (45). Moreover, the bioactive components of Ficus carica, specifically its fruit and leaves, have been shown to affect signaling pathways that lead to apoptosis in various tumor cells. For instance, the leaf extract of Ficus carica has been reported to enhance the activity of antioxidant enzymes, which may augment its anti-cancer effects by inhibiting proliferative signaling and promoting apoptotic pathways (46). Shaikh et al. (2020) indicate functional compounds within Ficus carica are important not only as cytotoxic agents but also as modulators of critical pathways, such as apoptosis, involved in cancer cell survival (47). Similarly, Hu, R. et al. (2019) reported that FSO inhibited the proliferation of colorectal cancer cells by modulating key signaling pathways involved in cell cycle regulation and apoptosis (17). The current study extends these findings by elucidating the specific role of FSO in modulating the mitochondrial apoptotic pathway in HepG2 cells. Furthermore, the observed effects on oxidative stress and apoptotic protein expression are supported by studies on other natural oils, such as olive oil and flaxseed oil, which have been shown to exert similar effects on cancer cells (48, 49).

Furthermore, while this study provides strong evidence for the central role of the mitochondrial pathway in FSO-induced apoptosis, it is important to consider the potential involvement of upstream regulatory signaling pathways. Key survival pathways, such as the PI3K/Akt and MAPK pathways, are often constitutively active in cancer cells, promoting cell survival by inhibiting pro-apoptotic proteins and activating anti-apoptotic proteins, including members of the Bcl-2 family (50). Various natural compounds, including those found in FSO, like PUFAs and phenolics, have been shown to modulate these pathways. Some flavonoids can inhibit the PI3K/Akt pathway, thereby reducing its anti-apoptotic signals and sensitizing cancer cells to apoptosis (51). Similarly, components of the MAPK pathway, such as ERK, JNK, and p38, can have dual roles in apoptosis, and their modulation by natural compounds is a common mechanism of anti-cancer

activity (52). Therefore, it is plausible that FSO-mediated apoptosis in HepG2 cells is not only a direct effect on mitochondria but also a result of the modulation of these upstream signaling cascades. While this study provides valuable insights into the anticancer potential of FSO, certain limitations must be acknowledged. First, the experiments were conducted in vitro, and the effects of FSO in vivo remain unknown. Future studies should investigate the efficacy and safety of FSO in animal models of hepatocellular carcinoma to validate its therapeutic potential. Second, while FSO is known to contain various bioactive compounds, a detailed chemical characterization of the specific oil sample used in this study was not performed. This limits our ability to correlate the observed effects directly with specific phytochemical constituents and accounts for potential variability in composition depending on source and processing methods. Therefore, future investigations should include comprehensive chemical profiling of the FSO. Third, the molecular mechanisms underlying FSO-induced apoptosis warrant further exploration, particularly the role of signaling pathways such as PI3K/Akt and MAPK, which are known to regulate apoptosis in cancer cells (53). Additionally, the potential synergistic effects of FSO with conventional therapies, such as chemotherapy and radiation, should be explored to enhance treatment outcomes.

Conclusion

In summary, this study demonstrates that fig seed oil exerts potent cytotoxic and pro-apoptotic effects on HepG2 cells through the mitochondrial apoptotic pathway. The observed modulation of Bcl-2 family proteins, cyt-c release, and caspase activation highlights the mechanistic basis of FSO-induced apoptosis. Furthermore, the reduction in oxidative stress underscores the protective role of FSO against cellular damage. These findings align with current literature on the anticancer properties of plant-derived oils and provide a foundation for future investigations into the therapeutic applications of FSO in hepatocellular carcinoma.

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Conflict of Interest Statement
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Availability of Data and Materials
The data supporting the findings of this study are not publicly available. However, the data can be requested from the corresponding author upon reasonable request, where necessary.

Artificial Intelligence Statement
The authors confirm that generative artificial intelligence was not used in the preparation of this manuscript, including the writing of the main text, figures, tables, or captions.

Authors Contributions
AY: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.; Writing- review & editing

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