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ORIGINAL ARTICLE

Determination of anti-cancer effects of *Nigella sativa* seed oil on MCF7 breast and AGS gastric cancer cells

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Abstract

Background This study aimed to investigate the cytotoxic, apoptotic, invasion, metastasis, and heat shock proteins (HSPs) effects of *N. sativa* oil on breast and gastric cancer cells.

Methods We assessed the cytotoxic and apoptotic effects of various concentrations of *N. sativa* oil (10-50-100-200 µg/mL) on MCF7 breast cancer and AGS, an adenocarcinoma of the gastric cell line, at 24, 48 and 72 h using the MTT test. Additionally, the expression of the Caspase-3, BCL2/Bax, MMP2-9 and HSP60-70 gene was examined using RT-PCR in cell lines treating with *N. sativa*.

Results The MTT experiments demonstrate that *N. sativa* has a time and dose-dependent inhibitory effect on the proliferation of MCF7 and AGS cancer cells. The vitality rates of MCF7 and AGS cells treated with *N. sativa* were 77.04–67.50% at 24 h, 65.28–39.14% at 48 h, and 48.95–32.31% at 72 h. The doses of 100 and 200 µg/mL were shown to be the most effective on both cancer cells. RT-PCR analysis revealed that *N. sativa* oil extract increased caspase-3 levels in both cell lines at higher concentrations and suppressed BCL2/Bax levels. Exposure of MCF7 and AGS cell lines to *N. sativa* caused a significant decrease in the expression of MMP2-9 and HSP60-70 genes over time, particularly at a dosage of 200 µg/mL compared to the control group ($p < 0.05$).

Conclusions Our findings indicate that *N. sativa* oil has a dose-dependent effect on cytotoxicity and the expression of apoptotic, heat shock proteins, and matrix metalloproteinases genes in breast and gastric cancer.

Keywords *N. sativa* · MCF7 · AGS · HSPs · MMPs · RT PCR

Introduction

Cancer poses a substantial health risk worldwide. This disease, which causes millions of deaths every year, is the second cause of death worldwide. In economically developing countries, cancer incidence is on the rise due to population growth and aging [1]. Since the 18th century, a wide variety of herbs have been utilized for cancer treatment [1, 2]. Anti-cancer herbs function therapeutically by inhibiting cancer-activating enzymes, stimulating DNA repair, increasing synthesis of protective enzymes, and boosting immune cell activity [3–5].

Herbal medicines are considered effective medicinal components for the cure and prevention of various illnesses. Several widely used anti-cancer medications are derived from organic sources [6, 7]. Natural products can enhance the efficacy of cancer therapies by acting on various targets and signaling cascades and anti-proliferative and anti-apoptotic effects as well as immunomodulatory and antioxidant properties [8, 9]. This provides a chemo-preventive property that can be used prophylactically and therapeutically, and it is safe for long-term use [8, 10]. Several studies reveal that a diet rich in healthy compounds found in fruits, vegetables, cereal grains, and spices can help to prevent various types of cancers [11, 12]. Natural plant compounds have demonstrated encouraging outcomes as anti-tumor and anti-cancer medications. Compared to various other anti-cancer

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treatments, reduced toxicity and fewer instances of recurrent resistance to hormonally targeted anti-cancer medications have been reported as additional indications of their efficacy [8, 10].

Since ancient times, *Nigella sativa (N. sativa)*, a plant native to Southeast Asia, has been utilized for medicinal purposes. Its oil and seeds are known by various names in the world such as black cumin, kalonji, kalojeera etc. [13]. Studies have been conducted on the anti-cancer properties of *N. sativa* and some of its active ingredients, like thymoquinone and alpha-hederin [14]. In many immunopharmacological investigations, it has become crucial to find and identify novel anti-tumour medications with minimal adverse effects on the immune system [15]. While *N. sativa* oil and seeds have rich health benefits, research on their holistic potential has not kept pace with studies on the individual components' potential to prevent life-threatening diseases like cancer [16].

According to some studies [17–19], *N. sativa* is beneficial against cancer of the blood system, lung, breast, cervix, colon, ovary, and skin.

Matrix metalloproteinases (MMPs) are a class of zinc-dependent proteolytic enzymes with different substrates but similar structural characteristics [20–22]. They are involved in the degradation and remodeling of the extracellular matrix (ECM) in various tissues throughout the body. MMPs dysregulation and/or overexpression promotes the course of various disorders [23–25]. MMPs play crucial roles in the initiation, development, and metastasis of tumors, and they can influence tumor cell behavior by cleaving pro-apoptotic agents and inducing an aggressive phenotype [26]. MMP-2 and MMP-9 which play important roles in several biological processes, including angiogenesis and neurogenesis [20] have been revealed to be the major ECM-degrading enzymes implicated in tumor invasion and metastasis [23, 27]. Several studies indicate that patients with breast cancer have a poorer prognosis when their MMP-2 and MMP-9 levels are elevated [28, 29]. These two enzymes were also linked with gastric cancer metastasis [24] and were said to be upregulated in patients with gastric cancer compared to controls [25].

Heat shock proteins (HSPs) are a class of proteins that are essential for protein folding. They are also known to modulate several crucial apoptotic factors. Several malignancies including breast, gastric, oral, and esophageal cancers have been associated with elevated levels of these proteins [30]. Due to the fact that most of these proteins inhibit apoptosis, cancer cells utilize HSPs as factors that promote survival [31]. Key components of the heat shock response (HSR) such as HSP and heat shock factor 1 (HSF1), have been associated with the progression of breast cancer [32]. Primary breast cancer tissues exhibited markedly elevated levels of HSP60 mRNA compared to normal breast tissues [33]. The HSP70 protein is detected in all cellular components, including the mitochondria, endoplasmic reticulum, the cytosol, and the nucleus [34]. Within the cell, HSP70 proteins act as molecular chaperones and are crucial for regulating cellular development, promoting proliferation, and inhibiting apoptosis [35]. HSP70 is constitutively expressed in healthy cells but is aberrantly expressed in many cancer types, aiding in these cells' survival in unfavorable circumstances. Therefore, inhibiting HSP70 in tumor cells is a promising cancer treatment strategy [36]. HSP60 plays an crucial role in the detection, prognosis, prevention, and therapy of several human malignancies [37]. Increased mRNA expression and HSP60 and HIF2 protein levels were detected in gastric cancer tissues [38]. HSP60 plays a crucial role in tumor aggressiveness and prognosis, and has potential as a target for predicting prognosis in patients with gastric cancer [37]. Inhibition of HSP70 has further intensified the growth arrest and apoptotic activation induced by *H. pylori* in gastric epithelial cells [39]. Although there is evidence of the significance of HSPs and MMPs in breast and gastric cancer, there are not many reports on the impact of *N. sativa* seed oil on these genes. Therefore, this paper will serve as a reference for future research.

This research aimed to explore the cytotoxic and apoptotic effects of cold-pressed oil from *N. sativa* seeds produced organically in the Bayburt region of Turkey on breast and gastric cancer cells. The study also aimed to explore the effects of *N. sativa* seed oil on genes of HSPs (HSP60 and HSP70) and invasion (MMP-2 and MMP-9).

Materials and methods

Oil extraction

The *N. sativa* seeds were cultivated in the experimental field of Bayburt University located in the Bayburt region of Turkey. The *N. sativa* seeds that had matured were collected and dried at room temperature in the absence of sunlight. Until extraction, seeds were stored at +4 °C. Oil was extracted from *N. sativa* samples using a screw press at temperatures around 40 °C, based on a unique technique developed by [40]. A total of 23.42 mL of oil was extracted from an average of 100 g *N. sativa* seed sample and kept at 4 °C for use in a cell culture assay.

Cell culture and cell cytotoxicity assessment

Fetal bovine serum (FBS), penicillin/streptomycin, trypsin- Ethylenediaminetetraacetic acid (EDTA), and Dulbecco's Modified Eagle Medium (DMEM), and cell culture

media were bought from GIBCO (Invitrogen Inc., NY, USA). A breast adenocarcinoma estrogen receptor-positive (MCF-7) and a Gastric Adenocarcinoma (AGS) cell lines were obtained from the American Type Culture Collection (ATCC) in the United States. The cell lines stored in the liquid nitrogen tank were removed. Subsequently, it was placed in a water bath at 37 °C briefly to facilitate melting. The cells were transferred into T75 cm^2 flasks. After 48 h, MCF-7 and AGS cells were counted at 5×10^3 cells/well in DMEM with 10% FBS, and then planted in 96-well plates. The sample was incubated at 37 °C with 5% CO_2. After 24 h, cells were treated with *N. sativa* seed oil extract at different concentrations (10–50-100-200 µg/ml) (dissolved with 1% Dimetil sülfoksit (DMSO)) [41]. These concentrations have been previously investigated in scientific studies.

MTT assays

The proliferation of MCF-7 and AGS cells was assessed by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) method after culturing them at a density of 1×10^4 cells/well in 96-well plates for 24, 48 and 72 h. The absorbance values at 570 nm were measured three times using a microplate reader spectrophotometer (Epoch Microplate Spectrophotometer, BioTek, USA) following the manufacturer instructions (Roche, Germany) [42]. A total of 10 µl of MTT solution at a concentration of 5 µg/ml was added to each well, after which the plate was incubated at 37 °C for 4 hours. Exactly 100 µl of solubilization buffer (SDS 10% in 0.01 N HCl) was added to the plate wells, which were then incubated at 37 °C overnight. The inhibition of cell growth was calculated using the formula [43]: (percentage survival of treated cells/percentage survival of control cells) × 100 (T/C%).

% Growth inhibition = 100 – [OD (test sample) – OD (blank)/OD (control) – OD (blank)]×100.

All cell culture procedures were conducted in compliance with the earlier cited standards and literature [42]. (All reagents and solutions for cell culture were sourced from Thermo Fisher-Germany).

Analyzing gene expression in cell lines

In each well of 6-well plates, 2×10^6 cells were seeded. The sample was incubated at 37 °C in an oven containing 5% CO_2 for 24 h. Following a 6-hour exposure to *N. sativa* seed oil extract at four concentrations (10–50-100-200 µg/mL), the cells were harvested from the wells using a scraper and then homogenized in the Tissue Lyser II device (Qiagen, Germany) (350 µL RLT solution was added to all groups).

The Qiagen RNA isolation kit (Cat No: 74,104-Qiagen, Hille, GERMANY) following homogenization was used for RNA isolation. The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) was utilized for cDNA synthesis. cDNA synthesis was conducted using a Veriti 96 Well Thermal Cycler (Applied Biosystem). The cDNA was kept at -20˚C.

Quantification of mRNA expression for Caspase-3 (Hs00234387_m1), Bax (Hs00180269_m1), Bcl-2 (Hs04986394_s1), MMP2 (Hs01548727_m1), MMP9 (Hs00957562_m1), HSP60 (Hs03924631_s1) and HSP70 (Hs00929524_g1) was achieved using the TaqMan Master Mix kit. Amplification and quantification were performed using the RT-PCR (StepOne Plus RT-PCR, Applied Biosystem). The reference gene utilized was β-actin (Hs01060665_g1). TaqMan® Gene Expression modify the PCR program according to the kit's instructions and initiate the process. All results were represented as fold-change in expression compared to groups using the $2^{-\Delta\Delta Ct}$ method. Each gene analysis was conducted in accordance with our previous study [44].

Statistical evaluation

Statistical analysis was performed using Graphpad 8.0.1 to calculate all data. The results are shown as mean +/- standard deviation. ANOVA was used to analyze the data, followed by the Duncan's test. Statistical significance was determined at $p<0.05$.

Results

In vitro cell toxicity

Various concentrations of *N. sativa* were assessed for their cytotoxic effects on MCF7 and AGS cell lines using the MTT assay at three time points: 24, 48, and 72 h. The data collected from the MTT test was calculated as a percentage to assess cell proliferation after the examination. The percentage of cell viability was calculated using absorbance readings at 570 nm for four concentrations (10-50-100-200 µg/mL). According to the data shown in Figs. 1 and 2, the cell viability in the control group was established as the reference point, with a percentage of 100%.

The results indicate that the propagation of the MCF7 and AGS cells in vitro was inhibited by *N. sativa* in a time- and dosage-dependent way (Figs. 1 and 2). Cell viability decreased at all *N. sativa* concentrations of 10-50-100-200 µg/mL after 24, 48, and 72 h at. It was found that the percentage of viable cells in the experimental groups significantly decreased as the amount of *N. sativa* seed oil used increased. The assessment of the MTT assay's data revealed that the IC50 values for *N. sativa* on MCF7 and

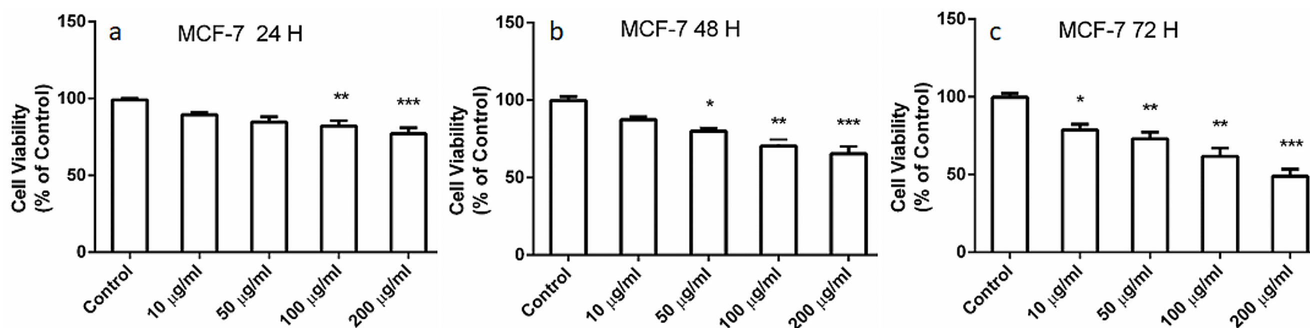

Fig. 1 Graphs showing the *N. sativa* viability test results on MCF7 cancer cells after 24 (**A**), 48 (**B**), and 72 (**C**) hours of MTT testing. (All groups were compared with the control group and * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$)

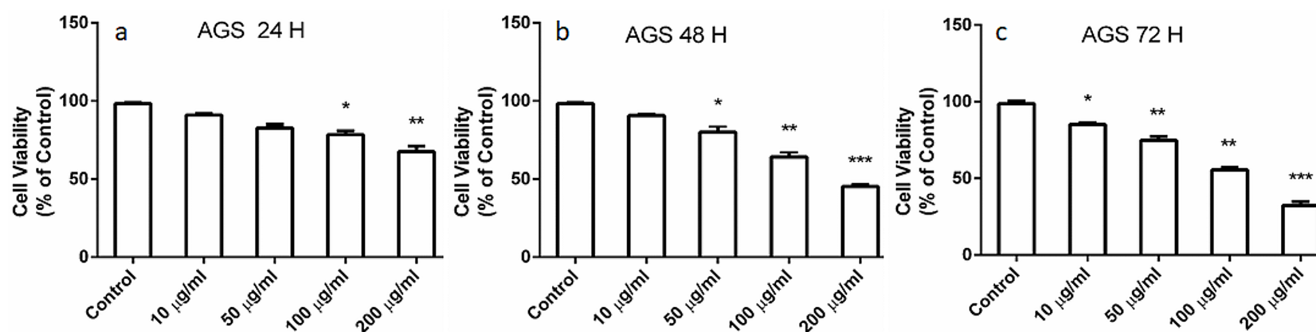

Fig. 2 Graphs showing the *N. sativa* viability test results on AGS cancer cells after 24 (**A**), 48 (**B**), and 72 (**C**) hours of MTT testing

AGS cells at 24, 48, and 72 h were 115.25- 105.19 µg/mL, 97.15–93.10 µg/mL, and 85.10-68.25 µg/mL, respectively. When *N. sativa* was studied, the viability rates of MCF7 and AGS cancer cells were 77.04–67.50% (24 h), 65.28–39.14% (48 h), and 48.95–32.31% (72 h).

A significant difference in viability rates was observed at the 24th hour when comparing MCF7 and AGS cells treated with 100 µg/mL and 200 µg/mL *N. sativa* to the control group ($p < 0.05$). However, at this same time, there was no difference ($p > 0.05$) seen in the viability rates of MCF7 and AGS cells treated with 10 µg/mL and 50 µg/mL than the control (Fig. 1A and 2A).

When compared with the control, there was a difference in the viability rates of MCF7 and AGS cells administered at doses of 50 µg/mL, 100 µg/mL, and 200 µg/mL at the 48th hour ($p < 0.05$). However, at this same time, there was no difference in viability rates of MCF7 and AGS cells treated with 10 µg/mL doses than the control ($p > 0.05$) (Figs. 1B and 2B).

At the 72-hour mark, differences were observed in viability rates between MCF7 and AGS cells and the control across all administered doses ($p > 0.05$) (Fig. 1C and 2C). Viability decreased significantly as the dose increased. The group of cells that were treated with the highest doses showed the lowest viability rates. *N. sativa* of 100 and 200 µg/mL effectively inhibited growth and proliferation in both cancer cell lines. *N. sativa* seed oil concentrations have demonstrated cytotoxic effects on both cancer cell lines. Based on these findings, it has been determined that the two cancer cell lines show a favourable response in reducing their viability rates.

Evaluation of the apoptotic effects of *N. sativa*

The impact of reduced cell viability at four concentrations (10–50 -100–200 µg/mL) on the MCF7 and AGS cell lines, resulting in apoptosis, was evaluated by examining the expressions of apoptosis-related genes (caspase-3, Bax and BCL2) using RT-PCR. The effects of *N. sativa* on the gene expressions of caspase-3, Bax, and BCL2 are shown in Fig. 3. It was shown that with *N. sativa* treatment, caspase-3 expressions were higher in MCF7 cancer cells than in the control group ($p < 0.05$). An increase in the dosage resulted in a clear elevation in caspase 3 expression. The highest expression was noticed in the group of cells that received the highest dose. A variation in caspase 3 gene expression was seen in MCF7 cancer cells between the control group at the four doses (Fig. 3A). But, at *N. sativa* of 10 µg/mL, no difference was seen in caspase 3 gene expression between AGS cancer cells and the control group. A rise in mRNA levels of the caspase-3 gene was found in both cancer cells at *N. sativa* of 200 µg/mL ($p < 0.05$). The expression of caspase-3 was found to be 4.73 ± 0.27 times higher in the MCF7 cell line treated with 200 µg/mL *N. sativa* group in. Moreover,

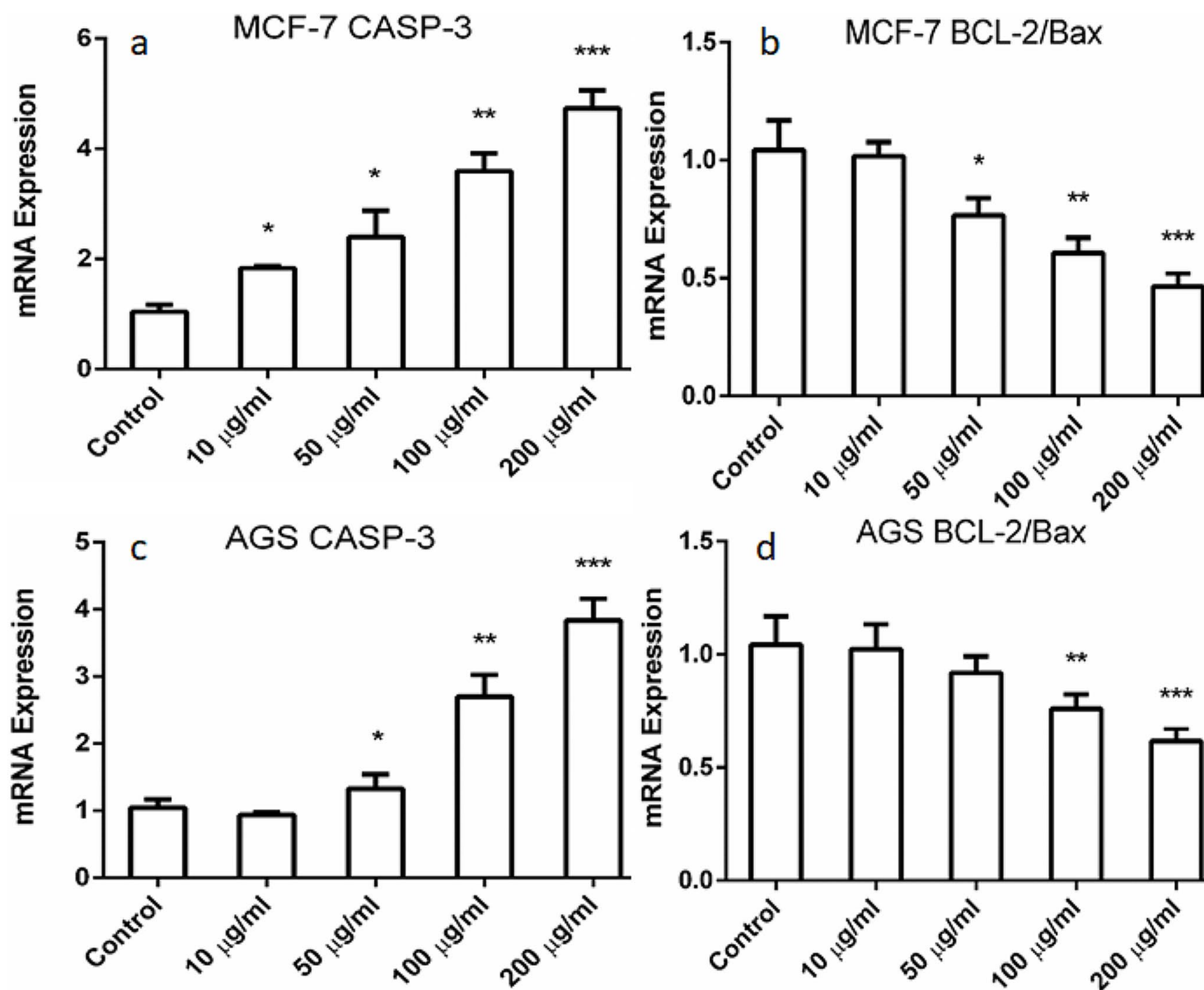

Fig. 3 **A-D.***N. sativa* decreased BCL2/Bax expression ratio and increased Caspase 3 expression level in MCF7 (**A-B**) and AGS (**C-D**) cancer cells

caspase-3 expressions were found to be 3.83 ± 1.19 fold in the *N. sativa* 200 μg/mL group in AGS cell line (Fig. 3C).

The BCL2/Bax ratio decreased with increasing dosage in MCF7 and AGS cancer cells. No difference was detected in reducing the BCL2/Bax ratio at a dose of 10 μg/mL dose in MCF 7 cancer cells than the control ($p > 0.05$) (Fig. 3B). However, a difference was noted when the BCL2/Bax ratio was reduced at doses of 50, 100, and 200 μg/mL in MCF7 cells ($p < 0.05$). The BCL2/Bax expressions were found to be 0.617 ± 0.91 fold in the *N. sativa* 200 μg/mL group in MCF7 cell line. Similarly, no significant difference was observed when the BCL2/Bax ratio was reduced at doses 10 and 50 μg/mL in AGS cancer cells ($p > 0.05$) (Fig. 3D). Conversely, a significant difference was found when the BCL2/Bax ratio was reduced at doses 100 and 200 μg/mL in AGS cells ($p < 0.05$). The BCL2/Bax expressions were determined to be 0.4647 ± 1.31 fold in the *N. sativa* 200 μg/mL group in AGS cell line (Fig. 3D**).**

Analysis of the effects of *N. sativa* on MMP-2, MMP-9, HSP60 and HSP70 gene expressions in MCF7 cell line

There was an inverse relationship between the MMP-2 levels and the dosage in MCF7 cancer cells. There was no difference in reducing the MMP2 level between the two doses (10–50 μg/mL) in MCF 7 cancer cells ($p > 0.05$). A significant reduction in MMP-2 expression was observed in MCF 7 cancer cells with two different dosages (100 μg/mL and 200 μg/mL) ($p < 0.05$). The level of MMP-2 was found to be 0.63 ± 0.75 fold in cancer cells treated with 200 μg/mL of *N. sativa*. The MMP-9 decreased as the dose increased in cancer cells. There was no difference observed

in decreasing the MMP-9 expression at a dose of 10 µg/mL in cancer cells ($p > 0.05$). A difference was seen in decreasing MMP9 expression across three doses (50-100-200 µg/mL) in cells ($p < 0.05$). MMP-9 expressions were found to be 50.63 ± 9.15 times higher in the *N. sativa* 200 µg/mL group compared to cancer cells (Fig. 4A-B.

A noticeable reduction in the mRNA level of the HSP60 and HSP70 genes was reported in the MCF7 cell line in response to varying doses of *N. sativa*. In MCF7 cancer cells, the mRNA expression of HSP60 and HSP70 reduced with increasing dosage. A notable decrease in HSP60 and HSP70 expression was seen in MCF 7 cancer cells with four different dosages (10-50-100-200 µg/mL) than the control group ($p < 0.05$). The expression of HSP60 was detected to be 0.54 ± 0.18 fold in the *N. sativa* 200 µg/mL group in the MCF7 cell line. HSP70 expressions were found to be 0.49 ± 0.08 fold in the *N. sativa* 200 µg/mL group in MCF7 cell line (Fig. 4C-D).

Analysis of the effects of *N. sativa* on MMP-2, MMP-9, HSP60, and HSP70 gene expressions in AGS cell line

A reduction in the mRNA level of MMP-2 and MMP-9 genes was seen in the AGS cell line in response to varying doses of *N. sativa*. The MMP-2 decreased as the dose increased in AGS cancer cells. There was no difference observed when the MMP2 expression was lowered at a dose of 10 µg/mL in cancer cells ($p > 0.05$). A decrease in MMP-2 expression was seen in cancer cells treated with three different dosages (50, 100, and 200 µg/mL) compared to the control group ($p < 0.05$). MMP-2 expression were found to be 50.63 ± 12.31 times higher in cancer cells treated with 200 µg/mL of *N. sativa* than the control group (Fig. 5A). The MMP-9 decreased as the dose increased in AGS cancer cells. A significant difference was found in the reduction of MMP-2 expression in cancer cells when exposed to dosages

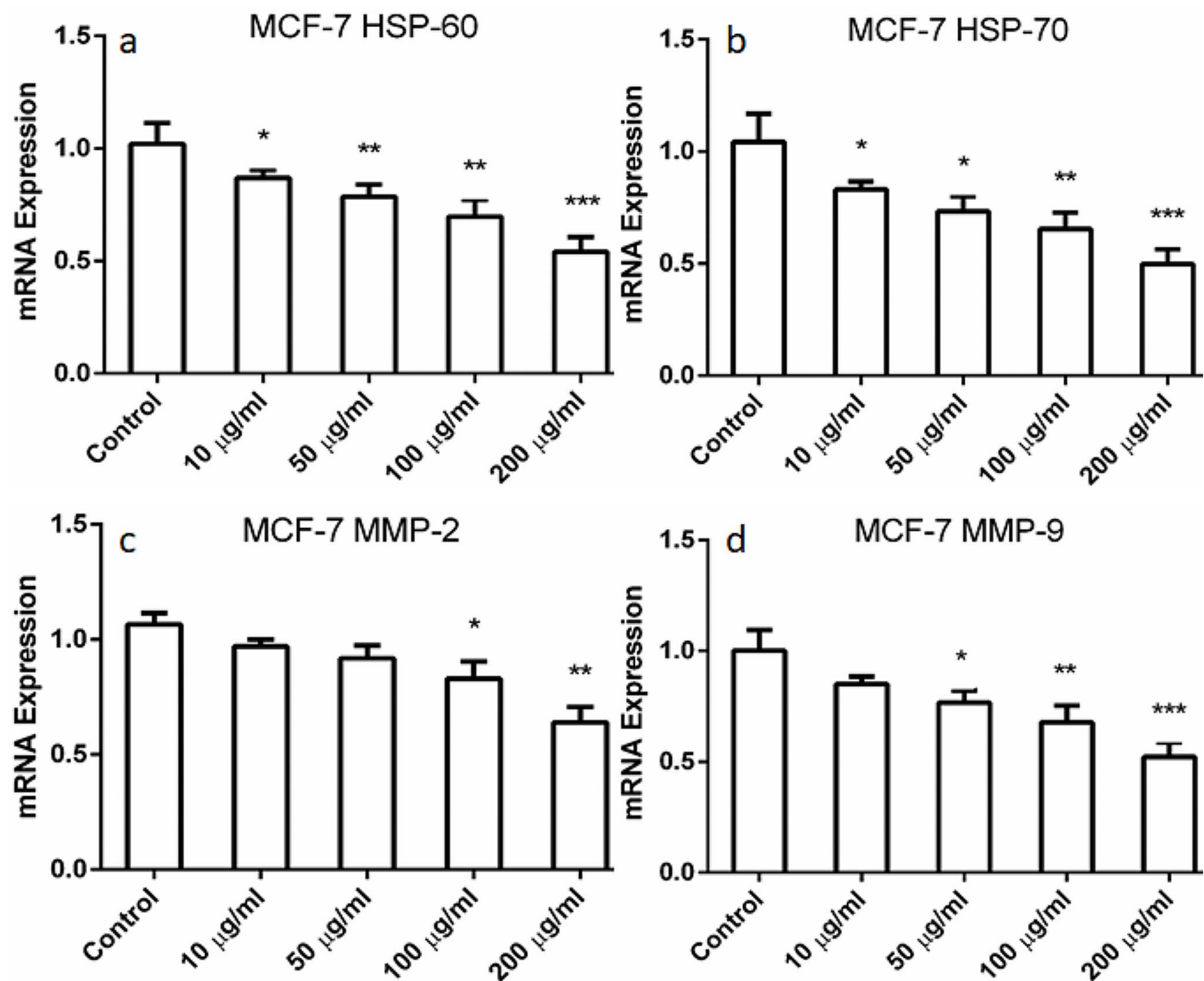

Fig. 4 **A-D.** *N. sativa* decreased MMP-2, MMP-9, HSP60 and HSP70 expression level in MCF7 cancer cells

of 10, 50, 100, and 200 µg/mL ($p < 0.05$). MMP-9 expression was found to be 43.91 ± 8.21 times higher in the *N. sativa* 200 µg/mL group compared to cancer cells (Fig. 5B).

A substantial reduction in the mRNA expression of HSP60 and HSP70 genes was reported in the AGS cell line in relation to the dosage of *N. sativa*. Decreased expression of HSP60 and HSP70 mRNA was seen in AGS cancer cells as the dose increased (Fig. 5C-D). A substantial decrease in HSP60 and HSP70 expression was seen in cancer cells at dosages of 10, 50, 100, and 200 µg/mL ($p < 0.05$). The HSP60 expression was found to be 36.10 ± 6.05 times higher in cancer cells treated with 200 µg/mL of *N. sativa*. Also, HSP70 expressions were found to be 38.17 ± 8.11 times higher in the *N. sativa* 200 µg/mL group in cancer cells (Fig. 5D).

Discussion

Each year, a significant number of people die from cancer [45]. For this reason, scientists conduct various research on the diagnosis, treatment, and prognosis of cancer. Owing to the adverse effects of chemotherapy treatments, scientists are interested in discovering natural herbal active components to treat cancer. This study researched the cytotoxic and apoptotic effects of *N. sativa* seed oil on breast and stomach cancer cells. We also analyzed their impact on the expression of HSP60 and HSP70 as well as the MMP-2 and MMP-9 genes, which play a role in processes like inflammation, cell proliferation, angiogenesis, and invasion. There have been limited studies on the impact of *N. sativa* seed oil on breast and gastric cancer cells. Our findings indicate that *N. sativa* has dose-dependent effects on both types of cancer cells.

N. sativa belongs to the Ranunculaceae family [46]. Pharmacological studies have identified that *N. sativa* has

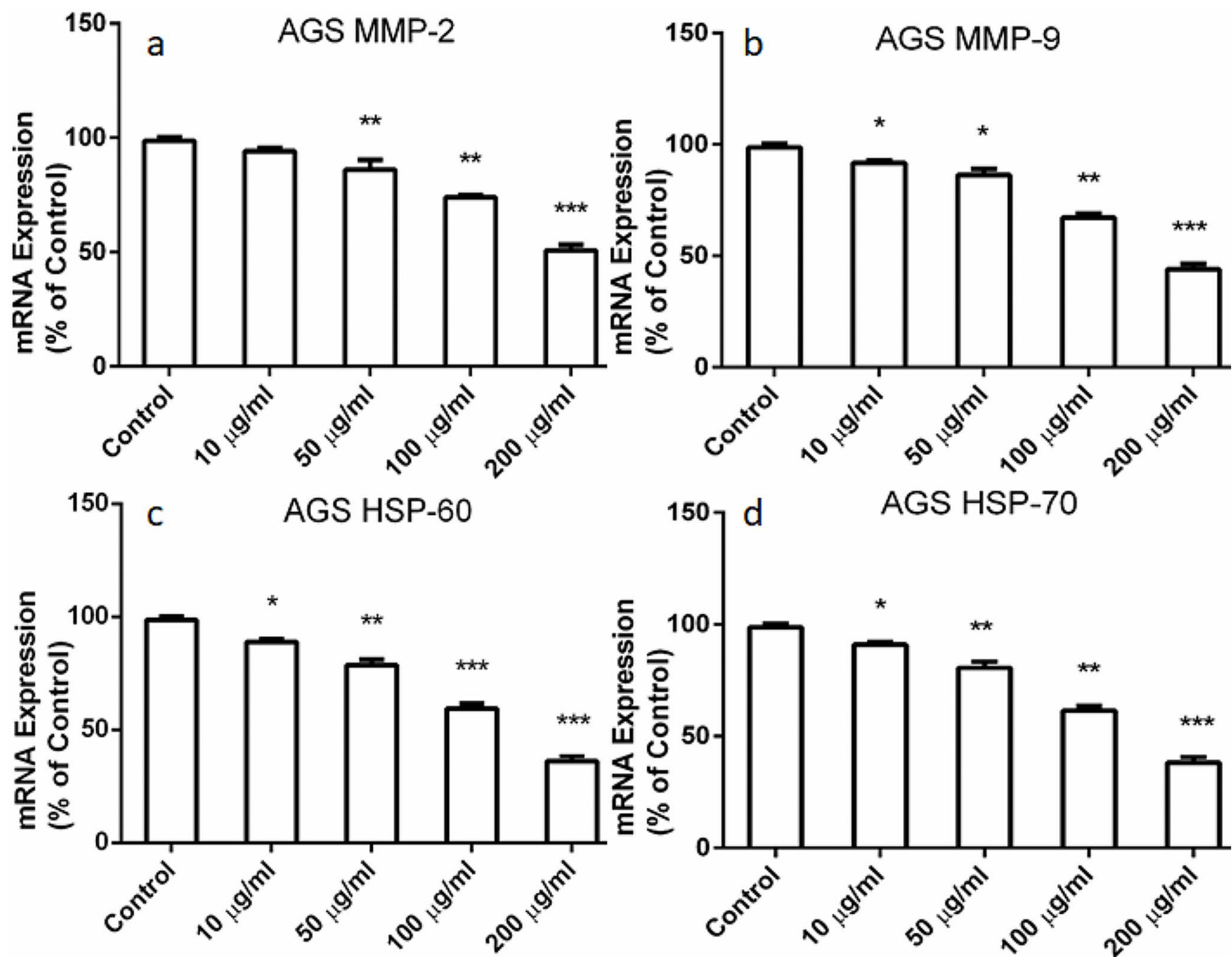

Fig. 5 **A-D.** *N. sativa* decreased MMP-2, MMP-9, HSP60 and HSP70 expression level in AGS cancer cells

a wide array of biological activity including anti-inflammatory, antibacterial, antiviral, anti-parasitic, anti-diabetic, and anti-ulcer properties [17]. Several studies have demonstrated that *N. sativa* is effective against cancer in the circulatory system, kidneys, lungs, prostate, liver, breast, and on various malignant cell lines. But, the molecular mechanisms responsible for this anti-cancer effect are still not fully understood [47].

N. sativa seed oil has been report to reduce cell viability and proliferation in MCF7 breast cancer cells [48]. A study discovered that *N. sativa* inhibited the growth of MCF-7 cancer cells in a manner that depended on both time and dosage [49]. *N. sativa*, whether used alone or in conjunction with oxidative stress, shown efficacy in vitro in influencing the lifespan of MCF-7 cells, suggesting promising possibilities for cancer prevention and treatment [50]. Administering *N. sativa* seed oil alone or in combination with doxorubicin resulted in reduced cell proliferation and viability in MCF-7 cells, indicating possible therapeutic benefits. This effect was showed at 50 µg/mL of *N. sativa*. *N. sativa* seed oil changed the morphology of breast cancer cells and decreased their survival and growth rate [48]. The lipid extract from *N. sativa* has demonstrated cytotoxic effects on MCF-7 cells, with an LC50 of 2.72 ± 0.232 mg/ml [51]. An assessment of the cytotoxic properties of *N. sativa* seed oil was conducted using the MTT assay on HepG2, MCF-7, A-549, and HEK293 cell lines. The cell viability percentages of HepG2, MCF-7, and A-549 cells all significantly decreased based on concentration in the data [52]. Cell viability was assessed at 50, 100, and 250 µg/mL concentrations of *N. sativa* seed oil in MCF-7 cells, resulting in viabilities of 46%, 32%, and 24% respectively. At the highest concentration, the viability rates of MCF7 cancer cells were found to be 77.04% (24 h), 65.28% (48 h), and 48.95% (72 h).

Gastrointestinal diseases can be prevented and treated by *N. sativa* [53]. The *N. sativa* seed oil treatment significantly decreased cancer cell proliferation than control group. AGS cells were exposed to dosages of 0.4 to 8.2 mg/mL for 24 h. The oil reduced the number of viable cells in the stomach cancer cells [54]. The literature has focused on more on exploring the impact components of *N. sativa* on gastric cancer rather than the individual effect of *N. sativa* seed oil. µµ TQ inhibited the proliferation of gastric cancer cells in a concentration- and time-dependent manner. TQ and doxorubicin significantly decreased the viability of gastric cancer cells [55, 56]. The viability rates of MCF7 cancer cells were 67.50% (24 h), 39.14% (48 h), and 32.31% (72 h) when exposed to the highest dose of *N. sativa*.

Many in vivo and in vitro reports have revealed that *N. sativa* volatile oil inhibits cancer growth by targeting multiple signaling pathways, particularly (including in cell proliferation and death etc.) [56–59]. The anti-cancer effects of *N. sativa* involve the generation of ROS, stimulation of caspases, and control of molecular targets such p53, p73, PTEN, STAT3, and PPAR-g [59, 60]. Most anticancer medications are related with the mitochondrial pathway in their mechanism of action [60, 61]. Higher Bax/Bcl-2 ratios have been linked to increased p53 expression, which in turn causes cytochrome c release, caspase activation, and eventually apoptosis [61, 62].

N. sativa oil inhibited the proliferation of MCF-7, HeLa, and Jurkat cells, inducing apoptosis in these cell lines [62, 63]. The viability of MCF7 cells decreased significantly to 90.67% and 82.6% when treated with 0.6% and 1.25% (V/V) N. sativa oil, respectively. The levels of Caspase-3, -8, -9, and p53 genes significantly rose in MCF-7 cells with higher doses and longer exposure times to *N. sativa* [49]. More investigations have been conducted on the correlation between thymoquinone (TQ) and octahydropyrazino [2,1-a:5,4-a′] diisoquinoline derivative (OM-90) components of *N. sativa* with apoptosis [56, 57]. TQ, produced from *N. sativa* oil, decreased the protein expression of anti-apoptotic genes Bcl-xL and Bcl-2 in breast cancer cells and breast tumor xenografts. TQ enhanced p38 phosphorylation and reactive oxygen species (ROS) production in breast cancer and have anti-proliferative and pro-apoptotic effects [1]. A report was conducted to analyze the impact of TQ on different cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-468, and T-47D) with varied levels of estrogen receptor, progesterone receptor, and p53 status [63–67].

Surprisingly, there are insufficient studies on *N. sativa* ‘s role in stomach cancer [56, 57]. In this paper, we determined that N. *sativa* seed oil had apoptotic effects on breast and gastric cancer cells. Our analysis of the mRNA level of apoptosis genes shows that Bcl-2 genes are downregulated and Bax, caspase-3, and caspase-9 genes are upregulated. It was determined that the effects at 24, 48 and 72 hours in MCF7 and AGS cell lines differed depending on the dose. The 200 µg/mL dose resulted in more than 50% cell death, particularly at the 72-hour mark. Also *N. sativa* has been shown to have apoptotic effects in these cancer cells. The 200 µg/mL dose significantly raised caspase-3 mRNA expression in both cell lines. The bcl2/Bax ratio is at its minimum at the given dose.

MMPs, particularly MMP-2 and MMP-9, have a vital role in the development and spread of tumors in a variety of malignancies [67, 68]. Two proteases, MMP-2 and MMP-9, have been linked to the development of tumor invasion and metastasis [68, 69]. These proteases have been identified in many forms of human cancer. Research suggests that breast cancer could be impacted by the MMP-2 and MMP-9 genes. Although their roles have been associated with breast cancer invasion and metastasis, it remains uncertain how they impact the disease's initiation [69, 70]. There were no clear

studies demonstrating the effect of *N. sativa* seed oil on the genes associated with invasion in breast and gastric cancer. Nevertheless, there have been limited studies demonstrating the impact of TQ on these genes across various cancer types.

Researchers observed an increase in E-Cadherin expression in MCF-7 and MDA-MB-231 cell lines treated with *N. sativa* [16]. They also noted a reduced in MMP-2 and MMP-9 expression, indicating their correlation with breast cancer prognosis [16]. TQ treatment suppressed Integrin-1, VEGF, MMP-2, and MMP-9 in TNBC cells [70, 71]. A study discovered that 30 µM TQ was more efficient at inhibiting the production of MMP-2 and −9 than 10 µM TQ in MDA-MB-231 cells. TQ treatment has demonstrated an inhibitory effect on Neuro-2a cell migration by suppressing the production of MMP-2 and MMP-9 [71, 72]. TQ decreased glioblastoma cell invasion by altering the release of MMP-2 and MMP-9 via alteration of the FAK-ERK signaling pathway [72, 73]. TQ decreased the expression of MMP2 and MMP9 mRNA and protein. TQ plays a function in inhibiting the growth, movement, and spread of A549 lung cancer cells [73, 74]. The current study found that *N. sativa* seed oil reduced the level of MMP-2 and MMP-9 genes in breast and stomach cancer cells. It was shown that the dose of 200 µg/ml lowered the level of MMP-2 and 9 the most.

We have not conducted any studies on the effects of *N. sativa* seed oil on HSPs in breast and gastric cancer. We identified a limited number of papers on the impact of TQ on HSPs in various disorders and different types of HSPs. TQ binds strongly to HSPA5/GRP78 to reduce its expression in patients with COVID-19 [74, 75]. TQ-loaded nanostructured lipid carriers modulated HSP70 to prevent the formation of ulcers generated by ethanol [75, 76]. Hsp70 dysregulation, an indicator of oxidative damage, has been linked to TQ-triggered ROS-mediated processes [76]. This research shown that the level of HSP60 and HSP70 genes was elevated in the control group of breast and gastric cancer cells. However, a decrease in the expression of these genes was noted with varying dosages of *N. sativa*. It was determined that the maximum decrease in these genes was at a dose of 200 µg/mL.

Conclusions

The study shows that *N. sativa* seed oils have cytotoxic effects on breast and stomach cancer cells and impact the mRNA expression of apoptotic genes, HSPs, and MMP genes, with variations based on the dosage. This work is noteworthy as it is the first to examine the impact of *N. sativa* seed oil on these genes collectively. However, our study had some limitations. First, we were able to separate *N. sativa* seed oil and analyze the effect of these seed oil on cancer cells. We did not have the opportunity to decompose it into its components. Secondly, we aim to validate these genes using techniques like immunohistochemistry or flow cytometry. Third, apart from exploring the effects of *N. sativa* on apoptosis, invasion, and HSPs, we would also like to examine other mechanisms that are crucial in cancer. Finally, due to budgetary constraints, we were unable to search the effects of *N. sativa* on other types of cancer.

We will attempt to find project support in the future and demonstrate other effects of *N. sativa* on various cancer cells. We think that our findings from this study will shed a guiding light for scientists who plan to conduct new studies on *N. sativa*.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Since the cell line was used in our study, the Ethics Committee approval was not required.

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