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Original article

Modulatory effects of white tea (*Camellia sinensis* L.) on genotoxicity in streptozotocin and cyclophosphamide-treated *Drosophila melanogaster*

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Summary. In the present study, it was aimed to determine the antigenotoxic activity of white tea against the genotoxic effect caused by two different alkylating antineoplastic cancer drugs streptozotocin (STZ) and cyclophosphamide (CP) in *Drosophila melanogaster* through the somatic mutation and recombination test (SMART). During the application of SMART, two different mutant strains were used in the genome of *D. melanogaster*, with the determinant genes being recessive flare (*flr*^{3}) and multiple wing hairs (*mwh*), respectively. Trans-heterozygous larvae of 72±hours, obtained through the crossbreeding of these two mutant strains, were chronically fed with white tea extract of different concentrations (0.625, 1.25, 2.50, and 5 mg/mL). In our study, the LD_{50} values of the relevant drugs were also found (STZ: 0.25 mg/mL, CP: 2.5 mg/mL), and it was determined that both drugs have a fairly high genotoxic effect ($p<0.05$). Afterward, white tea extracts of different concentrations were applied to larvae simultaneously with cancer drugs. From the data obtained, it was determined that white tea extract significantly reduced the clone induction frequency (CIF) in all treatment groups, by suppressing mutations enabling the formation of spots in wings, in parallel with the increasing concentration. Furthermore, when the inhibition percentage rates were subjected to examination in order to determine white tea's reduction rate of the genotoxic effect caused by cancer drugs, it was determined that the relevant rate was 55.79% in the 0.25 STZ+2.5 White Tea treatment group, and 60.00% in the 2.5 CP+5 White Tea treatment group. In conclusion, it was concluded that streptozotocin and cyclophosphamide drugs used in cancer treatments might create genotoxic effects even in healthy cells, and these effects might be reduced by additional white tea consumption.

Keywords: Antigenotoxicity, SMART, Streptozotocin, Cyclophosphamide, White tea, Cancer drug

Introduction

Camellia sinensis (Tea), which has been used in Far Eastern countries for many years and has spread to Europe, America and other regions over time, is the most consumed drink after water in the world (1,2). Tea drink is usually consumed in three different forms known as green tea, black tea, and oolong tea. White tea is the least produced and more expensive form of tea than others (3). Polyphenols, which constitute approximately 36% of the weight of dry tea, take a very important place among the components of tea types. Tea polyphenols, especially catechin derivatives, are strong antioxidant agents with positive effects on human health (4). Furthermore, tea has been reported to have many different pharmacological effects such as antioxidative, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, apoptotic, antiobesity,

hypocholesterolemic, antiatherosclerotic, antidiabetic, antibacterial, antiviral, and anti-aging effects (5).

The use of white tea, which is a special form of tea prepared from the leaves of some tea varieties that have not yet matured and opened and are covered with white hair, without undergoing any processes such as withering, baking, and fermentation, has been gradually increasing especially in the last decade (3,6). In the studies carried out, it was determined that white tea contains the highest amount of protein and catechin compared to other forms of tea, which is thought to be since the harvested part of white tea consists of only buds (7).

Although green tea and black tea are very beneficial for human health, white tea is known as the least produced form of tea with the highest level of antioxidants (8,9). It has been determined by many studies that white tea contains highly effective components against oxidative stress (10-13). It has been determined that flavonoids, which are a group of antioxidants found in white tea, have effective protection against many different types of cancers such as colon, prostate, and stomach cancers (14,15). Nevertheless, it has been found to have antibacterial and antiviral effects due to the antioxidants it contains (16,17) and to reduce cholesterol through catechins that are another group of antioxidants, and especially through epigallocatechin gallate (18).

In the study in which Komes et al. (19) compared 5 forms of tea including white, green, yellow, oolong, and black tea in terms of caffeine content, they listed the tea plant as white (3.62%)> yellow (3.18%)> black (2.79%)> oolong (2.77%)> green tea (2.35%) in terms of caffeine content. They reported that the amount of caffeine varied by origin, genetics, environmental factors, harvest time, processing method, and the age of the tea leaf. They indicated that young shoots contained higher amounts of caffeine. Therefore, white tea made from young shoots also had higher caffeine content compared to others (19).

Furthermore, as a result of recent studies in which different forms of tea have been compared, it has been determined that white tea has the strongest neuroprotective effect in the prevention of Alzheimer's disease (20) and the best protective effect on bone tissue and hyaline cartilage against heavy metal exposure (21). With all these properties, white tea stands out as a rare and special product among other forms of tea.

In the literature, no study was found on the protective effect of white tea against side effects to be caused by antineoplastic drugs. Antineoplastic drugs destroy both cancer cells and rapidly proliferating normal cells (intestinal and oral mucosal epithelium, bone marrow hematopoietic cells, hair follicle cells, germinative cells of the testis, embryo and fetus cells) (22). Alkylating agents are the most commonly used chemotherapeutic drugs that react with the alkyl group they contain to form a covalent bond with DNA and thus induce cell death by damaging DNA (23). Alkylating agents cannot distinguish cells during division and at rest but mainly have toxic effects on dividing cells. In addition to cytotoxicity, it has been determined that they are mutagenic and carcinogenic and may also lead to secondary cancers such as leukemia (24). Streptozotocin (STZ) is an N-nitroso-derived broad-spectrum alkylating chemotherapeutic drug, and cyclophosphamide (CP) is a nitrogen mustard-derived broad-spectrum alkylating chemotherapeutic drug (25,26).

In this study, it was aimed to determine possible genotoxic effects of streptozotocin and cyclophosphamide alkylating agents, which are among antineoplastic drugs, and the antigenotoxic activity of white tea on these effects using the SMART method.

Drosophila melanogaster which is also known as vinegar or fruit fly, is an ideal model organism that is frequently used in experimental research in many sciences, especially genetics due to its properties such as the fact that it has a very short life cycle of 9-10 days, lays many eggs at a time (40-50 eggs/day), is an organism in which a wide variety of natural and artificial variations (eye color, eye shape, wing shape, wing hair type, etc.) can be observed even with the naked eye, is grown in the laboratory easily and inexpensively, is one of the most suitable creatures with the ability of cross controlling, and having giant chromosomes (polytene chromosomes) which can be easily distinguished from mitotic chromosomes (27-29). Furthermore, features such as the fact that *D. melanogaster* enables *in vivo* study in a eukaryotic organism and has few chromosomes to eliminate complexity (one of the X/Y pairs and three autosomes), the high similarity of enzyme systems responsible for bioactivation with the enzyme

systems of mammals, and no need for ethics committee approvals are among the important reasons why it is preferred as a model organism in genetic studies (27). The somatic mutation and recombination test (SMART) with *Drosophila*, which has been frequently used in recent years, is an *in vivo* test system which is quite convenient and effective for the determination of mutagenic and recombinogenic activities of various chemicals (30).

Material and methods

Experimental animals

D. melanogaster's multiple wing hairs strain (*mwh/mwh*) and *flr*^{3} strain (*flr*^{3}*/ In(3LR)TM3* mutant strains were used in this study. The *mwh* gene (*multiple wing hair*) is a recessive gene and causes multiple wing trichomes instead of a wing trichome in the homozygous state (*mwh/mwh*) in somatic cells. The *flare* (*flr*^{3}) gene is also a recessive gene and causes the formation of shortened and darkened, blunted, or balloon-shaped amorphic trichomes instead of normal trichomes on the wings of *Drosophila*.

D. melanogaster mutant strains used in our study were obtained from Akdeniz University, Faculty of Science and Letters, Department of Biology, and grown in our laboratory in standard Lewis growth medium (31). *Drosophila* was cultured in incubators under ideal living conditions (25±1°C and 60% relative humidity). In the experimental part of our study, *Drosophila* Carolina Formula 4-24 Instant Medium (Burlington North Carolina, USA) was used for the application of chemicals to the larvae.

Chemicals

STZ 18883-66-4 coded streptozotocin and C-0768-1G coded cyclophosphamide used in our study were obtained from SIGMA. White tea, our plant material, was obtained by the brand "Beyaz İksir" (White Potion) from Turkey's General Directorate of Tea Enterprises (ÇAYKUR). 20 g of white tea was taken, and 500 mL of freshly boiled water was added to it, and then it was allowed to brew for 15-20 minutes. The brewed stock tea sample was frozen at -20°C for 24 hours and then lyophilized in a lyophilizer and dried, and the lyophilisates were stored in the refrigerator at +4°C until analysis.

Application of Genotoxicity and Anti-Genotoxicity Tests

For obtaining trans-heterozygous larvae, *flare* females were collected at 4-hour intervals when they were virgin, and they were taken to a new growth medium. Since egg yield in *flare* is better than *mwh*, virgin females of *flare* were used in crosses in our study. They were crossed so that each flask would include 40 *mwh* males and 40 *flare* females. The individuals with completed oogenesis were taken to a new growth medium for 8 hours and were allowed to lay eggs during this time so that the larvae to be treated would be in the same stage. The same individuals were used again for egg collection.

In our study, the LD_{50} doses of antineoplastic drugs on trans-heterozygous *D. melanogaster* larvae of 72±4 hours were determined. As a result of the preliminary applications, the LD_{50} dose was determined to be 0.25 mg/mL for STZ and 2.5 mg/mL for cyclophosphamide in *D. melanogaster* larvae. 0.3125, 0.625, 1.25, 2.5, and 5 mg/mL concentrations were selected for white tea applications. Furthermore, while distilled water was used for the negative control group, 1 mM concentration of ethyl methanesulfonate (EMS), which is an alkylating agent, was used for the positive control group.

Adult individuals developing from the larvae that were exposed to antineoplastic agents in the application environment were collected daily by etherizing, then they were placed in 70% ethyl alcohol to prepare wing preparations and kept in the refrigerator at +4°C. Afterward, these individuals were divided into two groups as normal wings (trans-heterozygous *mwh/flr*^{3}) and serrate wings (balanced heterozygous m*wh/TM3, BdS*) according to their wing morphology. Among them, although wings with the normal phenotype (*mwh/flr*^{3}) contained mutant clones resulting from both mutation and recombination, serrate wings (*mwh/TM3, BdS*) contained only clones resulting from mutation since the balancer chromosome suppressed recombination (32). Therefore, the preparations of wings in both phenotypes were separately prepared and evaluated.

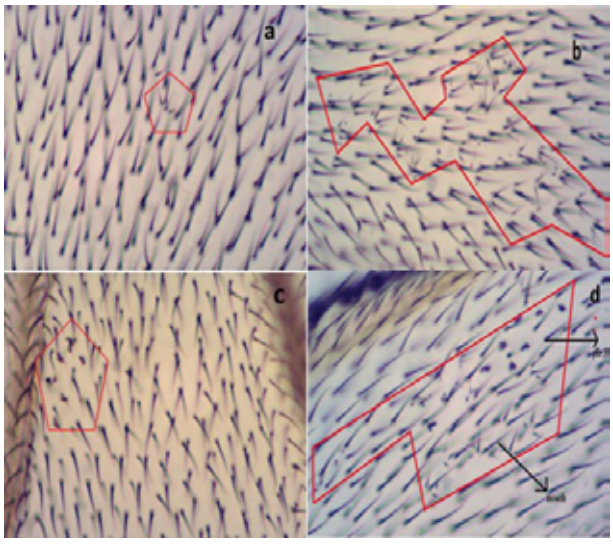

Figure 1. Various wing spot types in *Drosophila melanogaster* (10x40) **a:** Small single spots in the *mwh* genotype, **b:** Large single spots in the *mwh* genotype, **c:** Large single spots in the *flr*^{3} genotype, **d:** Twin spot

"Faure solution" used in the studies of Negishi et al. (33) and Schaik and Graf (34) were used to prepare wing preparations. Each sector on the wing surfaces of the prepared preparations was screened separately, and *mwh* and/or *flr*^{3} mutant clones were counted. These mutant clones were grouped into small single clones (1-2 *mwh* cells), large single clones (≥ 3 *mwh* or ≥ 4 *flr*^{3} cells), and twin clones (clones containing both *mwh* and *flr*^{3} cells side by side) (Figure 1). Clone induction frequency and inhibition percentages were calculated in all groups.

Data Evaluation and Statistical analysis

The LD_{50} doses determined for STZ and CP were used alone in trans-heterozygous larvae, and the results were statistically compared with the results of the negative control group. The data obtained from white tea combinations at different concentrations applied with STZ or CP were statistically compared with the only STZ-treated group or only CP-treated group.

The data were evaluated according to the multiple decision procedures of Frei and Würgler (35, 36). This method tests two alternative hypotheses: (i) the mutation frequency in the treated group is not higher than the mutation frequency in the control group and (ii) the frequency in the treated group is not less than m times (m: multiplication factor) as high as the observed spontaneous mutation frequency in the control. For statistical calculations, the conditional binomial test according to Kastenbaum and Bowman (37) was used at 5% significance levels. Based on the number of *mwh*, the number of wings was analyzed, and the number of cells scored in each wing (approximately 24.400), the clone formation frequency per cell cycle, and 10^5 cells were calculated (38).

Results

As a result of the preliminary studies, it was first concluded that the concentrations determined for white tea extracts had no genotoxic effect in *D. melanogaster*. When the wing spot data obtained and the data obtained from the negative and positive control groups, distilled water and EMS application were statistically compared, it was determined that there was no increase in the number of *mwh*, *flare,* or twin spots and no mutagenic effect occurred in any of the white tea (WT) application groups (Table 1). When the results were statistically analyzed, they were evaluated as either insignificant difference (i) or negative (-) ($p>0.05$).

When the data obtained from the SMART application performed with the previously determined LD_{50} concentrations of STZ and CP and the data obtained from the negative and positive control groups, distilled water and EMS application were statistically compared, a significant increase was observed in all spot types compared to the negative control group. The results were statistically evaluated as positive (+), and both drugs were found to have the genotoxic effect ($p<0.05$) (Table 2). Furthermore, based on the obtained data, it was observed that STZ had more genotoxic effects compared to CP (Table 2).

When the data obtained from STZ and white tea (STZ+ WT) application and the data obtained from only STZ-treated groups were compared, it was determined that there was a numerically significant decrease in all spot types depending on increasing white tea concentration ($p<0.05$) (Table 3). It was observed that a twin spot was never formed in the 0.25 STZ+5 WT group, which was the highest WT application,

Table 1. The data obtained from SMART with white tea application and statistical evaluation

Application Groups (mg/mL)	Number of wings	Small Single Spots (1-2 spots) (m=2)			Large Single Spots (>2 spots) (m=5)			Twin Spots (m=5)			Total mwh spots (m=2)			Total spots (m=2)			CIF (10^5 cells)
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	
Normal wings ***(mwh/flr³)***																	
Distilled Water	80	11	0.14		3	0.04		1	0.01		12	0.15		15	0.19		0.61
1 mM EMS	80	92	1.15	+	58	0.73	+	38	0.48	+	174	2.18	+	188	2.35	+	8.91
0.625 WT	80	11	0.14	i	2	0.03	-	1	0.01	i	14	0.18	i	14	0.18	-	0.71
1.25 WT	80	10	0.13	-	3	0.04	i	1	0.01	i	14	0.18	i	14	0.18	-	0.71
2.5 WT	80	10	0.13	-	1	0.01	-	1	0.01	i	12	0.15	i	12	0.15	-	0.61
5 WT	80	8	0.10	-	3	0.04	i	0	0.00	-	11	0.14	-	11	0.14	-	0.56
Serrate wings ***(mwh/TM3)***																	
Distilled Water	80	10	0.13		2	0.03					12	0.15		12	0.15		0.61
1 mM EMS	80	94	1.18	+	40	0.50	+				128	1.60	+	128	1.60	+	6.56
0.625 WT	80	10	0.13	i	3	0.04	i		*		13	0.16	i	13	0.16	i	0.67
1.25 WT	80	11	0.14	i	2	0.03	i				13	0.16	i	13	0.16	i	0.67
2.5 WT	80	8	0.10	-	0	0.00	-				8	0.10	-	8	0.10	-	0.41
5 WT	80	9	0.11	-	1	0.01	-				10	0.13	-	10	0.13	-	0.51

WT: White Tea, No: Number of spots, Fr: Frequency, D: Representation of statistical results (Frei and Würgler, 1995), +: positive; -: negative, i: insignificant difference, m: multiplication factor, CIF: Clone Induction Frequency, *: No twin spots occur due to the TM3 balancer chromosome. Probability level: $\alpha = \beta = 0.05$

and the total number of spots decreased from 1.38 to 0.63 (Table 3). When inhibition rates % were examined, it was observed that there were decreases in all application groups of individuals with the serrate wing phenotype except for the 0.25 STZ+0.625 WT application group. The inhibition rate % was calculated to be 55.79% in the 0.25 STZ+2.5 WT application group. Based on these data obtained, it was concluded that white tea extract nearly halved the STZ-induced genotoxicity rate (Table 3).

When the data obtained from CP and different concentrations of white tea (CP+WT) application and the data obtained from only CP-treated groups were statistically examined, it was determined that there was a numerically significant decrease in all spot types depending on increasing white tea concentration in the CP application as in the STZ group ($p<0.05$) (Table 4). When the inhibition rates % obtained by applying white tea to individuals treated with CP were examined, it was determined that the genotoxic effect was inhibited in all individuals with both normal and serrate wing phenotypes following the increase in concentration. In particular, while the inhibition rate % in normal wing individuals was 50.38% in the 2.5 CP+5 WT application group with the highest concentration, this rate increased up to 60.00% in serrate wing individuals (Table 4). Based on all these data obtained, it was determined that white tea extract significantly inhibited the induced genotoxicity in the CP application groups as in the STZ application group (Table 4).

Discussion

One of the most important side effects of anticancer drugs is the genotoxic effects they may cause in

Table 2. The data obtained from SMART with antineoplastic drugs application and statistical evaluation

Application Groups (mg/mL)	Number of wings	Small Single Spots (1-2 spots) (*m* = 2)			Large Single Spots (>2 spots) (*m* = 5)			Twin Spots (*m* = 5)			Total *mwh* spots (*m* = 2)			Total spots (*m* = 2)			CIF (10^5 cells)
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	
Normal wings *(mwh/flr³)*																	
Distilled Water	80	11	0.14		3	0.04		1	0.01		12	0.15		15	0.19		0.61
1 mM EMS	80	92	1.15	+	58	0.73	+	38	0.48	+	174	2.18	+	188	2.35	+	8.91
0.25 STZ	80	74	0.93	+	28	0.35	+	8	0.10	+	105	1.31	+	110	1.38	+	5.38
1.25 CP	80	69	0.86	+	27	0.34	+	8	0.10	+	99	1.24	+	103	1.29	+	5.07
Serrate wings *(mwh/TM3)*																	
Distilled Water	80	10	0.13		2	0.03					12	0.15		12	0.15		0.61
1 mM EMS	80	94	1.18	+	40	0.50	+		*		128	1.60	+	128	1.60	+	6.56
0.625 STZ	80	69	0.86	+	30	0.38	+				99	1.24	+	99	1.24	+	5.07
1.25 CP	80	71	0.89	+	29	0.36	+				100	1.25	+	100	1.25	+	5.12

STZ: Streptozotocin, CP: Cyclophosphamide, No: Number of spots, Fr: Frequency, D: Representation of statistical results, +: positive; -: negative, i: insignificant difference, m: multiplication factor, CIF: Clone Induction Frequency, *: No twin spots occur due to the TM3 balancer chromosome. Probability level: $\alpha = \beta = 0.05$

healthy tissues or cells. Therefore, it is quite important to determine the genotoxic activity of such drugs and to minimize these effects for cancer treatment. In this study, it was aimed to determine the potential genotoxic effects of streptozotocin and cyclophosphamide anticancer drugs by the somatic mutation and recombination test (SMART) and to reduce these effects with white tea extracts.

In the first part of our study, it was revealed that antineoplastic cancer drugs STZ and CP caused quite genotoxic effects in *D. melanogaster*. In the literature, many studies are indicating that these two antineoplastic drugs caused toxic effects, in parallel with our results (39, 40). For example, in a study on mice, it was determined that CP decreased the activity of the glutathione enzyme system and caused a toxic effect by increasing phase I enzyme activity metabolizing CP to toxic by-products and that this effect could be reduced by applying the extract of *Phyllanthus amarus* (Amla) plant (41). In another histopathological study on rat renal tissue in which the toxic effect of CP was investigated, it was observed by electron microscopy that severe damage occurred in renal cell organelles and glomerulus as a result of single-dose CP administration (42). In a SMART study carried out with *Drosophila,* the mutagenic and recombinogenic activities of 4 different compounds containing CP were investigated. In conclusion, it was determined that CP had both mutagenic and recombinogenic effects especially at a concentration of 5 mM in both standard strains and strains with high bioactivation (43).

As a result of the studies, it was determined that CP had two active metabolites, phosphoramide mustard (PM) and acrolein (ACR) (44). It is considered that the antineoplastic activity of CP was due to PM metabolite, PM suppressed cell division by binding to DNA and thus suppressed immunity, and caused antitumor activity (44). It was reported that the toxic effect of CP was caused by ACR, its active metabolite and that ACR had this effect by interfering with the tissue antioxidant defense system and leading to the formation of high levels of free oxygen radicals (FOR). FORs originating from ACR combine with molecules such as enzymes, receptors, and ion pumps,

Table 3. The data obtained from SMART with Streptozotocin and white tea (combined treatment) application and statistical evaluation

Application Groups (mg/ml)	Number of wings	Small Single Spots (1-2 spots) *(m=2)*			Large Single Spots *(>2 spots)* *(m=5)*			Twin Spots *(m=5)*			Total mwh spots *(m=2)*			Total spots *(m=2)*			CIF (10^5 cells)	% Inhibition
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D		
Normal wings (*mwh/flr³*)																		
Distilled Water	80	11	0.14		3	0.04		1	0.01		12	0.15		15	0.19		0.61	
0.25 STZ	80	74	0.93	+	28	0.35	+	8	0.10	+	105	1.31	+	110	1.38	+	5.38	
0.25 STZ+0.625 WT	80	70	0.88	-	25	0.31	-	8	0.10	i	98	1.22	-	103	1.29	-	5.02	6.52 ↓
0.25 STZ+1.25 WT	80	52	0.65	-	15	0.19	-	1	0.01	-	68	0.85	-	68	0.85	-	3.48	38.40 ↓
0.25 STZ+2.5 WT	80	32	0.40	-	15	0.19	-	2	0.03	-	48	0.60	-	49	0.61	-	2.46	55.79 ↓
0.25 STZ+5 WT	80	38	0.48	-	12	0.15	-	0	0.00	-	50	0.63	-	50	0.63	-	2.56	54.34 ↓
Serrate wings (*mwh/TM3*)																		
Distilled water	80	10	0.8		2	0.03					12	0.16		12	0.16		0.61	
0.25 STZ	80	69	0.86	+	30	0.38	+				99	1.24	+	99	1.24	+	5.07	
0.25 STZ+0.625 WT	80	66	0.83	-	35	0.44	i				101	1.26	i	101	1.26	i	5.17	0.00 ↓
0.25 STZ+1.25 WT	80	50	0.62	-	16	0.20	-		*		66	0.83	-	66	0.83	-	3.38	33.06 ↓
0.25 STZ+2.5 WT	80	36	0.45	-	10	0.13	-				46	0.58	-	46	0.58	-	2.36	53.22 ↓
0.25 STZ+ 5 WT	80	35	0.44	-	10	0.13	-				45	0.56	-	45	0.57	-	2.31	54.03 ↓

STZ: Streptozotocin, WT: White Tea, No: Number of spots, Fr: Frequency, D: Representation of statistical results, +: positive; -: negative, i: insignificant difference, m: multiplication factor, CIF: Clone Induction Frequency, *: No twin spots occur due to the TM3 balancer chromosome. Probability level: $\alpha = \beta = 0.05$.

disrupt their functions, and cause toxic effects (45). In our study, we considered that the toxic effects caused by antineoplastics might be due to the accumulation of FORs.

Although the actual mechanism and metabolic targets of STZ toxicity causing cell death in mammalian cells were not fully known over the last few years, nowadays, it has been exactly proven that STZ is a toxic beta-cell glucose analog and an alkylating agent (46). As a result of a study on the removal of STZ-induced oxidative stress and beta-cell degradation in rat pancreas with quercetin antioxidant, it was determined that STZ significantly increased lipid peroxidation and serum Nitric Oxide (NO) concentration and decreased antioxidant enzyme activities (47).

In the last part of our study, the fact that these two antineoplastic agents, the mutagenic, cytotoxic, and genotoxic activities of which were determined, would be applied with white tea extract and that its toxic effects could be reduced was demonstrated by SMART.

Table 4. The data obtained from SMART with Cyclophosphamide and white tea (combined treatment) application and statistical evaluation

Application Groups (mg/ml)	Wing Number (N)	Small single spots (1-2 spots) *(m=2)*			Large single spots (>2 spots) *(m=5)*			Twin spots *(m=5)*			Total mwh spots *(m=2)*			Total spots *(m=2)*			CIF (10^5 cells)	% Inhibition
		No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D		
Normal wings (*mwh/flr³*)																		
Distilled Water	80	11	0.14		3	0.04		1	0.01		12	0.15		15	0.19		0.61	
0.25 CP	80	69	0.86	+	27	0.34	+	8	0.10	+	99	1.24	+	103	1.29	+	5.07	
0.25 CP+0.625 WT	80	61	0.76	-	24	0.30	-	5	0.06	-	87	1.09	-	90	1.13	-	4.46	12.40 ↓
0.25 CP+1.25 WT	80	56	0.70	-	21	0.26	-	2	0.03	-	78	0.98	-	79	0.99	-	4.00	23.25 ↓
0.25 CP+2.5 WT	80	34	0.43	-	17	0.21	-	1	0.01	-	51	0.64	-	52	0.65	-	2.61	49.61 ↓
0.25 CP+5 WT	80	35	0.44	-	15	0.19	-	1	0.01	-	51	0.64	-	51	0.64	-	2.61	50.38 ↓
Serrate wings *(mwh/TM3)*																		
Distilled water	80	10	0.13		2	0.03					12	0.15		12	0.15		0.61	
0.25 CP	80	71	0.89	+	29	0.36	+				100	1.25	+	100	1.25	+	5.12	
0.25 CP+0.625 WT	80	66	0.83	-	26	0.33	-				88	1.10	-	88	1.10	-	4.51	12.00 ↓
0.25 CP+1.25 WT	80	61	0.76	-	21	0.26	-		*		82	1.03	-	82	1.03	-	4.20	33.06 ↓
0.25 CP+2.5 WT	80	37	0.46	-	12	0.15	-				49	0.61	-	49	0.61	-	2.51	51.20 ↓
0.25 CP+ 5 WT	80	32	0.40	-	8	0.10	-				40	0.50	-	40	0.50	-	2.05	60.00 ↓

CP: Cyclophosphamide, WT: White Tea, No: Number of spots, Fr: Frequency, D: Representation of statistical results, +: positive; -: negative, i: insignificant difference, m: multiplication factor, CIF: Clone Induction Frequency, *: No twin spots occur due to the TM3 balancer chromosome. Probability level: $\alpha = \beta = 0.05$.

In the literature, there are many studies in which the genotoxic and antigenotoxic effects of many plants commonly consumed in alternative medicine among people were investigated by SMART that we used in this study (48-58). For example, in a study carried out by Fernandes et al. (59), the antigenotoxic effect of vitexin, which is a flavonoid found in hawthorn leaf, was investigated in *Drosophila*. It was determined that vitexin flavonoid increased mutation-inducing agents such as doxorubicin and benzopyrene and showed antigenotoxic effect by statistically decreasing the frequency of wing spots. In another study, it was observed that the extracts of *Peumus boldus* and *Cryptocarya alba* plants, which are traditionally consumed by people in North America, had antimutagenic effects against Ethyl Methane Sulfonate (EMS) (60). In the study carried out by Prakash et al. (61), it was concluded that the mutagenic effects caused by interaction with radical groups of Methyl Methane Sulfonate (MMS) through the alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids contained in extracts of *Dioscorea pentaphylla* prepared with different solvents could be neutralized. The examination of all these studies carried out with SMART revealed how accurate and reliable the *Drosophila* wing spot test selected for our study or the SMART was.

The data obtained from STZ and CP and white tea (STZ+WT) and (CP+WT) application were separately compared with only STZ-treated groups and only CP-treated groups. When all the results obtained

by SMART were examined, it was determined that there was a numerically significant decrease in both drug groups in all spot types depending on increasing white tea concentration ($p<0.05$) (Table 3 and Table 4). Furthermore, when inhibition rates % were examined, it was determined that white tea extract inhibited mutations caused by antineoplastics at varying concentrations nearly by half (Table 3 and Table 4). We think that all this inhibitory activity resulted from the active compounds present in white tea and the antioxidative activity of these compounds. The results obtained from many studies on tea and white tea also support these data.

With the studies on the anticarcinogenic effect of tea, it has been revealed that tea consumption protects against chemical carcinogens that cause skin, lung, esophageal, stomach, liver, pancreas, breast, prostate, and colon cancers by reducing DNA damage in the cell (62). Furthermore, it is also considered that it provides this protection through the strong antioxidant properties of catechin-derived polyphenols contained in the tea content (63,64). Vinson and Dabbagh (65) found that the antioxidant power of tea catechins was higher compared to vitamins and listed the antioxidant activity of tea catechins from large to small as epigallocatechin gallate (EGCG)>epigallocatechin (EGC) > epicatechin gallate (ECG) (ECG) > epicatechin (EC) (65).

In another study in which results that were similar to the results obtained from our study were obtained, the antimutagenic effects of EGCG, one of the most important polyphenols in the content of the tea, were investigated by various test methods. As a result of the study, it was determined that the mutagenic effect of various substances decreased with the Salmonella AMES test by EGCG application, the mutagenic effect in FM3A cell cultures in mice was inhibited, and a suppressive effect was observed on various carcinogens by the *in vivo Drosophila* mutation test (SMART) (66).

In a review prepared from the studies in which the antitumor activity of EGCG was investigated, it was emphasized that EGCG had inhibitory effects on carcinogenic activity, tumorigenesis, proliferation, and angiogenesis, and induced cell death. In the study, it was demonstrated that these effects were associated with the modulation of reactive oxygen species production, EGCG-mediated inhibition of nuclear factor-κB signaling, and migration, angiogenesis, and cell viability inhibition. Furthermore, it was also concluded that EGCG could induce epigenetic modification by the inhibition of DNA methyltransferase activity and the regulation of acetylation on the histone, which may lead to an upregulation of apoptosis (67).

Despite the studies on various forms of tea, especially black and green tea, there are very few studies on the anticarcinogenic potential of white tea in the literature. However, studies reporting that white tea has antineoplastic effects in lung cancer cells (68) and may protect human skin from solar ultraviolet rays (69) have been carried out recently. In another study in which the antimutagenic activity of white and green tea was compared by the Salmonella test, it was emphasized that white tea had stronger antimutagenic activity and this power could be due to higher concentrations of white tea components such as caffeine, gallic acid, theobromine, EGC, and ECG (70).

In a study carried out to determine the antioxidative activity of white tea, significant increases were found in the amounts of antioxidative enzymes catalase, superoxide dismutase, and glutathione reductase after the administration of white tea to mice (71). In a similar study, the preventive activity of white tea against oxidative damage caused by anticancer drug adriamycin was investigated, and consequently, the protective properties of white tea against the degradation of different tissues in rats were revealed (72).

In a study in which the activity of white tea against three different types of bacteria forming the mouth plaque was examined, it was determined that white tea had antibacterial and antiplaque effects (73). In similar studies that were carried out previously, it was also emphasized that white tea had higher protective properties against bacteria, fungi, and viruses in the body compared to other forms of tea (17) and was good for teeth and gum diseases (74). In another antimicrobial study, it was determined that white tea had a quite high antibacterial effect against Gram+ bacteria such as *Staphylococcus aureus* (75).

As a result of a histopathological study carried out with mice, it was revealed that white tea extracts had hepatoprotective effects in mercury-induced acute liver injuries and that these results could be associated with antioxidant, antitoxic, and antiapoptotic properties of white tea (76).

Conclusions

As a result of our study, it was determined that two different antineoplastic cancer drugs streptozotocin and cyclophosphamide had genotoxic effects in somatic cells by the somatic mutation and recombination test (SMART). It is considered that the possible genotoxic effects of these drugs, which increase the frequency of somatic mutation, during treatment may be reduced by using some additional nutrients. It was revealed by our study that water extracts of white tea plants prepared by brewing were additional nutritional sources that could be used together with these and similar drugs. We think that they had this effect especially due to high antioxidative polyphenols in their content.

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