Ethan Cancer Research Journal
Volume 1 Issue 1 February 2024
ISSN: Pending…

FICUS CARICA L. EXTRACT: A BEACON OF HOPE FOR AGS HUMAN GASTRIC ADENOCARCINOMA

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ABSTRACT – *Objective:* Gastric cancer is one of the gastrointestinal tract tumors associated with a poor prognosis. *Ficus carica* L. is one of the most outstanding species among many traditional medicines. The aim of our study is to determine the antioxidant content of *Ficus carica* L., and its therapeutic effects on gastric cancer.
Patients and Methods: *Ficus carica* L. was extracted in a different solvent. The antioxidant parameters of the different concentrations of extracts were measured photometrically. The human gastric adenocarcinoma cells (AGS) were incubated with different concentrations of *Ficus carica* L. extract for 24 hours; cell viability by the ATP test, and intracellular reactive oxygen species levels were determined by H2DCF-DA fluorescent dye. Intracellular glutathione levels luminometrically, and mitochondrial membrane potential fluorometrically were detected. The apoptotic ef-
fect was analyzed with acridine orange/ ethidium bromide, and the genotoxic effect by the comet assay.
Results: It was found that methanol extract has higher antioxidant content compared to ethanol extract (p<0.001). In addition, it has been revealed that methanol extract has cytotoxic, genotoxic, and apoptotic effects on gastric cancer cells statistically significantly (p<0.001).
Conclusions: As a result of our study, it is thought that *Ficus carica* L., which has an anticancer effect, can be used together with routine treatments in gastric cancer.
KEYWORDS: Apoptosis, Cytotoxicity, *Ficus carica* L., Gastric adenocarcinoma, Genotoxicity.

INTRODUCTION

Gastric cancer is a heterogeneous disease with a variable combination in the sense of etiology such as chronic *Helicobacter pylori* infection, Epstein-Barr virus (EBV) coinfection involvement, en- vironmental, and genetic factors[1]. The cancer grades sixth among the most common cancer types and fourth among cancers that result in death worldwide. While the mean age is 68 at diagnosis, gastric cancer is more common in middle-aged and elderly populations, besides having an inci- dence and mortality of approximately 1.8 higher in male than female[2]. Although not specific to the disease, symptoms include nausea, vomiting, weight loss, anorexia, early satiety, epigastric pain, and dyspepsia. However, these symptoms may not appear as much as in the later stages of gastric adenocarcinoma[3]. Given the elevated metastatic propensity observed in advanced stages of gastric cancer, the overall prognosis of the disease is notably unfavorable[4]. Endoscopic resections, surgical resections, preoperative chemo radiotherapy, neoadjuvant chemotherapy, radiotherapy, immuno- therapy and targeted molecular therapies have become effective methods by courtesy of research that has been done to improve the prognosis of the disease and treatment recently[5]. Despite all these types of treatment, the prognosis of gastric cancer cannot be improved, and there is current- ly no established standard treatment[6]. Natural product compounds have demonstrated

potential in preventing the onset of diverse diseases and disorders, particularly gastrointestinal cancers, owing to their favorable impact on overall health. This assertion is supported by a plethora of preclinical, clinical, and epidemiological studies[7].

Integrative medicine is defined as the combination between standard medicine and complemen- tary treatments that have been represented to be safe and beneficial[8-10]. Although there are de- velopments in cancer treatment today, complementary treatment methods are utilized in addition to medical treatments as well[11]. The rationale behind this intervention is to ameliorate symptoms arising from the adverse effects of medical treatments administered to individuals with cancer and concurrently enhance their immune systems. These administrations contain natural nutrients, herbs and plants, vitamins, minerals, and probiotics[12,13]. Natural nutrients can provide cancer pa- tients to limit many side effects during therapy and allow the drug benefits to increase depending on the use of these products. However, these natural nutrients used during treatment can change the amount of drug reaching the target area and the therapeutic perspective of the drug used in the treatment that causes toxicity[14]. Drug-nutraceutical interactions may cause changes in the pharmacodynamics and/or pharmacokinetics of the active ingredients in the drug, thus affecting the positive outcome of the treatment process. The underlying mechanisms of the effects of phar- maceutically active substances in nutraceuticals which are difficult to understand, can improve health and reduce the risk of pathological conditions. Therefore, studies are required to evaluate them as therapeutically effective tools [8,15].

Ficus carica L. is the most outstanding species among many traditional medicines with therapeutic effects as a well-known *Ficus* (Moraceae) species[16]. Besides being rich in vitamins, minerals and fiber, it also contains sugar, phytochemicals, polyphenols, and organic acids[17]. Phenolic acids and flavo- noids such as gallic acid, chlorogenic acid, epicatechin, and quercetin-3-O-rutinoside, are the main types of phytochemical compounds found in *Ficus carica* L. especially[18]. Due to these components, it substantiates many biological effects such as antioxidant, anticancer, hepatoprotective, regulating blood glucose, antibacterial and antifungal, antispasmodic, and antiplatelet[19]. In this study, we aimed to substantiate the antioxidant content of *Ficus carica* L. extracts and its therapeutic effect in gastric adenocarcinoma where the prognosis cannot be improved yet, for which a specific treatment has not been provided.

MATERIALS AND METHODS

Preparation of Extracts

Ficus carica L. was obtained from Aydın Provincial Directorate of Agriculture and Forestry (Aydın, Tur- key). The materials cut into small pieces were mixed in 80% ethanol and 80% methanol for 24 hours in a closed amber beaker with a magnetic stirrer. The samples were filtered, then the organic phase was evaporated in the rotary evaporator, and the water phase was extracted by lyophilization.

Antioxidant Parameters

Total Antioxidant Status

The total antioxidant status of *Ficus carica* L. extracts was measured photometrically according to Erel et al[20] method. Several concentrations of the extract (1 -100 mg/mL) were measured in three times. One mM Trolox was used as the standard, and the results of the extracts were expressed as mmol Trolox equivalents/L.

Total Phenolic Content

The total phenolic content of *Ficus carica* L. extracts was determined using the Folin-Ciocalteu meth- od[21]. Extract samples prepared in different concentrations are mixed with Folin-Ciocalteu reagent (Sig- ma-Aldrich, St

Louis, MO, USA) and then 7.5% Na_2CO_3 (Sigma-Aldrich) and incubated for 2 hours at room temperature. The absorbance of the reaction mixture was measured at 760 nm with a multiplaque reader (Varioskan Flash Multimode Reader, Thermo Fisher Scientific, Waltham, MA, USA). Gallic acid (0 - 0.5 mg/mL) was used as the standard, and the total phenolic content was expressed in µg/mL gallic acid equivalents.

Total Flavonoid Content

The total flavonoid content of *Ficus carica* L. extract was determined photometrically. After the extract samples prepared at different concentrations were mixed with 5 % $NaNO_2$ (Sigma-Aldrich) solution, 10% $AlCl_3$ (Sigma-Aldrich) solution was added to the samples. After the reaction mixture was thoroughly mixed, it was incubated in the dark at room temperature for 40 minutes. The absorbance of the flavo- noid-aluminum complex formed was measured at 510 nm using a Varioskan Flash Multimode reader (Thermo Fisher Scientific). Quercetin (0 – 0.05 mg/mL) was used for the calibration curve. Total flavo- noid content is expressed as µg/mL quercetin equivalent.

Copper Ion Reducing Capacities

The copper ion-reducing effect of *Ficus carica* L. extracts were determined by the copper ion-reducing antioxidant capacity (CUPRAC) method[22]. Different concentrations of the extracts were added to 10 mM $CuCl_2$ (Sigma-Aldrich) solution, 7.5 mM neocuproin reagent (Sigma-Aldrich), and 1 M NH_4Ac pH:7 buffer, mixed well and incubated for 30 minutes at room temperature. 1 mM Trolox was used as standard. After incubation, the absorbance of the samples was measured at 450 nm with a multi-plate reader (Varioskan Flash Multimode Reader, Thermo Scientific). Results were expressed as mmol Trolox equivalents/L.

Free Radical Scavenging Capacity

The free radical scavenging capacity of different concentrations of *Ficus carica* L. extracts was deter- mined according to the Blois method[23]. One mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) solution as free radical, and trolox and gallic acid as standard antioxidants, were used. The color change in the DPPH radical was determined by measuring the decrease in absorbance at 517 nm against pure methanol as blank. Results were expressed as percent inhibition (%) using percent inhibitions and calcu- lated according to the formula below.

Inhibition (%) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100$

Prooxidant Activity

The prooxidant activities of methanol and ethanol extract of *Ficus carica* L. at different concentrations (10 - 100 mg/mL) were determined using total antioxidant status and free radical scavenging activ- ities. Gallic acid was used as a standard for prooxidant activity, and results were expressed as mM gallic acid.

Maintenance of Cell

Human gastric adenocarcinoma cell line AGS (American Type Culture Collection, Manassas, VA, USA; CRL-1739™) was cultured with a complete medium containing F-12K Medium 89 % (Gibco Invitrogen Corporation, Carlsbad, CA, USA), fetal bovine serum 10% (Sigma-Aldrich), and 100 µg/mL penicillin/ streptomycin (Sigma Aldrich). Cells were incubated at 5 % CO_2, and 37°C temperature.

Cell Viability

The cytotoxic activity of *Ficus carica* L. extracts were determined by the luminometric ATP method. Af- ter seeding, 1.5×10^4 cells/well were incubated overnight in 5% CO_2 at 37°C, and the cells were exposed to different concentrations of the extracts (1-100 mg/mL) for 24 hours. A total of 0.1% DMSO was given to the cells as a control. After 24 hours, the medium was aspirated, and the ATP assay kit (Cell-Titer-Glo Luminescent Cell

Viability Assay, Promega, Madison, WI, USA) was applied according to the kit protocol. The light emitted in the presence of ATP was read luminometric by a Varioskan Flash Multimode Reader (Thermo-Fisher Scientific). Half maximal growth inhibitory concentration (IC_{50}) values were calculated from the concentration-response curves.

Intracellular Reactive Oxygen Species

Reactive oxygen species (ROS) production activity of *Ficus carica* L. extracts was determined using the 2,7-dichloride-hydrofluorescein-diacetate (H_2DCF-DA; Sigma, Saint Louis, MO, USA) fluorometric dye[24]. According to the method, 1.6×10^4 cells/well were seeded in 96-well black opaque plates. Then the cells were incubated at 37°C, 5 % CO_2 in darkness, with various concentrations of *Ficus carica* L. extracts for 24 hours. After incubation, the medium was withdrawn from the wells, and the cells were washed with 1x dPBS. After washing, the cells were incubated with H_2DCF-DA dye (100 mM) for 30 minutes at room temperature and read at Ex/Em: 488/495 nm with a fluorometric by Varioskan Flash Multimode Reader (Thermo Scien- tific, Waltham, MA, USA). Results are expressed as % relative fluorescence (RFU) to control.

Intracellular Glutathione

Changes in intracellular glutathione levels associated with the *Ficus carica* L. extracts were measured luminometrically using GSH/GSSG-Glo™ Assay (Promega, Madison, WI, USA). AGS cells were seeded in white opaque 96-well plates as 1.6×10^4 cells/well. Then the cells were incubated at 37°C in 5% CO_2 for 24 hours with the different extract doses below the IC_{50}. After the treatment, the cells were incubated with the Glutathione Reagent included in the kit for 5 minutes; Luciferin Detection Reagent was added, and the occurring luminescence emit was measured by a multimode reader (Varioskan Flash Multimode Reader, Thermo Fisher Scientific). Results were expressed as % relative luminescence (RLU) compared to control and were expressed as µM.

Mitochondrial Membrane Potential

The effect of the extract on the mitochondrial membrane potential (MMP) in AGS cells was measured with the fluorometric 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$) dye[25] (Sigma-Aldrich). Cells were seeded in 96-well black opaque plates at 5.10^3 cells/well, and extracts were applied to the cells at doses below the IC_{50}. After 24 hours of treatment, cells were incubated with $DiOC_6(3)$ dye (40 nM) for 15 minutes at 37°C and washed with 1x dPBS after incubation. Measurement was taken with a fluorescence plate reader (Varioskan Flash Multimode Reader, Thermo-Fisher Scientific) at a wavelength of Ex/Em: 484/500 nm.

Apoptosis

Acridine orange-ethidium bromide (AO/EB; Sigma-Aldrich) dual staining method[26] was used for the microscopic determination of apoptotic cell ratios after different concentrations of the extracts were applied to 6-well plates on AGS cells seeded with $1x10^6$ cells/ well for 24 hours. Cell examination was performed under a fluorescent microscope (Leica DM 1000, Solms, Germany).

DNA Damage

DNA damage related to the extracts on AGS cells was determined by the alkaline single cell gel electrophoresis assay (comet assay) method[27]. 2×10^5 cells/well were seeded in six-well plates for 24 hours. Af- ter the incubation, the extracts at doses under IC_{50} were added to the cells and incubated again at 37°C, 5% CO_2 for 24 hours. Then the media of the cells were aspirated, and the cells were washed once with ice-cold 1x dPBS. Cells removed with 0.025% trypsin/EDTA were centrifuged at 500 x g for 5 minutes to obtain pellets. Cells were mixed with 0.6% low melting agarose (Sigma-Aldrich) and placed on slides cov- ered with 1% normal melting agarose (Sigma-

Aldrich). The slides were then electrophoresed at 72 V/cm (26 V, 300 mA) for 0.25 min at 4°C. After electrophoresis, the slides were neutralized by holding in 0.4 M Tris (pH:7.5) for 5 minutes and fixed with ethanol. After all these procedures, the slides were stained with ethidium bromide dye and analyzed with the Comet Assay IV program (Perceptive Instruments, Suffolk, UK) with a fluorescent microscope (Leica DM 1000, Solms, Germany). Images were captured from ran- domly selected areas, and a minimum of 100 cells were counted. Results were expressed as % tails.

Statistical Analysis

Statistical Package for the Social Sciences version 25.0 (SPSS Inc., Armonk, NY, USA) was used for the analysis of all data obtained. Parametric data are expressed as mean ± standard deviation. A nonlinear regression analysis was performed to calculate the IC_{50} value for the cell. Differences between *Ficus car- ica* L. extracts were analyzed for statistical significance with one-way ANOVA, Student's *t*-test or Mann Whitney-*U* test. Pearson, correlation coefficient test was used to evaluate the relationships between parameters. A value of $p < 0.05$ was considered statistically significant, and all experiments in the study were performed in quadruplicate.

RESULTS

Antioxidant profile of *Ficus carica* L. methanol and ethanol extracts

The antioxidant status of *Ficus carina* L. methanol and ethanol extract concentrations 1 mg/mL -100 mg/ mL were determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The extracts were expressed as mmol in terms of Trolox equivalent. As the dose increased, the total antioxidant levels increased significantly ($p<0.001$; Figure 1).

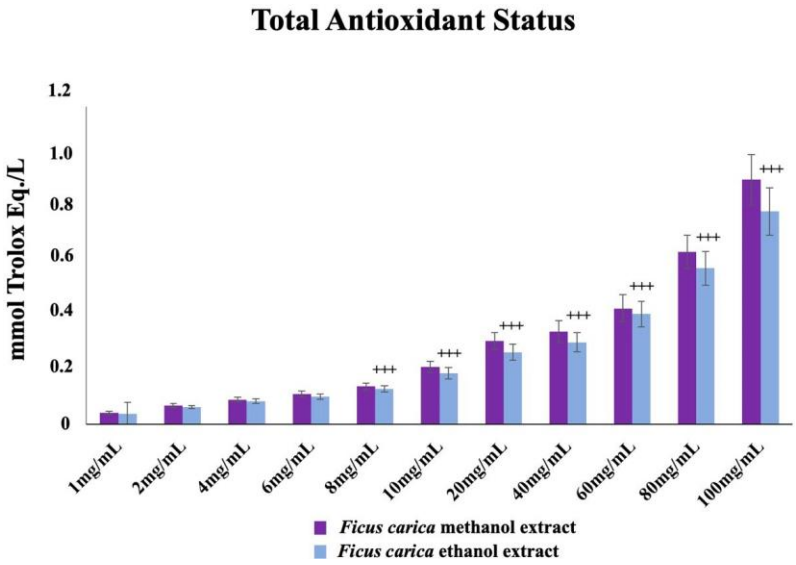

Figure 1. Total antioxidant status (TAS) levels of Ficus carina L. methanol and ethanol extracts. The data is expressed as mmol Trolox equivalent per liter. Differences for Ficus carica L. ethanol extract $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ values were considered statistically significant.

The total antioxidant levels of all parameters are presented in Figure 2. It was observed that the methanol extract of *Ficus carica* L. had higher phenolic and flavonoid content than the ethanol extract, and the total antioxidant

capacity increased significantly (p<0.001) as the dose increased. Radical scav- enging activity and copper reducing activity were also found to be strong.

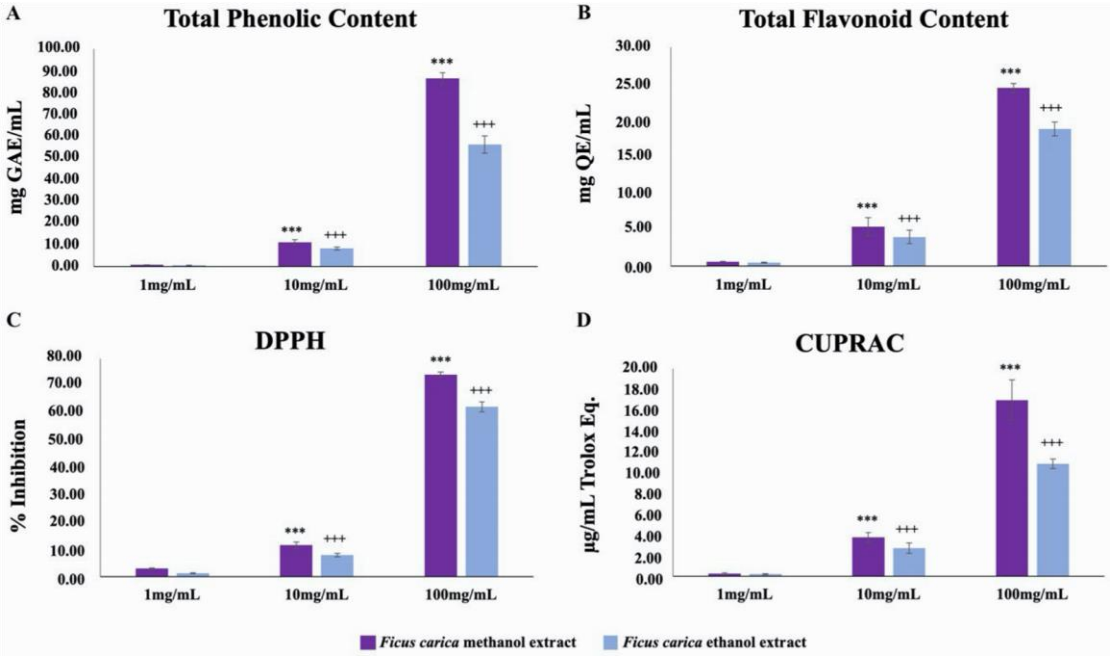

Figure 2. **A**) Total Phenolic content, **B**) Total Flavonoid content, **C**) 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, and **D**) Cupric Reducing Antioxidant Capacity (CUPRAC) of Ficus carica L. ethanol and methanol extracts in concentrations between 1 mg/mL, 10 mg/mL and 100 mg/mL. Differences for Ficus carica L. ethanol extract ^{+}p <0.05, ^{++}p <0.01, ^{+++}p <0.001 and for methanol extract $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 values were considered statistically significant.

In the methanol and ethanol extract of *Ficus carica* L., a specific dose of antioxidant showed a pro-ox- idant effect after a certain concentration (20 mg/mL; Figure 3). Doses after this critical dose will be the concentrations to be determined for cancer treatment.

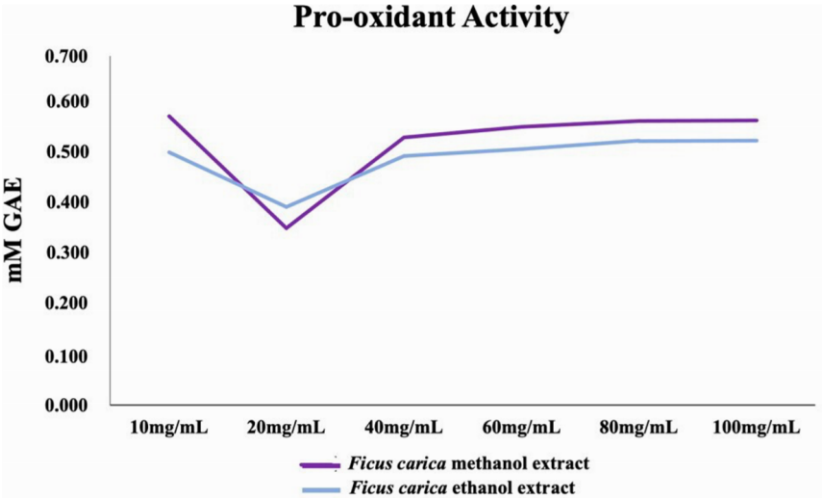

Figure 3. Pro-oxidant activity of Ficus carica L. methanol and ethanol extract with concentrations be- tween 10 mg/mL and 100 mg/mL. The extracts were calculated and expressed as mM GAE equivalent.

Cell viability and intracellular ROS (iROS) levels of *Ficus carica* L. methanol extract on cells

The results of the cytotoxicity and iROS assays can be observed in Figure 4. The methanol extract of *Ficus carica* L. inhibited the proliferation and significantly reduced iROS production of the AGS cells ($p<0.001$). The level of ATP viability increases up to 2 mg/mL in the cell and then decreases dose-depen- dent manner. When the cytotoxicity results were examined, the ROS levels in cancer cells decreased at low doses, while ROS increased as the dose increased. Since the metabolic activity of the cancer cell is high, the increased ROS originating from the fruit extract showed a synergistic effect.

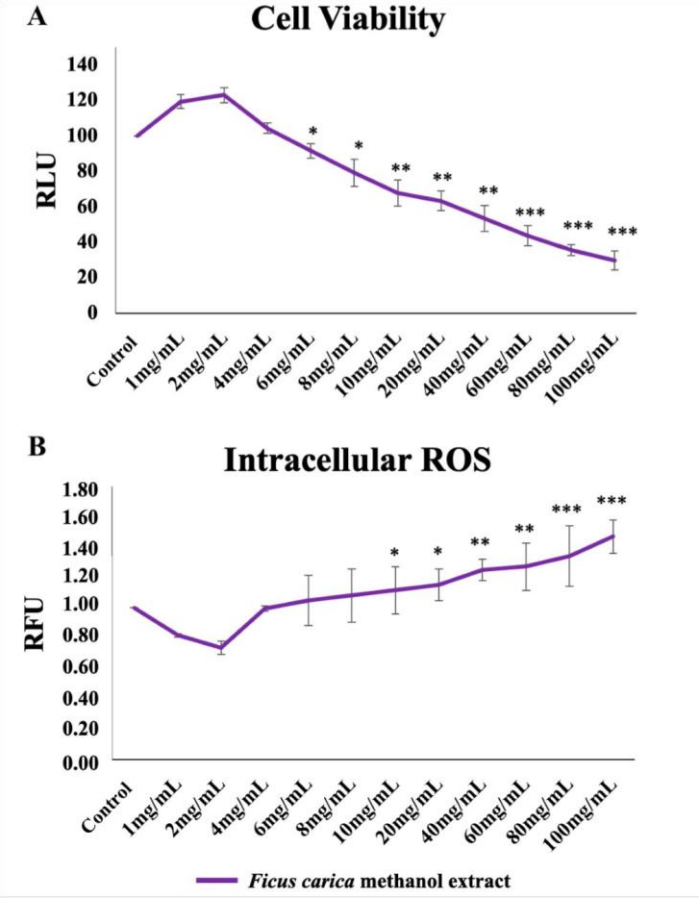

Figure 4. Effect of treatment by *Ficus carica* L. methanol extract on **A**) cell viability and **B**) intracellular reactive oxygen species (iROS) production after 24 hours in gastric adenocarcinoma cells (AGS). Differences for *Ficus carica* L. methanol extract $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 values were considered statistically significant.

Glutathione and MMP levels of *Ficus carica* L. methanol extract on cells

The reduced glutathione (GSH) levels and the induction of apoptosis in cell line investigated via mi- tochondrial pathways are shown in Figure 5. The results show that intracellular glutathione level and mitochondrial membrane potential decreased with increasing *Ficus carica* L. doses. This caused the cells to be taken to apoptosis.

Apoptosis of *Ficus carica,* L. methanol extract on cells

When apoptosis, one of the cell death mechanisms, was examined, there was a significant and concentration-dependent increase in the apoptotic cell population of gastric cancer cells upon treatment with *Ficus carica* L. methanol extract compared to control (Figure 6).

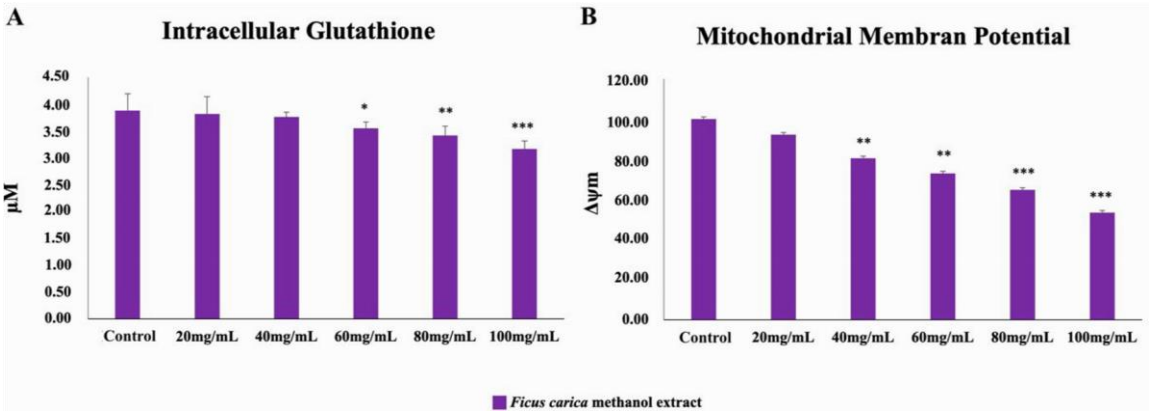

Figure 5. A) Glutathione (GSH) and B) mitochondrial membrane potential (MMP) levels of Ficus carica L. methanol extract concentrations 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL in gastric adenocarcinoma cells (AGS). $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 values were considered statistically significant.

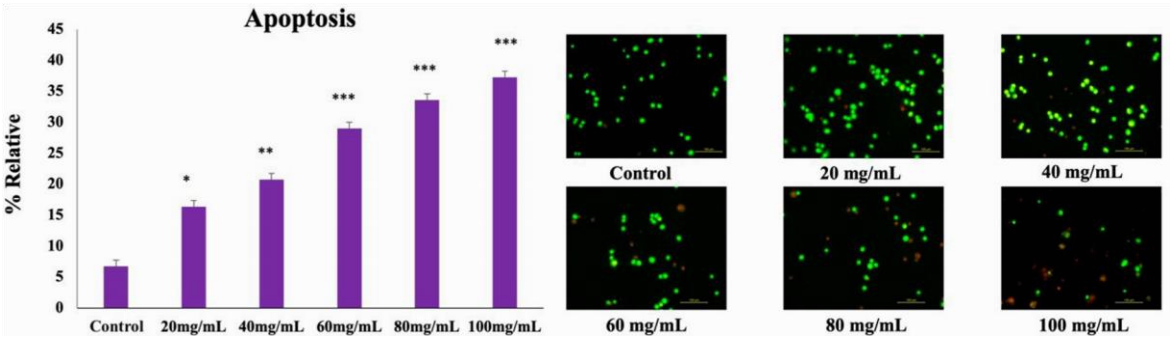

Figure 6. Apoptotic effect of *Ficus carica* L. methanol extract. The cells were treated with concentrations 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL 100 µg/mL in AGS cells. Results are given as % apoptosis and expressed as mean ± SD. By representative immunofluorescence images, apoptotic cells have shown in orange with fragmented apoptotic bodies and chromatin. The green color is the normal morphology of living cells. Yellow early apoptotic cells indicate nuclear restriction and chromatin thickening. Differenc- es for *Ficus carica* L. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 values were considered statistically significant.

DNA damage of *Ficus carica* L. methanol extract on cells

The parameters related to the tail length and the percentage of DNA in the tail were determined quanti- tatively by Comet assay. This assay is based on the principle of releasing damaged DNA from the nucleus by electrophoresis. If the DNA contains breaks, the damaged DNA migrates towards the nucleus, and when stained with a fluorescent binding dye such as ethidium bromide, these damaged cells take on a comet-like appearance (Figure 7). At 24 hours treatment time, DNA damage levels were significantly increased at all *Ficus carica* L. methanol concentrations compared to the control treatment in a dose-de- pendent manner (p<0.001).

DISCUSSION

Gastric cancer is one of the gastrointestinal tract tumors characterized by epidemiological and his- topathological differences and associated with poor prognosis[28]. Although the chemotherapy appli- cation used in current

treatment methods in advanced stage gastric cancer affects the course of the disease in a good way, the development of resistance of cancer cells to chemotherapy drugs and the formation of cytotoxic side effects affect the treatment negatively. Therefore, in addition to existing

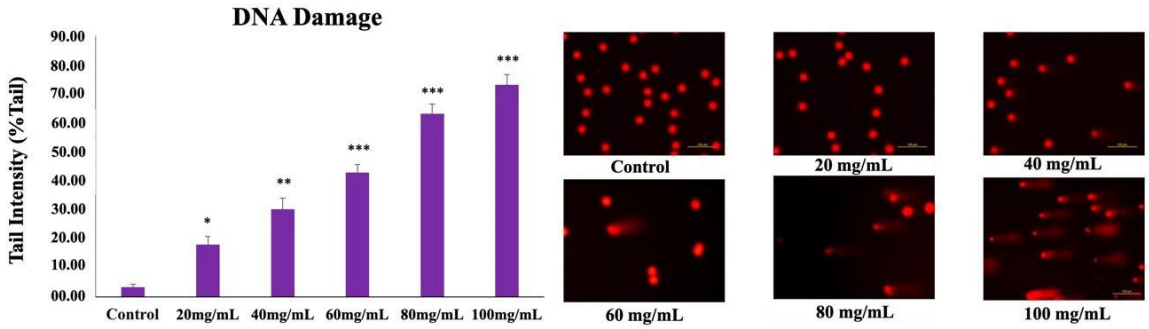

Figure 7. The effect of Ficus carica L. methanol extract concentrations 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL 100 µg/mL on DNA damage in AGS cells. Results are given as % tail density and expressed as mean ± SD. By representative immunofluorescence images, damaged DNAs have been shown in bright nuclei and comet-like nuclei. The undamaged DNAs were round. Differences for Ficus carica L. methanol extract $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 values were considered statistically significant.

treatment methods, there is a need to develop new therapeutic targets with higher therapeutic effi- cacy and less cytotoxic side effects. This study investigated the antioxidant profile and the cytotoxic, genotoxic, and apoptotic effects of *Ficus carica* L. extracts in different organic solvents on AGS gastric cancer cells.

The fruits of *Ficus carica* L. contain plenty of vitamins, sugars, carbohydrates, minerals, phenolic compounds and organic acids. Its fruits, leaves, roots, shoots, and parts, such as latex, are used to treat several human diseases[29]. Some studies have determined the antioxidant and high phenolic contents of different parts of the *Ficus carica* L. plant, showing that it can be used as one of the medicinal plants in treatment support[30]. In this study, the results are supported by previous studies showing the antioxidant effects of *Ficus carica* L. extracts. When the antioxidant profiles of *Ficus carica* L. methanol and ethanol extracts were examined, it was found that the methanol extract showed more antioxidant effects. Radical scavenging activity and copper reducing activity were also found to be strong.

Prooxidants are toxic substances that cause oxidative damage to diseases. Oxidative stress may oc- cur due to an imbalance of antioxidants and prooxidants in the organism which leads to the formation of reactive species such as ROS. Reactive nitrogen and phenoxy radicals can damage cellular macromol- ecules and cause mutations by affecting DNA and rapidly dividing cells, resulting in the emergence of cancer [31,32]. As a result of these mutations, cancer development becomes more accessible. The antiox- idant system cleans damaged molecules, prevents mutations, repairs oxidative damage, and further- more prevents radical formation before damage[33]. In the methanol and ethanol extract of *Ficus carica* L., a specific dose of antioxidant showed a prooxidant effect after a certain dose. In the evaluation of biological activity, it was revealed that *Ficus carica* L. methanol extracts increased cytotoxicity and iROS levels with increasing doses on gastric cancer cell line AGS.

In a study by Khodarahmi et al[34], the cytotoxic effects of ethanolic fruit and leaf extracts on the HeLa cell line were examined. Another study reported that seed, fruit and leaf extracts of *Ficus carica* L. were cytotoxic against

A549, BT549 and MCF-7 cell lines[35]. The level of ATP viability increases up to 2 mg/mL in the cell and then decreases dose-dependent manner in our study. This suggests that the extract has a cytotoxic effect against also AGS cells.

As a result of changes in the antioxidant system, an increase in the basal iROS level occurs. This high ROS can cause dysfunction of the mitochondrial pathway and induce cellular apoptosis[36]. Glutathione is necessary to maintain the antioxidant defense system and scavenge ROS. It also plays a role in DNA and protein synthesis, enzyme activity, and gene expression. The imbalance of the glutathione system has a vital role in cancer and its progression[37]. Besides, disruption of mitochondrial membrane integrity causes depolarization of MMP and plays an important role in programmed cell death[34,38]. Intracellular glutathione reduction is closely associated with apoptotic cell death triggered by a wide variety of stim- uli, including activation of death receptors, cytotoxic drugs, stress, and environmental agents[39]. This study observed that *Ficus carica* L. methanol extract treatment caused apoptosis in dose dependent manner. In addition, *Ficus carica* L. methanol extract caused apoptosis in AGS cells by reducing MMP and glutathione in a dose-dependent manner.

CONCLUSIONS

In the genotoxic activity of *Ficus carica* L. methanol extract evaluated by alkaline single-cell electropho- resis, after incubating the doses below IC_{50} in cancer cells for 24 hours, the comet assay method was applied to evaluate the DNA damage percentage. This study showed that *Ficus carica* L. methanol ex- tract induced DNA damage dose-dependently. Significant decreases were observed in the absorbance values of all concentrations compared to the control. In other studies, genotoxic activity was evaluated by gastric cancer cells[40], and it was found to be compatible with our results. As a result of our study, it is thought that *Ficus carica* L., which has an anti-cancer effect, can be used together with routine treat- ments in the treatment of gastric cancer.

FINANCIAL SUPPORT:
None.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE: None.

CONSENT TO PUBLICATION:

The Authors approved the manuscript and give their consent for submission and publication.

AVAILABILITY OF DATA AND MATERIAL:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST:

The Authors declare no conflict of interest.

FUNDING:
None.

AUTHOR CONTRIBUTIONS:

Eray Metin Guler contributed to the design and experimental process of the research; Beyza Nur Ozkan and Kubra Bozali contributed to data collection and manuscript writing; Tunahan Dundar and Abdurrahim Kocyigit contributed to the interpre- tation and analysis of the data. All authors critically reviewed the manuscript and agreed to be fully responsible for ensuring the integrity and accuracy of the work.

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REFERENCES

Song M, Rabkin CS, Camargo MC. Gastric Cancer: an Evolving Disease. Curr Treat Options in Gastroenterol 2018; 16: 561-569.

Cordova-Marks FM, Carson WO, Monetathchi A, Little A, Erdrich J. Native and Indigenous Populations and Gastric Cancer: A Worldwide Review. Int J Environ Res Public Health 2022; 19: 5437.

Johnston FM, Beckman M. Updates on Management of Gastric Cancer. Curr Oncol Rep 2019; 21: 67.

Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumor Biol 2017; 39: 1010428317714626.

Joshi SS, Badgwell BD. Current treatment and recent progress in gastric cancer. CA Cancer J Clin 2021; 71: 264-279.

Kitayama J, Ishigami H, Yamaguchi H, Sakuma Y, Horie H, Hosoya Y, Lefor AK, Sata N. Treatment of patients with peritoneal metastases from gastric cancer. Ann Gastroenterol Surg 2018; 2: 116-123.

Roshani M, Jafari A, Loghman A, Sheida AH, Taghavi T, Zadeh SST, Michael RH, Homayounfal M, Mirzaei H. Applications of resveratrol in the treatment of gastrointestinal cancer. Biomed Pharmacother 2022; 153: 113274.

Berretta M, Dal Lago L, Tinazzi M, Ronchi A, La Rocca G, Montella L, Di Francia R, Facchini BA, Bignucolo A, Montopoli M. Evaluation of Concomitant Use of Anticancer Drugs and Herbal Products: From Interactions to Synergic Activity. Cancers 2022; 14: 5203.

Berretta M, Rinaldi L, Taibi R, Tralongo P, Fulvi A, Montesarchio V, Madeddu G, Magistri P, Bimonte S, Trovò M, Gnagnarella P,

Cuomo A, Cascella M, Lleshi A, Nasti G, Facchini S, Fiorica F, Di Francia R, Nunnari G, Pellicanò GF, Guglielmino A, Danova M, Rossetti S, Amore A, Crispo A, Facchini G. Physician Attitudes and Perceptions of Complementary and Alternative Medicine (CAM): A Multicentre Italian Study. Front Oncol 2020; 10: 594.

Berretta M, Montella L. Integrative medicine in the cancer setting: a new challenge for physicians and patients. WCRJ 2022; 9: e2405.

Berretta M, Della Pepa C, Tralongo P, Fulvi A, Martellotta F, Lleshi A, Nasti G, Fisichella R, Romano C, De Divitiis C, Taibi R, Fiorica F, Di Francia R, Di Mari A, Del Pup L, Crispo A, De Paoli P, Santorelli A, Quagliariello V, Vincenzo Iaffaioli R, Tirelli U, Facchini G. Use of Complementary and Alternative Medicine (CAM) in cancer patients: An Italian multicenter survey. Onco- target 2017; 8: 24401.

Jones E, Nissen L, McCarthy A, Steadman K, Windsor C. Exploring the use of complementary and alternative medicine in cancer patients. Integr Cancer Ther 2019; 18: 1534735419854134.

Inci H, Inci F. Complementary and alternative medicine awareness in cancer patients receiving chemotherapy. WCRJ 2020; 7: e1752.

Fasinu PS, Rapp GK. Herbal interaction with chemotherapeutic drugs—a focus on clinically significant findings. Front Oncol 2019; 9: 1356.

Daliu P, Santini A, Novellino E. From pharmaceuticals to nutraceuticals: bridging disease prevention and management. Expert Rev Clin Pharmacol 2019; 12: 1-7.

Morovati MR, Ghanbari-Movahed M, Barton EM, Farzaei MH, Bishayee A. A systematic review on potential anticancer activ- ities of Ficus carica L. with focus on cellular and molecular mechanisms. Phytomedicine 2022; 154333.

Nuri Z, Uddin MS. A review on nutritional values and pharmacological importance of Ficus carica. J Curr Res Food Sci 2021; 2: 7-11.

Arvaniti OS, Samaras Y, Gatidou G, Thomaidis NS, Stasinakis AS. Review on fresh and dried figs: Chemical analysis and oc- currence of phytochemical compounds, antioxidant capacity and health effects. Food Res Int 2019; 119: 244-267.

Mawa S, Husain K, Jantan I. Ficus carica L. (Moraceae): Phytochemistry, Traditional Uses and Biological Activities. Evid Based Complement Alternat Med 2013; 2013: 974256.

Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112-119.

Slinkard K, Singleton VL. Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enol Vit 1977; 28:

49.

Apak R, Guclu K, Ozyurek M, Esin Karademir S, Ercag E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int J Food Sci Nutr 2006; 57: 292-304.

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-1200.

Kim SJ, Park YS, Paik HD, Chang HI. Effect of anthocyanins on expression of matrix metalloproteinase-2 in naproxen-induced gastric ulcers. Br J Nut 2011; 106: 1792-1801.

Kocyigit A, Guler EM. Curcumin induce DNA damage and apoptosis through generation of reactive oxygen species and re- ducing mitochondrial membrane potential in melanoma cancer cells. Cell Mol Bio 2017; 63: 97-105.

McGahon AJ, Martin SJ, Bissonnette RP, Mahboubi A, Shi Y, Mogil RJ, Nishioka WK, Green DR. The end of the (cell) line: methods for the study of apoptosis in vitro. Methods Cell Biol 1995; 46: 153-85.

Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184-91.

Röcken C. Molecular classification of gastric cancer. Expert Rev Mol Diagn 2017; 17: 293-301.

Mawa S, Husain K, Jantan I. Ficus carica L. (Moraceae): Phytochemistry, Traditional Uses and Biological Activities. Evid Based Complement Alternat Med 2013; 2013: 974256.

Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, Altman A, Kerem Z, Flaishman AM. Antioxidant activities and anthocyanin content of fresh fruits of common fig (Ficus carica L.). J Agric Food Chem 2006; 54: 7717-7723.

Vickers NJ. Animal communication: when i'm calling you, will you answer too? Curr Bio 2017; 27: R713-R715.

Veberic R, Jakopic J, Stampar F. Internal fruit quality of figs (Ficus carica L.) in the Northern Mediterranean Region. Ital J Food Sci 2008; 20: 255-262.

Kryston T, Georgiev A, Pissis P, Georgakilas A. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. Mutat Res Fundam Mol 2011; 711: 193-201.

Khodarahmi GA, Ghasemi N, Hassanzadeh F, Safaie M. Cytotoxic effects of different extracts and latex of Ficus carica L. on HeLa cell line. Iran J Pharm Res 2011; 10: 273.

Alanazi AM. The cytotoxicity effect of rosemary and myrrh extracts on cancer cell lines. 2016, Tennessee State University.

Jarvis RM, Hughes SM, Ledgerwood EC. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free Radic Biol Med 2012; 53: 1522-1530.

Corso CR, Acco A. Glutathione system in animal model of solid tumors: From regulation to therapeutic target. Crit Rev Oncol Hematol 2018; 128: 43-57.

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Front Pharmacol 2021; 12: 643972.

Franco R, Cidlowski JA. Apoptosis and glutathione: beyond an antioxidant. Cell Death Differ 2009; 16: 1303-1314.

Hashemi SA, Abediankenari S, Ghasemi M, Azadbakht M, Yousefzadeh Y, Dehpour AA. The effect of fig tree latex (Ficus carica) on stomach cancer line. Iran Red Crescent Med J 2011; 13: 272.