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Lemongrass Extract and Anticancer Impact: mRNA Levels of Expressing Apoptosis and Mitochondrial Fission in MCF7 Cells

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ABSTRACT

This study investigated the potential inhibitory effects of lemongrass leaves extract on the proliferation of MCF7 breast cancer cells and its underlying mechanisms. MCF7 cells were exposed to increasing concentrations of lemongrass leaves extract (0, 50, 100, 200, and 300 µg/mL) for 24 h, and cell viability was assessed using the MTT assay. The results demonstrated a concentration-dependent reduction in the survival of MCF7 cells when treated with lemongrass leaves extract, indicating its selective cytotoxicity towards cancer cells. The calculated IC_{50} for lemongrass leaves extract was approximately 200 µg/mL. Furthermore, the study explored the impact of lemongrass leaves extract on mitochondrial morphology in MCF7 cells. It was observed that mitochondrial fragmentation increased with higher concentrations of the extract, accompanied by changes in nuclear morphology and DNA fragmentation. To elucidate the molecular mechanisms underlying these effects, the study examined the mRNA expression levels of apoptotic genes in MCF7 cells treated with lemongrass extract. The results revealed that lemongrass extract significantly upregulated the expression of CASPASE-7, p53, and DR4 genes in a concentration-dependent manner. Notably, a substantial decrease in Bcl2 mRNA levels was observed at higher concentrations of lemongrass extract. In summary, our findings suggest that lemongrass leaves extract possesses anti-proliferative properties against MCF7 breast cancer cells, likely mediated through the induction of apoptosis and modulation of apoptotic gene expression. These results contribute to a better understanding of the potential therapeutic benefits of lemongrass extract in cancer treatment.

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INTRODUCTION

Breast cancer is a prevalent and life-threatening disease that affects both women and, less frequently, men. It is characterized by the uncontrolled growth of malignant cells in breast tissues, often forming tumors. Breast cancer is a major global health concern, with millions of cases diagnosed each year. Its impact on individuals, families, and healthcare systems underscores the urgent need for effective treatment options. Current breast cancer treatments, such as surgery, chemotherapy, radiation therapy, and targeted therapies, have made significant progress in improving survival rates and quality of life for patients. However, these treatments can be associated with various side effects, and drug resistance can develop over time, limiting their long-term effectiveness. Therefore, there is a continuous quest to discover new treatment approaches that are more effective, less toxic, and have fewer side effects.

Lemongrass (*Cymbopogon citratus* (DC.) Stapf) is a natural tropical plant. The plant's leaves are widely used as healing infusions in Brazilian traditional medicine and culture. Their main purpose is to manage a range of illnesses, as lemongrass incorporates anti-spasmodic, analgesic, anti-inflammatory, antipyretic, diuretic as well as sedative properties (Bidinotto *et al*., 2011;). Moreover, lemongrass essential oil (LGEO) provides different *in-vitro* and *in-vivo* pharmacological effects, namely anxiolytic and anticonvulsant ones (Silva *et al*., 2008). There are also notions about variety of antibacterial, antifungal and antiprotozoal effects ensured by lemongrass (Silva *et al*., 2008; Duarte *et al*., 2007; Oliveria *et al*., 2009). In the recent decades, research has provided evidence on antimutagenic and antioxidant effects of lemongrass extracts or their various mixtures (including citral, b-myrcene and geraniol) in relation to diverse biologic environments (both *in-vitro* and *in-vivo*) (Choi *et al*., 2000; Rabbani *et al*., 2006; Tapia *et al*., 2007; Pereira *et al*., 2009). It was also noted that geraniol could decrease the proliferative properties of

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specific cell lineages Caco-2 human colon cells as well as MCF7 human breast cancer cells (Duncan *et al*., 2004). Furthermore, ethanolic lemongrass extract orally provided to male Fischer 344 rats demonstrated vivid inhibition patterns in both colonic aberrant crypt foci (ACF) and hepatic glutathione S-transferase placental form (GST-P) positive foci development, which were originally initiated by carcinogens such as azoxymethane and diethylnitrosamine, accordingly (Puatanachokchai *et al*., 2002). Cancer chemical prevention (chemoprevention) can be described as a management approach designed to prevent, suppress or alternatively reverse cancer processes by managing and applying natural or synthetic substances (Stoner *et al*., 1997; Aggarwal *et al*., 2006). Chemopreventive substances are able to suppress cancer progression either by restricting the exposure and vulnerability to carcinogens (for instance, by developing blocking agents or carcinogen inhibitors) or by reducing tumor distribution/development phases (for example, applying suppressing substances and agents) (Stoner *et al*., 1997). Various elements taken from dietary plants or natural medicine potentially incorporate chemopreventive qualities sufficient to minimize or alleviate DNA corruption; some of them are possibly able to reverse the carcinogenic activities (*in-vitro* and *in-vivo*) (Aggarwal *et al*., 2006). Meanwhile, a variety of epidemiological researches indicate of positive correlation between reduced risk of selected cancer types development and consumption of teas, dietary fruit, vegetables, and cereals (Khan *et al*., 2008). Thus, the optimistic and vast findings from laboratory research and clinical trials are a good foundation for searching and identifying potentially useful and efficient cancer chemoprevention substances and its derivatives. The research described in this study seeks to investigate the potential of lemongrass extract, specifically lemongrass essential oil (LGEO), as a novel treatment option for breast cancer, using the MCF7 cell line as a model system.

MATERIALS AND METHODS

Lemongrass essential oil extraction

Lemongrass leaves (*Cymbopogon citratus* Stapf) were purchased from the local medicinal plants markets in November 2021 and deposited with a voucher specimen (237), Riyadh, Saudi Arabia. The extract was performed from fresh lemongrass leaves through 3h of boiling hydrodistillation using a Clevenger apparatus and as described (Bidinotto *et al*., 2011). The extract was stored at 4 °C in a dark receptacle until the moment of use as.

Cell culture

Human MCF7 breast cancer cell line was bought from the American Type Culture Collection (ATCC, Manassas, VA, USA) and kept in DMEM/Ham's F-12 (1:1 v/v) medium enhanced with 100 mL/L FBS, 1.5 g/L sodium bicarbonate, 400 µg/mL hydrocortisone, 10 mL/L penicillin and streptomycin (0.1 mg/mL).

Cell treatment

From the humidified hatchery, cells seeding was additionally done at 1×10^6 cells/well or 1×10^5 cells/well in 96 well tissue culture plates separately. Cultivated MCF7 cells were divided into five clusters to establish an effective cancer management protocol. The extract from lemongrass was put on to a culture media and the cells were then treated with 4 separate fluctuated concentrations of 0, 50, 100, 200 and 300 µg/mL for 24h. These concentrations were determined according to the findings of Bidinotto *et al*. (2011).

Cytotoxicity assay

The extract from lemongrass (0.10 mL) were dissolved in 9.90 mL of DMSO to get a working concentration of 1 mg/mL. The active concentration was prepared freshly and filtered through 0.45 µ filter before each assay. In brief, 10 mL of extract was prepared in a concentration of 1 mg/mL. For each sample, 500 µL were poured in ten Eppendorf tubes. The samples were syringe-filtered using 0.45 µM filter to remove contaminants. 500 µL of the sample's working concentration was further added to the first Eppendorf tube and mixed well. Then, 500 µL of this volume was transferred from first to last tube by serial dilution to obtain the desired concentration of the lemongrass extract. As a result, the volume remains constant, but there was a gradual change in concentration. The cytotoxicity assessment was performed using MTT assay. For this assay, MCF7 cells were plated in 96-well culture plates ($1x10^4$ cells/well). The cells were exposed to concentrations of 0, 50, 100, 200 and 300 µg/mL of lemongrass extract for 24 h. The measurements were performed in triplicate. The colors developed in the plates were read at 550 nm by using DMSO as a blank. The percentage of cell viability was expressed using the following formula:

% Cell viability = mean absorbance of treated cells/mean absorbance of control cells × 100

Mitochondrial fission

MCF7 cells were exposed for 30 min using 250 nM of MitoTracker Deep-Red FM (Invitrogen) in a free culture. After two time-washing with PBC, nuclei were recolored by Hochest 33342 for 10 min. A microscope (Zeiss LSM700 confocal) was used to view the mitochondrial morphology and following the methods described in

previous report (Alkhateeb *et al*., 2001).

RNA extraction and cDNA synthesis

All out RNA was separated utilizing Invitrogen-TRI-zol reagent as indicated by the maker's guidelines and evaluated by estimating the absorbency at 260 nm. The quality of RNA was controlled by estimating 260/280 proportions. From that point, the synthesizing of the cDNA-strand was produced utilizing the High-Amplitude cDNA turn around interpretation pack (Applied Biosystems) as indicated by the maker's directions (Zordoky *et al*., 2008).

Measurement of mRNA expressions by real-time polymerase chain reactions (RT-PCR)

The primers were utilized in the present examination (Table I) were bought from (Invitrogen, USA). Measure controls were consolidated in separated wells but onto a similar plate, to be more specific. All the samples and controls were run in triplicates on an ABI 7500 Fast Real-time PCR. The quantitative RT-PCR data was breaking down by a near edge (Ct) strategy, and the overlap acceptances of treated examples were contrasted and the untreated examples. Relative quality expression (i.e., ΔΔCT) strategy as earlier outlined was used to analyse the data on the RT-PCR (Livak and Schmittgen, 2001). *GAPDH* was utilized as an interior reference gene to standardize the declaration of the selected genes.

Table I. The primers' sequences.

Genes	Primer sequence 5`→3`
CASPASE-7	F CGAAGGCCCATACCTGTCACTTTATC
	R CTACCGCCGTGGGAACGATGGCAGA
p53	F GCCCCCAGGGAGCACTA
	R GGGAGAGGAGCTGGTGTTG
BCI2	F CATGTGTGTGGAGAGCGTCAA
	R GCCGGTTCAGGTACTCAGTCA
DR4	F AGTACATCTAGGTGCGTTCCTG
	R GTGCTGTCCCATGGAGGTA
GAPDH	FCGTCCCGTAGACAAAATGGT
	R TCAATGAAGGGGTCGTTGAT

Statistical analysis

Analytical examinations were performed by use of SigmaStat programming adaptation 3.5 (Systat Software, San Jose, CA, USA). Quantitative outcomes were presented as mean standard deviations. Esteems of p being lower than 0.05 were deemed statistically imperative.

RESULTS

Effect of lemongrass leaves extract on MCF7 cell proliferation

To determine the ability of lemongrass leaves extract to inhibit growth and proliferation of MCF7 breast cancer cells were treated with steadily increasing concentrations of lemongrass leaves extract (0, 50, 100, 200 and 300 µg/mL) for 24 h, after which cell reasonability and expansion were determined using MTT assay. Figure 1 exhibits that endurance of MCF7 cells were altogether diminished after incubation with lemongrass leaves separate in a focus subordinate way when contrasted with untreated MCF7 cells (Fig. 1), proposing that lemongrass leaves extract is tumor cell selective. The determined IC_{50} for lemongrass leaves extract is around 200 µg/mL.

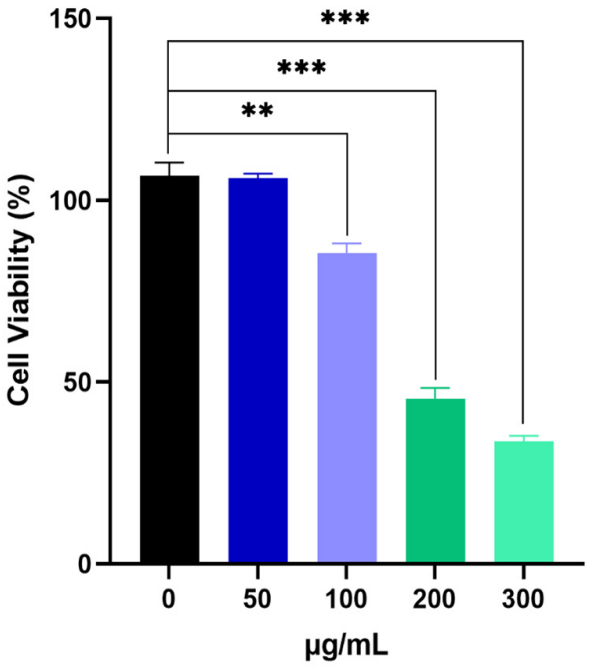

Fig. 1. Cytotoxicity assessment by MTT assay on MCF7 breast cancer cells exposed to various concentrations of lemongrass leaves extract for 24 h. Stars indicate statistically significant differences of cytotoxicity and cell viability assessment between the concentrations (0, 50, 100, 200 and 300 µg/mL). Values are put as percentages of the control (mean ± SEM, n = 5) ***P < 0.001, **P < 0.01, *P < 0.05 in comparison to the control (0 µg/mL).

Mitochondrial activity

The mitochondrial morphology was changed by lemongrass leaves extract in MCF7 breast cancer cells, mitochondrial splitting levels were increased in lemongrass

leaves extract treated groups with increase the extract of lemongrass leaves concentrations (0, 50, 100, 200 and 300 µg/mL) for 24 h (Fig. 2). Hochest staining indicates changed nuclear shape or/and neighboring nuclei' DNA fragments. The control cells indicated healthy shape having round nucleus with normal mitochondrial morphology (0 µg/mL).

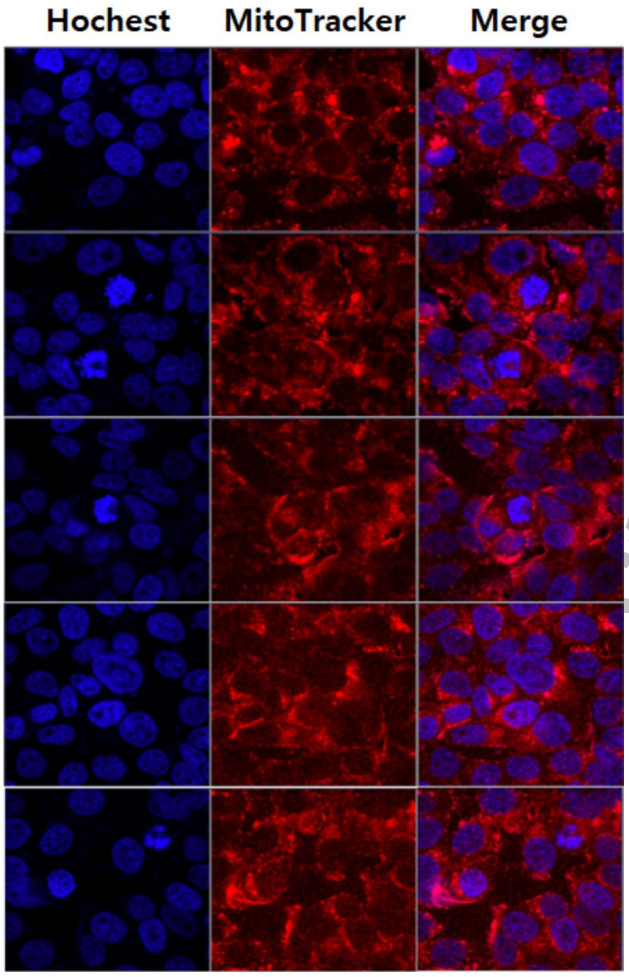

Fig. 2. The effect of lemongrass extract on MCF7 cells showing abnormal shape of nuclei by (Hochest 33342-blue stain) and mitochondrial fusion by (100 nM MitoView™ 405- Blue stain). A, represents untreated MCF7 cells with normal structure and undamaged mitochondrial status. In contrast, other MCF7 cells were treated by lemongrass extract had mitochondrial changes in shape with fragmented patterns and punctiform morphology when MCF7 cells treated at four different concentrations (50, 100, 150 and 200 µg/mL) as seen in B, C, D, and E, respectively.

Effect of lemongrass extract on the mRNA expression levels of apoptotic genes in MCF7 cells

To look at whether the inhibitory impact of lemongrass extract on MCF7 cells expansion and development is an apoptotic-interceded treatment, we decided the limit of lemongrass extract to regulate the outflow of anti-apoptotic and apoptotic genes. For this reason, MCF7 cells were treated for 24 h with expanding the concentrations of lemongrass extract (0, 50, 100, 200 and 300 µg/mL), as dictated by the outcomes in the cells' suitability (Fig. 3), from there on *CASPASE-7*, *p53*, *DR4*, and *Bcl2* mRNA expression levels were controlled by *GAPDH* gene, respectively.

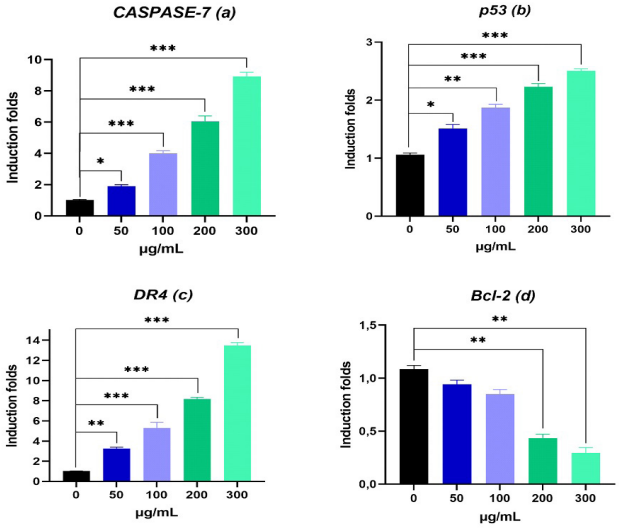

Fig. 3. Effect of lemongrass extract on apoptotic markers *CASPASE-7* (a), *p53* (b), *DR4* (c), and *Bcl-2* (d) mRNA levels in MCF7 cells were put under treatment for 24 h with different groupings of lemongrass extract (0, 50, 100, 200 and 300 µg/mL). From that point, the mRNA levels of *CASPASE-7*, *p53*, *DR4*, and *BcL2* were evaluated utilizing RT-PCR and standardized to *GAPDH* housekeeping gene. Values were recorded as means ± SEM ± SEM (n = 5) of three tests. ***P < 0.001, **P < 0.01, *P < 0.05 contrasted with control (0 µg/mL).

Figure 3 show that lemongrass extract essentially actuated *CASPASE-7*, *p53*, and *DR4* mRNA expression levels in a focus subordinate way (Fig. 3). The greatest enlistment was seen at the most elevated fixations tried (50, 100, 200 and 300 µg/mL) in *DR4* mRNA expression levels and at (100, 200 and 300 µg/mL) in *p53* and *CASPASE-7* mRNA expression levels. Interestingly, a significant decrease was seen in *Bcl2* mRNA levels at the most markedly fixation tried (200 and 300 µg/mL) because of lemongrass extract treatment (Fig. 3).

DISCUSSION

This research aims to build upon the existing body of knowledge on the anticarcinogenic properties of lemongrass extract by specifically focusing on its impact on mitochondrial dynamics and apoptosis in MCF7 breast cancer cells. The findings from this study may contribute to the development of novel therapeutic and chemopreventive strategies for breast cancer, potentially offering patients more effective and less toxic treatment options. The essential culturing of lemongrass depends on its oil's pharmacological factors. In fact, LGEO incorporates a vast number of diverse bioactive elements, including citral (mix of geranial and neral), isoneral, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol, not to mention other significant bioactive agents. The above-mentioned elements aim to impart different pharmacological reactions in LGEO, stimulating antifungal, antibacterial, antiviral, anticancer, and antioxidant effects in return (Livak *et al*., 2001).

Our findings revealed that the application of lemongrass extract decreased cell viability of MCF7 cells. The reduction was gradual with elevation of extract's concentrations, specifically upon reaching 200 µg/mL compared to baseline/control value (0 µg/mL), where the determined IC_{50} for lemongrass leaves extract was around 200 µg/mL. The reduction in the living cells amounts was explained by glucose uptake inhibition factor that regulates ATP development and cell proliferation, which are heavily relevant in the cell growing process. Subjective phytochemical analysis of lemongrass extract revealed that the major part of biological activity pertaining to cancer prevention has been associated with plant's specific elements (namely citral and b-myrcene) that demonstrated high antioxidant and antigenotoxic/antimutagenic effects in suppressing various mutagens (Bidinotto *et al*., 2011; Pan *et al*., 2021).

Additionally, increased levels of ROS in mitochondria might lead to attacks of free radicals on membrane phospholipids moving prior to film depolarization in mitochondria. This depolarization phase is considered an irrevocable threshold in the apoptosis process (Kimura *et al*., 2021). The enhancement of ROS formation induced extensive and frequent apoptosis scenarios (Fig. 2). It was found that mitochondrial morphology causes energy imbalances; moreover, it can continually transform through fission and fusion processes. It means that close regulation between inter-organelle interactions and mitochondrial course of development is crucial. Similarly, mitochondrial splitting results in the deactivation of insulin-subordinate glucose take-up (Alkhateeb *et al*., 2021). Therefore, it is stated that apoptosis is a strongly controlled process essentially impacted by a several marked pathways - for instance, mitochondrial caspases and related pathways (Zhivotovsky and Kroemer, 2004).

Apoptosis stimulation with ROS formation by malignant growth under chemoprotective elements, for instance, doxorubicin (Elbekai *et al*., 2004), induces disease cell passing; moreover, it contributes to DNA damage and endangers genomic safety (Manosroi *et al*., 2006). Still, a vast number of malignant growths chemoprotective agents are cytotoxic, which means inevitable toxicity upon their use. Thus, the formation of novel chemopreventive agents aimed at suppressing cell expansion and regulating apoptosis in malignant growth cells with minimal or zero reactions is relevant and can be potentially achieved in the future. With the purpose to measure in vivo effects and parameters, this study focuses on applying human breast malignancy MCF7 cells. The goal was to observe human reactions toward lemongrass extract with defining the capacity of the extract to reduce MCF7 cells growth and proliferation. In addition, the purpose was to learn the actual role of apoptosis in lemongrass extracts extricate-interceded relation.

The study's outcomes are in accord with findings from recent research where lemongrass extract was orally applied to APCmin/+ transgenic mice with consequential decrease of intestinal tumors (Ruvinov *et al*., 2019). Importantly, the research demonstrated that lemongrass extract was tolerated appropriately, and it was found effective at suppressing the growth of colon cancer xenograft, although in mice experiments only. The impact of lemongrass extract on oxidative pressure gene HO-1 mRNA level in MCF7 cells was measured by applying different concentrations (50, 100, 200 and 300 µg/mL) of the extract throughout the 24-h period. It revealed essential increase with elevating the concentration level.

Furthermore, mRNA levels in MCF7 cells were processed with diverse concentrations (50, 100, 200 and 300 µg/mL) of lemongrass extract during 24 h and greatest enlistment was seen at the most elevated fixations tried (50, 100, 200 and 300 µg/mL) in *DR4* mRNA expression levels and at (100, 200 and 300 µg/mL) in *p53* and *CASPASE-7* mRNA expression levels. Interestingly, a significant decrease was seen in *Bcl2* mRNA levels at the most markedly fixation tried (200 and 300 µg/mL) due to intense exposure to lemongrass extract. These findings imply that the increased concentrations of the extract restored mRNA gene expression to the lower rates, i.e., normal conditions. Past researches actualized that *DR4*, *p53* and *CASPASE-7* gene expression levels were increased in several human cancer types, namely breast, lung, colon and colorectal types following the treatment with anticancer agents (Yadav *et al*., 2018). Additionally,

Alkhateeb *et al*. (2021) reported that *Bcl2* mRNA levels was showed a huge decrease after applying the treatment (24).

CONCLUSION

This study's findings imply that lemongrass extract demonstrated an identifiable and relatively stable anticarcinogenic impact (inhibition effect) *in vitro*. The outcomes were achieved in terms of applying human breast cancer cell line (MCF7) and by stimulating mitochondrial fission, ROS generation, and apoptosis along with glucose uptake inhibition in a dose-dependent manner with higher extract concentrations. Nonetheless, the outcomes achieved need for additional testing to learn if this impact takes place in case of healthy cell lines. Moreover, further research efforts are necessary for identifying LGEO's other possible uses in cancer treatment discipline, specifically lemongrass' capacity to treat other cancer types, as well as prevent and secure from potential relapse.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Statement of conflict of interests

The author has declare no conflict of interest.

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