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TRPV1 channel-mediated apoptotic effects of *Liquidambar orientalis* Miller Gum on gastric cancer cell line

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Abstract

TRP channels play important roles in various cellular functions, such as proliferation, migration, invasion, and the ability to induce apoptosis in digestive system cancers. *Liquidambar orientalis* Miller Gum is used in the treatment of skin problems, peptic ulcers and parasitic infections, as an antiseptic, as well as a supportive therapy for the treatment of some digestive tract cancers. In our study, we aimed to investigate the TRPV1 channel-mediated apoptotic effects of *Liquidambar orientalis* Miller Gum on HGC-27 Human Gastric Cancer Cell line.

HGC-27 cells were cultured and divided into three main groups as control, *Liquidambar orientalis* (Gum) and *Liquidambar orientalis*+capsazepine. The cells were incubated with *Liquidambar orientalis* along with a TRPV1 channel inhibitor and stimulator together. The effects of *Liquidambar orientalis* Miller Gum via TRPV1 channels were investigated in relation to apoptosis, mitochondrial depolarization, ROS and caspase-3 & -9 enzyme activations.

The levels of apoptosis in the *Liquidambar orientalis* group were significantly higher in cancer cells compared to the control group. TRPV1 channel stimulator administration resulted in significantly increased apoptosis, ROS, caspase-3 and -9 levels in the *Liquidambar orientalis* group compared to the *Liquidambar orientalis*+capsazepine group.

The apoptotic effects of *Liquidambar orientalis* Miller Gum on gastric cancer cells have been shown to be associated with increased activation of TRPV1 channels due to increased intracellular reactive oxygen species.

Keywords: *Liquidambar orientalis*, Gum, TRPV1, Gastric cancer, Apoptosis.

Introduction

Gastric cancer remains one of the most common and deadly cancers worldwide, especially among older males [1]. Gastric cancer is responsible for one to 1 in every 12 deaths globally and is the fifth most frequently diagnosed cancer [2]. Generally, gastric cancer is a consequence of the multifactorial interplay between host genetics, microbial factors, nutrition, and environmental milieu, where it is thought that oxidative stress plays a crucial role in the occurrence and development of gastric cancer [3]. Over the past few decades, researchers have focused on drug discovery from herbal medicines or botanical sources, an important group of complementary and alternative medicine therapies. With a long history of herbal usage and clinical management of a variety of diseases in indigenous cultures, the success rate of developing a new drug from herbal medicinal preparations should, in theory, be higher than that from chemical synthesis [10]. Bioactive metabolites found in plants are potential anticancer drugs [5]. *Liquidambar orientalis* Miller (LOR) is an endemic species belonging to the family of Hammamelidaceae. It is commonly named the “günlük” or “sığla” tree and is widely widespread in the southwest border ports in Turkey. Although the studies about LOR in our country are mostly on the balsam of the plant, its leaves and fruits are quite limited [5,6]. LOR is a herbaceous plant known to have medicinal and cosmetic properties and is widely used in phytotherapy in the Mediterranean region [4]. Mostly, the traditional therapeutic use of LOR balsam is the treatment of injuries such as gastritis and ulcers [5]. The intracellular Ca^{2+} attenuates a variety of basic cellular mechanisms, including cell proliferation and cell death. The mechanisms contributing to the regulation of the cytosolic Ca^{2+} concentration include the entry of Ca^{2+} from outside of cells following the activation of cation channels [7]. The transient receptor potential (TRP) family is an important nonselective cation channel, and the family contains 7 subfamilies [8]. TRP vanilloid type-1 TRPV1, Ca^{2+} permeable cation channels are activated by different stimuli, such as low pH, oxidative stress and noxious heat (≥43). Capsaicin is the natural pungent ingredient of hot chilli peppers, which activates the TRPV1 channel [9].

In this study, the aim was to determine the existing apoptotic activity of LOR balsam in gastric cancer cells via TRPV1 channels.

Materıals and Methods

Reagents/Stains and Cell Culture

Dulbecco’s modified Eagle’s medium (DMEM), Trypsin–EDTA, Fetal bovine serum, penicillin-streptomycin, dimethyl sulfoxide, Dihydrorhodamine-123 (DHR 123) was procured from Sigma Aldrich (St. Louis, MO). Probenecid and a mitochondrial stain 5.50, 6.60-tetrachloro-1.10.3.30-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) was procured from Santa Cruz (Dallas, Texas, USA). Fura 2 (AM) florescent calcium stain was procured from Calbiochem (Darmstadt, Germany), and Pluronic® F-127 was procured from Biovision (San Francisco, USA). APO percentage assay with a release buffer was procured from Biocolor (Belfast, Northern Ireland). Caspase-3 (AC-DEVD-AMC) and caspase-9 (AC-LEHD-AMC) substrates were procured from Enzo (Lausanne, Switzerland).

Cell Culture

Human gastric cancer cells (HGC-27) were a gift from the Laboratory of the Department of Medical Genetics, Yeditepe University Faculty of Medicine. HGC-27 cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium. All mediums containing 10% FBS (fetal bovine serum) (Fisher Scientific), and 1% pen. /strep, Antibiotic combination in 8–10 flasks (5 ml, 25 cm^2 sterile with filter cap). The cells were incubated at 37°C at 5% CO_2 in a humidified incubator. After cells reached 75–85% confluence, they were incubated with the chemical compounds described in the section on groups. Cells were examined daily for evidence of contamination. After chemical treatments, washed cells were detached with 0.25% Trypsin–EDTA from T25 flasks, and then 4 ml fresh medium was added per flask. Cell suspensions were collected using a recharged automatic pipette and transferred into the 15-ml falcon tubes. Cells were centrifuged (100g, 5 min), then the supernatants were removed, and centrifugation was repeated by adding fresh medium into the sterile falcon tubes to wash the cells and make them ready for use in experiments.

Study Groups

Group 1 (Control): The HGC-27 cells were not incubated with *Liquidambar orientalis Mill.* gum and capsazepine (TRPV1 channel inhibitor) were kept in a flask containing the same cell culture medium and conditions.

Group 2 (LOR): HGC-27 cells in the group were incubated with 50 µg/ml *Liquidambar orientalis Mill.* gum for 24 hrs [11].

Group 3 (LOR+Cpz): HGC-27 cells in the group were incubated with 50 µg/ml *Liquidambar orientalis Mill.* gum for 24 hrs and then incubated with capsazepine (TRPV1 channels antagonist, 0.1 mM, 30 min).

During calcium signalling analysis (Fura-2/AM), cells were stimulated on 20th cycles with 0.1 mM capsaicin (Cap, 0.1 mM TRPV1 stimulator) in the existence of 1.2 mM calcium and calcium-free buffer in the extracellular environment. For apoptosis, intracellular reactive oxygen species, mitochondrial depolarisation, caspase-3 and caspase-9 experiments, the cells were further treated with TRPV1 channel agonist capsaicin (Cap, 0.1 mM, 10 min) for activation of TRPV1 channel before related analysis

Measurement of Intracellular Free Calcium Concentration ([Ca^{2+}]i)

The Calcium ion concentration was measured by UV light-excitable Fura-2 acetoxymethyl ester (Fura-2-AM) as an intracellular calcium ion indicator. After the cells were incubated with the chemical compounds described in the groups' section, HGC-27 cells were detached with %0.25 Trypsin–EDTA from T25 flasks. After centrifuged (100G, 5 min), The medium was removed and replaced with HEPES-buffered saline [HBS; 5 mM KCl, 145 mM NaCl, 10 mM D-glucose, 1 mM $MgCl_2$, 1 mM $CaCl_2$, (1.2 mM). 10 mM HEPES and 0.1% (w/v) bovine serum albumin (BSA); pH 7.4] containing 5 µM fura-2 AM and 0.05% (w/v) Pluronic F-127, and cells were incubated for 1 h at 37˚C in the dark. The loaded cells were washed twice with HBS and covered with 1000 µL of HBS supplemented with 2.5 mM probenecid for at least 20 min at 37˚C in the dark to allow for Fura-2 AM de-esterification. The cells were seeded in clear flat-bottom black 96-well culture trays (Grainer Cell Star, Life Sciences USA) at a density of 3×10^4 cells/per well. Fluorescence emission intensity at 510 nm was determined in individual wells using a plate reader equipped with an automated injection system (SynergyTM H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 s for 50 acquisition cycles (cycle: 3s; gain: 120) in response to agonists (Cap, 0.1 mM) added with the automated injector. [Ca^{2+}]i in cells was expressed as the average emission at 510 nm in individual wells in response to excitation at 340/380 nm normalized to initial fluorescence emission obtained during the first 10-20 cycles. Measurement of $[Ca^{2+}]_i$ was performed as modified by Uguz et al., 2009 and Martinez et al., 2016 [12,13].

Apoptosis and Intracellular ROS Production Measurement

The APOPercentageTM, which is used as an assay for the detection and quantification of apoptosis (Biocolor Ltd., Belfast, Northern Ireland), was performed according to the manufacturer's instructions. The APOPercentageTM assay is a dye-uptake assay, which stains only the apoptotic cells with a red dye. When the membrane of apoptotic cell loses its asymmetry, the APOPercentage dye is bonded to phosphatidyl serine lipids actively and transported into cells, staining apoptotic cells red, thus allowing the detection of apoptosis by a multiple reader as previously described elsewhere [14, 15]. After the cells were incubated with the chemical compounds described in the groups' section, cells were washed with 1xPBS and resuspended in 50 µl 1xPBS per group. After adding 950 µl 1xPBS and 10 µl APOPecerntage dye (Biocolor, Belfast, Northern Ireland), the cells were incubated in a shaker for 30 min at 37 °C in a humidified CO_2 incubator in the dark. After the incubation, cells were washed twice by re-suspension in 500 µl of 1xPBS and centrifugation for 5 min at 1x100 g. After removing the supernatant, 200 µl percentage release solution was added. Cells were transferred to the transparent plates at 50 µl volume per well and allowed the detection of apoptosis by spectrophotometry at 550 nm (Synergy™ H1, Biotek, USA).

In reactive oxygen radical's production analysis, Rhodamine 123(Rh 123) is a non-fluorescent, non-charged dye, which is a cell membrane permanent green florescent dye that can easily pass the cell membranes where it is oxidized to cationic rhodamine 123, which localizes in the mitochondria and exhibits green fluorescence. It was sequestered by mitochondria. The cells (10^6 cells/ml per group) were incubated with 20 µm DHR 123 as florescent oxidant dye at 37 °C for 25 min. The Rh123 fluorescence intensities were determined by using an automatic microplate reader (Synergy™ H1, Biotek, USA). The excitation and emission wavelengths of the analyses were 488 nm and 543 nm, respectively. Data are presented as fold-change over the pretreatment level [14,15].

Assay for Caspase-3 and Caspase-9 Activities

The determinations of caspase-3 and caspase-9 activities were based on methods previously reported [14,15]. HGC-27 cells were sonicated, and cell lysates were incubated with 2 ml of substrate solution (20 mM HEPES [pH 7.4], 2 mM EDTA, 0.1% CHAPS, 5 mM DTT and 8.25 lM of caspase substrate) for 1 h at 37°C. Caspase-3 substrate (NAcetyl-Asp-Glu-Val-Asp-7-amido-4-Methylcoumarin) (ACDEVD-AMC) and caspase-9 substrate (Ac-Leu-Glu-His-Asp-7-Amino-4-methylcoumarin) (AC-LEHD-AMC) cleavages were measured with the microplate reader (Synergy™ H1, Biotek, USA) with excitation wavelength of 360 nm and emission at 460 nm. The data were calculated as fluorescence units/mg protein and presented as fold-change over the pretreatment

level (experimental/control).

Mitochondrial Membrane Potential (JC-1) Analyses.

The cells were incubated with JC-1 (1 µM concentration of 5,50,6,60-tetrachloro-1,10,3,30-tetraethylbenzimidazolylcarbocyanine iodide) at 37 °C for 45 min. JC-1 fluorescence was measured by a single excitation wavelength (488 nm) with dual emission, green (520 nm) and red (596 nm), using the microplate reader (Synergy^{TM} H1, Biotek, USA). The lipophilic cationic dye, JC-1, exhibits potential-dependent accumulation in mitochondria. It indicates mitochondrial depolarization by a decrease in the red-to-green fluorescence intensity ratio. After incubation with JC-1, the dye was removed, and the cells were washed in PBS. The green JC-1 signal was measured at the excitation wavelength of 485 nm and the emission wavelength of 535 nm, and the red signal at the excitation wavelength of 540 nm and the emission wavelength of 590 nm. Fluorescence changes were analyzed using a fluorescence spectrophotometer (Synergy^{TM} H1, Biotek, USA) [13,14]. Data are presented as emission ratios (590/535). Changes in mitochondrial membrane potential were quantified as the integral of the decrease in JC-1 fluorescence ratio of experimental/control [14,15].

Statistical Analyses

All results were expressed as means±standard deviation (SD). Significant values in the groups were assessed with one-way ANOVA. Statistical Analyses were calculated using GraphPadPrism version 7.04 for Windows (GraphPad Software, San Diego, California, the USA). P <0.05 was considered significant.

Additional Information

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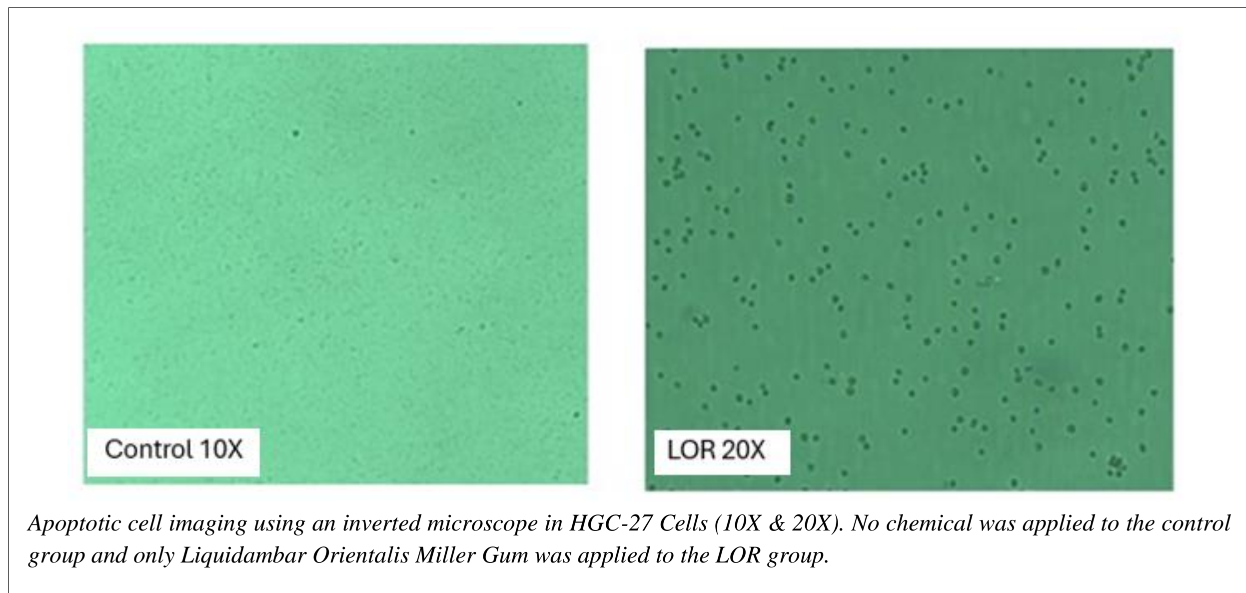
Results

Microscopic Image of HGC-27 Cells and Cytosolic Calcium Signalling (Fura-2 AM) Results



Apoptotic cell imaging using an inverted microscope in HGC-27 Cells (10X & 20X). No chemical was applied to the control group and only Liquidambar Orientalis Miller Gum was applied to the LOR group.

The effect of Anatolian sweetgum tree (Liquidambar orientalis) gum on TRPV1 channels mediated cytosolic calcium levels in human gastric cancer cells is shown in Figure 1 A-B. TRP Vanilloid 1 channel stimulator capsaicin and blocker capsazepine were used to investigate the role of TRPV1 channels on intracellular cytosolic calcium ion increase. As shown in the line graph in Figure 1 A, it was observed that the calcium influx in gastric cancer cells was triggered in the group using Liquidambar orientalis compared to the control group (p<0.001). Again, when the TRPV1 channel inhibitor group was compared with the control group, cytosolic calcium level increased in the Liquidambar orientalis+Capsazepine group, and a significant difference was found between them compared to the control group (p<0.001). There was no statistically significant difference between the group using TRPV1 channel stimulator capsaicin and the group using inhibitor.

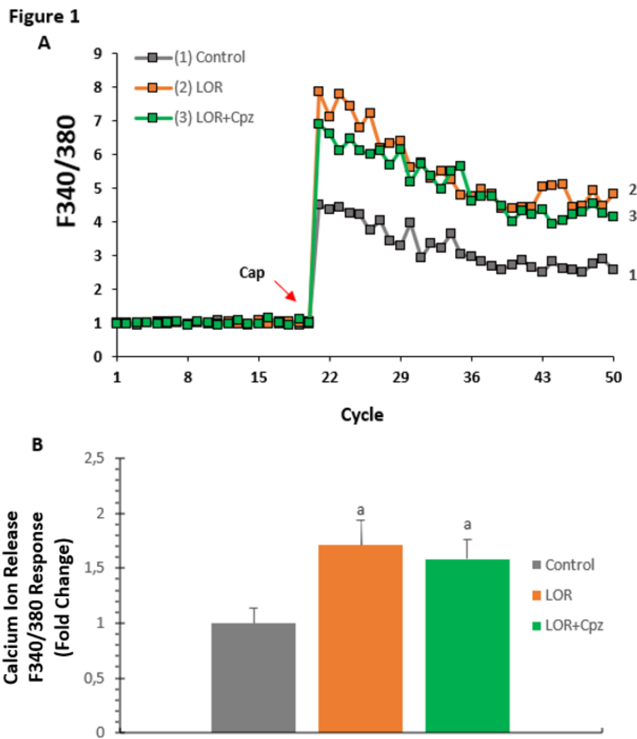


Figure 1. *Effects of Liquidambar orientalis gum (50 µg/ml, 24 h) on free intracellular cytosolic calcium increase ($[Ca^{2+}]_i$) (A) and cellular calcium ion release (B) via TRPV1 channels in gastric cancer (HGC-27) cells. All groups were stimulated with capsaicin (0.1 mM capsaicin at cycle 20) for calcium signalling analysis. Only the Liquidambar orientalis+capsazepine group was incubated with 0.1 mM capsazepine 30 min before the analysis and then stimulated with 0.1 mM capsaicin. (n=3 and mean ± SD). $^a p<0.001$ vs control, $^b p<0.05$ vs LOR.*

Programmed Cell Death (Apoptosis), ROS, Mitochondrial Depolarisation, Caspase-3 and Caspase-9 Activation Analysis Results

The results of apoptosis (A), Reactive Oxygen Species (B), Mitochondrial depolarisation (C), caspase-3 activation (D) and caspase-9 activation (E) in gastric cancer cells are shown in Figure 2 (A-E). In the analyses performed, apoptosis, ROS, Mitochondrial depolarisation levels, caspase-3 activation, and caspase-9 activation were statistically higher in the group using *Liquidambar orientalis* compared to the control group (p<0.001). When the group using TRPV1 channel inhibitor was compared with the control group, the same analysis results increased in the *Liquidambar orientalis*+capsazepine group, and a significant difference was found between them compared to the control group (p<0.001). When the *Liquidambar orientalis* treated group and Liquidambar orientalis+capsazepine (TRPV1 inhibitor) treated group were compared, apoptosis, ROS, mitochondrial depolarisation levels, caspase-3 activation and caspase-9 activation levels were statistically lower in *Liquidambar orientalis*+capsazepine group (p<0.001).

Discussion

Although *Liquidambar orientalis* extracts are not widely known in many communities, people in certain regions of the world frequently use *Liquidambar orientalis* plant and gum for skin diseases, cough and stomach problems [16]. In the literature, it has been reported in some studies that it has apoptotic activity on various cancer cells such as colorectal, laryngeal, breast and lung cancer in *in-vitro* studies [17,18]. However, there are no studies investigating its apoptotic activity on gastric cancer. Only literature reviews suggest that it may have potential benefits for gastric cancer. In our study, we concluded that *Liquidambar orientalis* gum also induces cell death in gastric cancer cells.

In the study conducted by Duran and Tuncer on Hep-2 cells in 2023, it was reported that caspase-3, caspase-8, and caspase-9 activation levels were higher compared to the control group. In our study, the results of caspase-3 and caspase-9 activation were similarly found to be higher compared to the control group. In the same study, it was reported that the level of programmed cell death using Annexin V/FITC Apoptosis Kit was higher in the *Liquidambar orientalis* group compared to the control group [17]. In our study, similar to the study conducted by Duran and Tuncer in 2023, apoptosis results were found to be higher in the liquidambar orientalis group compared to the control group.

TRP channels are calcium, sodium and potassium permeable functional ion channels that are generally found in the cell membrane and organelles and can be activated by oxidative stress, especially oxidative stress, except for some sub-members of TRPV, TRPM, TRPA, TRPC members such as capsaicin, menthol, heat, cold, etc. [19]. While these channels regulate some physiological processes in the cell, on the other hand, the pathophysiology of diseases, as well as the increased intracellular oxidative stress after the use of chemotherapeutic drugs in cancer cells, cause activation of these channels and indirectly proapoptotic activities are also known [20,21]. TRPV1 channels are also activated by oxidative stress due to the increase in intracellular free radicals. In Hep-2 cancer cells, it has been reported that the production of free radicals increased due to the use of *Liquidambar orientalis* [17], but there is no study in the literature on the TRP channels-mediated effects of *Liquidambar orientalis* gum due to increased intracellular radical levels. In our current study, it was found that *Liquidambar orientalis* increased the level of free oxygen

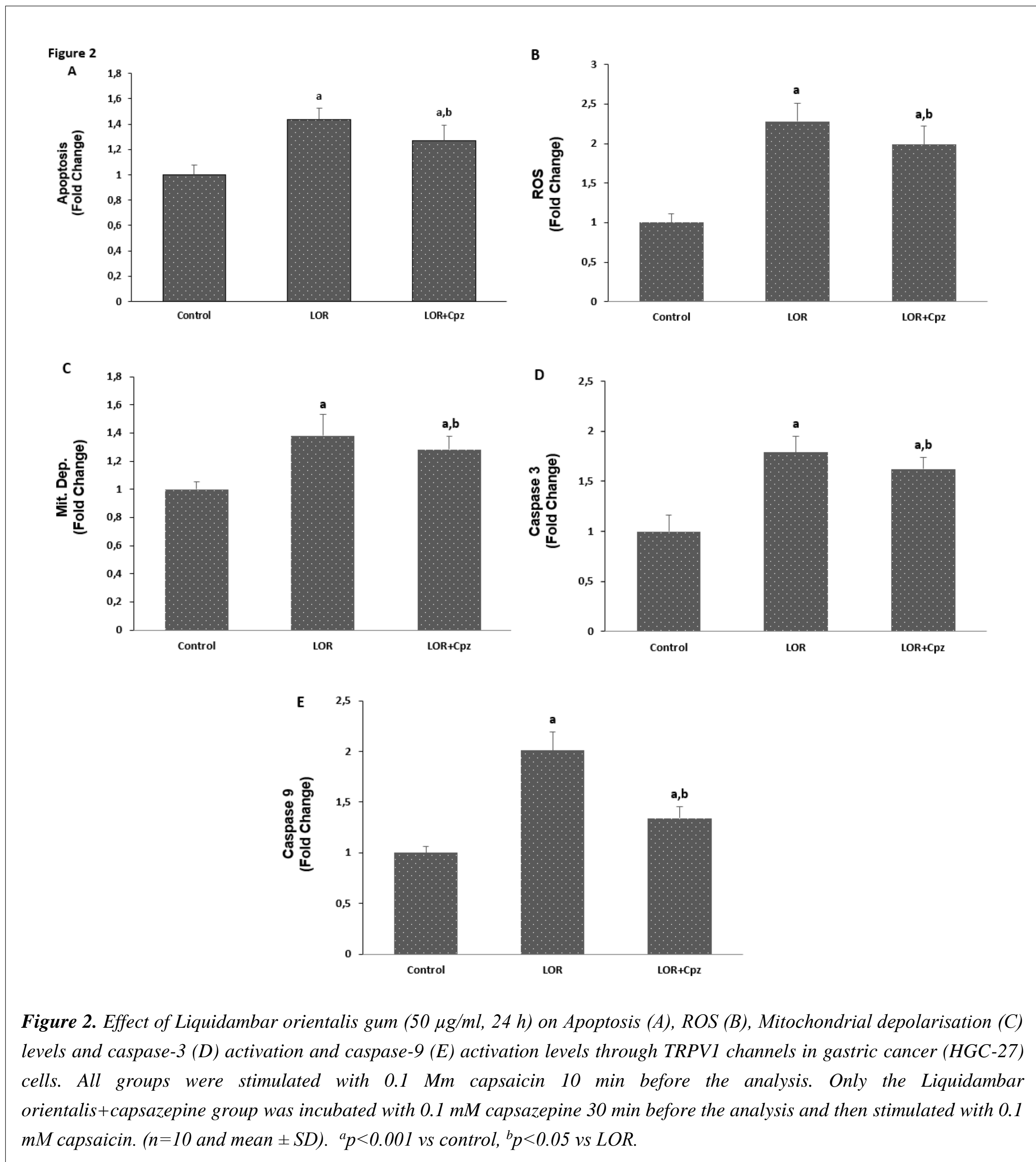


Figure 2. *Effect of Liquidambar orientalis gum (50 µg/ml, 24 h) on Apoptosis (A), ROS (B), Mitochondrial depolarisation (C) levels and caspase-3 (D) activation and caspase-9 (E) activation levels through TRPV1 channels in gastric cancer (HGC-27) cells. All groups were stimulated with 0.1 Mm capsaicin 10 min before the analysis. Only the Liquidambar orientalis+capsazepine group was incubated with 0.1 mM capsazepine 30 min before the analysis and then stimulated with 0.1 mM capsaicin. (n=10 and mean ± SD).* $^{a}p<0.001$ *vs control,* $^{b}p<0.05$ *vs LOR.*

radicals in gastric cancer cells and increased oxidative stress level increased the activation of TRPV1 channels.

In conclusion, although it was concluded that *Liquidambar orientalis* gum increased free radical levels by increasing the amount of intracellular calcium in *in-vitro* gastric cancer cells, it is necessary to establish a gastric cancer model on *in-vivo* mice or rats and repeat the same treatment protocol.

Financial Disclosure

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Ethics committee approval

This research was carried out using cells propagated through commercially available cell culture. Ethics committee approval is not required in this study. The study was conducted following the international declaration, guidelines, etc.

Referee Evaluation Process

Externally peer-reviewed

Conflicts of interest

The authors report no conflicts of interest.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

References

1. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Gastroenterology Review. 2019;14(1):26–38. https://doi.org/10.5114/pg.2018.80001
2. Bray F, Ferlay J, Soerjomataram I, Siegel R. L, Torre L. A, & Jemal A. Global Cancer Statistics GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2018. https://doi.org/10.3322/caac.21492
3. Liu Y, Shi Y, Han R, Liu C, Qin X, Li P & Gu R Signaling pathways of oxidative stress response: the potential therapeutic targets in gastric cancer. Front. Immunol. 2023;14:1139589. https://doi.org/10.3389/fimmu.2023.1139589
4. Sagdic O, Ozkan G, Ozcan M, Ozcelik S. A Study on Inhibitory Effects of Sigla Tree (Liquidambar orientalis Mill. var. orientalis) Storax Against Several Bacteria. Phytother. Res. 2005;19;549–551. https://doi.org/10.1002/ptr.1654
5. Cetinkaya S, Cinar Ayan I, Suntar I, Dursun HG. The Phytochemical Profile and Biological Activity of Liquidambar orientalis Mill. var. orientalis via NF-κB and Apoptotic Pathways in Human Colorectal Cancer. Nutrition and Cancer, 2021;1-15. https://doi.org/10.1080/01635581.2021.1952455
6. Atmaca H, Camli Pulat C, Cittan M. Liquidambar orientalis Mill. gum extract induces autophagy via PI3K/Akt/mTOR signaling pathway in prostate cancer cells. International Journal of Environmental Health Research, 2020;1–9. https://doi.org/10.1080/09603123.2020.1818187
7. Zitt C, Halaszovich CR, Lückhoff A. The TRP family of cation channels: probing and advancing the concepts on receptor-activated calcium entry. Prog Neurobiol. 2002;66(4):243-64. https://doi.org/10.1016/s0301-0082(02)00002-3
8. Steinberg X, Lespay-Rebolledo C, Brauchi S. A structural view of ligand-dependent activation in thermoTRP channels. Front Physiol. 2014;5:171. https://doi.org/10.3389/fphys.2014.00171
9. Alsalem M, Millns P, Altarifi A, El-Salem K, Chapman V, Kendall DA. Anti-nociceptive and desensitizing effects of olvanil on capsaicin-induced thermal hyperalgesia in the rat. BMC Pharmacol Toxicol. 2016;17(1):31. https://doi.org/10.1186/s40360-016-0074-9
10. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, Ko KM. New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013;1–25. https://doi.org/10.1155/2013/62737
11. Atmaca H, Camli Pulat C, Cittan M. Liquidambar orientalis Mill. gum extract induces autophagy via PI3K/Akt/mTOR signaling pathway in prostate cancer cells. Int J Environ Health Res. 2022;32(5):1011-1019. https://doi.org/10.1080/09603123.2020.1818187
12. Uğuz AC, Nazıroğlu M, Espino J, et al. Selenium modulates oxidative stress-induced cell apoptosis in human myeloid HL-60 cells through regulation of calcium release and caspase-3 and -9 activities. J Membr Biol. 2009;232(1-3):15-23. https://doi.org/10.1007/s00232-009-9212-2
13. Martinez NA, Ayala AM, Martinez M, Martinez-Rivera FJ, Miranda JD, Silva WI. Caveolin-1 Regulates the P2Y2 Receptor Signaling in Human 1321N1 Astrocytoma Cells. J Biol Chem. 2016;291(23):12208-12222. https://doi.org/10.1074/jbc.M116.730226
14. Öz A, Çelik Ö. Curcumin inhibits oxidative stress-induced TRPM2 channel activation, calcium ion entry and apoptosis values in SH-SY5Y neuroblastoma cells: Involvement of transfection procedure. Mol Membr Biol. 2016;33(3-5):76-88. https://doi.org/10.1080/09687688.2017.1318224
15. Uguz AC, Cig B, Espino J, et al. Melatonin potentiates chemotherapy-induced cytotoxicity and apoptosis in rat pancreatic tumor cells. J Pineal Res. 2012;53(1):91-98. https://doi.org/10.1111/j.1600-079X.2012.00974.x
16. Özbek AG., Ersus Bilek S. Properties and usage of Liquidambar orientalis. Clin Nutr Metab, 2018;(5)1-3 https://doi.org/10.15761/CNM.1000111
17. Duran T, Tuncer Z. Investigation of Cytotoxic and Apoptotic Effects of Styrax Liquidus Obtained From Liquidambar orientalis Miller (Hamamelidaceae) on HEp-2 Cancer Cell with Caspase Pathway. Eurasian J Med. 2023;55(3):185-191. https://doi.org/10.5152/eurasianjmed.2023.23130
18. Mutlu D, Gunal B, Secme M, Bozbeyoglu Kart NN, Abban Mete G, Mercan Dogan N, Semiz G, Melahat Fenkci S and Arslan Ş. Biological activities of Liquidambar orientalis: antibiofilm, cytotoxicity, apoptosis, and miRNA expressions. Botanica Serbica, 2024;48(1):17–26. https://doi.org/10.2298/BOTSERB2401017M
19. Piciu F, Balas M, Badea MA, Cucu D. TRP Channels in Tumoral Processes Mediated by Oxidative Stress and Inflammation. Antioxidants (Basel). 2023;12(7):1327. https://doi.org/10.3390/antiox12071327
20. Banner KH, Igney F, Poll C. TRP channels: emerging targets for respiratory disease. Pharmacol Ther. 2011;130(3):371-84. https://doi.org/10.1016/j.pharmthera.2011.03.005
21. Zhong T, Zhang W, Guo H, Pan X, Chen X, He Q, Yang B, Ding L. The regulatory and modulatory roles of TRP family channels in malignant tumors and relevant therapeutic strategies. Acta Pharm Sin B. 2022;12(4):1761-1780. https://doi.org/10.1016/j.apsb.2021.11.001