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Investigation of the anticancer effects of some plant seed oils with medicinal uses

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ABSTRACT: Cancer is an important health problem and responsible for a significant number of deaths worldwide. Chemotherapy resistance and related side effects prevent cancer treatment from reaching the desired level. In this study, we aimed to investigate the anti-cancer properties of *Carthamus tinctorius*, *Salvia hispanica* and *Linum usitatissimum* seed oils on hepatocellular (HepG2), colorectal (HCT-116) and gastric carcinoma (AGS) cell lines. Human umbilical vein endothelial cells (HUVECs) were used as control cell group. Cell viability of HepG2, HCT-116, AGS and HUVECs was determined by MTT method after treatment with various concentrations of *Carthamus tinctorius*, *Salvia hispanica* and *Linum usitatissimum*. Colorimetric elisa kit was used for quantitative determination of caspase-3 activation. Anti-migratory effect of *Carthamus tinctorius* and *Salvia hispanica* were investigated by wound healing assay. *Carthamus tinctorius, Salvia hispanica* and *Linum usitatissimum* seed oils significantly inhibited the proliferation of cancer cells. There was an insignificant decrease in the viability of HUVEC cells after seed oils treatments. Migration of HepG2 and AGS cells significantly suppressed by treatment of *Salvia hispanica* and *Carthamus tinctorius* respectively. *Salvia hispanica* and *Carthamus tinctorius* significantly increased caspase-3 activation in cancer cells. Our results showed that *Carthamus tinctorius, Salvia hispanica* and *Linum usitatissimum* exhibited a selective cytotoxicity against HepG2, HCT-116 and AGS cells without damaging normal cells.

KEYWORDS: *Carthamus tinctorius*; *Salvia hispanica*; *Linum usitatissimum*; anticancer; migration; caspase-3.

1. INTRODUCTION

Cancer refers to a group of diseases include that have many characteristics such as uncontrolled cell division, ability to metastasize, having abnormal cell structures and morphology (1). Although advances in diagnosis and treatment, cancer incidence and mortality are increasing rapidly worldwide (2). Over the years, traditional medicine has been used with traditional cancer treatment to promote therapeutic effects and reduce toxicity (3). Natural compounds and their ingredients are crucial sources for anti-tumor agent discovery (4). *Carthamus tinctorius*, also called safflower, is a seed oil widly used in Chinese medicine to improve blood circulation and prevent blood clotting (5). In the recent times, it has also been shown that anticancer functions of *Carthamus tinctorius* (6). Because of rich content *Carthamus tinctorius* extracts and oil has been often preferred in drug development with many pharmacological activities worlwide (1,7).

Various studies have reported the many active constituents such as Kaempferol 7-O-β-D glucopyranoside, N-(p-coumaroyl)serotonin, N-feruloylserotonin, oleic acid, linoleic acid, palmitic acid have reported in the content of *Carthamus tinctorius* (8). The anti-carcinogenic properties of some of these compounds well defined in previous studies (1,9-10). It has been reported that some extracts derived from *Carthamus tinctorius* suppress tumor development in skin cancer or melanoma (11-12).

Salvia hispanica, commonly known as "chia", is an annual herbaceous plant with rich ingredients that have beneficial effects on human health (13). *Salvia hispanica*, belonging to the Lamiaceae family, has been traditionally used in the treatment of tuberculosis, bronchitis and microbial infections since ancient times (13). Many phenolic compounds (caffeic acid, ferulic acid, rosmarinic acid, chlorogenic acid) effective against various diseases, including cancer, have been isolated from chia seeds (13). In addition to these phenolics, high amount of flavonoids (myricetin, quercetin, kaempferol, rutin, daidzin, genistein) and fatty acids (α-linolenic acid, linoleic acid, palmitic acid, stearic acid, oleic acid) were identified in many studies (13). For this composition, Chia seeds are an excellent source for cancer research (13).

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Due to its rich content such as omega-3, fiber and proteins has been the focus of attention in cancer studies (14). Output data from many studies have emphasized that *Salvia hispanica* exhibit anti-cancer activity in some cancer cell lines (HeLa, HCT-15, HCT-116, MCF7, MDA-MB-231, MCF7/Vin, MCF7/Vin+, MCF7/Vin- cells, Vero, and HepG2 cells) (14).

*Linum usitatissimum (*also knownas flaxseed*)* has been utilised for oil and textile fibers productions, while also for human nourishment or medical purposes since long time. It is cultivated in a wide geographical area including many countries (15). Beneficial effects of *Linum usitatissimum* on diseases such as cancer is of great interest for cancer therapy lately (15). Many studies have reported that flaxseed and its components have a toxic effect against cancer cells (15-18). It has been reported to *Linum usitatissimum* has a large number of phenolic (gallic, vanillic, chlorogenic, caffeic, p-coumaric, sinapic, ferulic, and trans-o hydroxy cinnamicacid) and flavonoid (secoisolariciresinol diglucoside quercetin and rutin,) constituents (19). Zhou et al. determined high amounts of phenolic acids such as gallic, ferulic, and vanillic acid in flaxseed hydroalcoholic extracts (19). These compounds constitute the main factor underlying the anti-carcinogenic effect of *Linum usitatissimum* (19). Lignin, a class of phytoestrogens, is the major constituent of *Linum usitatissimum*. Antitumor activity of lignans against breast, prostate and colon cancer was reported in previous studies (19). Joseph et al. prepared the seed extracts with evaporation method and identified a mixture of phytoconstituents containing flavonoids, terpenoids, glycosides, alkaloids, steroids, phenols, and anthraquinones from ethyl acetate extract of *Linum usitatissimum* seeds (20).

In our study, we aimed the investigate the possible inhibitory activity of *Carthamus tinctorius, Salvia hispanica* ve *Linum usitatissimum* seed oils on HepG2, HCT-116 and AGS cells proliferation, migration and caspase -3 activation.

2. RESULTS

2.1. *Carthamus tinctorius, Salvia hispanica, Linum usitatissimum* inhibited proliferation of HepG2, HCT-116 and AGS cancer cell lines cancer without effecting normal cells

The cytotoxic effect of *Carthamus tinctorius*, *Salvia hispanica*, *Linum usitatissimum* on HepG2, HCT-116 and AGS cancer cell lines was assesed by the MTT assay ($p < 0.001$) (Figure 1). Our results showed that all the oils effectively reduced the cell vibility in a dose dependent manner. Among other oils, especially *Salvia hispanica* showed a strong cytotoxic effect against HepG2 cells. At a concentration of 20 µl, *Salvia hispanica* reduced HepG2 cell viabilty by % 50.81. Highest concentration of chia (80 µl) seed significantly decreased HepG2 cell viability (%30.08) compared with the control. 40 µl of *Linum usitatissimum* and 50 µl of *Carthamus tinctorius* concentrations reduced the cell viability of HepG2 cells under %50. *Salvia hispanica* concentrations of 30 and 50 µl, caused a % 40.1 and % 57.36 decrease in cell viability of HCT-116-cells compared with untreated control group, respectively ($p < 0.001$). Higher concentrations of *Carthamus tinctorius* and *Linum usitatissimum* than 50µl were more effective in reducing cell viability of HCT-116 cells compared to lower doses. *Carthamus tinctorius* showed a stronger inhibitory effect against the proliferation of AGS cells compared to other oils. Treatment with 40µl *Carthamus tinctorius* concentration was reduced the cell viability of AGS by ~50%. The highest decrease (62.55%) in cell viability of AGS cells was detected in 80µl *Carthamus tinctorius* concentration. *Salvia hispanica was more effective in terms of anticarcinogenic effect against AGS cells, at high doses (higher than* 50µl). *Linum usitatissimum* exhibited a weaker cytotoxic effect against AGS cells compaired with *Carthamus tinctorius* and *Salvia hispanica*. There was no significant alteration between AGS cell viability over a concentration range of 10µl to 50µl. However, a significant decrease was observed at 80µl concentration of *Linum usitatissimum*. All of the three seed oils did not exhibit a cytotoxic effect against HUVECs on the tested concentrations. Some doses of *Salvia hispanica* and *Linum usitatissimum* induce small increases in HUVECs proliferation.

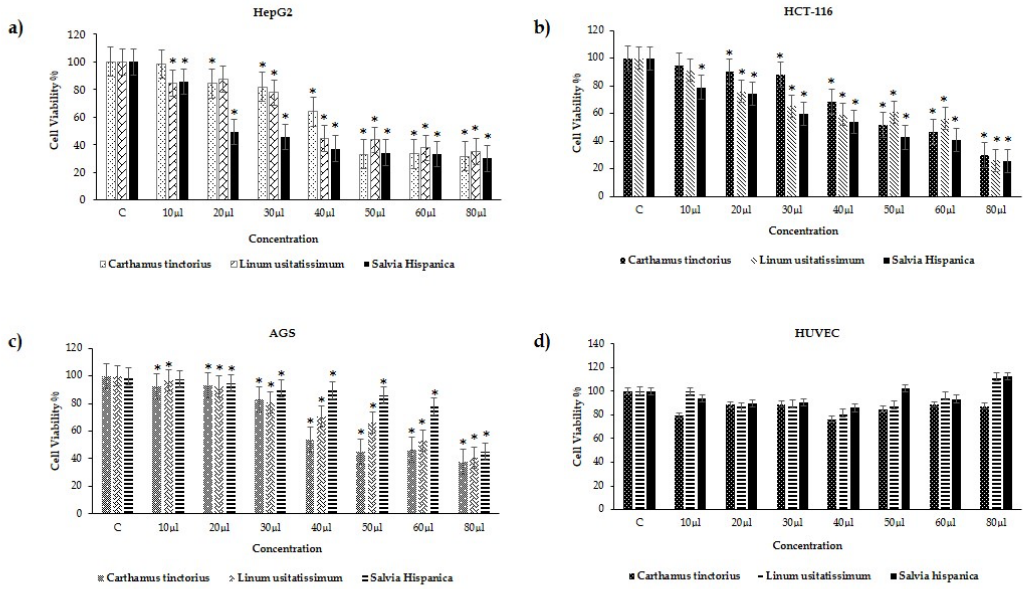

Figure 1. Cell viability of HepG2 (a), AGS (b), HCT-116 (c) and HUVECs (d) after 24h treatment with different concentrations of *Carthamus tinctorius*, *Salvia hispanica* and *Linum usitatissimum* are demonstrated. *Carthamus tinctorius, Salvia hispanica* and *Linum usitatissimum* inhibited the viability of HepG2, HCT-116 and AGS cells. Only a little cytotoxicity effect on HUVECs was observed with at the tested concentrations of seed oils treatment * $p<0.001$ vs. control group.

2.2. Caspase-3 activation induced by *Carthamus tinctorius* and *Salvia hispanica*

According to the MTT assay results, two major doses of seed oils were selected for caspase 3 assay. Treatment concentrations of *Salvia hispanica* for HepG2 cells were 20 µl and 80 µl, for HCT-116 cells were 30 µl and 50 µl. AGS cells were treated with 40 µl and 80 µl concentrations of *Carthamus tinctorius*. Caspase 3 activity was determined after treatment cells with seed oils for 24h. In general, a dose-dependent increase was observed in the level of caspase 3 in all the three cell lines. 30 µl *salvia hispanica* concentration caused an insignificant alteration in caspase 3 activity compared with the control in HCT-116 cells ($p=0.415$). Conversely, 50 µl of *salvia hispanica* significantly increased the activity of caspase-3 in comparison to control group ($p=0.016$). Both concentrations of *Carthamus tinctorius* significantly increased caspase-3 activity in AGS cells ($p < 0.001$). The results of caspase 3 activity are presented in figure 2. These results suggest that caspase 3 is one of the mechanisms involved in cell death induced by *Salvia hispanica* and *Carthamus tinctorius*.

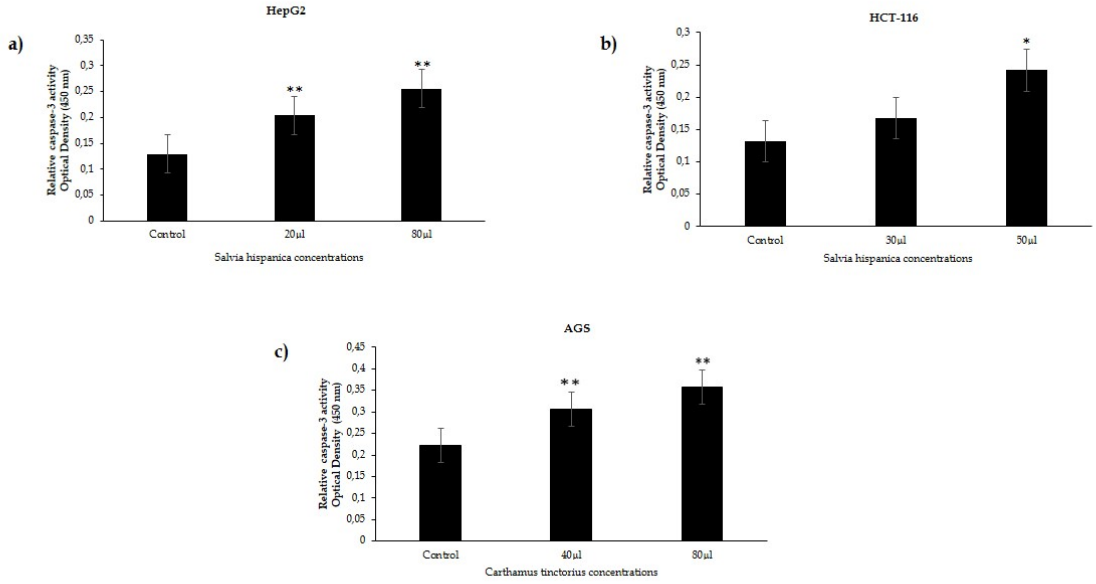

Figure 2. Caspase-3 activities are demonstrated in HepG2 (a), HCT-116 (b) and AGS (c) cells after 24 hours of treatment with Salvia hispanica and Carthamus tinctorius at the indicated concentrations. *p=0.016 **p < 0.001

2.3. *Salvia hispanica* and *Carthamus tinctorius* inhibited the HepG2 and AGS cells migration

Salvia hispanica and *Carthamus tinctorius* tested for their anti-migration activity on HepG2 and AGS cells, respectively. For wound healing assay, two concentrations of seed oils were selected that caused a significant reduction in cell viability in MTT assay. After cell layers wounded; AGS and HepG2 cells were incubated with *Carthamus tinctorius* and *Salvia hispanica* respectively for 12 and 24 hours. Wound closure area was photographed with inverted microscope (magnification, x100) at 0, 12 and 24 h of seed oil treatments (Figure 3). Our results showed that wound gaps in the treatment groups were larger than in the control groups. Migration inhibition rates of 40 µl *Carthamus tinctorius* were observed %3 and %32 at 12 and 24 h respectively. İnhibition rates of 60 µl *Carthamus tinctorius* treated AGS cells were up to %15.83 and % 46.8 respectively with the the same time period ($p<0.001$). It was found that 60 µl *Salvia hispanica significantly* suppressed the migratory potential of HepG2 cells with % 16.27 to % 29.6 at 12 and 24 h respectively ($p<0.001$). Data from the present study revealed that *Salvia hispanica* and *Carthamus tinctorius* significantly inhibited migration capacity of HepG2 and AGS cells respectively.

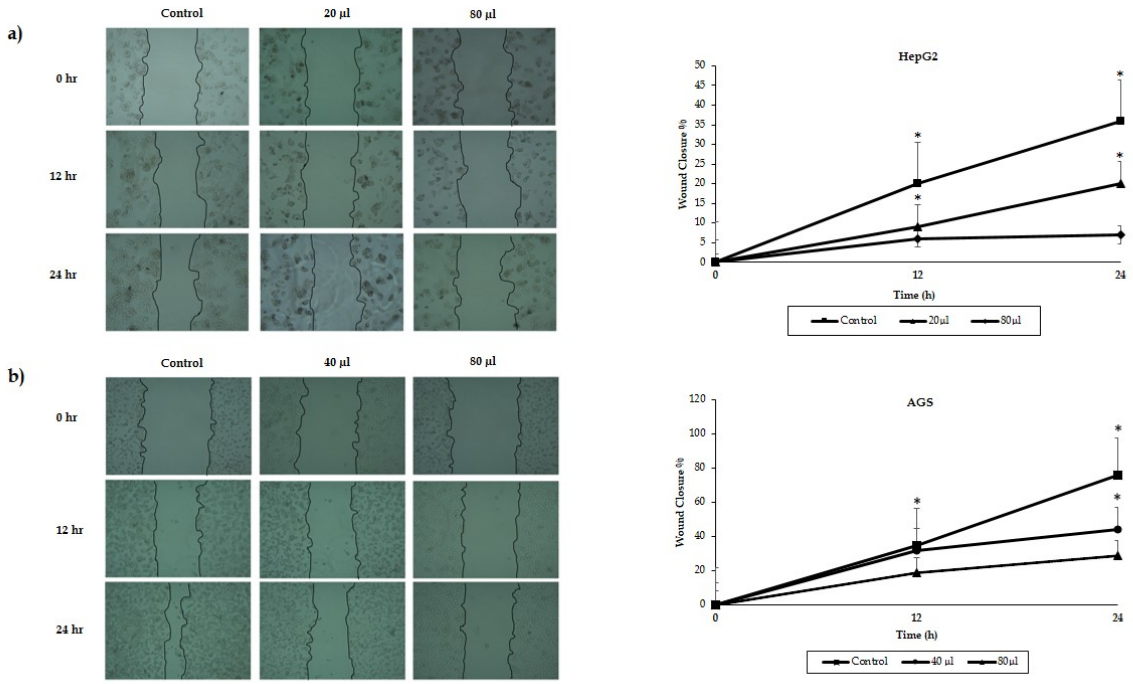

Figure 3. Anti-migration effects of *Salvia hispanica* and *Carthamus tinctorius* on HepG2 (Panel A) and AGS (Panel B) cells are demonstrated, respectively. (Magnification, x100). Cells (2 × 10^5 cells/well) were incubated in a 6 well plate until reach confluency and wounded. Cells were incubated with indicated doses of *Salvia hispanica* and *Carthamus tinctorius* for 24h. Images of wound closure area were photographed at 0, 12 and 24 h of *Salvia hispanica* and *Carthamus tinctorius* treatment. Graphs represent the percentage of wound closure area at 0, 12 and 24 h of *Salvia hispanica* and *Carthamus tinctorius* treatment. Results were presented as percent of wound closure area compared with untreated control. *p < 0.01 vs. control.

3. DISCUSSION

Many studies have reported that the natural compounds derived from plants is promising as they are more potent and less toxic antitumor agents (22). Due to the inadequate and undesirable side effects of existing cancer treatments, the development of natural therapeutics has been accelerated (1,23-24). This study was performed to investigate antiproliferative and antimigration potential of *Carthamus tinctorius*, *Linum usitatissimum* and *Salvia hispanica* seed oils on human hepatocellular, colorectal and gastric carcinoma. The data from our results showed that the seed oils of *Carthamus tinctorius*, *Linum usitatissimum* and *Salvia hispanica* inhibited the proliferation of hepatocellular cancer cells, colon cancer cells and gastric cancer cells in a dose-dependent manner. On the other hand, these oils exerted a slight cytotoxicity effect on non-malignant HUVEC cells. These seed oils selectively exerted toxic effects on cancer cells.

Carthamus tinctorius (Safflower, Compositae) is a self-fertile, annual and diploid herb that grows in a dry and hot climate (1). *Carthamus tinctorius* has a rich content used for medicinal and biological purposes (1). It has the potential bioresources of development new agents against human cancers (1). Anticancer effects of *Carthamus tinctorius* against several cancers such as lung, colorectal and cervix cancer were reported in previous studies (1,25). Compounds with potential anticancer effects such as alkane-6,8-diols, N-feruloylserotonin and N-(p-coumaroyl) serotonin were isolated from the methanol extract of *Carthamus tinctorius* seed (5). Linoleic acid and oleic acid, which are important components of *Carthamus tinctorius*, are accepted as an important agent with therapeutic potential (1,26). Especially Hydroxysafflor yellow A (HSYA) is an important component of safflower and it has been shown to inhibit the proliferation of many cancer cells through different mechanisms (22,27-29). Another safflower flavonoid Kaempferol reduced cell viability by activating apoptosis of breast cancer cells (30). Hyperoside, another safflower content, has been shown to reduce the NF-kB pathway and ROS formation (31) and stimulate apoptosis in non-small cell lung cancer cells (32). Kızılşahin et al. investigated the synergistic activity of taxol (Tax), a chemotherapy agent, and flaxseed oil on prostate cancer cells. They showed that the combined use of Tax and safflower seed oil increased the toxicity on cancer cells (33).

To the best of our knowledge, we have showed for the first time that *Carthamus tinctorius* inhibited both proliferation and migration of AGS cells. In our study, *Carthamus tinctorius* inhibited the growth of AGS cells in a dose dependent manner. This result is consistent with other researches which showed that *Carthamus tinctorius* significantly inhibited proliferation of human gastric cancer cells (SGC-7901 and BGC-823 (33-35).

Salvia hispanica has been recently attracted attention a lot of attention for its health benefits and anti-cancer properties.

A number of previous studies showed that cytotoxic effects of *Salvia hispanica* on many cancer cells such as MCF-7 (breast cancer), Caco2 (colorectal cancer), PC3 (prostate cancer), HepG2 (liver cancer), HCT-116 (colon cancer), AGS (gastric cancer) (14,36-37). Güzel et al. investigated the ethanol extracts of *Salvia hispanica* extracted by evaporation on A549 human lung cancer cell line. They showed antiproliferative effect of Chia seeds against lung cancer cells (13).

Quintal-Bojórquez, et al showed the anticancer effect of protein fractions isolated from *Salvia hispanica* against HepG2 cells (36). Rosas-Ramirez et al., reported the effects of chia oligosaccharide mucilages in decreasing proliferation of HepG2 cells (14). Tawfik, S. A., et al showed that the *Salvia hispanica* increased the sensitivity of HepG2 cells to the chemotherapy agent doxorubicin (38). Our results are support of those previous studies regarding the inhibitory effect of *Salvia hispanica* on HepG2 cell proliferation.

Apoptosis is the main death pathway that has been the focus of cancer searches. Caspases are a family of cysteine proteases that play crucial role in initiation and execution of apoptosis signaling pathways. Both the extrinsic and the intrinsic apoptotic pathways are gathered together in caspase-3 and this cascade resulted with caspase 3 activation. Then activated caspase 3 trigger a a series of downstream caspases cascade reactions and finally induces apoptosis (35). The role of *Salvia hispanica* on caspase 3 activation in cancer cells has not been investigated before and evaluated for the first time by this study. In previous studies, it has been reported that *Carthamus tinctorius* has anti-cancer activity through caspase activation (1,22, 39). Our findings on caspase 3 activation after treatment with safflower support previous studies. In our study, caspase 3 elisa kit was used to measure to determine of crucial apoptotic factor caspase-3 in cancer cells after seed oil treatments. A dose-dependent increase in caspase 3 activity was observed in cancer cells treated with *Salvia hispanica* and *Carthamus tinctorius*. Our results indicated that caspase 3 play an active role in cell death caused by *Salvia hispanica* and *Carthamus tinctorius*.

Linum usitatissimum has attracted great interest in the field of cancer research with its bioactive constituents in recent years (40). Previous studies were documented its anticancer properties in cervical (TC1 induced papilloma model) , liposarcoma (SW872, SW982), breast (MCF-7), ovarian (A2780), and colon (LOVO, HT-29) cancers (18, 41-45). Lehraiki et al investigated the anti-cancer effects of lignans which they isolated from flaxseed on MCF-7 and MDA-MB-231 cell proliferation. In their study, they showed that anhydrosecoisolariciresinol, a lignan isolated from flaxseed, significantly reduced cell proliferation in MCF-7 and MDA-MB-231 breast cancer cells (46).

According to our literature knowledge, we have for the first time evaluated the anti-carcinogenic property of *Linum usitatissimum* in the colon, hepatocellular and gastric cancer cell lines. Compared with other oils, it was less effective against cancer cells and showed proliferation-reducing activity only at high doses. This low effectiveness may be due to chemical composition of Linum usitatissimum. Because the chemical composition of seed oils can be changed by many factors such as harvest time, growing and storage conditions in different geographical regions of the world (44). And, also anti-cancer potential of *Linum usitatissimum* may be different depending upon the type of cancer cell.

Uncontrolled cell migration is involved in cancer formation and subsequent processes like invasion, angiogenesis and metastasis. It is an important reason in the development of malignant tumors (45). Therefore, suppression of cell migration is an important therapy target for the controlling of tumor growth (45). Cancer cell migration is one of the crucial steps of metastatic cascade (45). Shaer et al demonstrated the anti- migration potencial of *Salvia hispanica* extract prepared with PLGA-PEG coated nanoparticles system (47). In some studies, the inhibitory effect of *Carthamus tinctorius* on the migration of various cancer cells has been shown (3,48). In our study we found that *Salvia hispanica* and *Carthamus tinctorius* dose-dependent inhibited migration capability of HepG2 and AGS cells respectively. Our findings are consistent with other studies which showed anti-migration effect of *Salvia hispanica* and *Carthamus tinctorius* against cancer cells.

4. CONCLUSION

Due to the inadequate or side effect of present cancer treatment options, the development of new anti-cancer agents has been accelerated. Many researchers have focused on alternative cancer treatment methods. Herbal-derived agents are important resources to increase the effectiveness of standard cancer treatment methods. *Carthamus tinctorius*, *Salvia hispanica* and *Linum usitatissimum* have been used in traditional medicine in various parts of the world since ancient times due to their beneficial effects on human health. There has been an increasing interest in all these seed oils in recent years due to their rich anti-carcinogenic content. This study was designed for anti-cancer activity of *Carthamus tinctorius*, *Salvia hispanica* and *Linum usitatissimum* seed oils on HepG2, HCT-116 and AGS cancer cells. Our findings suggest that *Carthamus tinctorius, Salvia hispanica* and *Linum usitatissimum* seed oils exert strong anticarcinogenic effects against HepG2, HCT-116 and AGS cancer cell lines without effecting normally cultured HUVECs. This study provides an overview of the effects of these seed oils on cancer cells. These seed oils are likely to aid the development of new antitumor agents. The anti-cancer activities of these seed oils need to be confirmed by further in vivo and clinical studies. Our study will be a reference for future research on the medicinal applications of these plants.

5. MATERIALS AND METHODS

5.1. Reagents

Carthamus tinctorius, *Linum usitatissimum*, *Salvia hispanica* seed oils were commercially purchased. RPMI 1640 medium, DMEM high glucose medium, fetal bovine serum (FBS), penicillin–streptomycin, phosphate-buffered solution (PBS), and 0.25% trypsin were purchased from (Capricorn Scientific, Germany), MTT assay kit bought from Biotium (San Francisco, USA), caspase assay kit purchased from Bt Lab (Shanghai, China).

5.2. Sample Preparation

Firstly, seed oils were sterilized by passing through syringe filters. Since treatments with essential oils might be deleterious to cells, the oils were diluted with 10% DMSO. A stock solution was prepared by dissolving the seed oils in 10% DMSO at a ratio of 1:25 v/v and the final concentration of DMSO used in the experiments was reduced below 1% by diluting with the medium (16). DMSO (%1) was used as control to determine its effect against cells. Therefore, the effect of DMSO on cell viability was also analyzed. All experiments were repeated three times.

5.3. Cell lines and cell culture

Human liver cancer cell line HepG2 and Human umbilical vein endothelial cells (HUVECs) were kindly supplied by Dr. C. Verda Bitirim (Ankara University Stem Cell Institute). Colon cancer cell line Hct-116 was kindly provided by Prof. Dr.Ferda Arı (Bursa Uludag University, Department of Biology). A human gastric adenocarcinoma cell-line AGS cell line was kindly provided by Assoc. Prof. Rabia Çakır Koç. HUVEC and AGS cell lines were cultured in 10% FBS-containing DMEM high glucose cell culture media. HepG2 and HCT-116 cell lines were cultured in 10% FBS-containing DMEM low glucose and RPMI-1640 cell culture media respectively. All media were supplemented with L-glutamine, 10% inactivated fetal bovine serum,1% penicillin–streptomycin and cells were incubated in incubator which supply humidified atmosphere at 37 °C with 5% CO_2.

5.4. Cell Viability

MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) was used to assess the cell viability. MTT assay was performed as per the manufacturer's protocol. Cancer cell lines (AGS, Hct-116 and HepG2) and HUVECs were incubated in RPMI-1640 or DMEM medium containing 10% FBS and then seeded into each well ($5x10^3$ cells /well) of a 96-well plate and incubated overnight. After the incubation, cell media was removed and cells were treated with various dilutions of seed oils in media (1/2500; 1/1250; 3/2500; 1/625; 1/500; 3/1250; 2/625 equivalent to 10; 20; 30; 40; 50; 60; 80 μl, respectively) for 24h. After 24 hours incubation, the medium was replaced with fresh medium and 10 µL of MTT solution (5 mg/mL in PBS) was added each well and cells were further incubated for 4 h at 37 °C. Then 200 µL DMSO was added to each well to dissolve formazan crystals. Lastly, the absorbance of each sample was recorded at 570 nm wavelength using a microplate reader. The viability of untreated cells was accepted as 100% and the effect of seed oils to the cell viability was measured by dividing the absorbance of the treated cells by that of the treated with DMSO as control cells.

5.5. Caspase 3 assay

A commercially available kit (Bt-lab, Chine) was used for caspase-3 activity. Caspase-3 avtivity was measured according to manufacturer's protocol. According to the results of MTT assay, two concentration of seed oils were selected for use in the caspase 3 assay procedure. Cancer cells were seeded into 6-well cell culture plates with $5x10^5$ cells per well and incubated until they covered the well surface. After this time, cells were treated with selected doses of seed oils for 24 hours. After treatment procedure cells were scraped with scraper and transferred in to the 15 mL conical tubes. Cell lysets were centrifuged at 2000 rpm for 5 minutes at 4°C. Supernatant was discarded and cell pellet was dissolved in 0.3ml of chilled RIPA lysis buffer. Cell lysates were passed through syringe for further cell lysis and to form homogeneous lysate. Cell suspension was centrifuged at 14.000 rpm for 10 minutes and ıncubated on ice for 30 minutes. Supernatants were transferred to a new microcentrifuge and stored at -20^0C. Optical density (OD value) of each well were measured with a microplate reader (Epoch BioTeK, Agilent Technologies, U.S.A.) at 450 nm.

5.6. Wound healing assay

Wound-healing assay was used to assess the effects of seed oils on cell migration (32). HepG2 and AGS cells were seeded at a density of 2×10^5 cells into 6-well plates in 2 ml medium and incubated until they formed a confluent monolayer. Then a straight scratch was generated with a sterile pipette tip on the the cell monolayer in each well. Cellular debris was removed by PBS and the wounded monolayers of HepG2 and AGS cells were treated with *Salvia hispanica* (20 µl and 80 µl) and *Carthamus tinctorius* (40 µl and 80 µl) oils respectively for 24h. PBS added to remove the floating cells, and the remaining cells were imaged immediately (at 0 h) using an inverted microscope (Euromex Arnhem, The Netherlands). The new medium that including different concentrations of seed oils was added to the wells, except for the control. Following 24 h incubation at 37˚C, images were photographed and cell migration was calculated as a percentage of the area covered by cells at 24 h compared to the initial wound area at 0 h Experiments were repeated 3 times.

5.7. Statistical analyses

Descriptive statistics of the quantitative variables in the study are given as mean and standard deviation. All data were expressed as mean ± standard deviation (SD) of three independent experiments. The conformity of the variables to the normal distribution was examined using the Shapiro Wilk test. One way analysis of variance (one-way anova) was used in the mean comparison of groups with more than two categories, and comparisons with the control group for the variables that differed were examined by Tukey and Dunnett tests. In the comparison of the mean between different periods, the analysis of variance method in repeated measures (repeated measures anova) was applied. The sphericity assumption was examined by the Mauchly test. $p < 0.05$ was accepted as the statistical significance level. SPSS (version 28) and GraphPad Prism 9.4.1 were used in the analysis.

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Conflict of interest statement: The authors declared no conflict of interest

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