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Positive effects of white tea on breast cancer: N-methyl-N-nitrozourea intraductal induced breast carcinoma model

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ABSTRACT

An objective of this study was to investigate the chemopreventive and therapeutic effects of white tea (WT) on breast cancer.

Fifty Sprague-Dawley rats were divided into five groups: control, MNU, taxol, low dose white tea (LWTE) and high dose white tea (HWTE). Either tap water or WT was given by oral gavage for 4 weeks. At the end of this period, mammary carcinoma was induced in all groups except the control group. Then oral gavage continued for 14 weeks. For laboratory analyses, blood and mammary tissues were collected.

LWTE was determined to prevent lipid peroxidation, DNA and protein damage, reduce inflammatory cytokine levels, prevent cancer cell proliferation by inducing apoptosis, inhibit tumour growth by blocking M-CSF release and NRP-2 activity. Hyperplasia and histopathological changes increased by MNU exposure were successfully repaired with WT.

The results of our study show that WT has positive effects in preventing and treating breast cancer.

1. Introduction

According to World Health Organization statistics for 2022, breast cancer, one of the three most common cancers in the world, is the most commonly diagnosed cancer in women, accounting for 11.6 % of total breast cancer cases in women, and is the leading cause of cancer deaths, with an estimated 2.3 million new cases of breast cancer detected and 666 thousand women dying from breast cancer (Swanton et al., 2024).

Surgery, targeted therapies, radiotherapy and chemotherapy are the main treatments for breast cancer. Taxol is one of the most widely used chemotherapeutic agents in the treatment of breast cancer (Gong et al., 2023). Chemotherapy drugs used to treat breast cancer not only destroy cancerous cells but also damage healthy cells, resulting in numerous side effects that negatively impact patients' quality of life (Kurt & Kapucu, 2018). Compared with chemical drugs, plant-derived bioactive compounds have the advantages of being safer, bioavailable and less toxic (Das et al., 2023).

7,12-Dimethylbenz(a)anthracene (DMBA) and N-methyl N-nitrosourea (MNU) are chemical carcinogens that cause breast cancer (Arivazhagan & Sorimuthu Pillai, 2014). Compared to MNU, DMBA has slower carcinogenic activity, so DMBA-induced mammary tumors take longer to develop (Budán et al., 2009). Although it is easy to inject and prepare MNU because it is water soluble, it is more difficult to use DMBA because it is fat soluble (Tsubura, Lai, Miki, et al., 2011). Breast cancer induced chemically by MNU is widely used because it has many similarities with human breast cancer (Gao et al., 2021; Tsubura, Lai, Kuwata, et al., 2011). With traditional methods, namely, intraperitoneal (i.p.) or intravenous injection of MNU for model generation, mammary

Abbreviations: 8-OhdG, 8-Hydroxy-2′ deoxyguanosine; C, Control; CA 15-3, Cancer antigen 15-3; DCIS, Ductal Carsinoma *in situ*; DMBA, 7,12-dimethylbenz(a) anthracene; DMSO, Dimethyl sulfoxide; DNA, Deoxyribonucleic acid; EGCG, Epigallocatechin gallate; ER-α, Estrogen Receptor-α; GAE, Gallic acid equivalent; GSH, Glutathione; H+E, Harris haematoxylin and Eosin G; HPLC, High-Performance Liquid Chromatography; HWTE, High dose white tea extract; IDC, Invasive Ductal Carcinoma; IL-6, Interleukin-6; ILC, Invasive Lobular Carcinoma; LWTE, Low dose white tea extract; M-CSF, Macrophage-Colony Stimulating Factor; MDA, Malondialdehyde; MNU, N-methyl-N-nitrozourea; MMP-9, Matrix metalloproteinase-9; NRP-2, Neuropilin-2; PC, Protein carbonyl; QE, Quercetin equivalent; TAX, Taxol; TBARS, Thiobarbituric acid reactive substances; TIMP-1, Tissue inhibitors of metalloproteinas-1; TNF-α, Tumor necrosis factor; VEGF, Vascular endothelial growth factor; WT, White tea.

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tumors arise randomly in six pairs of mammary glands, resulting in tumor induction in unpredictable locations (Gao et al., 2021; Murata et al., 2006; Stearns et al., 2011). With intraductal (i.duc) administration of MNU, the agent is placed directly into the mammary ductal system so that tumors can be induced in predictable locations (Teo & Sukumar, 2014).

Camellia sinensis which belongs to Theaceae family, is the most consumed beverage after water in the world (Üstün and Demirci, 2013). It has been reported that white tea (WT) produced from the tea plant by minimal processing has superior properties in some respects compared to other tea varieties, especially with its bioactive components, and is associated with many diseases including cancer due to the strong antioxidant activities of phenolic substances in its content (Hinojosa-Nogueira et al., 2021; Salman & Üniversitesi, 2018). Although many studies have been carried out on green tea and catechins, especially Epigallocatechin-3-gallate (EGCG), the existing studies on WT are limited (Farhan, 2022).

In recent studies, it has been reported that WT inhibits apoptosis by inhibiting lipid peroxidation, reducing oxidative stress and caspase-3 expression (Ylldlrlm et al., 2020). It has also been shown to effectively suppress the proliferation of HeLa and BEL-7402 cells, inhibit the proliferation of HT-29 cells, activate caspases and protect the DNA of healthy cells against oxidative damage, and induce apoptosis in non-small cell lung cancer (NSCLC) cell lines (Habanjar et al., 2023; Liu et al., 2018; Mao et al., 2010).

In a comparison of all tea types, EGCG, which is the predominant catechin, was found to be highest in WT (Zhao et al., 2019). EGCG has been shown to inhibit breast cancer tumors and enhance the effects of anticancer drugs. It has also been reported to be a complementary and versatile adjuvant candidate for breast cancer treatment (Marín et al., 2023). Previous research has revealed that taking compounds naturally occurring in foods has a significant impact on antioxidant potency, bioavailability and safety. Experimental research using a dietary matrix rather than isolated compounds is needed on the chemoprevention benefits of natural products. Green tea has been shown to suppress cell migration and breast cancer formation more effectively than EGCG, and in this context, it has been reported that the mixture of phytochemicals may increase the anticancer potential (Santos et al., 2024).

Despite the fact that there are many treatments for breast cancer today, none of them offer a complete cure and there are still many people who suffer from breast cancer and die despite the treatments applied. The addition of functional foods to the diet may contribute to the treatment or prevent the formation of cancer. In our literature review, we found no studies on the preventive or therapeutic effects of WT, which is one of such functional foods and can be used as a routine beverage in daily life, on breast cancer. The objectives of this research are to determine whether WT has chemopreventive and therapeutic effects. If our findings are positive, it will contribute to the decrease in the incidence of breast cancer, which is an important health problem especially for women all over the world, and thus to the prevention of cancer-related complications. We also aim to shed light on different perspectives on the treatment or prevention of breast cancer in the future through the results obtained.

2. Materials and methods

2.1. Antioxidant activity studies of white tea

2.1.1. Supply of white tea samples

Pesticides are used against agricultural pests in teas produced outside Türkiye. In teas grown in Türkiye, pesticides are not used because snow and cold kill agricultural pests naturally. Since chemical pesticides and additives are not used in the production of tea in Türkiye, only one hundred percent of natural and healthy tea is produced. This feature makes Turkish tea more invaluable. The WT samples used in the study were obtained from the WT shoots harvested in the spring of 2022 in the province of Rize by the General Directorate of ÇAYKUR.

2.1.2. Determination of the total polyphenol and flavonoid contents in white tea extracts and HPLC analysis

To determine the total polyphenol content of the extracts, 0.1 g of WT was weighed and added to 50 mL 100 °C of boiled water. The mixture was infused for 10 min on a magnetic stirrer and filtered. The modified Folin-Ciocalteu spectrophotometric method was used to determine the total polyphenol content (Horžić et al., 2009).

In order to quantify the total flavonoid content of the extracts, 0.05 g of WT was weighed and added to 50 mL 100 °C of boiled water. The mixture was infused for 10 min on a magnetic stirrer and filtered. The aluminum chloride method was used to determine the total flavonoid content (Chang et al., 2002; Lin & Tang, 2007).

To perform the HPLC analysis of the WT extracts, 1.5 g of WT was weighted and added to 100 mL 100 °C boiled hot water, brewed for 10 min and then filtered. The ISO14502-2/2005 standard method was used to conduct the analysis.

2.2. Animal studies

2.2.1. Preparation of N-methyl-N-nitrosourea and white tea

The weight of the rats were measured weekly and the required WT was adjusted accordingly. WT was brewed according to the procedure we used to determine polyphenol and flavonoid content. Freshly prepared white teas were brought to room temperature daily and used.

N-Methyl-N-nitrosourea (MNU) was obtained from TRC (Canada). MNU was dissolved in dimethyl sulfoxide (DMSO) or 0.9 % saline at a ratio of 1 (g):10 (mL):10 (mL). The human dose equivalent was calculated using the formula ($k \times w^{2/3}$) $\times 10^{-4}$ (Gao et al., 2021).

2.2.2. The experimental animals and study groups

Fifty female Sprague–Dawley rats aged 3 weeks and weighing 120–130 g were used in the study. The rats were housed in transparent polyethylene cages under standard conditions (24 ± 2 °C, 45 ± 5 % relative humidity, and a 12 h light/dark cycle). Their diet consisted of standard pellet feed and access to tap water *ad libitum*. The experimental protocol was approved by the Local Ethics Committee of Recep Tayyip Erdogan University Animal Experiments (Decision number: 2021-17/02.12.2021).

Rats were randomly divided into 5 groups with 10 animals in each group. Oral gavage applications, which were started after one week of adaptation, were continued at the doses and frequencies specified in Table 1 until the end of the study.

The doses of WT to be given to rats were determined as 50 mg/kg/mL and 200 mg/kg/mL based on the literature (Cui et al., 2012). For the first 4 weeks of the study, 1 ml tap water was administered to control (C), N-Methyl-N-Nitrosourea (MNU) and Taxol (TAX) groups, 50 mg/kg/mL to

Table 1
Animal groups and practices.

Groups	Number of Rats	Intraductal Injection	Oral Gavage Applications	
			Dose and Agent	Frequency of Administration
1. Group (C)	10	DMSO+ % 0.9 Salin	1 mL (water)	5 days a week
2. Group (MNU)	10	MNU	1 mL (water)	5 days a week
3. Group (TAX)	10	MNU	1 mL (water)	5 days a week
4. Group (LWTE)	10	MNU	50 mg/kg/mL (WT)	5 days a week
5. Group (HWTE)	10	MNU	200 mg/kg/mL (WT)	5 days a week

C: Control, MNU: N-Methyl-N-Nitrosourea,TAX: Taxol, LWTE: Low-dose white tea extract, HWTE: High-dose white tea extract.

the low dose white tea (LWTE) group and 200 mg/kg/mL to the high dose white tea (HWTE) group by oral gavage. At the end of the 4th weeks, 100 mg/kg ketamine hydrochloride (Ketalar, 100 mg/10 mL, Pfizer, Istanbul, Türkiye) and 10 mg/kg rompun (Rompun, 2 % 25 mL, Bayer, USA) were administered i.p. and the nipples of the rats were exposed with depilatory foam to avoid damage. During the anesthetization of the rats, sterile artificial tear drop was administered to prevent dry eyes (Bu & Li, 2020). The C group received 25 μl of MNU solvent (DMSO+0.9 % NaCl), the other groups received 25 μl of MNU (1.5 mg/25 μl) injected i.duc into the third right nipple. To monitor i.duc migration, sesame seed-sized bromophenol blue was added to the MNU solution (Bu & Li, 2020).

After MNU administration, oral gavage administration was continued in all groups for 14 weeks as indicated in Table 1. Once tumor formation had commenced, the TAX group was administered 5 mg/kg taxol i.p. on a weekly basis for a period of three weeks (Merz et al., 2011).

2.3. Termination of the experiment and collection of samples

2.3.1. Taking the vaginal swab

During the final week of the study, vaginal smears were collected from the rats to determine their menstrual cycle. It is based on the principle of gently swabbing the vaginal wall by rotating a cotton-tipped swab moistened with saline in the vagina (Mohammed et al., 2018). The preparations stained with the Papanicolaou method were evaluated under a microscope, and those in the proestrus or diestrus phase were identified and sacrificed. The same procedure was applied to rats in other cycles by waiting for the appropriate day.

2.3.2. Sample collection and preparation

At the conclusion of the experiment, the rats were anaesthetised with a combination of ketamine (100 mg/kg, i.p.) and Rompun (10 mg/kg, i.p.). The hind foot was squeezed with pliers to control the depth of anaesthesia. The mammary tissues of rats that were sacrificed under anesthesia were preserved in a 10 % formaldehyde solution for histopathological and immunohistochemical analyses. Blood samples from rats were collected in tubes without anticoagulant tubes from the intracardiac and kept at room temperature for one hour to allow fibrin to form. The samples were then centrifuged at 3500 rpm for 15 min at 2–8 °C. The obtained supernatants were stored at −80 °C until the biochemical parameters were studied.

2.4. Biochemical, histopathological and immunohistochemical analyses

2.4.1. Biochemical analyses

2.4.1.1. Malondialdehyde (MDA) and glutathione (GSH) analysis. The MDA was measured using a modified version of the Draper and Hadley method (Draper & Hadley, 1990). The GSH were determined by the Ellman reagent method (Ellman, 1959).

2.4.1.2. ELISA measurements in serum. The levels of VEGF (E0659Ra), NRP-2 (E2180Ra), MMP-9 (E0321Ra), PC (E0870Ra), TNF-α (E0764Ra), IL-6 (E0135Ra), and CA 15-3 (E1139Ra) were determined using Bioassay Technology Laboratory (BT-LAB) brand kits, while estrogen receptor-α (E-EL-R1006), M-CSF (E-EL-R0601), and TIMP-1 (E-EL-R0540) levels were determined using elabscience brand kits. Enzyme-linked immunosorbent assay (ELISA) was used in accordance with the manufacturer's recommendations.

2.4.2. Histopathological analysis

The samples of mammary gland tissue removed from rats were trimmed to a volume of 1.5 cm^3 and fixed in 10 % phosphate-buffered formalin solution for 24–36 h. After the fixation stage, the specimens were placed in tissue tracking cassettes and kept for two hours in dehydration (with increasing ethanol series), mordanting stage, soft paraffin embedding and finally hard paraffin for routine histological follow-up procedures using a fully automatic tissue tracking device.

After routine histological tissue follow-up, the specimens were embedded in tissue embedding cassettes using a tissue embedding device and hard paraffin. Paraffin blocks of mammary gland tissue were sectioned at 3–4 μm thickness with a rotary microtome and stained with Harris hematoxylin and eosin G.

2.4.3. Immunohistochemistry (IHC)

In our study, Caspase-3 (rabbit polyclonal, ab32351, Abcam, UK) was used to detect apoptotic cells, Ki-67 primary antibodies (rabbit polyclonal, ab15580, Abcam, UK) were used to observe cell proliferation, and 8-OHdG primary antibodies (rabbit polyclonal, BS-1278R, Thermo Scientific, Germany) were used to detect DNA damage caused by free oxygen radicals. Along with the primary antibody, secondary antibody (goat anti-rabbit IgG H&L (HRP), ab205718, Abcam, UK) kits were used.

Sections of breast tissue were deparaffinized in accordance with the manufacturer's guidelines, and then antigen retrieval was performed using a Leica IHC/ISH (Leica Biosystems, Leica, Germany) device and incubated with primary and secondary antibodies for 60 min. The breast tissue sections were stained with diaminobenzidine tetrahydrochloride and Harris hematoxylin.

2.4.4. Semi-quantitative analysis

Mammary gland epithelial cells showing Caspase-3, Ki-67 and 8-OHdG positivity were scored as shown in Table 2. The experimental group of the study was analyzed by a double-blind histopathologist. In each preparation, 30 different areas were randomly selected and scored.

2.5. Statistical analyses

The data obtained by biochemical, histopathological and immunohistochemical analyses were evaluated by the Shapiro–Wilk test, skewness–Kurtoisis values, Q–Q plots and Levene's test for normally distributed data. One-way ANOVA and Tukey's LSD tests were used to evaluate whether there was a significant difference between the groups for the data fitting a normal distribution. Nonparametric data were calculated as the median-25 %–75 % interquartile range and analyzed by the Bonferroni and Kruskal–Wallis tests (Mann–Whitney *U* test for pairwise comparisons). p values < 0.05 were regarded as significant.

3. Results

3.1. Body weight changes of the animals

The animals' total body weight gain was measured weekly throughout the study period. The percent weight gain was calculated with the formula (Last weight − First weight)/First weight * 100. The animals' body weight and percent weight gain are given in Table 3.

When the final weights of the animals were compared, it was determined that there was a statistically significant difference between C group and MNU group ($p = 0.001$), MNU group and TAX, LWTE, HWTE groups ($p = 0.001$, $p = 0.000$, $p = 0.000$, respectively). Similarly, it was determined that there was a significant difference between the C group

Table 2
Immune positivity scores.

Score	Finding
0	Less than <5 %
1	Between 6 % and 25 %
2	Between 26 % and 50 %
3	Less than >50 %

Table 3
Body weight and % weight gain.

Study Groups	Body weight and % weight gain		
	[1]Initial Weight (g)	[2]Finish Weight (g)	[2]Weight Gain (%)
C	123 (23)	192 ± 3,2[a,**]	79 ± 0,8[a,**]
MNU	128 (26)	171 ± 1,6	40 ± 7,6
TAX	124 (14)	189 ± 3,8[a,**]	70 ± 7,4[a,**]
LWTE	125 (20)	201 ± 3,3[a,***]	79 ± 6,1[a,***]
HWTE	118 (13)	196 ± 2,9[a,***]	72 ± 4,5[a,**]

[a] Significantly different compared with the MNU group.
[1] Kruskal Wallis,
[2] One-way ANOVA, LSD.
** p < 0,01.
*** p < 0,001.

and MNU ($p = 0.001$) group, MNU group and TAX, LWTE, HWTE groups ($p = 0.003$, $p = 0.000$, $p = 0.001$, respectively) n the percentage weight gain. The highest weight gain was found in the LWTE group and the lowest weight gain was found in the MNU group.

3.2. Biochemical results

3.2.1. Total phenolic and flavonoid contents of white tea extracts and HPLC analysis results

The total phenolic content of the WT extracts was determined to be 226 ± 3.13 mg GAE/g dry weight using gallic acid as a standard. The total flavonoid content was determined by the standard graph using quercetin at a concentration of 10.6 ± 2.8 mg QE/g dry weight. HPLC analysis of the WT extracts used in our study revealed that the total catechin content was 12.74 %, of which 9.2 % was EGCG. The results of the HPLC analysis of the extracts are presented in Table 4.

3.2.2. Results of the analysis of serum samples

3.2.2.1. Serum CA15-3, VEGF, NRP-2, M-CSF, MMP-9, ER-α and TIMP-1 levels. Serum CA 15-3 levels were higher in the MNU group compared to the C group. Compared with the MNU group; TAX and LWTE groups were lower and HWTE group was higher. However, the differences were not significant.

Serum MMP-9 levels were significantly higher in MNU, TAX and HWTE groups ($p = 0.035$, $p = 0.009$ and $p = 0.004$, respectively) compared to C group. Conversely, MMP-9 levels were observed to be reduced in the LWTE group and elevated in the TAX and HWTE groups in comparison to the MNU group; however, these differences were not significant.

Serum NRP-2 levels were higher in the MNU group compared to the C group. In comparison to the MNU group, the TAX and LWTE groups exhibited lower NRP-2 levels, while the HWTE group demonstrated higher levels, but there was a significant difference only with LWTE group ($p = 0.008$). Furthermore, NRP-2 levels were significantly lower in the TAX and LWTE ($p = 0.041$ and $p = 0.002$, respectively) groups compared to the HWTE group.

Table 4
HPLC analysis results for white tea.

White Tea Analysis (HPLC) Results	
	% Dry Matter
Gallic acid	0.11
Caffeine	5.01
EGC	0.38
C	0.00
EC	0.87
EGCG	9.20
ECG	2.28
Total Catechin	12.74

Serum VEGF levels were significantly higher in MNU, TAX, LWTE and HWTE ($p = 0.007$, $p = 0.001$, $p = 0.002$ and $p = 0.000$, respectively) groups compared to C group.

Serum TIMP-1 levels were higher in MNU and TAX groups and lower in LWTE and HWTE groups compared to C group, but the differences were not significant.

Serum M-CSF levels were significantly higher in the MNU and TAX ($p = 0.001$, $p = 0.049$, respectively) groups compared to the C group. Compared to MNU group, M-CSF levels were lower in TAX, LWTE and HWTE groups, but there was a significant difference only between LWTE and HWTE groups ($p = 0.000$, $p = 0.000$, respectively). Compared to the TAX group, M-CSF levels were significantly lower in the LWTE and HWTE groups ($p = 0.000$, $p = 0.008$, respectively).

ER-α levels were greater in the MNU group than in all other groups and were the same in the WT and control groups, but there was no significant difference between the groups.

The serum CA15-3, VEGF, NRP-2, M-CSF, MMP-9, ER-α and TIMP-1 levels are presented in Table 5.

3.2.2.2. Serum TNF-α and IL-6 levels. Serum IL-6 levels were significantly higher in the MNU and HWTE groups ($p = 0.019$, $p = 0.020$, respectively) compared to the C group. Compared to the MNU group, they were significantly lower in the TAX and LWTE groups ($p = 0.025$, $p = 0.020$, respectively). Similarly, when compared to the HWTE group, IL-6 levels were significantly lower in the TAX and LWTE groups ($p = 0.002$, $p = 0.001$, respectively).

Serum TNF-α levels were significantly higher in MNU, TAX and HWTE groups ($p = 0.005$, $p = 0.001$ and $p = 0.004$, respectively) compared to C group. Furthermore, when compared to the LWTE group, it was significantly higher in the MNU, TAX and HWTE groups ($p = 0.041$, $p = 0.011$ and $p = 0.04$, respectively).

The serum TNF-α and IL-6 levels are presented in Table 6.

3.2.2.3. Serum MDA, GSH and PC levels. Serum MDA levels were significantly higher in the MNU group ($p = 0.010$) compared to the C group. Compared to the MNU group, MDA levels were lower in TAX, LWTE and HWTE groups, but were significantly different only in the LWTE and HWTE groups ($p = 0.013$, $p = 0.023$, respectively).

Serum GSH levels were significantly lower in the MNU group ($p = 0.029$) compared to the C group. Compared to the MNU group, GSH levels were higher in TAX, LWTE and HWTE groups, but there was a significant difference only in the LWTE group ($p = 0.000$). In contrast, when comparing the LWTE group to the TAX and HWTE groups ($p = 0.005$, $p = 0.001$, respectively), GSH levels were significantly lower.

Serum PC levels were significantly higher in MNU, TAX and HWTE groups ($p = 0.003$, $p = 0.019$, $p = 0.002$ and $p = 0.027$, respectively) compared to C group. Compared to the MNU group, PC levels were lower in the TAX, LWTE and HWTE groups, but there was a significant difference only with the LWTE group ($p = 0.046$).

The serum MDA, GSH and PC levels are presented in Table 7.

3.3. Histopathological findings

The cancer types observed in the mammary gland sections of the MNU-treated groups were analyzed in accordance with the pathological diagnostic guidelines, as shown in Table 8. When mammary gland tissue sections stained with Harris hematoxylin and eosin G (H+E) were examined under a light microscope, we found that 62 % of the subjects had ductal carcinoma *in situ* (DCIS) type cancer caused by hyperplasic epithelial cells that filled the lumen of the gland (Table 8, Fig. 1*a*). In addition, we observed that in 28 % of the subjects, hyperplasic epithelial cells spread to the surrounding tissues along the lumen of the mammary gland, and invasive ductal carcinoma (IDC) type cancer formed (Table 8, Fig. 1*b*). In 10 % of the subjects, we observed that hyperplasic mammary gland epithelial cells spread to the surrounding tissues of the mammary

Table 5
Serum MMP-9, CA 15-3, NRP-2, VEGF, TIMP-1, ER-α and M-CSF levels.

Study groups	Measured Parameters						
	[1]CA 15-3 (ulU/mL)	[1]MMP-9 (ng/mL)	[1]NRP-2 (ng/mL)	[1]VEGF (ng/L)	[1]TIMP-1 (ng/mL)	[1]M-CSF (pg/mL)	[2]ER-α (ng/mL)
C	8.34 ± 0.6	0.85 ± 1.82	4.75 ± 0.12	245 ± 21	22.7 ± 0,74	54 ± 1.55	0,25 (0,01)
MNU	8.98 ± 0.5	1.35 ± 1.32[a,*]	5 ± 0.32	322 ± 16[a,**]	24.06 ± 0.32	70 ± 2.32[a,**]	0,27 (0,03)
TAX	8.38 ± 0.64	1.47 ± 0.2[a,**]	4.37 ± 0.17	338 ± 13[a,**]	24.4 ± 1.28	63 ± 2.3[a,*]	0,26 (0,03)
LWTE	8.51 ± 0.3	1.21 ± 1.34	4.05 ± 0.21[b,**,c,**]	338 ± 12[a,**]	21.5 ± 1.45	42 ± 3.34[b,***,c,***]	0,25 (0,02)
HWTE	10.57 ± 0,9	1.57 ± 0.75[a,**]	5.02 ± 0.24[c,*]	342 ± 25[a,***]	21.3 ± 0.98	52 ± 2.63[b,***,c,**]	0,25 (0,04)

[a] Significantly different compared with the C group.
[b] Significantly different compared with the MNU group.
[c] Significantly different compared with the TAX group.
[1] One-way ANOVA, LSD.
[2] Kruskal Wallis.
* $p < 0,05$.
** $p < 0,01$.
*** $p < 0,001$.

Table 6
Serum IL-6 and TNF-α levels.

Study Groups	Measured Parameters	
	IL-6 (ng/L)	TNF-α (ng/L)
C	3,15 ± 0,28	128 ± 17
MNU	3,99 ± 0,23[a,*]	166 ± 4[a,**,c,*]
TAX	3,25 ± 0,28[b,*,d,**]	172 ± 8[a,**,c,*]
LWTE	3,21 ± 0,18[b,*,d,**]	140 ± 4
HWTE	4,22 ± 0,84[a,*]	164 ± 8[a,**,c,*]

One-way ANOVA, LSD.
[a] Significantly different compared with the C group.
[b] Significantly different compared with the MNU group.
[c] Significantly different compared with the LWTE group.
[d] Significantly different compared with the HWTE group.
* $p < 0,05$.
** $p < 0,01$.

Table 7
Serum MDA, GSH and PC levels.

Study Groups	Measured Parameters		
	MDA (nmol/mL)	GSH (mmol/mL)	PC (ng/mL)
C	1,24 ± 0,8	341 ± 23	36 ± 6,3
MNU	2,08 ± 0,3[a,*]	268 ± 11[a,*]	54 ± 4,1[a,**]
TAX	1,66 ± 0,2	305 ± 23[c,**]	51 ± 0,76[a,*]
LWTE	1,34 ± 0,15[b,*]	385 ± 18[b,***]	43 ± 4,3[b,*]
HWTE	1,38 ± 0,16[b,*]	294 ± 16[c,**]	50 ± 2,24[a,*]

One-way ANOVA, LSD.
[a] Significantly different compared with the C group.
[b] Significantly different compared with the MNU group.
[c] Significantly different compared with the LWTE group.
* $p < 0,05$.
** $p < 0,01$.
*** $p < 0,001$.

Table 8
Breast cancer type in the MNU-induced breast cancer group.

Type	Percentages
Ductal Carsinom In situ *(DCIS)*	**62 %**
Adenoid cystic	55 %
Metaplastic	4 %
Adenocarcinoma with lymphoid-rich stroma	3 %
Invasive Ductal Carcinoma (IDC)	**28 %**
Adenocarcinoma with lymphoid-rich stroma	28 %
Invasive Lobular Carcinoma (ILC)	**10 %**
Lobular classical	8 %
Micropapillary	2 %

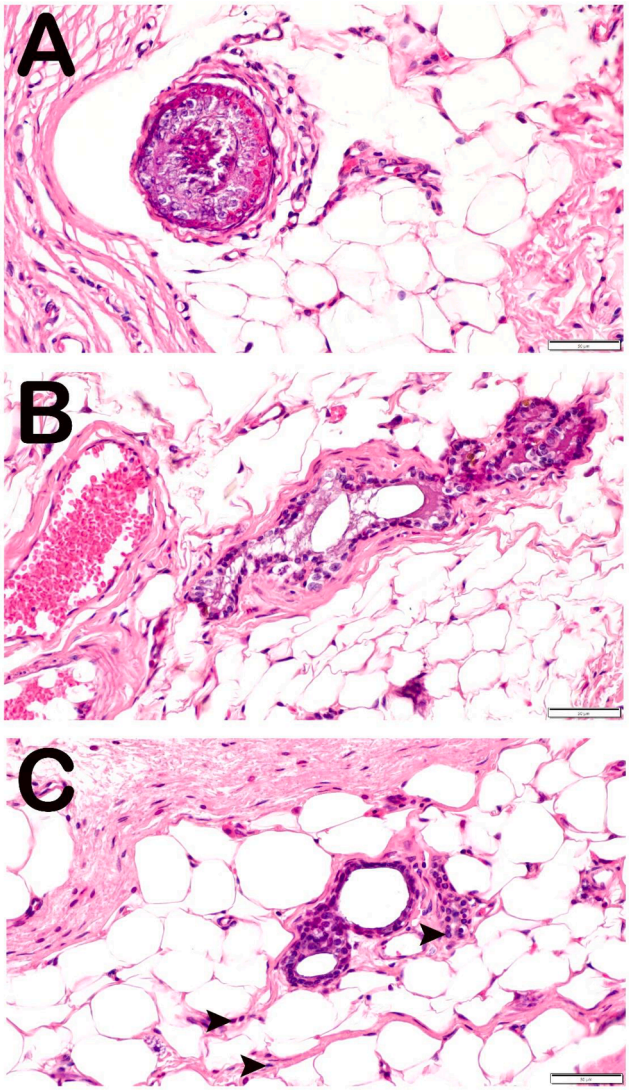

Fig. 1. Representative light microscopy images of cancer types observed in mammary gland sections from the MNU-treated groups. **A (×20):** Hyperplasic epithelial cells of the mammary gland fill the gland lumen and form DCIS type cancer (in 62% of the subjects). **B (×20):** Hyperplasic epithelial cells spread to the surrounding tissues along the lumen of the mammary gland, forming IDC type cancer (in 28% of the subjects). **C (×20):** Hyperplasic mammary gland epithelial cells spread to the surrounding tissues of the mammary gland and form ILC type cancer (in 10% of the subjects).

gland and formed invasive lobular carcinoma (ILC) type cancer (Table 8, Fig. 1*c*).

When the sections stained with H&E were examined under a light microscope, we observed that the sections of the C group contained mammary glands consisting of normal epithelial cells and myoepithelial cells (Fig. 2*a–b*). In contrast, we observed that the mammary glands of the MNU group contained hyperplasic epithelial cells. In mammary glands containing hyperplasic epithelial cells, we commonly observed DCIS type cancer formations (Fig. 2*c*). We detected reduced hyperplasic epithelial cells (spiral arrow) in the mammary glands of the TAX group (Fig. 2*d*). Similarly, we observed that the number of epithelial cells showing DCIS-type hyperplasia decreased in the mammary glands of the LWTE group (Fig. 2*e*). We observed that the number of mammary glands containing hyperplasic epithelial cells was reduced in the HWTE group (Fig. 2*f*).

3.4. Immunohistochemical (IHC) findings

3.4.1. Caspase-3 primary antibody

When we examined representative mammary gland sections incubated with a Caspase-3 primary antibody under a light microscope to identify apoptotic epithelial cells, we observed that the sections of group C contained mammary glands consisting of normal Caspase-3-negative epithelial cells and myoepithelial cells (Caspase-3 positivity score: 0 (0–0.5, Table 9, Fig. 3*a*)). Similarly, in mammary glands with DCIS cancer formations in the MNU group, hyperplasic epithelial cells were Caspase-3 negative (Caspase-3 positivity score: 0 (0–1), Table 9, Fig. 3*b*). In contrast, we detected an increase in the number of apoptotic hyperplasic epithelial cells that showed intense caspase-3 positivity in the TAX group (caspase-3 positivity score: 2 (1–2), Table 9, Fig. 3*c*). Similarly, we observed that the number of epithelial cells showing Caspase-3 positivity decreased in the mammary glands containing DCIS type hyperplastic epithelial cells in the LWTE group (caspase-3 positivity score: 2 (2–2) Table 9, Fig. 3*d*). In mammary gland sections from the HWTE group, an increased number of apoptotic hyperplastic epithelial cells were positive for caspase-3 (caspase-3 positivity score: 2(2–2), Table 9, Fig. 3*e*).

Table 9
Immune-Positivity Score Results (median (25 %–75 % interquartile range)).

Groups	Caspase-3	Ki-67	8-OHdG
Control	0(0–0)	0(0–0)	0(0–0)
MNU	0(0–1)	3(2–3)[a]	2(2–3)[a]
TAX	2(1–2)[a,b]	0(0–0.5)[b]	1(1–1)[b]
LWTE	2(2–2)[a,b]	0(0–1)[b]	1(0–1)[b]
HWTE	2(2–2)[a,b]	0(0–1)[b]	1(0–1)[b]

Mann–Whitney *U* test with Bonferroni corrections.
[a] $P < 0.001$ versus to C Group.
[b] $P < 0.001$ versus to MNU Group.

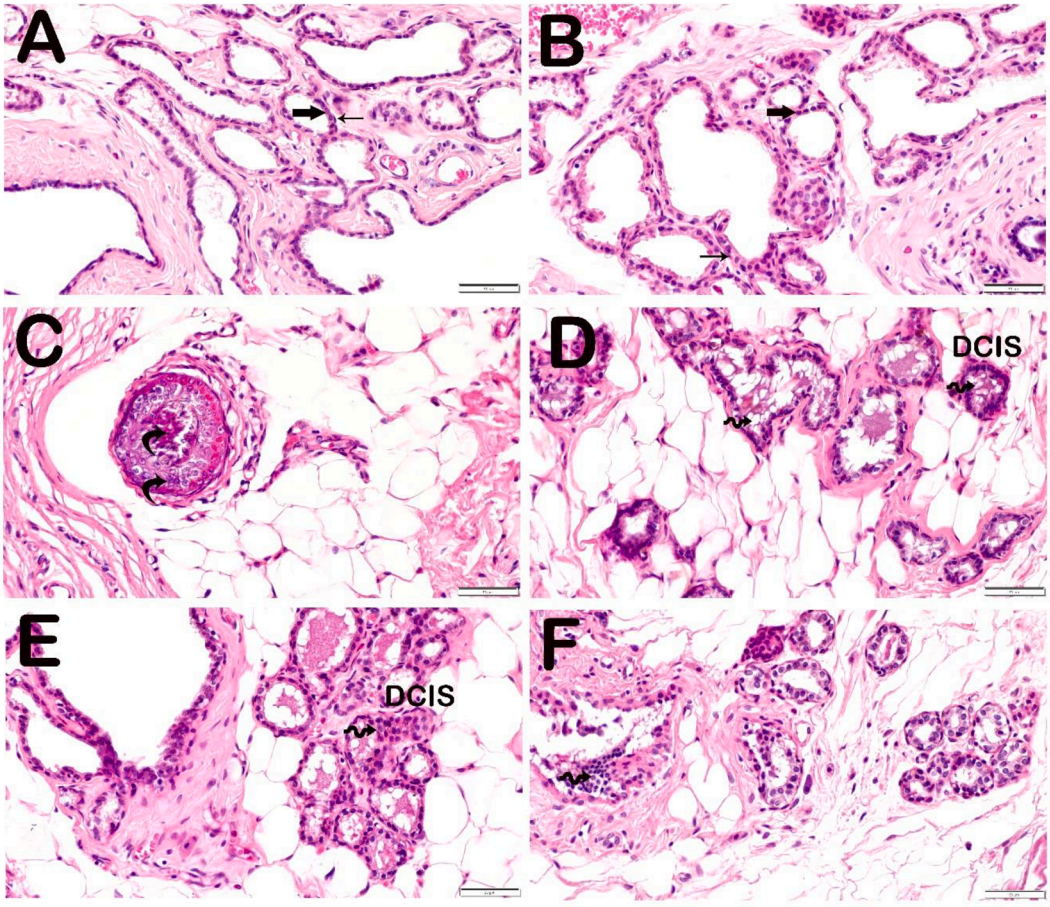

Fig. 2. Representative light microscopy images of mammary gland sections stained with Harris hematoxylin and eosin G. **A(×20)-B(×40) C Group:** Sections from group C showing mammary glands composed of normal epithelial cells (arrow) and myoepithelial cells (thin arrow). **C(×40) MNU Group:** Numerous mammary glands were observed to contain hyperplasic epithelial cells. In mammary glands containing hyperplasic epithelial cells, hyperplasia, usually caused by filling the lumen, and DCIS type cancer formations are observed. **D (×40) TAX group:** A decrease in mammary glands containing hyperplasic epithelial cells (spiral arrow) was observed. **E (×40) LWTE Group:** In the DCIS group, a reduced number of mammary glands (spiral arrow) containing hyperplasic epithelial cells was observed. **F (×40) HWTE group:** The number of mammary glands containing hyperplasic epithelial cells (spiral arrow) was reduced.

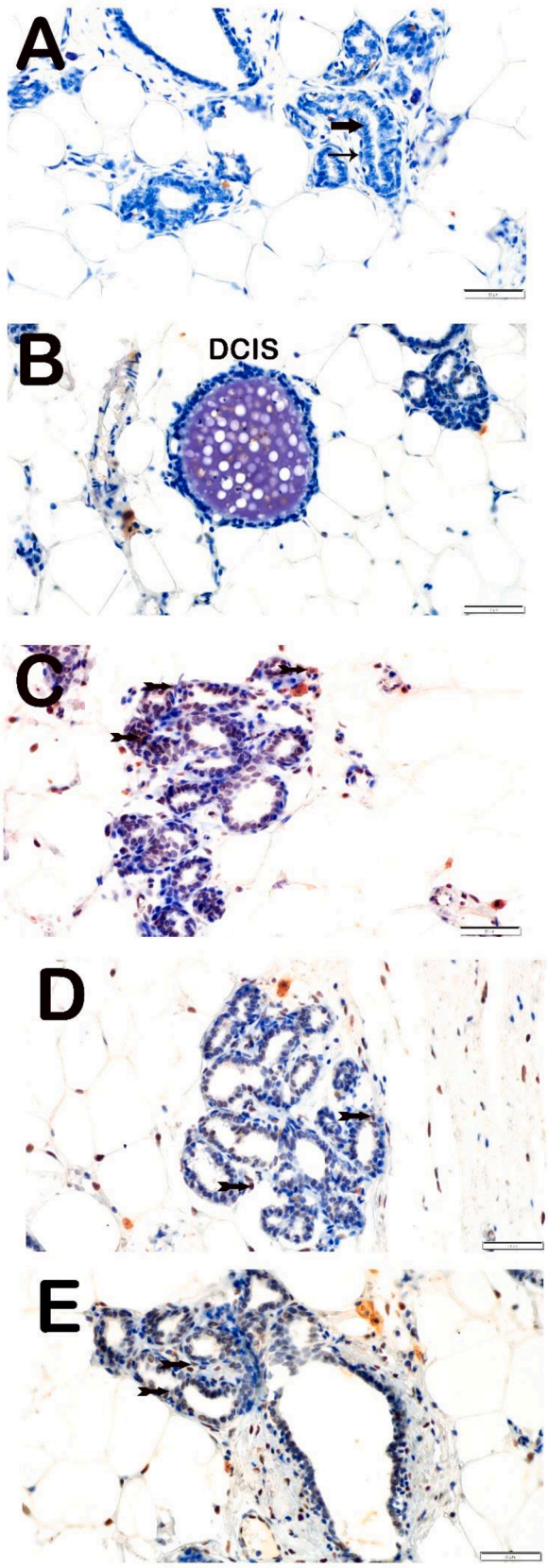

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Fig. 3. Representative light microscopy images of mammary gland sections incubated with a caspase-3 primary antibody. **A (×40) C Group:** In the sections of group C, mammary glands consisting of normal immune-negative epithelial cells (arrow) and myoepithelial (thin arrow) cells were observed (caspase-3 positivity score: 0 (0–0.5)). **B (×40) MNU Group:** In the mammary glands where DCIS type cancer formations are observed, hyperplasic epithelial cells are Caspase-3-negative (Caspase-3 positivity score: 0 (0–1)). **C (×40) TAX Group**: Mammary glands (spiral arrow) containing hyperplasic epithelial cells showing intense Caspase-3 positivity (Caspase-3 positivity score: 2 (1–2)). **D (×40) LWTE Group:** The number of apoptotic epithelial cells showing Caspase-3 positivity (spiral arrow) decreased in the mammary glands containing DCIS type hyperplasic epithelial cells (Caspase-3 positivity score: 2(2–2)). **E (×40) HWTE Group**: Many hyperplasic epithelial cells (spiral arrow) were positive for Caspase-3 (caspase-3 positivity score: 2 (2–2)).

3.4.2. Ki-67 primary antibody

When we examined mammary gland sections incubated with Ki-67 primary antibody to observe cell proliferation under a representative light microscope, we observed that the sections belonging to the C group contained mammary glands consisting of immune-negative epithelial cells with a normal structure (Ki-67 positivity score: 0 (0–0.5); Table 9, Fig. 4*a*). In contrast, we found an increased number of hyperplasic epithelial cells showing intense Ki-67 positivity in the mammary glands of patients with DCIS type cancer in the MNU group (Ki-67 positivity score: 3 (2–3) Table 9, Fig. 4*b*). On the other hand, we observed a decrease in hyperplastic epithelial cells showing Ki-67 positivity in the mammary glands of the TAX group (Ki-67 positivity score: 0 (0–0.5), Table 9, Fig. 4*c*). Similarly, we detected that the number of hyperplasic epithelial cells showing Ki-67 positivity in the DCIS type decreased in the mammary glands of the LWTE group (Ki-67 positivity score: 0 (0–0), Table 9, Fig. 4*d*). We observed a decrease in the number of hyperplasic epithelial cells that were Ki-67 positive in HWTE group breast tissue sections, and immune-negative epithelial cells were also prevalent (Ki-67 positivity score: 0 (0–0), Table 9, Fig. 4*e*).

3.4.3. 8-OHdG primary antibody

After mammary gland sections incubated with 8-OHdG primary antibody were examined under a light microscope to observe DNA damage caused by free oxygen radicals, we observed mammary glands consisting of normal immune-negative epithelial cells in sections from the C group (8-OHdG positivity score: 0 (0–0); Table 9, Fig. 5*a*). In contrast, we found that the number of mammary glands containing hyperplasic epithelial cells showing intense 8-OHdG positivity increased in the MNU group (8-OHdG positivity score: 2(2–3) Table 9, Fig. 5*b*). We detected a decrease in the number of hyperplasic epithelial cells that were 8-OHdG positive in the mammary glands of the TAX group compared to the MNU group (8-OHdG positivity score: 1 (1–1), Table 9, Fig. 5*c*). Similarly, we observed that the number of hyperplasic epithelial cells showing 8-OHdG positivity decreased in the LWTE group mammary glands (8-OHdG positivity score: 1 (0–1), Table 9, Fig. 5*d*). We observed that a large number of hyperplasic epithelial cells showing 8-OHdG positivity were reduced in the mammary gland sections of the HWTE group (8-OHdG positivity score: 1 (0–1), Table 9, Fig. 5*e*).

4. Discussion

Breast cancer, which affects women in particular, is a disease with high mortality and morbidity rates worldwide (Kubatka et al., 2018). In general, conventional cancer treatment options are not completely effective. In recent years, the use of some types of complementary medicines by women with a history of breast cancer has significantly increased such as phytotherapeutic products and nutritional supplements (Lopes et al., 2017). Although WT is the least processed tea compared to other teas and therefore the richest in catechins and antioxidants, there are very few studies evaluating WT. In the current study, we hypothesize that WT, which is high in antioxidants, can be used in

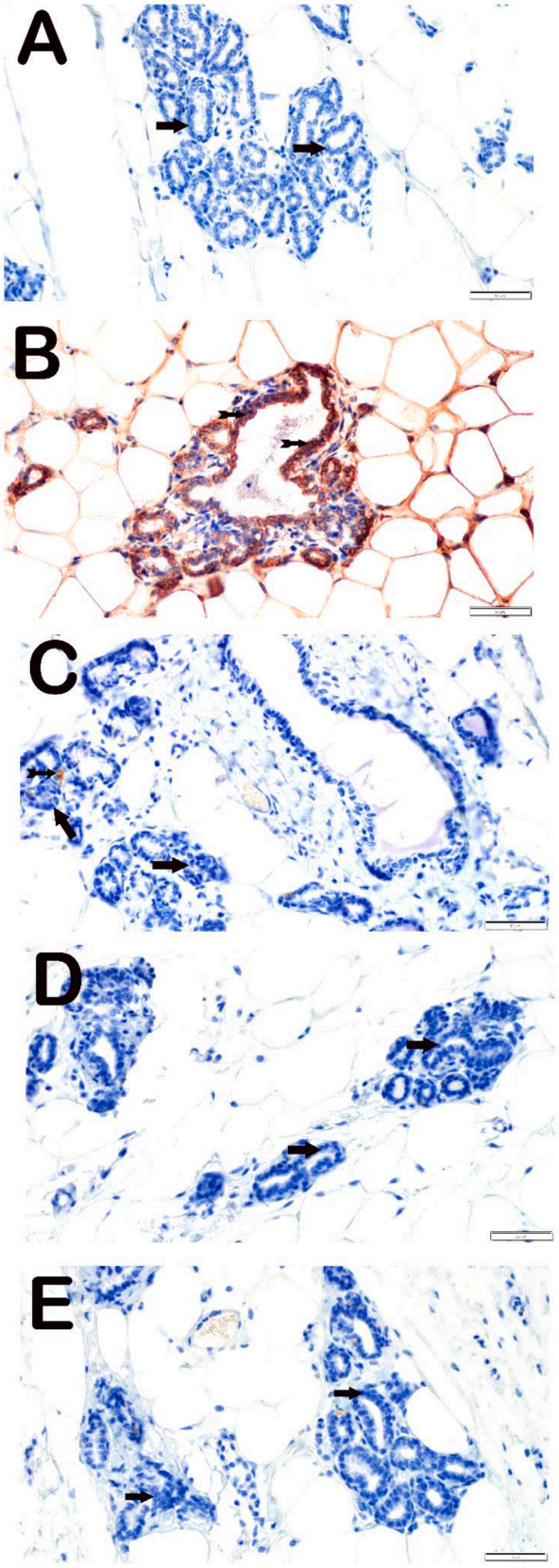

Fig. 4. Representative light microscopy images of mammary gland sections incubated with Ki-67 primary antibody. **A (×40) C Group:** Sections belonging to the C group show mammary glands with normal structure and Ki-67-negative epithelial cells (arrow) (Ki-67 positivity score: 0 (0–0)). **B (×40) MNU Group:** Hyperplasic epithelial cells with intense Ki-67 positivity were observed in the mammary glands of patients with DCIS-type cancer (Ki-67 positivity score: 3 (2–3)). **C (×40) TAX Group:** In the mammary glands, Ki-67-positive hyperplasic epithelial cells were reduced (spiral arrow) (Ki-67 positivity score: 0 (0–0.5)). **D (×40) LWTE Group:** Ki-67-positive hyperplasic epithelial cells (spiral arrow) in the DCIS type were reduced in number (Ki-67 positivity score: 0 (0–0)). **E (×40) HWTE Group:** The number of hyperplasic epithelial cells (arrow) showing Ki-67 positivity decreased, and immune-negative epithelial cells were widespread (Ki-67 positivity score: 0 (0–0)).

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the prevention and treatment of breast cancer. Our findings suggest that WT may prevent breast cancer formation by inhibiting proliferation, preventing DNA damage, and inducing cancer cell apoptosis in addition to its anti-inflammatory and antioxidant effects.

It is reported that there is a strong relationship between total phenolic matter content and antioxidant activity of tea leaf (Akbulut et al., 2020). The WT used in our study was determined to have higher total phenolic and flavonoid content than the white teas used by Zhao et al. and Peiro et al. (Peiró et al., 2014; Zhao et al., 2019). The mineral, elemental, catechin, total phenolic content and antioxidant activity of tea are subject to variation depending on the type of tea, the growing season, the brewing time and temperature (Akbulut et al., 2020). As a result of HPLC analysis of our WT extracts, it was determined that the majority of the total catechin amount was EGCG. In previous studies, EGCG has been shown to be a potent chemo-preventive agent (Filippini et al., 2020). Based on these data, the high amount of EGCG in WT may be associated with the prevention of the development or progression of breast cancer.

Cancer has been shown to cause disturbances in protein, carbohydrate and lipid metabolism (Heber et al., 1992). MDA and GSH are considered a good indicator of oxidative stress, PC protein damage (Arauz et al., 2016; Reznick & Packer, 1994; Soares et al., 2021). In a previous study, it was shown that WT increased GSH levels and decreased MDA levels; similarly, in another study, tea polyphenol significantly decreased MDA levels and increased GSH levels (Cui et al., 2012; Liu et al., 2018). In the present study, it was determined that GSH levels, which decreased in addition to increased MDA and PC levels with MNU exposure, changed in the opposite direction, especially with low dose white tea. Our results support our idea that WT with high antioxidant content may help prevent cancer by reducing oxidative stress in the body.

EGCG, an important component of WT, has been shown to reduce the formation of 8-OHdG (Yang & Wang, 2016). In the present study, while immune-negative epithelial cells were observed in the C group, the increase in 8-OHdG positivity in the MNU group revealed that DNA damage occurred with i.duc MNU injection, and the decrease in 8-OHdG positivity in the treatment groups revealed that WT prevented DNA damage.

Cachexia, the most common complication of cancer, is an important cause of cancer deaths (Tosun & Köksal, 2012). In our study, we found that the weight change in the MNU group was almost half that of the other groups. We can say that LWTE, which provides the same level of weight gain as group C, has an optimum protective or therapeutic effect against breast carcinogenesis. Since WT inhibits tumor growth, the animals in the groups given WT may have gained the same amount of weight as healthy animals.

M-CSF, which regulates the growth and differentiation of haematopoietic progenitor cells, induces cancer cell growth under pathological conditions (Tatar et al., 2019). The overexpression of circulating M-CSF is associated with a poor prognosis and is used to predict the prognosis of patients with cancer (Chambers et al., 1997). EGCG has been reported to have an inhibitory effect on M-CSF release (Lee et al., 2013). In the

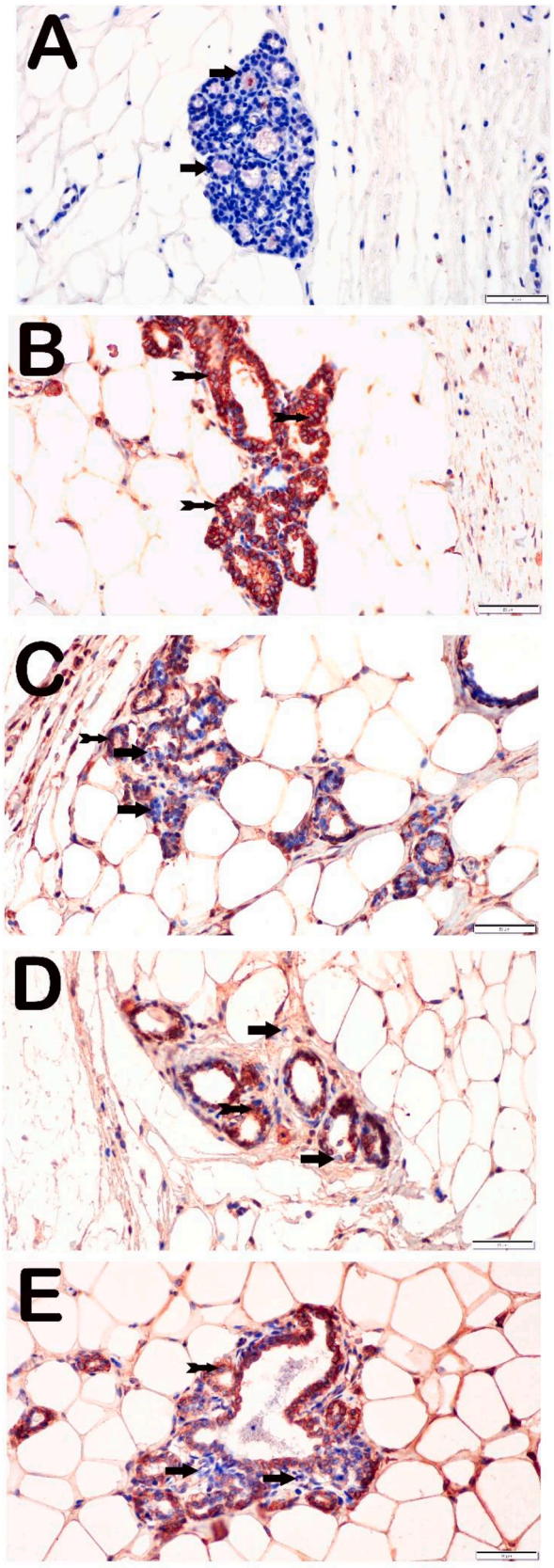

Fig. 5. Representative light microscopy images of mammary gland sections incubated with 8-OHdG primary antibody. **A (×40) C Group**: In the sections of group C, mammary glands consisting of immune-negative epithelial cells (arrow) with a normal structure were observed (8-OHdG positivity score: 0 (0–0)). **B (×40) MNU Group:** The number of mammary glands containing hyperplasic epithelial cells showing intense 8-OHdG positivity increased (8-OHdG positivity score: 2(2–3)). **C(×40) TAX Group:** In the mammary glands, hyperplasic epithelial cells showing 8-OHdG positivity were reduced (spiral arrow) (8-OHdG positivity score: 1(1–1)). **D (×40) LWTE Group:** Hyperplasic epithelial cells showing 8-OHdG positivity (spiral arrow) were reduced in number (8-OHdG positivity score: 1 (0–1)). **E (×40) HWTE Group:** Reduced numbers of hyperplasic epithelial cells showing 8-OHdG positivity (spiral arrow) are observed (8-OHdG positivity score: 1(0–1)).

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current study, the fact that M-CSF levels were significantly lower in the WT groups among all groups supports our idea that WT may have a positive effect in preventing or treating breast cancer by inhibiting M-CSF release and therefore tumor growth. Overexpression of NRP-2 has been associated with increased angiogenesis and tumor cell survival (Zhao et al., 2019). In our study, NRP-2 levels were significantly lower in the LWTE group compared to the MNU group. Although there are many reports proving the role of NRP-2 in worsening cancer prognosis, there is no effective inhibitor on the market to block the NRP-2 signalling pathway (Islam et al., 2022). In particular, by blocking NRP-2 activity with LWTE, the growth of cancer cells can be inhibited, cancer progression can be prevented, and metastasis-free survival can be prolonged.

Breast cancer originates from ductal and epithelial cells and progresses progressively from hyperplasia to atypical hyperplasia and from *in situ* carcinoma to early and advanced invasive carcinoma (Zhang & Gu, 2020). Gao et al. determined that 89 % of the tumors in breast cancer induced by i.duc MNU were IDC, as papillary, cribriform, ductal, and tubular carcinomas (Gao et al., 2021). In our study, the rate of ductal carcinoma was 62 %, much lower than the percentage of IDC and ILC. In the studies conducted by Gao et al. and in our study, differences in the types of cancer occurred due to the small sample size. In previous studies, rats were shown to exhibit marked cellular hyperplasia in mammary tissue as a result of MNU induction (El-Beltagy et al., 2021). Similarly, hyperplasic areas increased with MNU exposure in our study. The histopathological changes and hyperplasia caused by MNU in breast tissue have been shown to be successfully reversed by the use of WT.

WT has been shown to increase caspase-3 activity and inhibit the proliferation of cancer cells through apoptosis induction (Filippini et al., 2020; Liu et al., 2018). Similarly, in our study, chemotherapy and WT inhibited the proliferation of cancer cells by induction of apoptosis. Ki-67 is used as a prognostic indicator in many cancer types including breast cancer (Davey et al., 2021). Tea catechins have been shown in previous studies to be potent inhibitors of proliferation (Singh et al., 2011). In the MNU group, the number of Ki-67 hyperplasic epithelial cells increased in areas where DCIS cancer formations were observed, and their decrease in the WT groups supports our opinion that WT prevents the formation of cancer by reducing proliferation which is an important part of cancer development and progression.

Research shows that cancer development is closely related to the inflammatory response and that many cancers are caused by chronic inflammation (Deng et al., 2023). Elevated levels of TNF-α and IL-6 promote the progression of breast cancer (Habanjar et al., 2023). Tea polyphenols have been reported to significantly reduce proinflammatory cytokine levels, including TNF-α and IL-6, significantly alleviating inflammation (Tang et al., 2019). In our study, IL-6 and TNF-α levels increased with MNU exposure. The significant decrease in inflammatory cytokine levels in the LWTE group suggests that low dose white tea has a better anti-inflammatory effect than high dose white tea and may contribute to the prevention of tumour growth by suppressing cytokine levels.

According to our results, WT with high antioxidant content inhibited

lipid peroxidation, prevented DNA, protein and cell damage caused by free radicals, decreased inflammatory cytokine levels, prevented proliferation of cancer cells by inducing apoptosis, as well as inhibited tumour growth by blocking M-CSF release and NRP-2 activity.

Although our current study was based on a small group of participants, our findings suggest that WT has favourable effects on the prevention or treatment of breast cancer and greatly reduces cancer-induced complications such as cachexia. In this study, we investigated which dose was more effective by giving two different doses of WT. As a result, we determined that low dose of WT prevented breast carcinogenesis better than high dose. We also shed light on the potential use of WT as a dietary intervention to guard against breast cancer and prevent its progression. WT cannot replace therapy, but it can be used as an aid to support breast cancer prevention because of its beneficial properties.

Our study is the first experimental investigation on the protective effects of WT in breast cancer induced by i.duc MNU induction. Our apoptosis findings need to be supported by studies focusing on mechanisms such as intracellular Ca^{+2} and mitochondrial Ca^{+2}. In addition, a general evaluation of oxidative stress due to lipid peroxidation was made with MDA, GSH and PC levels in our study. In this context, detailed emphasis of our study with other studies dealing with oxidant and antioxidant enzymes and/or proteins will carry the subject further. Further research is required to fully elucidate the molecular mechanisms involved in the treatment of breast cancer. In our study, certain cancer types were observed; this protective effect should be supported by studies on different breast cancer subtypes.

Consent for publication

Not applicable.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used DeepL in order to translation support. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Sibel Mataraci Karakas: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Adnan Yilmaz:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tolga Mercantepe:** Methodology, Formal analysis, Data curation, Conceptualization. **Atilla Topcu:** Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Esra Pinarbas:** Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No datasets were generated or analyzed during the current study.

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Not applicable.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106462.

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