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ARAŞTIRMA / RESEARCH

Effect of geraniol and vitamin C on diethylnitrosamine induced experimental hepatocellular carcinogenesis

Geraniol ve vitamin C'nin dietilnitrozamin kaynaklı deneysel hepatoselüler karsinogenez üzerindeki etkisi

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Abstract

Purpose: This study aimed to investigate the protective effect of geraniol and vitamin C on the experimental hepatocellular carcinogenesis (HCC) model by inducing FL83B hepatocyte cell lines with diethylnitrosamine (DENA).

Materials and Methods: The cells prepared in the medium were incubated with DENA (5 µM), geraniol (5 µM), and vitamin C (50 µM) for 48 hours in an incubator at 37 °C and 5% CO2. Groups were designed as follows: Group 1 (Control), group 2 (DENA Control), group 3 (DENA+Geraniol), group 4 (DENA+Vitamin C), and group 5 (DENA+Geraniol+Vitamin C) on standard cell culture plates. Six plates from each experimental group were studied. After the homogenization was centrifuged, analyses of pathway mediators NF-κB, AIF, caspase-3, BCL-2, bax, gadd153, GRP78, and COX were performed by the Elisa method.

Results: The expression of Bax, caspase-3, COX-2, NFkB, GADD153, AIF, and GRP78 increased in cancer cells when compared to group 1 and decreased in other groups where antiproliferative agents were applied. Bcl-2 expression is decreased when compared to group 1, and expression is increased in other groups where antiproliferative agents are applied.

Conclusion: There was a significant hepatoprotective effect in the groups administered geraniol+vitamin C on pathway mediators in a DENA-induced HCC model.

Keywords: Cancer, cell culture, hepatocyte, liver, mediators, pathway

Öz

Amaç: Bu çalışmanın amacı dietilnitrozamin (DENA) ile FL83B hepatosit hücre hattını indükleyerek oluşturulan deneysel hepatosellüler karsinogenezis (HSK) modelinde geraniol ve vitamin C'nin koruyucu etkisini araştırmaktır.

Gereç ve Yöntem: Bu amaçla çalışmamızda besiyerinde hazırlanan hücreler DENA (5 µM), geraniol (5 µM) ve vitamin C (50 µM) ile 37 °C sıcaklıkta ve %5'lik CO_2'li etüvde 48 saat inkübe edildi. Hücreler inkübe edildikten sonra gruplar standart hücre kültürü plakalarında Grup 1 (Kontrol), grup 2 (DENA Kontrol), grup 3 (DENA+Geraniol), grup 4 (DENA+Vitamin C) ve grup 5 (DENA+Geraniol+Vitamin C) olmak üzere beş gruba ayrıldı. Her deney grubundan altı plaka çalışıldı. Homojenatlar santrifüj edildikten sonra yolak mediyatörlerinden NF-κB, AIF, caspase-3, BCL-2, bax, gadd153, GRP78, and COX-2 analizleri Elisa metodu ile yapıldı.

Bulgular: Kanser hücrelerinde Bax, caspase-3, COX-2, NFkB, GADD153, AIF ve GRP78 ekspresyonu grup 1 ile kıyaslandığında artmakta, geraniol ve vitamin C'nin uygulandığı diğer gruplarda ekspresyonun azalmakta olduğu belirlenmiştir. Bcl-2 ekspresyonu grup 1 ile kıyaslandığında azalmakta, geraniol ve vitamin C'nin uygulandığı diğer gruplarda ekspresyon artmaktadır.

Sonuç: Bu çalışmada, DENA ile indüklenen HCC modelinde geraniol ve vitamin C uygulanan gruplarda yolak mediyatörleri aracılığıyla önemli bir hepatoprotektif etki olduğu gözlendi.

Anahtar kelimeler: Hepatosit, hücre kültürü, kanser, karaciğer, mediyatör, yolak

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INTRODUCTION

The liver is the body's largest organ. The liver is located in the upper part of the abdominal cavity; it is located in the right hypochondriacal and epigastric region. It also covers a part of the left hypochondriac region. The liver is located between the planum subcostal and the 5th costa; It is separated from the pleura, lung, pericardium, and heart via the diaphragm. The level of the liver is between T8-L3 according to the column vertebrae. It is attached to the stomach, duodenum, diaphragm, and anterior abdominal wall thanks to its ligaments. The liver secretes bile, and the bile fluid pours into the second part of the duodenum and mixes with the digestive tract[1–4]. The liver can store various minerals and vitamins. In addition, it has a wide range of effects by synthesizing protein and removing harmful substances from the body Because it is an organ with limited tension, it can be easily torn. It is the second organ in the body that can be easily ruptured after the spleen. In ruptures and injuries (especially due to blunt trauma), excessive bleeding is seen as a result of the rich vascular system. There is also upper quadrant pain. Due to the tissue of the liver, tight sutures are not possible[1–3].

Cancer, which can affect various parts of the body, basically occurs when cells multiply uncontrollably. The hallmarks of cancer are the rapid growth of abnormal cells that grow outside the normal limits of the cells, invade neighboring structures, and spread to other tissues and organs. The second feature is the ability to metastasize. Metastases are one of the main causes of death from cancer [1–3]. Inflammation is seen primarily in the liver, regardless of the cause. Later, necrosis, fibrosis, and regeneration develop. Dysplastic nodules and early absolute hepatoma develop from regeneration nodules. Regeneration in the liver plays an important role in the development of hepatocellular carcinogenesis (HCC). HCC, known as malignant neoplasia of the liver, originates from hepatocytes[2,5]. HCC, a primary malignant tumor, is the most common and deadliest type of liver cancer[1]. Approximately one out of every 100 cancer cases encountered has been diagnosed with liver cancer. Liver tumors do not show any clinical findings other than fever, abdominal pain, jaundice, and hepatomegaly[2,3,5].

Diethylnitrosamine (DENA), which is a hepatocarcinogen; is known to disrupt nuclear enzymes involved in DNA repair and replication and is used as a carcinogen to induce liver cancer[6,7]. N-nitrosamines have been proven to cause various tumor types in all animal species, and these compounds are considered to pose serious health hazards to humans[1,2]. The use of DENA to induce tumorigenesis in the liver hepatocyte cell line as an experimental model of human hepatocarcinogenesis yields very successful results[8–11].

The geraniol compound, which is an isoprene derivative, is found in the essential oils of fragrant plants such as geranium and lemon. Geraniol appears as a pale light-yellow clear oil that is insoluble in water but soluble in most organic solvents. It is found as the main ingredient of rose and palmarosa oil while lemon ginger, rose, orange, etc. It is present in plants as an active ingredient[12,13]. Geraniol has a characteristic rose scent and a sweet, waxy, citrus-like flavor like a rose. It is often found together with oxidation products (geranial and neral)[12,13].

Besides its cosmetic use, geraniol has been shown to have some bioactive properties, including anti-inflammatory, anti-angiogenesis, and anti-tumor effects[14–19]. The anti-tumor effect of geraniol has been demonstrated in cancer types of various organs such as the liver, lung, pancreas, and colon[12,13,16–22]. This mechanism of geraniol, acyclic monoterpenoid alcohol, is by suppresses HMG-CoA reductase activity and stops the growth of co-cultured tumor cells[12,14]. The basement membrane surrounding cells is made of collagen[21,23,24].

Vitamin C, also known as ascorbic acid, is synthesized from D-glucose in many animal species. The presence of microorganisms was not required in the synthesis of ascorbic acid. In different species, for example, humans, monkeys, guinea pigs, some birds, and fish do not synthesize vitamin C. Because these organisms do not have the enzyme L-glonolactone oxidase genetically. Absorption of ascorbic acid from the small intestine is like monosaccharides. Vitamin C is absorbed mostly in the stomach. It is 20-30 times higher in most tissues than in plasma in glands such as the hypophysis, suprarenal cortex, corpus luteum, and thymus. It is also used as an antioxidant because it is a strong reducing agent during reduction and oxidation. Vitamin C can be converted into dehydroascorbic acid and enter and leave the cell via the glucose transport protein. When dehydroascorbic acid enters the cancer cell, it turns back to ascorbic acid thanks to glutathione. This ascorbic acid turns into dehydroascorbic acid again before it can leave the cell, and in the meantime, it releases hydrogen peroxide. In this way, it destroys cancer cells[21,23,24].

Vitamin C increases collagen synthesis. It somewhat reduces the growth of cancer cells. It causes early apoptosis and necrosis. Cancer cells secrete collagenase. Collagenase dissolves collagen between cells or tissues. The basement membrane surrounding the cells is made of collagen[21,23,24]. Cancer cells metastasize when the basement membrane breaks down. For cancer cells to metastasize, the basement membrane surrounding the cell must be broken down. To protect this basement membrane consisting of collagen, vitamin C strengthens the basement membrane by increasing collagen synthesis and maintaining its mechanical integrity. As a result, the proliferation of cancer cells is prevented[21,23,24].

Since the tumor begins to threaten the patient in a very short time after its formation, we think that studies on this subject should be increased, and new treatment combinations should be studied in experimental settings. No study showing the protective effect of geraniol and vitamin C together has been found to our knowledge.

For this reason, our study was carried out to determine the protective effect of geraniol and vitamin C on the liver structure in the HCC model induced by DENA in the FL83B hepatocyte cell line. For this purpose, the expression of nuclear factor kappa-B (NF-κB), apoptosis-inducing factor (AIF), caspase-3, BCL-2, bax, gadd153, 78-kDa glucose-regulated protein (GRP78), cyclooxygenase-2 (COX-2) were analyzed.

MATERIALS AND METHODS

The inclusion criteria are no contamination during the incubation or homogenization steps, the exclusion criterion is contamination during the incubation or homogenization stages, and the inability to prepare the cell culture medium required for the growth of cells. This study was carried out at Cukurova University, Faculty of Medicine, Department of Anatomy and Medical Pharmacology. In this study, FL83B (ATCC® CRL-2390TM) (Manassas, Virginia, USA) monolayer mouse hepatocyte cell culture obtained from the American Type Culture Collection Organization (ATCC) was used. Approval of the ethics committee is not required because the cells are purchased commercially.

Cell culture

The provided cells were grown in a single layer which has 1×105 live cells in 1 ml, in standard cell culture plates of 75 cm^2. The cells prepared in the medium were incubated in an incubator at 37°C and 5% CO2 for 48 hours with DENA (5 µM), geraniol (5 µM), and vitamin C (50 µM). After homogenization of these cells, NF-κB, AIF, caspase-3, BCL-2, bax, gadd153, GRP78, and COX-2 (Awareness Technology Inc., ChroMate Elisa Reader, US) analyzes were performed by Enzyme-Linked Immunosorbent Assay (ELISA) method. The values obtained as a result of the analysis were calculated in µg/µl according to the standard curve drawn in the GraphPad Prism 8.1.2. (CA, USA) program.

Cell culture medium

Cell culture medium was prepared using 10% Fetal Bovine Serum (FBS) (Hyclone), 1% L-Glutamine (Hyclone), 1% Penisilin-Streptomisin (Hyclone), Dulbecco's modification of Eagle's medium (DMEM) (GIBCO) to support the growth of cells.

Cell incubation

The cells prepared in the medium were incubated with DENA (5 µM), geraniol (5 µM), and vitamin C (50 µM) for 48 hours in an incubator (Nuve EN400) with 37 °C and 5% CO2. After the cells were replicated, groups were designed as follows: Group 1 (Control), group 2 (DENA Control), group 3 (DENA+Geraniol), group 4 (DENA+Vitamin C), and group 5 (DENA+Geraniol+Vitamin C) on standard cell culture plates. Six plates from each experimental group were studied. After the incubation step, the experimental groups were frozen in an Eppendorf tube at -20 ºC.

Tissue homogenization

To homogenize cells, 3 ml RIPA (Radio-Immunoprecipitation Assay) buffer, 30 µl phenylmethanesulfonylfluoride (PMSF), 30 µl sodium vanadate, 30 µl protease inhibitor were added per 1 gram on cells frozen at -20 ºC in an Eppendorf tube and homogenates were obtained from the cells by shredding on ice with an ultrasonic lysing device. The homogenates were centrifuged (Nuve NF200) at 10,000 rpm for 10 minutes, and the supernatants separated from the upper part were removed, while the lower precipitates, ie pellets, were discarded.

Elisa test

Bradford solution was added to the homogenized

cells and absorbances of the expression of pathway markers were read at 590 nm wavelength in the Elisa reader.

Statistical analysis

Relaxation responses of tissues are expressed as a percentage of contractions. It is shown with standard errors. The values obtained for drawing graphs and statistical analysis were calculated in µg/µl according to the curve drawn in GraphPad Prism 8.1.2. (CA, USA) program on the computer. One-way (ANOVA) and posthoc test (Bonferroni) were used for statistical comparisons. The purpose of ANOVA analysis of variance is to determine the difference between the groups to be compared and the number of groups to be more than two. The purpose of using posthoc test (Bonferroni) analysis is to compare the variance between groups. Results were evaluated at a 95% confidence interval and significance level of $p < 0.05$.

RESULTS

NF-κB expression increased in the DENA group compared to the control group, and the group with the highest inhibition compared to the DENA applied group was the group to which DENA + Geraniol + Vitamin C was applied. AIF expression increased in the DENA applied group when compared to the control group, and the group with the highest inhibition compared to the DENA applied group was the group to which DENA + Vitamin C was applied. Caspase-3 expression increased in the DENA group when compared to the control group and the group with the highest inhibition compared to the DENA applied group was the group to which DENA + Geraniol + Vitamin C was applied. Bcl-2 expression decreased in the DENA applied group when compared to the control group and the group with the highest expression levels compared to the DENA applied group was the group to which DENA + Geraniol + Vitamin C was applied. Bax expression increased in the DENA applied group compared to the control group, and the group with the highest inhibition compared to the applied DENA group was the group to which DENA + Geraniol + Vitamin C was applied. Gadd153 expression increased in the DENA applied group compared to the control group and the group with the highest inhibition compared to the DENA applied group was the group to which DENA + Geraniol + Vitamin C was applied. GRP78 expression increased in the DENA applied group when compared to the control group, and the group with the highest inhibition, when compared to the applied DENA group, was the group to which DENA + Geraniol + Vitamin C was applied. COX-2 expression increased in the DENA applied group compared to the control group, and the group with the highest inhibition compared to the DENA applied group was the group to which DENA + Geraniol + Vitamin C was applied.

This finding suggests that geraniol ve vitamin C can reduce DENA-induced HCC and it can be used as a supplement to protect. All the findings were found statistically significant ($p<0.05$). The mean±standard error values of the results were obtained respectively in five groups (Group 1; Control, Group 2; DENA Control, Group 3; DENA + Geraniol, Group 4; DENA + Vitamin C and Group 5; DENA + Geraniol + Vitamin C) and are shown in Table 1 and Figure 1 (n = 6, ANOVA, Post hoc: Bonferroni. *; $p < 0.05$ # for the control group; $p < 0.05$ for the DENA group).

Table 1. Values of expression analysis of pathway markers according to experimental groups, respectively.

	Control	DENA Control	DENA+ Geraniol	DENA+ Vitamin C	DENA+ Geraniol+ Vitamin C
NF-κb* (ng/ml)	1.208±0.071	5.217±0.325	3.017±0.209	3.333±0.208	2.583±0.172
AIF† (pg/ml)	0.518±0.033	1.490±0.036	1.012±0.119	0.968±0.175	1.037±0.084
Caspase-3 (ng/ml)	3.520±0.356	16.170±1.352	9.830±0.833	10.970±0.912	7.500±0.764
Bcl-2 (pg/ml)	19.330±1.542	8.000±0.856	14.000±0.516	13.670±0.882	15.330±1.085
Bax (ng/ml)	1.180±0.076	8.350±0.999	5.033±0.518	6.333±0.882	3.167±0.477
Gadd153 (ng/ml)	0.409±0.041	1.463±0.124	0.891±0.047	0.777±0.087	0.617±0.069
GRP78‡ (pg/ml)	0.760±0.060	1.830±0.087	1.290±0.052	1.210±0.083	0.910±0.184
COX-2§ (pg/ml)	1268±542	3962±1384	2315±02153	2602±01480	1864±1671

*Nuclear factor kappa-B, †Apoptosis-inducing factor, ‡78-kDa glucose-regulated protein, and §Cyclooxygenase-2.

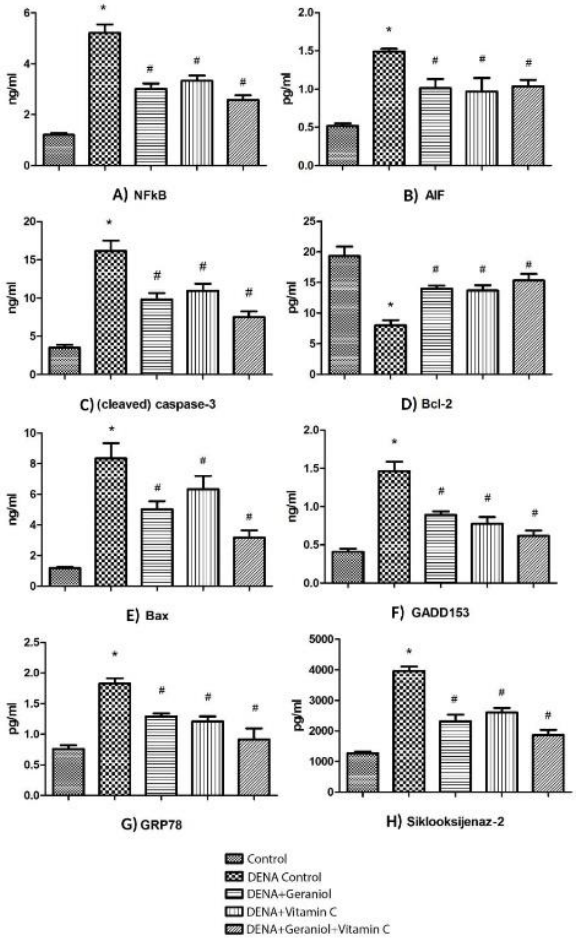

Figure 1. Graphical distribution of expression analysis of pathway markers according to groups.

*Difference between control and DENA control group is significant with $p<0.05$. # Difference between DENA control and other groups, except for control, is significant with $p<0.05$.

A) Nuclear Factor kappa-B (NF-κB): The highest inhibition concerning the NF-Kb expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

B) Apoptosis-Inducing Factor (AIF): The highest inhibition concerning the AIF expression was observed in DENA+Vitamin C, compared to DENA control.

C) Caspase-3: The highest inhibition concerning the Caspase-3 expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

D) Bcl-2: The highest expression concerning the Bcl-2 expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

E) Bax: The highest inhibition concerning the NF-kB, Caspase-3, Bax, Gadd153, GRP78, and COX-2 expressions were observed in DENA+Geraniol+Vitamin C, compared to DENA control.

F) Gadd153: The highest inhibition concerning the Gadd153 expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

G) 78-kDa glucose-regulated protein (GRP78): The highest inhibition concerning the GRP78 expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

H) Cyclooxygenase-2 (COX-2): The highest inhibition concerning the COX-2 expression was observed in DENA+Geraniol+Vitamin C, compared to DENA control.

DISCUSSION

Today, studies in cell culture have a vital place in research activities[10,13,17,19,21,22,25–29]. With the acceleration of cancer research, the use of cancer cell lines has gained great importance, especially in the development of cancer drugs and in determining their effects[22].

Previous studies include the ones examining the protective effect of geraniol against liver damage in cell lines, humans, and experimental animals [12,14,16–19]. There was no study in which geraniol and vitamin C were used together in hepatocellular carcinoma formed by DENA in mouse hepatocyte cell line. Therefore, in our article, discussions, and comparisons are necessarily made on different experimental models designed using DENA, geraniol, or vitamin C.

Also in previous studies,[8–11,23,30,31], 15 different hepatotoxic agents, including carbon tetrachloride (CCL4), allyl alcohol, aroclor 1254, methotrexate, diquat, carbamazepine, methacrylate, arsenic, DENA, monocrotaline, dimethylformamide, amiodarone, amiodarone, were administered to rat hepatocytes and evaluated for carcinogenicity. As a result, it was shown that DENA caused changes in the expression of many genes that none of the other hepatotoxins regulate[8–11].

For this reason, DENA is often used as a carcinogen in experimental applications. In this study, hepatocellular carcinoma formation was provided by inducing FL83B hepatocyte cell lines with DENA. In a study, Azap et al. conducted on rats, the effects of geraniol on CCl4-induced liver fibrosis were investigated and as a result, it was stated that its hepatoprotective upshot on liver fibrosis [16].

The antiproliferative effects of geraniol were mainly due to the perturbation of the ion channels and membrane causing modifications of membrane-bound protein activity and changes in intracellular signaling pathways. In their study, Mohammed et al. investigated the effects of geraniol on cyclophosphamide-induced liver toxicity and the expressions of NFK-B and COX-2 in rats and stated that it had a hepatoprotective effect[18]. In addition, geraniol also showed antioxidant activities[18]. This study also found that NFK-B decreased in the groups given therapeutically effective agents and COX-2 expression increased in the DENA induced group. Ortiz et al. demonstrated that geraniol had an antiproliferative effect against the growth of cancer cells[13]. Also in the literature, detected in vivo and in vitro cells that it caused changes in cell permeability, membrane, and ion channel perturbation[17,20]. The fact that it has no cytotoxic effect and low toxicity is among the reasons why geraniol is preferred for therapeutic uses[17,20].

In this way, the effect of essential oils would be better understood and thus, the use of the drug would contribute to the fight against cancer and/or cardiovascular diseases. In our study, which was similar to the results of previous studies on geraniol[13,16–20], it was observed that geraniol suppressed cells by showing an antiproliferative effect in DENA sourced hepatocellular carcinoma cells. It is believed that geraniol may be beneficial in the development of cancer treatments and will provide a scientific basis for future research.

In a study[32] investigating the hepatoprotective effects of vitamins B_{12}, C, and E against hepatotoxicity created in hepatocyte cell culture obtained from rat hepatocytes, Abdulkhalek et al. suggested that it was beneficial in preventing hepatic oxidative stress. It was also stated that vitamin C alone showed superior protection against acetaminophen-induced liver damage compared to other vitamins in rats [32]. Rahmouni et al. evaluated carbon tetrachloride-induced hepatotoxicity and oxidative stress in male albino Wistar rat liver showed that vitamin C had a protective effect[23].

In a study, Abdulrazzaq et al. conducted on rats, the hepatoprotective effects of ascorbic acid, alpha-lipoic acid, and silymarin, a flavonoid obtained from the seeds of the plant called Silybum marinum, which is a hepatoprotective agent, or their combination on acetaminophen-induced hepatotoxicity were investigated and as a result, it was stated that its hepatoprotective effect on liver[31]. In addition, the measured liver function tests indicated an augmented hepatoprotection of the combination preparation than when compared individually[31]. In studies against hepatotoxicity in vivo and in vitro models, it was emphasized that AIF was important for DNA damage and liver damage since it was involved in DNA fragmentation[26,30,33,34].

The increase in AIF expression seen as a result of liver damage in the mentioned studies[26,30,33,34] was supported by the AIF expression observed in the

DENA-induced group in this study. In a study conducted in an FL83B mouse liver cell line on oxidants, which are important toxic substances that play a role in the emergence and development of liver diseases, caspase-3 expression was investigated after the treatment of FL83B cells with tert-butyl hydroperoxide, which is an organic peroxide and consequently, it was stated to increase the expression of apoptotic molecules, cause cytochrome-c release, and induce BCL-2, bax and IRE1α/TRAF2 complex formation[29].

In addition, as a result of the t-BHP application, it was noted to induce liver FL83B cell viability and apoptosis by upregulating PDIA6 levels[29]. In this study, it was found that caspase-3 and Bax's expression decreased and observed an increase in anti-apoptotic Bcl-2 expression as a result of hepatotoxicity.

Al-Hrout et al. examined the anticarcinogenic effect of safranal which is a monoterpenoid, as geraniol, formally derived from beta-cyclocitral by dehydrogenation, in HCC therapy on HepG2 cells through gadd153 were shown to be directly effective in initiating apoptosis. It was also emphasized that cell death is generally promoted by apoptosis, and they have been shown to possess antitumorigenic and proapoptotic activities in vitro[25].

Gadd153 expression levels conducted by Ala'a-Hrout et al. were similar to the expression levels in this study[25]. In a study investigating the anticancer effect of caudatin in a diethylnitrosamine-induced HCC rat model, Song et al. examined expression levels of GRP78 in rats with hepatocellular carcinoma[8]. It was stated that overexpression of GRP78 in cells increased the invasion of cancer cells[8]. It was also emphasized that the overexpression of GRP78 accelerated the cell proliferation process[8].

In this study, it was observed that there was a significant hepatoprotective effect in the groups administered DENA+Geraniol+Vitamin C on pathway mediators in a diethylnitrosamine (DENA)-induced HCC model. The results of our study support the literature studies. To the best of our knowledge, this is the first study to demonstrate the anti-proliferative effects of geraniol and vitamin C on FL83B cells with experimental HCC-induced apoptosis with DENA.

In conclusion, the present study revealed that geraniol and vitamin C have a beneficially hepatoprotective impact against hepatocellular damage induced by DENA. Moreover, this study emphasizes that cell death is generally promoted through apoptosis and shows potential for the development of chemopreventive agents in HCC. Geraniol and vitamin C's protective effect on HCC may be due to their antioxidant and anti-inflammatory effects. And also, it is the first study in the literature showing that geraniol and vitamin C have a hepatoprotective effect when used together through specific pathway mediators. These upshots may be useful in developing hepatocellular carcinoma prevention strategies. Future studies should focus on cell cycle, protective mechanisms, and synergistic effects, in different experimental models (animal models - rats, mice, humans), and also these studies should be continued in different types of cancer.

Yazar Katkıları: Çalışma konsepti/Tasarımı: RGTŞ, HMK, NB, EŞ; Veri toplama: RGTŞ, HMK, NB, ÖO, EŞ; Veri analizi ve yorumlama: RGTŞ, HMK, NB, ÖO, EŞ; Yazı taslağı: RGTŞ, HMK, NB; İçeriğin eleştirel incelenmesi: RGTŞ, HMK, NB, ÖO, EŞ; Son onay ve sorumluluk: RGTŞ, HMK, NB, ÖO, EŞ; Teknik ve malzeme desteği: RGTŞ, HMK, NB, ÖO, EŞ; Süpervizyon: RGTŞ, HMK, NB, ÖO, EŞ; Fon sağlama (mevcut ise): Yok.
Etik Onay: Bu çalışmada FL83B (ATCC ® CRL-2390TM) tek tabakalı fare hepatosit hücreleri kullanılmıştır. Hücreler ticari olarak satın alındığı için etik kurul onayı gerekli değildir.
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Ethical Approval: In this study, FL83B (ATCC® CRL-2390TM) monolayer mouse hepatocyte cells were used. Approval of the ethics committee is not required because the cells are purchased commercially.
Peer-review: Externally peer-reviewed.
Conflict of Interest: None of the authors have any conflict of interest related with this study.
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