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Using green coffee and cinnamon extracts for Regulation of the proliferation signaling and inflammatory events during treating breast cancer

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ABSTRACT

According to NCI, one-third of all female cancer diagnoses in Egypt are due to breast carcinoma. This research investigates the use of low-cost, ecologically-friendly plant extracts (green coffee and cinnamon) dissolved in Ethanol for the treatment of breast cancer. Many women prefer natural medicines and conventional ones to manage their illness, reduce sideeffects from medication, or slow the progression of the disease. The potential effects of these plant extracts on MCF-7 cells were analyzed using MTT assay, qRT-PCR, and ELISA. The results showed that cells were substantially destroyed by green coffee extract. The cells exposed to green coffee, like those exposed to cinnamon extracts, generated considerably more LDH. Research compared the effects of cinnamon and green coffee extracts on MCF-7 cells and found that the latter had a greater impact on apoptosis. The proteins p53 and caspase-3, as well as the ERK signaling cascade, were influenced by the green coffee components.RAF/MEK/ERK pathway in MCF-7 cells was inhibited. By altering critical apoptotic signaling pathways, green coffee may exhibit anti-cancer effects and promote apoptosis in breast cancer cells. The average levels of IL-1α and IL-1β, measured by ELISA, were found to have risen dramatically after 24-hours. While IL-6 and IL-8 mean concentration shows a steady state. Finally, green coffee extract was shown to inhibit the production of pro-inflammatory cytokines.

***Keywords*:** Green coffee, cinnamon, P53, ERk, Nf-Kb, Casp3, IL-1α, IL-1β, IL-6, IL-8

INTRODUCTION

Cancer is a major international health concern. As a result of demographic shifts, an estimated 420 million additional instances of cancer will be diagnosed yearly over the world by the year 2025 (Zugazagoitia *et al*., 2016). Among the many strategies used to defeat cancer is doing fundamental scientific study to better understand the molecular and biochemical processes of cancer development, methods for identifying and eliminating cancer-causing chemicals, early tumor detection and removal strategies, and the development of novel cancer drugs and therapies are all part of this approach. Most cancer research funding and time over the last 40 years has gone toward creating new treatments for existing cancers (Carbone and Pass, 2004).

In response to malignant or benign situations, tumor cells release tumor markers. Predictive and prognostic biomarkers do exist. Biomarkers allow for a factor-free quantification of prognosis. The presence or absence of these indicators is highly predictive of the disease's recurrence or mortality risk. Patients' responses to therapies may be predicted by markers (Łukasiewicz *et al*., 2021).

In terms of cancer-related mortality throughout the globe, breast cancer ranks fifth on the list compiled by GLOBOCAN 2020. According to estimates, there will be over 2,300,000 new instances of breast cancer worldwide. Therefore, it is both a prominent cause of cancer-related fatalities in women and one of the most common kinds of cancer overall (Łukasiewicz *et al*., 2021). Preventative strategies and screening programs are essential to lowering the breast cancer incidence rate and allowing for earlier treatment. Developing appropriate recommendations and methods to give the most effective ways of managing breast cancer on a worldwide scale is the mission of the Breast Health worldwide Initiative (BHGI) (Łukasiewicz *et al*., 2021).

Female breast cancer is now the most frequent disease in women (Abas *et al*., 2022). Diet and nutrition have recently gained popularity as a preventative measure against cancer, with several in vitro and in vivo scientific studies examining the efficacy of different natural substances and extracts. In addition, several natural components in the diet have been linked to potential benefits in cancer prevention and therapy (Hefni *et al*., 2022).

Coffee's complex chemical composition may have a wide range of impacts on the human body, from boosting antioxidant activity to energizing the neurological system. Recent studies have shown that drinking coffee on a daily basis may reduce the likelihood of getting serious illnesses including type II diabetes, Parkinson's disease, Alzheimer's disease, and even liver cancer (Stelmach *et al*., 2015). Green coffee infusions, which are prepared from unroasted coffee beans, have become popular due to its positive health effects associated with consuming antioxidant-rich foods and beverages. Besides it may assist in weight reduction and the prevention or treatment of obesity due to their capacity to increase metabolic rate (Stelmach *et al*., 2015). Moreover, it contains phenolic chemicals, and more particularly chlorogenic acids (CGAs), which have antioxidant qualities (Stelmach *et al*., 2015). Chlorogenic acids (CGAs) have found widespread use in the healthcare and supplement industries and have possible health benefits (Gouthamchandra *et al*., 2017). Treatment with chlorogenic acid has showed promise in reducing tumor growth in many types of cancer, including colon, brain, breast, lung, and chronic myelogenous leukemia, according to preclinical and phase I clinical research. However, further research is needed to completely understand the molecular processes behind the anti-cancer benefits of chlorogenic acid (Gouthamchandra *et al*., 2017).

There have been no reported side effects from using cinnamon for millennia, and as a result, its bark, essential oils, bark powder, phenolic compounds, flavonoids, and separated components have been the subject of much research. Each of these attributes has been linked to various health benefits for humans. Antioxidants and antimicrobials may exert their effects directly on oxidants and bacteria, respectively, whereas the anti-inflammatory, anticancer, and antidiabetic actions are believed to be mediated via receptors (Rao and Gan, 2014). Numerous studies have examined cinnamon's positive effects on human health. Many biological mechanisms contribute to cinnamon's anti-cancer effects. The ability to hinder cancer cell survival is vital for any potential anti-carcinogenic agent. Cinnamon's anti-cancer properties have been shown in several researches (Sadeghi *et al*., 2019).The previous results demonstrated that when cinnamon extract was applied to cell cultures, it was significantly reduced tumor cell growth and induced active tumor cell death through an uptick in pro-apoptotic chemicals. As a

result, genes including Bcl-2, Bcl-xL, and survivin, which are controlled by NF-kB and AP1, was less active. By the same method as in vitro, oral treatment of the cinnamon extract significantly reduced tumor development in a melanoma transplanting model. In addition, HPLC analysis revealed that both the aqueous extract and fraction of cinnamon procyanidins and cyanidins inhibited the kinase activity of vascular endothelial growth factor subtype 2 (VEGFR2). In light of these results, cinnamon deserves further investigation as a possible cancer preventative (Zhang *et al*., 2017). Angiogenesis inhibitory properties of synthetic cinnamaldehydes have been investigated and it was found that it inhibited NF-B activity and interleukin-8 (IL-8) production in A375 cells stimulated by tumor necrosis factor alpha (TNF-□) (Rao and Gan, 2014). These results provide credence to the underappreciated potential of cinnamic acid as an anticancer treatment (Cabello *et al*., 2009).

The purpose of the current research is to look at the data connecting green coffee beans, cinnamon, and their main bioactive constituents to a reduced chance of developing breast cancer. Existing modes of action that may help provide such safety are the primary focus of this investigation.

MATERIALS AND METHODS

Plant extraction

Green coffee and cinnamon powder (10 mg each) were combined, then sterilized in 70% ethanol and allowed to dry at room temperature to produce the plant extract. Two days were spent incubating each sterile extract in 1 ml of 70% ethanol at room temperature while regularly vortexed. The supernatant was concentrated to 500µg/µl and incubated at 4 degrees Celsius in a clean, new tube (Wendakoon *et al*., 2012).

Cell lines

The breast cancer research cell line MCF-7 was purchased from VACSERA in Giza, Egypt. 5% heat-treated bovine serum albumin (BSA), 4 mM L-glutamine, and 4 mM sodium pyruvate were added to Roswell Park Memorial Institute (RPMI) 1640 medium for cell culture. The cells were cultured in a 75ml cell-culture flask at 37°C with 5% CO2. Research in this area has been mixed (Abd El Maksoud *et al*., 2019; Khalil *et al*., 2017). Zeiss A-Plan 10X lens objective was used for inverted microscopy to get pictures of the cultivated cells.

Proliferation assay

The shape of the cells was examined using a microscope turned upside down. To stimulate cell growth, cells were planted at a density of $10x10^4$ cells per well on a 6-well plate. After two washes in PBS, the cells were trypsinized by adding a sufficient amount of trypsin and incubated at 37 degrees Celsius for three minutes. After the trypsinized cells were re-suspended in a suitable amount of complete RPMI medium, their morphology was observed under an inverted microscope

High performance liquid chromatograph (HPLC) analysis:

The Agilent 1260 series equipment was used for the HPLC analysis, and the separation was carried out on an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm).The flow rate of the mobile phase was 0.9 ml/min, and the solvents used were water (A) and acetonitrile with 0.05% trifluoroacetic acid (B). The mobile phase was optimized using a linear gradient algorithm, and the parameters were as follows: 0 minutes (82% A), 5 minutes (80% A), 8 minutes (60% A), 12 minutes (82%

A), 15 minutes (82% A), and 20 minutes (82% A). The 280 nm wavelength was selected on the multi-wavelength detector, and 5 l of each sample solution was injected. Throughout the examination, a constant temperature of 40 degrees Celsius was maintained in the column.

Cytotoxic concentration 50% (CC_{50})

The cytotoxic effect and CC50 of the extracted material were calculated for the MCF-7 cell line. $10X10^3$cells were placed in each well of a 96-well plate, and the plates were placed in a CO2 incubator at 37 degrees Celsius. The cells were exposed to extracts at doses ranging from 0.3 to 3 milligrams per milliliter after an overnight incubation. The MTT cell growth test kit (Sigma-Aldrich, Germany) was used to determine cell viability and cytotoxicity by quantifying the quantity of formazan dye released by viable cells. The cytotoxic concentration and cell viability were calculated using the absorbance at 570 nm.

Lactate dehydrogenase (LDH) production

The (Abc-65393) LDH assay kit was used to evaluate LDH generation in the culture media obtained from cells treated with 600 µg/ml of each extract. 100µl of lysed cells and 100µl of LDH reaction mix were incubated for 30 minutes at room temperature, as recommended by the manufacturer. A plate reader set to OD450nm was used to assess LDH activity. The average LDH production of the treated cells was compared to that of fake cells, and the result was expressed as a fold change (Khalil, 2012; Khalil *et al*., 2019).

Quantitative real time PCR (qRT-PCR)

TriZol (Invitrogen, USA) was used to extract total cellular RNA, and an RNA purification kit (Invitrogen, USA) was used to purify the RNA before it was used in qRT-PCR to assess gene expression. M-MLV reverse transcriptase (Promega, USA) was used to convert 1 µg of total RNA into complementary DNA (cDNA). The QuantiTect-SYBR-Green PCR Kit (Qiagen, USA) and the particular primers found in Table (1) (Khalil *et al*., 2016; Morikawa *et al*., 2015) were used to quantify mRNA expression levels of ERK, Nuclear factor Kappa B (NF-kB), tumor suppressor gene P53, and Casp3. Normalization of the real-time PCR data was performed by comparing the expression level of target genes to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The final volume of the PCR reaction was 25 µl, and it included 10 µl SYBR green, 0.25µl RNase inhibitor (25 U/µl), 0.2 µM of each primer, 2 µL of synthesized cDNA, and nuclease-free water. PCR was performed at 94 degrees Celsius for 5 minutes, then at 60 degrees Celsius for 15 seconds, and finally at 72 degrees Celsius for 30 seconds, for a total of 35 cycles (El-Fadl *et al*., 2021; Khalil *et al*., 2019)**.**

Enzyme-linked immune-sorbent assay (ELISA)

Human ELISA kits (Abcam, 46028, 214025, 100572, and 100575, respectively) were used to perform ELISA tests to detect and evaluate the quantities of released interleukin-1 alpha (IL-1a), IL-1b, IL-6, and IL-8. Mcf-7 cells were grown in 96-well plates overnight before being treated with each extract at a concentration of 600 g/ml for varied amounts of time (0, 6, 12, 24, 36, 48, and 72 hours). At each time point, 1X cell lysis buffer (Invitrogen, USA) was used to lyse the cells. Following two-hour incubation at room temperature with 100 l of the lysed cells, 100 l of the control solution, and 50 l of 1X biotinylated antibody were added to an ELISA plate reader. Next, 100 l of a 1X streptavidin-HRP solution was added to each well after the samples had been placed in individual ones. After that,

the samples were incubated with 100 l of a chromogen TMB substrate solution at room temperature and out of the light for 15 minutes. After adding 100 l of stop solution to each sample well, the reaction was interrupted, and the absorbance at 450 nm was determined (Khalil *et al*., 2020; Khalil *et al*., 2017)

Table 1: The sequences of oligonucleotides utilized for quantifying the mRNA levels of the specified genes.

Genes	**Primer sequences (5'-3')**		
ERK-sense	AACATCAGACCTACTGCCAGCGC	**Casp3-sense**	GGACAGCAGTTACAAAATGGA
ERK-antisense	CGCAGGATCTGGTAGAGGAAGT	**Casp3-antisense**	CGGCAGGCCTGAATGATGAAG
NF-kB1-sense	AATGGCAGAAGATGATCCATAT	**GAPDH-sense**	TGGCATTGTGGAAGGGCTCA
NF-kB1-antisense	CTGTGGGCATGCAGGTGGATAT	**GAPDH-antisense**	TGGATGCAGGGATGATGTTCT
P53-sense	GCGAGCACTGCCCAACAACA	**Casp3-sense**	GGACAGCAGTTACAAAATGGA
P53-antisense	GGTCACCGTCTTGTTGTCCT	**Casp3-antisense**	CGGCAGGCCTGAATGATGAAG

Data analysis

All charts and histograms were made in Microsoft Excel. Quantitative analysis of qRT-PCR results for mRNA expression was performed using delta-Delta Ct analysis, with the following equations (Khalil H *et al*., 2017; X. Rao *et al*., 2013):

(1) delta-Ct = Ct value for target gene - Ct value for GAPDH,

(2) delta-delta Ct = delta-Ct value for experimental group - delta-Ct value for control group

(3) Quantification fold change = ($2^{-\text{delta-delta ct}}$).

Statistical significance was determined using the student's two-tailed t-test, with a p-value ≤ 0.05.

1. **Green coffee and cinnamon quantitative analysis by HPLC:**

The phenolic and flavonoid components in the ethanol extract for green coffee and cinnamon vary, as shown in Tables (2 & 3). The phenolic compounds in both extracts that was found were chlorogenic and Ferulic acid but other specific compounds were found only in green coffee extract such as Pyro catechol and Daidzein. P-coumaric was found in cinnamon only. According to the current

RESULTS

results, it was determined that the highest concentrations found in green coffee was chlorogenic (1810.41µg/ml), Pyro catechol and Daidzeinalso (299.48 and 115.12 µg/mL, respectively). While the highest concentration found in cinnamon was Catechin (35.16 µg/mL). The major compounds found in the study were agreed with those given by the national research center (NRC) as a result of using Agilent 1260 series.

Table (2): Results of HPLC test for phenolic and flavonoid chemicals extracted from green coffee in accordance with a certain retention time (RT).

Group	RT (min)	Compound	Conc. (µg/mL)	Group	RT (min)	Compound	Conc. (µg/mL)
Phenols	3.3	Gallic acid	3.64	**Flavonoid**	7.9	Rutin	3.46
	4.2	Chlorogenic acid	1810.41		9.1	Coumaric acid	1.12
	5.5	Methyl gallate	2.46		10.4	Naringenin	241.88
	6.0	Caffeic acid	2.72		12.7	Quercetin	6.43
	6.5	Syringic acid	11.12		14.5	Apigenin	0.12
	6.7	Pyro catechol	299.48		15.0	Kaempferol	0.35
	8.8	Ellagic acid	2.45		15.6	Hespretin	0.00
	9.1	Coumaric acid	1.12		4.6	Catechin	23.68
	9.7	Vanillin	0.08		14.0	Cinnamic acid	0.03
	10.2	Ferulic acid	125.22		12.2	Daidzein	115.12

Table (3): Results of HPLC test for phenolic and flavonoid chemicals extracted from cinnamon in accordance with a certain retention time (RT).

Group	RT (min)	Compound	Conc. (µg/mL)	Group	RT (min)	Compound	Conc. (µg/mL)
Phenols	5.0	Pyrogallol	10.33	**Flavonoid**	4.4	Rutin	9.14
	7.0	Catechin	35.16		5.2	Naringine	8.16
	8.0	Chlorogenic	6.47		7.0	Quercetin	15.23
	9.8	p-PH benzoic	8.16		7.9	Kaempferol	6.17
	12.0	Ferulic	9.12		9.1	Luteolin	7.46
	13.0	P-coumaric	17.36		10.0	Apigenin	14.56
	15.6	Vanillic	8.66		12.0	Catechin	9.52

2. Cytotoxic effect of plants extracts on MCF-7cells:

Cell lines MCF-7 were treated with plant extracts (green coffee and cinnamon) at concentrations ranging from 0 to 20 mg/mL and compared to cells pre-treated with 70% ethanol. To investigate the possible cytotoxic effects of the plant extract, the CC50 was determined by testing the extract on MCF-7 cells for 48 hours. Cell proliferation and cytotoxicity were measured using the MTT test. Figure (1A) depicts the mild cytotoxic effects of cinnamon extracts on the treated cells.

Micrographs show that green coffee significantly decreased cell viability of breast cancer cells. The plant extracts were compared to 70% ethanol and a control sample in terms of their effects on cancer cell viability (Fig.1B). It was obvious from Figure (2), the cytotoxic effects of green coffee extraction increased with increasing concentration. The cytotoxicity of green coffee against MCF-7 was substantial, with a CC50 (concentration inhibiting 50% of cells) of over 1.25 mg/ml. Table (4) shows the statistical analysis performed on the data using the T-Test hypothesis and a significance level of $P \leq 0.05$.

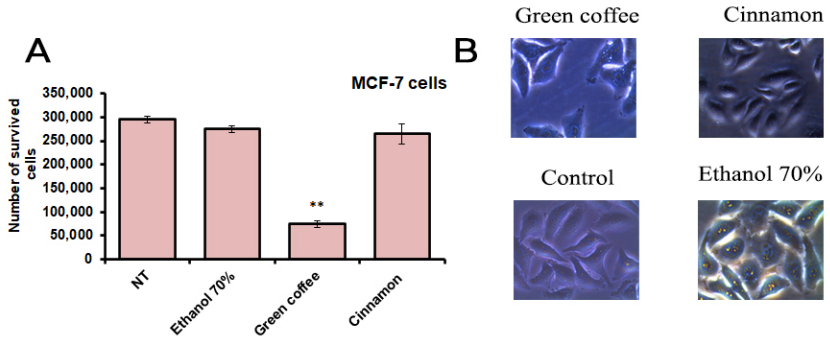

Fig. (1): After treating MCF-7 cells with extracts of green coffee and cinnamon, the number of surviving cells was counted to determine the cytotoxic impact of the plant compounds. The standard deviation of four separate experiments is shown by the error bars. Two-tailed t-tests were used to determine statistical significance, and differences with a p value of *p ≤ 0.05 and **p ≤ 0.01 representing significant differences. were judged to be meaningful. Two days following treatment, the morphology of cells exposed to green coffee extract, cinnamon extract, or ethanol was compared to that of untreated cells and cells exposed to ethanol using representative inverted microscope pictures (NT).

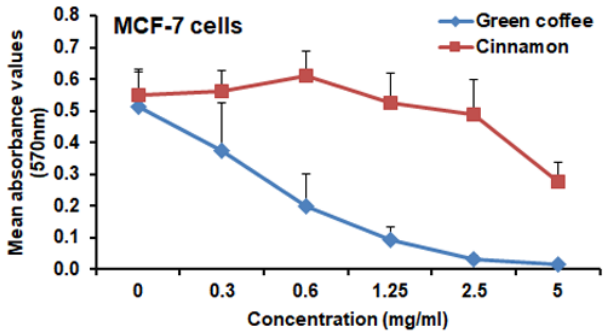

Fig. (2): Plants extraction cytotoxic effect.

Table (4): Resultsof statistical parameters of cell viability rate of treated cells.

Statistical measurements	Control	Plants extract	
	Ethanol 70%	Green coffee	Cinnamon
Mean absorbance	275000	75000	265000
STD	7071.07	7071.07	21213.20
P values	0.106	0.001*	0.198

3. Production of LDH in treated cells:

Since the soluble cytosolic enzyme LDH is released into the culture medium following injury or loss of integrity of the cell membrane, its detection is a frequently used approach for assessing cell viability. Elevated levels of LDH are associated with the necrosis of these cells. Figure 3 shows that relative LDH production was significantly higher after green coffee treatment (18.5-fold increase) and lower after cinnamon treatment (7.25-fold reduction).

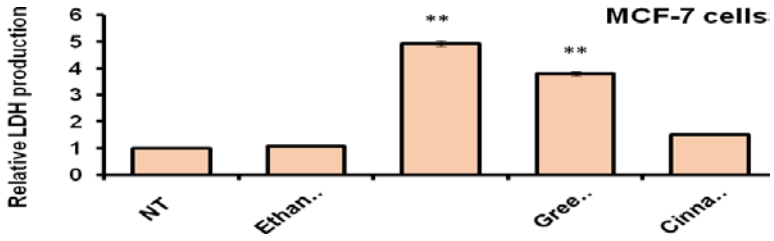

Fig. (3). Error bars reflect the standard deviation (SD) of four independent replicates, and show the relative amounts of LDH production between treated and Triton 100-X treated cells. The significance and P-values of the LDH production levels were calculated using a two-tailed Student's test.

4. Determination of gene expression using Real-Time qRT-PCR:

The mRNA levels of ERK, P53, Caspase-3, and NF-KB in treated MCF-7 cells were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) to determine the impact of natural plant extraction on cellular signaling. Total RNA was collected and transcribed into cDNA after cells were treated with green coffee and cinnamon extracts. Figure (4C, D) shows that after 0.25 mg/mL green coffee administration, ERK (P = 0.02091) and NF-KB (P=0.01767) were significantly inhibited, whereas P53 expression was increased more than 5-fold (P = 0.01787) (Fig. 4A). More than 4-fold (P = 0.01787) upregulation of Caspase-3 was also observed in Figure (4B).

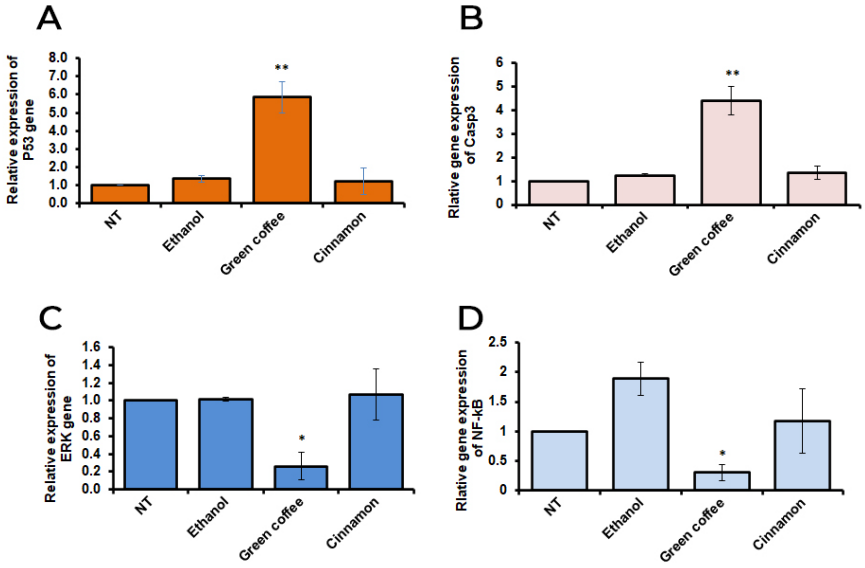

Fig. (4): P53, Caspase 3,ERK andNF-KB expression levels were measured after treating MFC-7 cells. (A), (B), (C), and (D) depict, using GAPDH-mRNA levels as an internal reference, the fold change in steady-state mRNA of the P53, ERK, and Caspase-3 genes in MCF-7 treated cells relative to ethanol-treated cells. The significance of cycle threshold (Ct) values was determined using a two-tailed Student's t-test, and the error bars represent the standard deviation (SD) from three separate trials., with *p ≤ 0.05 and **p ≤ 0.01 indicating statistical significance.

5. Quantitative secretion of IL-1α, IL-1β, IL-6 and IL-8 by ELISA:

After being exposed to green coffee and cinnamon, human tumor cells were tested for their production of inflammatory and anti-inflammatory cytokines. After 24 hours, compared to untreated cells or cells treated with ethanol, the mean amounts of IL-1**α** (Fig. 5A) and IL-1**β** (Fig. 5B) considerably increased. Figure (6A) shows that mean concentrations of IL-6 were stable, and Figure (6B) shows that mean concentrations of IL-8 were also stable, although the rate of rise was not statistically significant. Based on these results, green coffee extract seems to boost anti-inflammatory cytokine release without substantially altering pro-inflammatory cytokine regulation.

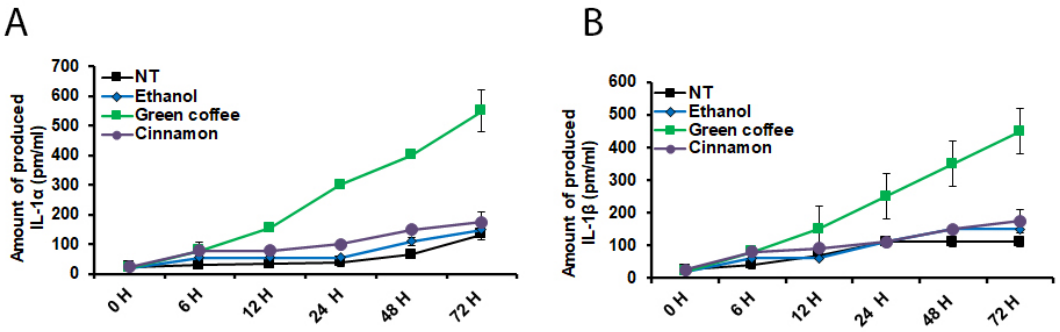

Fig. (5 A, B): Cells treated with green coffee or cinnamon extracts showed high responding to treatment in terms of production of IL-1α (A) and IL-1β (B) released into the medium at various time periods.

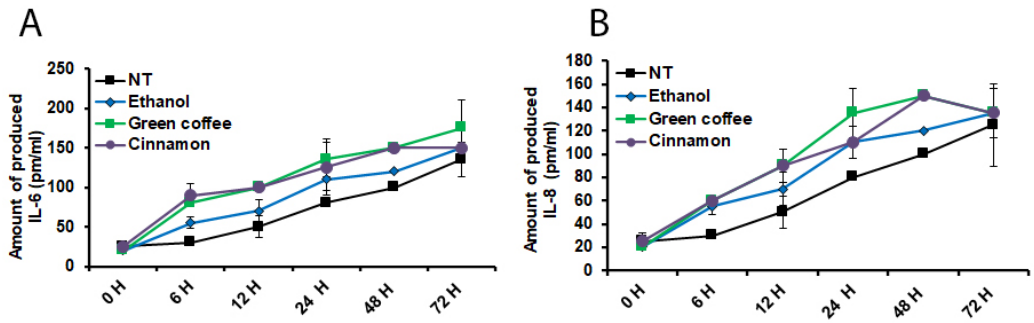

Fig. (6 A, B): Cells treated with green coffee or cinnamon extracts showed high responding to treatment in terms of production of IL-6 (A) and IL-8 (B) released into the medium at various time periods.

DISCUSSION

Breast cancer is the second most common disease in women and is difficult to avoid on a worldwide scale since its development includes many different kinds of cells. However, there has been significant progress in the recent decade in understanding breast cancer and developing preventative methods (Sun *et al*., 2017). The researchers' current efforts are focused on creating less harmful versions of existing cancer treatments (Li *et al*., 2016; Efferth *et al*., 2007). The value of plant-based medicines is enormous. Polyphenols, flavonoids, and terpenoids are just a few examples of plant-based substances that have been shown to have important health effects. The potential of these compounds to improve human health and standard of living has been the subject of much research (Pan *et al*., 2008). Plant-based diets increase bioavailability of plant-based chemicals, which in turn may stimulate several important biological functions (Kanti and Syed, 2009).

The purpose of this research was to determine whether or not the extracts of green coffee and cinnamon may be used in the treatment of breast cancer. In comparison between the results conducted from the quantitative analysis of green coffee and cinnamon by using HPLC according to the species employed, it was found that the current test findings for Green Coffee species often have the same photochemical components in varying levels like phenolic, flavonoid, chlorogenic acid, pyro-catechol, ferulic acid and naringenin. While cinnamon extract content catechol, ferulic acid, caffeic acid and apigenin. These findings support the growing interest among researchers in exploring green coffee bean extracts as a potential health-promoting supplement (Nuhu, 2014). Green coffee beans consist mostly of chlorogenic acids (CGAs), which account for 5-14 % of their content (Roshan *et al*., 2018). However, extraction solvents, time and temperature, and storage stability may all considerably affect the CGA concentration of various formulations. To assess this range, 54 widely available green coffee bean extracts were analyzed using a straightforward and low-cost HPLC technique (Vinson *et al*., 2019). The use of fewer animals in experiments and the prevention of artifacts are only two of the many reasons why in vitro testing was so important for the planning of future investigations. To determine whether an ingredient has therapeutic potential, pharmacological activity tests like cell proliferation and cytotoxicity assays must be performed. While several in vitro cell viability assays exist, it is essential to remember that they all evaluate biological processes in somewhat different ways (Méry *et al*., 2017; Kepp *et al*., 2011).

The current experments showed that the green coffee substances have anti-proliferative and cytotoxic properties. The use of an inverted microscope and a hemocytometer demonstrated significant changes in cell shape and the quantity of viable cells after green coffee therapy. The results of Bender and Atalay (2018) are consistent with these findings.

Through a series of tests, we determined the effect of green coffee extracts on cancer cells by measuring cell survival after incubation with green coffee extracts dissolved in RPMI 1640 medium for 24 hours at various doses. It was obvious that the viability of human breast cancer cell lines significantly decreased as a function of green coffee extract concentration. Notably, cinnamon did not have the same result.

Moreover, the results showed that growth of human breast cancer MCF7 cell lines was slowed by exposure to green coffee extract. The IC50 was roughly 1.25 mg/mL, indicating that even at these high doses, cell viability was maintained. Caffeine inhibits the growth and overall number of ER+ MCF7 and ER- MDA-MB-231 breast cancer cells with the greatest effect shown at 5 mmol/L as shown by Rosendahl *et al*. (2015).

However, there was no evidence found in the current study to ensure that chlorogenic acid was cytotoxic to breast cancer cell lines. A similar anti-proliferative tendency was also shown by the growth curves and IC50 values, but with some small differences (Bender and Atalay, 2018).

Also the present results conducted to investigate the cytotoxic effect of the green coffee and cinnamon on MCF-7 cells showed cytotoxicity in cancer cells treated by cinnamon extract while it was more significant on cells treated with green coffee extract which is compatible with the work of Schuster *et al*. (2022) who studied the effect of combination of the natural products arctigenin, chlorogenic acid, and cinnamaldehyde on breast cancer cells.

In the current research MTT tests were used, which quantify mitochondrial activity in live cells, to evaluate cell viability. The results showed that chlorogenic acid (CGA) has a dose- and time-dependent effect on cell viability in a variety of cancer cell lines (as shown in Figure 1). Based on these findings, it seems that CGA may have an effect on cell survival through altering mitochondrial structure and metabolism. Chlorogenic acid may have anti-cancer benefits, however this has not been well studied (Burgos-Morón *et al*., 2012).

Assays for lactate dehydrogenase (LDH) are helpful for assessing cell-mediated cytotoxicity and identifying substances that trigger cell death. Lactate dehydrogenase (LDH) is released into the extracellular environment during both necrosis and apoptosis as a result of a compromised or ruptured cell membrane. As a result Hiebl *et al*. (2017) suggested that the presence of LDH in cell supernatants may be used as a wide test for assessing cell viability and as a marker for compromised cell membrane integrity. The present results demonstrated that LDH levels were significantly elevated in MCF-7 cells treated with green coffee extracts. This was in agreement with the results of Bandyopadhyay *et al*. (2004); Belkaid *et al*. (2006) and Xu *et al*. (2013).

Nwafor *et al*. (2022) and Olthof *et al*. (2001) emphasize the growing interest in phenolic compounds as a therapeutic option for the treatment of cancer and other pathological disorders. About 8,000 phenolic compounds, including flavonoids, simple phenols, lignans, coumarins, tannins, and xanthones, have been isolated and identified so far (Farah *et al*., 2008). Gallic acid (Roskoski, 2012) caffeic acid (Chaowuttikul *et al*., 2020) and ferulic acid (Gonthier *et al*., 2003) have all been the subject of previous reviews focusing on their anticancer effects. Some of these phenolic compounds have been shown to protect cells against a wide variety of forms of damage (Stalmach *et al*., 2010; Naso *et al*., 2014) and their anticancer effects have been proven to be particularly impressive. The current results reveal that treatment with green coffee bean extract for 24 and 48 hours increases p53 gene expression while decreasing ERK and NF-KB gene levels in MCF-7 cells. Green coffee's phenolic and flavonoid components may be responsible for slowing the development of MCF-7 cells by elevating these two cell-cycle regulator proteins. Treatment with green coffee bean

extract not only inhibited MCF-7 cell growth, but also lowered cell viability after 72 hours, indicating that cell death was induced. Polyphenols have been shown to have anticancer effects by inducing cell cycle arrest, inhibiting signaling pathways involved in cell division, apoptosis, and angiogenesis, modulating reactive oxygen species (ROS) levels, activating tumor suppressor proteins like p53, and promoting cancer cell differentiation and transformation into normal cells (Gouthamchandra *et al*., 2017).

Chlorogenic acid has been shown to have anticancer effects (Gupta *et al*., 2022) by interrupting the cell cycle, causing apoptosis, and decreasing the multiplication of cancer cells. Immune pathway genes like nuclear factor of activated T cells 2 (NFATc2) and NFATC3 are upregulated by chlorogenic acid, while B cell-specific genes like Moloney murine leukemia virus integration site 1 protein and SRY-box transcription factor 2 are downregulated. This leads to the death of cancer cells. Another mechanism by which chlorogenic acid contributes to apoptosis is by inducing intracellular DNA damage and the subsequent production of topoisomerase I- and topoisomerase-II-DNA complexes (Gupta *et al*., 2022).

Deregulation of the transcription factor NF-B. (Dolcet *et al*., 2005) has been shown in many different kinds of cancer cells, making it less effective at regulating proliferation and survival. TNF receptor-associated factor 1 (TRAF1) and TRAF2 are essential components of the apoptotic process and are regulated by NF-κB. According to the present findings, 24 hours of exposure to green coffee extract raised NF-B in the cytoplasm and lowered NF-κB in the nucleus in MCF-7 cells, whereas the overall expression level of NF-κB dropped with higher concentrations of green coffee extract. The results also showed that nuclear NF-κB levels decreased while cytoplasmic levels increased. Cell cycle progression, cell size, and cell viability are all influenced by NF-κB (Joyce *et al*., 2001). Cancer cells may undergo apoptosis when EGCG inhibits nuclear factor kappa B (Gupta *et al*., 2004) which is accomplished by the activation of caspases 3, 8, and 9.

The presence of a correlation in the present investigation between decreased nuclear NF-κB levels and growth arrest and Caspase-3 activation may indicate that this phenomenon led to the apoptosis of MCF-7 cells. Cinnamon extract, on the other hand, resulted in a general alteration in P53, ERK, NF-kappa B, and Caspase-3 levels. Previous studies on NPC cells have shown that EGCG mediates NF-κB suppression via blocking the phosphorylation and degradation of IκBα (Zhao *et al*., 2004).

Yoon *et al*. (2018) found that coffee extract upregulates cellular proliferation, growth factor/RAS signaling, cellular protection, p53-mediated apoptosis, angiogenesis, and antioxidant and protection-related proteins in mouse livers, while downregulating NFkB signaling proteins, inflammatory proteins, and oncogenic proteins in mouse livers. They related this effect to the sequestration of NF-κB in an inactive state in the cytoplasm.

The current results indicated that green coffee and cinnamon extracts increased the expression of P53 and caspase3, while decreasing the expression of ERK and NFκB. These indicate that these extracts may have triggered apoptosis through the intrinsic route.

In this study, we evaluated the inflammatory response in breast cancer cells treated with green coffee natural extracts by monitoring several cytokines that are produced by tumor-infiltrating inflammatory cells during the carcinogenesis process. These cytokines can be either pro-inflammatory or anti-inflammatory (Yoon *et*

al., 2018).In the present study we monitored Some of these inflammatory cytokines to evaluate the inflammatory reaction in the breast cancer cells treated with green coffee natural extracts.

According to Shin *et al*.(2015) the intestinal epithelial-like cells, such as Caco-2 and HT-29, were used to study inflammatory reactions or regulatory elements in the intestine, such as inflammatory chemokine secretion chlorogenic acid treatment of Caco-2 cells results in elevated levels of IL-8, a cytokine that initiates an inflammatory response. While treating ovarian cancer ES-2 and SKOV3 cell lines with ginger extract as a source of phenolic compounds drastically reduced NF-κb-regulated gene products such IL-8 and VEGF (Rhode *et al*., 2007). In addition, IL-6 expression was suppressed in human periodontal ligament cells treated with chlorogenic acid, as reported by (Yu, Zhang, and Wang 2016). (Weng *et al*., 2014) reported a decrease in IL-6 levels in cultures of keratinocytes treated with TNF- and luteolin, which is consistent with our findings; we also found that MCF-7 cells treated with green coffee extract showed almost a steady state of IL-8 and IL-6, implying for no significant inflammatory response of pro-inflammatory cytokines.

Wound healing and both innate and adaptive immunity depend on the interleukin-1 (IL-1) family of inflammatory cytokines. Members of this family, including the closely related IL-1α and IL-1 β, have been linked to tumorigenic phenotype promotion and cancer therapy resistance. Transactivation of transcription factors like Nuclear Factor Kappa B (NF-κB) and Activator protein 1 (AP-1) is a critical node in IL-1β signaling pathways. Therefore, it is critical to create efficient inhibitors of NF-κB to block its activity, which may benefit patients with IL-1and NF-κB-driven illness in breast cancer (Diep *et al*., 2022). The majority of the literature on IL-1β and cancer has focused on its pro-tumorigenic effects. Figure 5A and 5B indicate that after 24 hours of exposure to green coffee extract, IL-1α (compared to untreated cells) and IL-1β (compared to untreated cells) levels were considerably higher. Exposure to cinnamon extract resulted in a non-significant increase in the production rate of IL-6 IL-8 as shown in (Figure 6A and 6B). Perhaps this effect is attributable to IL-1β potential to enhance the differentiation of CD4+ T cells into Th17 cells. Cancers of the breast, colon, lung, head, and neck, and melanoma have all been studied in mouse tumor models, and the results reveal that IL-1β considerably enhances inflammation, leading to enhanced invasiveness (Apte *et al*., 2006).

Conclusion:

The current findings suggest that green coffee beans and cinnamon extracts containing Chlorogenic acid are promising as a dietary, chemo-preventive, and therapeutic agent for the prevention and treatment of breast cancer.

Recommendations:

It was recommended to carry out more investigations on human by administration of green coffee beans and cinnamon extracts to assess their capacity to decrease the growth rate of breast tumor in females.

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Using green coffee and cinnamon extracts for Regulation of the proliferation signaling and inflammatory events during treating breast cancer

استخدام مستخلصات البن الأخضر والقرفة لتنظيم إشارات الانتشار والأحداث الالتهابية أثناء علاج سرطان الثدي

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المستخلص

وفقًا للمعهد القومي للسرطان ، فإن ثلث إجمالي تشخيصات سرطان الإناث في مصر ناتج عن سرطان الثدي. يبحث هذا البحث في استخدام مستخلصات نباتية منخفضة التكلفة وصديقة للبيئة (القهوة الخضراء والقرفة) المذابة في الإيثانول لعلاج سرطان الثدي. تفضل العديد من النساء الأدوية الطبيعية والتقليدية لإدارة مرضهن أو تقليل الآثار الجانبية للأدوية أو إبطاء تقدم المرض. تم تحليل التأثيرات المحتملة لهذه المستخلصات النباتية على خلايا MCF-7 باستخدام مقايسة MTT و qRT-PCR و ELISA. أظهرت النتائج أن الخلايا السرطانية تم تدميرها بشكل كبير بواسطة مستخلص البن الأخضر. أنتجت الخلايا المعرضة لمستخلصات البن الأخضر والقرفة المزيد من LDH. قارنت الدراسة بين تأثيرات مستخلصات القرفة والقهوة الخضراء على خلايا MCF-7 ووجد أن الأخير كان له تأثير أكبر على موت الخلايا المبرمج. تأثرت البروتينات p53 و caspase-3 بالإضافة إلى سلسلة إشارات ERK بمكونات القهوة الخضراء ، وتم منع مسار RAF / MEK / ERK في خلايا MCF-7. من خلال تغيير مسارات إشارات موت الخلايا المبرمج الحرجة ، قد تظهر القهوة الخضراء تأثيرات مضادة للسرطان وتعزز موت الخلايا المبرمج في خلايا سرطان الثدي. وجد أن متوسط مستويات IL-1α و IL-1β ، المقاسة بواسطة ELISA ، قد ارتفع بشكل كبير بعد ٢٤ ساعة. بينما يظهر متوسط التركيز IL-6 و IL-8 حالة مستقرة. أخيرًا ، تبين أن مستخلص البن الأخضر يثبط إنتاج السيتوكينات المحفزة للالتهابات.