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Research Article

EFFECT OF BITTER MELON AGAINST CISPLATIN AND VALPROIC ACID-INDUCED GENOTOXICITY IN *Drosophila melanogaster* Meigen

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Abstract: In human body, which is exposed to number of chemicals and physical agents in daily life, malfunctions may occur from time to time in detoxification mechanisms that will prevent the harmful effects of genotoxic agents, whose negative effects cannot be eliminated. In this study, the genoprotective effects of bitter melon (*Momordica charantia* L.) seed and fruit extracts against the genotoxic potential of the antineoplastic agent cisplatin and the antiepileptic drug valproic acid in 72±4 hours old transheterozygous larvae of the fruit fly *Drosophila melanogaster* Meigen were determined by the Somatic Mutation and Recombination Test (SMART). The results showed that the mutation frequencies approached the negative control values by inhibiting the formation of chemical agent-induced mutant clones in all doses in the experimental groups in which plant extracts were applied at doses of 1.25, 2.5 ve 5 mg/mL, while the mutation frequency increased only in the groups that applied cisplatin and valproic acid. These results show that co-administration of cisplatin and valproic acid reduces the genotoxic effect of *M. charantia.*

Özet: Günlük hayatta birçok kimyasal ve fiziksel ajana maruz kalan insan vücudunda, genotoksik ajanların zararlı etkilerini önleyecek detoksifikasyon mekanizmalarında zaman zaman bozukluklar oluşabilmekte ve bu ajanların olumsuz etkileri ortadan kaldırılamamaktadır. Bu çalışmada, antineoplastik bir ajan olan cisplatin ve bir antiepileptik ilaç olan valproik asidin 72±4 saatlik *Drosophila melanogaster* Meigen transheterozigot larvalarındaki genotoksik potansiyeline karşı acı kavun (*Momordica charantia* L.) tohum ve meyve ekstraktlarının genoprotektif etkileri Somatik Mutasyon ve Rekombinasyon Testi (SMART) ile belirlenmiştir. 1,25; 2,5 ve 5 mg/mL dozlarında bitki ekstraktları uygulanan deney gruplarında, mutasyon frekanslarının tüm dozlarda kimyasal madde kaynaklı mutant klon oluşumunu engelleyerek negatif kontrole yaklaşırken mutasyon frekansının sadece sisplatin ve valproik asit eklenen gruplarda arttığı gözlenmiştir. Bu sonuçlar, sisplatin ve valproik asitin birlikte uygulanmasının *M. charantia*'nın genotoksik etkisinin azalttığını göstermektedir.

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Introduction

Cancer is the uncontrolled growth and proliferation of cells as a result of mutations occurring in their genetic material, with the pressure of the hereditary forces and environmental effects that individuals are exposed to throughout their lives (Nenclares & Harrington 2020). Surgery, immunotherapy, radiotherapy, and chemotherapy are among the main treatment methods for cancer, which occupies an important place among health problems in the world. Although the treatment methods aim to increase the life span of the patients and lead to a better life quality, depending on the method used, the treatment may have application difficulties and lead to the occurrence of toxic damage (Abbas & Rehman 2018). Cancer is not only a long-term and complex disease, but it can also bring along mental and neurological diseases (Ateşci *et al.* 2003, Stein *et al.* 2008). Cisplatin (CP) is a drug in the class of strong chemotherapeutics and triggers cytotoxicity and causes damage by interacting with DNA depending on the cell type and content, in addition to its healing effects in cancer cells (Gomez-Ruiz *et al.* 2012). It was determined that the induction of apoptosis caused by the anticancer action mechanism of CP also constitutes the mainline of the toxicity mechanism (Dasari & Tchounwou 2014). On the other hand, as a chronic neurological disorder that is both affected by mental states and affects the mental state, epilepsy takes its place in

many studies. Epilepsy often requires lifelong treatment with antiepileptic drugs (AEDs) (Fiest *et al.* 2017).

It has been determined that the long-term use of AEDs is critical for most of the patients of which some are exposed to toxic effects of AEDs due to the long-term use (Gaitatzis & Sander 2013). Being antiepileptic, valproic acid (VPA) is used as an anticonvulsant and mood regulator in various nervous system disorders (Ornoy *et al.* 2020). Side effects such as teratogenicity, cytotoxicity, hepatotoxicity, and pancreatitis have been reported in the lifetime use of VPA (Adewole *et al.* 2021). Neurological problems such as epileptic seizures observed in cancer patients may develop depending on both the type of the cancer and the treatment administered (Aydın & Sohtaoğlu 2015).

In addition to the detoxification mechanisms naturally found in organisms, phytochemicals and antioxidants with antigenotoxic effects taken into the body through diet are effective in preventing the harmful effects of genotoxins (Christen *et al.* 2000, Prakash *et al.* 2021). *Momordica charantia* L. (MC), known as bitter melon, contains different carotenoids during its ripening stage and shows antioxidant properties based on its tocopherol content (Chekka & Mantipelly 2020, Poovitha & Parani 2020, Khalid *et al.* 2021a, b). The medicinal properties of MC such as antidiabetic, anti-inflammation, anticancer, antiviral, and cholesterol-lowering in herbal treatment were reported to be caused by the phenolic compounds showing the antioxidant and antimutagenic potential of the plant (Anilakumar *et al.* 2015, Li *et al.* 2020, Khalid *et al.* 2021a, b).

In the present study, the protective role of MC which is increasingly important against toxic effects caused by the combined use of CP and VPA was investigated in the model organism *Drosophila melanogaster* Meigen, the fruit fly, with Somatic Mutation and Recombination Test (SMART) technique. SMART, also known as the wing spot test in *D. melanogaster*, is an *in vivo* safe, versatile, inexpensive, and easily applicable mutagenicity test technique that is frequently used to detect genotoxic/antigenotoxic effects of various agents on fruit fly breeds (Idaomar *et al.* 2002, Çolak & Uysal 2021). The SMART technique is more preferable than other similar techniques since it shows the mutation in a phenotype (Zimmering *et al.* 1997), allows observing of mitotic recombination, determines the loss of heterozygousness in flies with appropriate gene markers (Würgler 1986), can give results in one generation and allows analyzing many cells with a single fly (Sarıkaya & Çakır 2005).

The increasing incidence of diseases such as cancer and neurological disorders, combined with the growing global population and stress-related factors, has led to a higher utilization of CP and VPA. Consequently, the potential for toxicity and related health risks associated with these drugs have become a significant concern. With this study, it is thought that the use of MC will reveal the positive or negative aspects of toxicity during the treatments in which CP and VPA are used together. Besides, it is thought that it will bring a new perspective in terms of the regulation of antineoplastic and antiepileptic use in treatments.

Materials and Methods

Materials

Cisplatin (CAS: 15663-27-1), valproic acid (CAS: 9966-1), and ethyl methanesulfonate (M0880) were obtained from Sigma Aldrich. The plant material was obtained commercially. The maceration method was applied to obtain the methanol extracts of the seeds (MCS) and fruits (MCF). According to the LD_{50} dose determined as a result of the 24-hour applications of CP, VPA, and MC on *D. melanogaster* larvae, the working concentrations were determined as 0.05 mM for CP and VPA. Methanol extracts at concentrations of 1.25, 2.5, and 5 mg/mL were used for MC seeds and fruits.

Somatic mutation and recombination test

For this test, 1-3 day virgin females of the *flare*^{3} (*flr*^{3}; *flr*^{3}*/In* (*3LR*) *TM3 Bd*^{S}) strain and the same age of the males of the *multiple wing hair (mwh)* strain of *D. melanogaster* were used. The 72±4 hour transheterozygote larvae obtained from the standard cross between *mwh/mwh* and *flr*^{3}*/TM3, Bd*^{s} which were grown in Erzincan Binali Yıldırım University Basic Sciences Application and Research Center Laboratory, were separated from the standard medium, wetted with 5 mL test solution and transferred to glass bottles containing 1.5 g ready-made *Drosophila* medium (Instant *Drosophila* Medium Formula 4-24, Carolina Biological Supply Co., Burlington, NC, USA). 100 larvae were applied in each test group and each application was repeated 3 times. Fruit and seed extracts of MC were administered individually at 1.25, 2.5 ve 5 mg/mL doses to evaluate their genotoxic effect, and each dose was in combination with 0.05 mM CP and VPA to evaluate its antigenotoxic effect. The larvae were grown on a medium with distilled water in negative control trials and on a medium with 1 mM EMS in positive control trials. Stocks and application groups were kept in incubators providing 25±1ºC and 40- 60% humidity. Among the emerging adults, those who had a normal wing shape phenotypically were reserved for preparation. The selected individuals were taken into bottles containing 70% ethanol and stored at +4°C until the wing preparations (Mollet & Würgler 1974, Graf *et al.* 1984, Graf *et al.* 1989).

For microscopic investigations of the wings, they were separated from the body with the help of forceps, placed on slides, and fixed with Entellan (Merck). For each application, the wings of 40 individuals were screened under a light microscope (Leica DM 500 model) and mutant clones were recorded. In the evaluation of mutant clones, if there is 1- 2 *mwh* in the mutant cell cluster, it is called a small single spot, if there are 3 or more *mwh* or 4 or more *flr*^{3}, it is called a large single spot and if *mwh* and *flr*^{3} phenotypes coexist, it is called a twin clone. Small single clones are formed by point mutation, deletion, non-

separation, and mitotic recombination between two determining genes (*mwh* and *flr*^{3}), while twin clones are formed by mitotic recombination between the centromere of the third chromosome and the *flr*^{3} gene (Graf *et al.* 1984).

Statistical analysis

We analyzed data obtained from the groups using the conditional binomial test with a 5% confidence interval. To evaluate the antigenotoxic effect of MC extracts, we compared them with the negative control group. Meanwhile, to assess the genotoxic effect of CP and VPA groups, we compared them with the positive control group (Kastenbaum & Bowman 1970). The results obtained were determined as negative, positive, or inconclusive (Frei & Würgler 1988). The average induction frequency in each wing cell was calculated according to the formula f= n/NC×10^5 (Szabad *et al.* 1983). If only *flr*^{3} clones are taken into account, "f" in the equation indicates the average frequency of *flr*^{3} clones induction, "n" indicates the total number of *flr*^{3} clones observed, "N" indicates the number of wings analyzed, and "C" indicates the number of cells that can be examined on a wing. The percentage of inhibition used to evaluate the antigenotoxic effect was calculated according to the formula % Inhibition = [(a-b)/a]×100. In this formula, "a" corresponds to the total clone frequency in CP and VPA applications and b corresponds to the total clone frequency in the application of CP and VPA and plant extracts together.

Results

We conducted a pilot study to test the toxicity, due to the limited research on the toxicity of CP, VPA, and MC extracts in the context of SMART. We aimed to assess the potential toxic effects of CP and VPA. The concentrations used were determined based on survival assays in *D. melanogaster* (Fig. 1)

Our results for the survival test indicated that at 0.05 mM CP and VPA, there was high toxicity. Even though it is more noticeable in CP+VPA individuals, using VPA was less toxic than CP. However, as a result of the use of CP and VPA with fruit and seed extracts, the survival rate increased and the values approached the control.

Firstly, the antigenotoxic effect of plant extracts was evaluated (Tables 1-2). All concentrations of MCF and MCS did not alter the frequency of mutant spots when compared to the negative control.

For the genetic toxicity and genoprotectivity evaluation, 0.05 mM CP and VPA, and three dilutions of each of MCF and MCS (1.25, 2.5, and 5 mg/mL) were applied to larvae from the crosses of the wing SMART assay. The genotoxicity of CP and VPA was compared with EMS used as a positive control. The data regarding the effects of the extract on marker-heterozygous larvae are presented in Tables 1-2 as the number of flies analyzed, the frequency of different mutant clones, and the total spots scored for the experimental groups. The total spots scored serve as an indicator of the overall genotoxicity of the compound being tested. Although CP and VPA significantly increased the frequencies of mutant spots in *mwh/flr*^{3} and *mwh/TM3*, the frequencies of total spots showed a decrease in both CP and VPA with MCF and MCS.

Our findings indicate that the extracts derived from MCF and MCS do not exhibit direct or indirect mutagenic or recombinogenic effects in SMART. When assessing the antigenotoxic effects of MCF and MCS, we observed that their post-exposure significantly modified the genotoxicity induced by CP and VPA. This suggests that MCF and MCS have an impact on the mechanisms involved in the repair and processing of DNA lesions induced by these genotoxic agents.

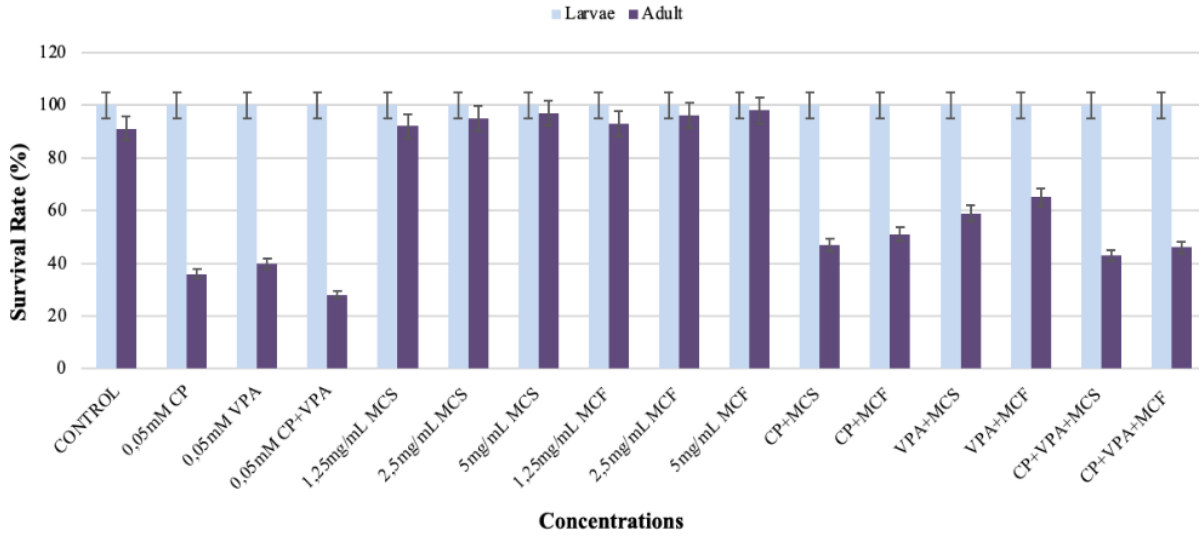

Fig. 1. The survival rate of individuals from the crossings treated with different concentrations of CP, VPA, and MC extracts.

Table 1. Results obtained with the SMART in *Drosophila melanogaster* wing cells with CP, VPA, and MCS.

	Genotypes and experimental groups	Number of wings (N)	Small single spots (1–2 cells) (*m = 2*)			Large single spots (>2 cells) (*m = 5*)			Twin spots (*m = 5*)			Total *mwh* spots (*m = 2*)			Total spots (*m = 2*)			Clone induction frequencies (per 10^5 cells per cell division)	% Inhibition
			No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D	No	Fr.	D		
mwh/flr³	**Distilled water**	80	24	(0.30)		2	(0.03)		1	(0.01)		26	(0.33)		27	(0.34)		1.33	
	MCS (1.25 mg/mL)	80	15	(0.19)	-	2	(0.03)	i	1	(0.01)	i	16	(0.20)	-	18	(0.23)	-	0.82	
	MCS (2.5 mg/mL)	80	13	(0.16)	-	2	(0.03)	i	1	(0.01)	i	14	(0.18)	-	16	(0.20)	-	0.72	
	MCS (5 mg/mL)	80	12	(0.15)	-	1	(0.01)	-	0	(0.00)	i	13	(0.16)	-	13	(0.16)	-	0.67	
	EMS (1 mM)	80	181	(2.26)	+	119	(1.49)	+	29	(0.36)	+	192	(2.40)	+	329	(4.11)	+	9.84	
	CP (0.05 mM)	80	215	(2.69)	+	62	(0.78)	+	30	(0.38)	+	241	(3.01)	+	307	(3.84)	+	12.35	
	VPA (0.05 mM)	80	87	(1.09)	+	41	(0.51)	+	17	(0.21)	+	92	(1.15)	+	145	(1.81)	+	4.71	
	CP+VPA	80	227	(2.84)	+	71	(0.88)	+	33	(0.41)	+	258	(3.23)	+	331	(4.14)	+	13.22	
	CP+MCS (1.25 mg/mL)	80	155	(1.94)	-	60	(0.75)	i	25	(0.31)	-	167	(2.09)	-	240	(3.00)	-	8.56	21.88↓
	CP+MCS (2.5 mg/mL)	80	115	(1.44)	-	50	(0.63)	-	20	(0.25)	-	126	(1.58)	-	185	(2.31)	-	6.45	39.84↓
	CP+MCS (5 mg/mL)	80	95	(1.19)	-	39	(0.49)	-	16	(0.20)	-	102	(1.28)	-	150	(1.88)	-	5.23	51.04↓
	VPA+MCS (1.25 mg/mL)	80	66	(0.83)	-	28	(0.35)	-	11	(0.14)	-	75	(0.94)	-	105	(1.31)	-	3.84	27.62↓
	VPA+MCS (2.5 mg/mL)	80	36	(0.45)	-	17	(0.21)	-	8	(0.10)	-	43	(0.54)	-	61	(0.76)	-	2.20	58.01↓
	VPA+MCS (5 mg/mL)	80	28	(0.35)	-	12	(0.15)	-	6	(0.08)	-	34	(0.43)	-	46	(0.58)	-	1.74	67.96↓
	CP+VPA+MCS (5 mg/mL)	80	74	(0.93)	-	29	(0.36)	-	15	(0.19)	-	93	(1.16)	-	118	(1.48)	-	4.76	64.25↓
vh/TM3	**Distilled water**	80	20	(0.25)		1	(0.01)					21	(0.01)		21	(0.01)		1.08	
	MCS (1.25 mg/mL)	80	14	(0.18)	-	2	(0.03)	i				16	(0.20)	-	16	(0.20)	-	0.82	
	MCS (2,5 mg/mL)	80	13	(0.16)	-	1	(0.01)	i				14	(0.18)	-	14	(0.18)	-	0.72	
	MCS (5 mg/mL)	80	11	(0.14)	-	1	(0.01)	i				12	(0.15)	-	12	(0.15)	-	0.61	
	EMS (1 mM)	80	152	(1.90)	+	97	(1.21)	+				249	(3.11)	+	249	(3.11)	+	12.76	
	CP (0.05 mM)	80	203	(2.54)	+	51	(0.64)	+				254	(3.18)	+	254	(3.18)	+	13.01	
	VPA (0.05 mM)	80	78	(0.98)	+	35	(0.44)	+				113	(1.41)	+	113	(1.41)	+	5.79	
	CP+VPA	80	219	(2.74)	+	68	(0.85)	+				287	(3.59)	+	287	(3.59)	+	14.70	
	CP+MCS (1.25 mg/mL)	80	131	(1.64)	-	51	(0.64)	i		*		182	(2.28)	-	182	(2.28)	-	9.32	40.63↓
	CP+MCS (2.5 mg/mL)	80	100	(1.25)	-	43	(0.54)	-				143	(1.79)	-	143	(1.79)	-	7.33	53.39↓
	CP+MCS (5 mg/mL)	80	82	(1.03)	-	32	(0.40)	-				114	(1.43)	-	114	(1.43)	-	5.84	62.76↓
	VPA+MCS (1.25 mg/mL)	80	53	(0.66)	-	20	(0.25)	-				73	(0.91)	-	73	(0.91)	-	3.74	49.72↓
	VPA+MCS (2.5 mg/mL)	80	24	(0.30)	-	11	(0.14)	-				35	(0.44)	-	35	(0.44)	-	1.80	75.69↓
	VPA+MCS (5 mg/mL)	80	17	(0.21)	-	8	(0.10)	-				25	(0.31)	-	25	(0.31)	-	1.28	82.87↓
	CP+VPA+MCS (5 mg/mL)	80	61	(0.76)	-	21	(0.26)	-				82	(1.03)	-	82	(1.03)	-	4.20	75.12↓

No: the number of clones, Fr.: frequency, D: statistical diagnosis according to Frei and Würgler (1985), *: balancer chromosome TM3 does not carry the *flr³*mutation. +: positive, -: negative, i: inconclusive, m: multiplication factor, probability levels α=β=0.05.

Table 2. Results obtained through the SMART in *Drosophila melanogaster* wing cells with CP, VPA, and MCF.

	Genotypes and experimental groups	Number of wings (N)	Small single spots (1–2 cells) (*m* = 2)			Large single spots (>2 cells) (*m* = 5)			Twin spots (*m* = 5)			Total *mwh* spots (*m* = 2)			Total spots (*m* = 2)			Clone induction frequencies (per 10^5 cells per cell division)	% Inhibition
			No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*		
mvh/flr^3	**Distilled water**	80	24	(0.30)		2	(0.03)		1	(0.01)		26	(0.33)		27	(0.34)		1.33	
	MCF (1.25 mg/mL)	80	13	(0.16)	-	2	(0.03)	i	1	(0.01)	-	13	(0.16)	-	16	(0.20)	-	0.67	13
	MCF (2.5 mg/mL)	80	10	(0.13)	-	1	(0.01)	-	0	(0.00)	-	10	(0.13)	-	11	(0.14)	-	0.51	10
	MCF (5 mg/mL)	80	8	(0.10)	-	1	(0.01)	-	0	(0.00)	-	9	(0.11)	-	9	(0.11)	-	0.46	8
	EMS (1 mM)	80	181	(2.26)	+	119	(1.49)	+	29	(0.36)	+	192	(2.40)	+	329	(4.11)	+	9.84	
	CP (0.05 mM)	80	215	(2.69)	+	62	(0.78)	+	30	(0.38)	+	241	(3.01)	+	307	(3.84)	+	12.35	
	VPA (0.05 mM)	80	87	(1.09)	+	41	(0.51)	+	17	(0.21)	+	92	(1.15)	+	145	(1.81)	+	4.71	
	CP+VPA	80	227	(2.84)	+	71	(0.88)	+	33	(0.41)	+	258	(3.23)	+	331	(4.14)	+	13.22	
	CP+MCF (1.25 mg/mL)	80	149	(1.86)	-	57	(0.71)	i	21	(0.26)	-	163	(2.04)	-	227	(2.84)	-	8.35	26.04↓
	CP+MCF (2.5 mg/mL)	80	109	(1.36)	-	46	(0.58)	-	17	(0.21)	-	124	(1.55)	-	172	(2.15)	-	6.35	44.01↓
	CP+MCF (5 mg/mL)	80	90	(1.13)	-	35	(0.44)	-	14	(0.18)	-	97	(1.21)	-	139	(1.74)	-	4.96	54.69↓
	VPA+MCF (1.25 mg/mL)	80	61	(0.76)	-	23	(0.29)	-	9	(0.14)	-	74	(0.93)	-	93	(1.16)	-	3.79	38.34↓
	VPA+MCF (2.5 mg/mL)	80	32	(0.40)	-	14	(0.17)	-	6	(0.11)	-	39	(0.49)	-	52	(0.65)	-	2.00	64.09↓
	VPA+MCF (5 mg/mL)	80	26	(0.33)	-	10	(0.13)	-	3	(0.04)	-	31	(0.39)	-	39	(0.49)	-	1.59	72.93↓
	CP+VPA+MCF (5 mg/mL)	80	73	(0.91)	-	22	(0.28)	-	11	(0.14)	-	89	(1.11)	-	106	(1.33)	-	4.56	67.87↓
mvh/TM^3	**Distilled water**	80	20	(0.25)		1	(0.01)					21	(0.01)		21	(0.01)		1.08	
	MCF (1.25 mg/mL)	80	12	(0.15)	-	1	(0.01)	i				13	(0.16)	-	13	(0.16)	-	0.67	
	MCF (2.5 mg/mL)	80	8	(0.10)	-	0	(0.00)	-				8	(0.10)	-	8	(0.10)	-	0.41	
	MCF (5 mg/mL)	80	6	(0.08)	-	0	(0.00)	-		*		6	(0.08)	-	6	(0.08)	-	0.31	
	EMS (1 mM)	80	152	(1.90)	+	97	(1.21)	+				249	(3.11)	+	249	(3.11)	+	12.76	
	CP (0.05 mM)	80	203	(2.54)	+	51	(0.64)	+				254	(3.18)	+	254	(3.18)	+	13.01	
	VPA (0.05 mM)	80	78	(0.98)	+	35	(0.44)	+				113	(1.41)	+	113	(1.41)	+	5.79	
	CP+VPA	80	219	(2.74)	+	68	(0.85)	+				287	(3.59)	+	287	(3.59)	+	14.70	
	CP+MCF (1.25 mg/mL)	80	127	(1.59)	-	48	(0.60)	i				175	(2.20)	-	175	(2.20)	-	8.97	42.71↓
	CP+MCF (2.5 mg/mL)	80	96	(1.20)	-	40	(0.50)	-				136	(1.70)	-	136	(1.70)	-	6.97	55.73↓
	CP+MCF (5 mg/mL)	80	77	(0.96)	-	28	(0.35)	-				105	(1.31)	-	105	(1.31)	-	5.38	65.89↓

Table 2. Results obtained through the SMART in *Drosophila melanogaster* wing cells with CP, VPA, and MCF (Continued).

Genotypes and experimental groups	Number of wings (N)	Small single spots (1–2 cells) (*m = 2*)			Large single spots (>2 cells) (*m = 5*)			Twin spots (*m = 5*)			Total *mwh* spots (*m = 2*)			Total spots (*m = 2*)			Clone induction frequencies (per 10^5 cells per cell division)	% Inhibition
		No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*	No	Fr.	*D*		
VPA+MCF (1.25 mg/mL)	80	51	(0.64)	-	16	(0.20)	-		*		67	(0.84)	-	67	(0.84)	-	3.43	53.59↓
VPA+MCF (2.5 mg/mL)	80	23	(0.29)	-	7	(0.09)	-				30	(0.38)	-	30	(0.38)	-	1.54	79.01↓
VPA+MCF (5 mg/mL)	80	14	(0.18)	-	5	(0.06)	-				19	(0.24)	-	19	(0.24)	-	0.97	86.74↓
CP+VPA+MCF (5 mg/mL)	80	58	(0.73)	-	16	(0.20)	-				74	(0.93)	-	74	(0.93)	-	3.79	77.54↓

No: the number of clones, Fr.: frequency, D: statistical diagnosis according to Frei and Würgler (1985), *: balancer chromosome TM3 does not carry the *flr*^{3} mutation. +: positive, -: negative, i: inconclusive, m: multiplication factor, probability levels α=β=0.05.

Discussion

Antineoplastic agents used in the treatment of cancer can damage healthy cells and tissues, in addition to their healing effects (Dillard & German 2000, Olusanya *et al.* 2018). Nervous system disorders result from agents that induce neuronal death and damage through the disruption of numerous pathways, such as protein aggregations, oxidative stress, and neuroinflammation, are yet another type of disease whose effects have grown in recent years (Yavarpour-Bali *et al.* 2019). As knowledge of neuroscience increased, the use of anticonvulsant agents for nervous system diseases also increased (Pal *et al.* 2021). To reduce cytotoxic and genotoxic side effects due to drugs requiring long-term use such as antineoplastics and anticonvulsants, it became a necessity to carry out studies containing supportive alternative food supplements that can be obtained from plants. Plants are used as preventive and curative for the prevention and treatment of various diseases in traditional medicine for a long time (Dillard & German 2000). Even if medicated treatment methods provide rapid relief, their long-term use can create toxic effects and cause serious health problems such as allergic reactions, and liver and kidney damage. Thanks to its components without any side effects, herbal sources that act in a healing way against diseases are preferred in developing countries (Gunjan *et al.* 2015). In this context, it is important to determine the toxic/genotoxic potential of plants that do not cause side effects, are reliable, natural, and economical, and are used for therapeutic purposes (Vale *et al.* 2013).

Drosophila melanogaster has been used as a living model organism to study toxicity and genotoxicity related to its development, disease genes, and its relevance to humans (Schneider 2000, Marsh & Thompson 2006). In the present study, it was aimed to determine the possible genotoxic effects of CP and VPA and to eliminate these effects with methanol extracts of MC fruits and seeds. For this purpose, the antigenotoxic effect of the MC plant against CP and VPA genotoxicity was determined in *D. melanogaster* by SMART. It was determined that SMART was the most efficient test technique performed with *D. melanogaster* mutant strains which also allowed mutations and recombination caused by chemicals to be seen in phenotype (Frölich & Würgler 1990, Sarıkaya & Çakır 2005). The most important reason for choosing VPA, which is used as an antiepileptic with CP as an antineoplastic agent, is that they have previously been shown to have genotoxic effects (Katz 1987, Denli *et al.* 2000). MCF and MSC, which are selected as preservatives, were preferred because they are extracts of a plant frequently used in medical treatments.

Stimulation of oxidative stress mechanisms is known to be responsible for the toxic damage caused by CP (Dugbartey *et al.* 2016). VPA is an antiepileptic agent used in the treatment of nervous system diseases such as migraine, bipolar disorder, schizophrenia, and neuropathic pain, mainly epilepsy (Shona *et al.* 2018). The increasing incidence of diseases such as cancer and neurological disorders, combined with the growing global population and stress-related factors, has led to a higher utilization of CP and VPA. Consequently, the potential for toxicity and related health risks associated with these drugs has become a significant concern. They can cause many side effects in the body due to their strong toxic effects after overdose intake and long-term treatment (Lheureux & Hantson 2009, Chateauvieux *et al.* 2010). Recently, it has been reported that the frequency of use of these two drugs in many countries increased with increasing population density (Cauli 2021, Singh *et al.* 2021).

Previous *in vivo* studies demonstrated that CP and VPA are toxic to the percentage of survival and lifespan in *D. melanogaster* (Ersöz & Çolak 2019). Becit *et al.* (2021) evaluated the anticancer and cell viability effects of phenolic compounds of pycnogenol against CP toxicity

in hepatocellular carcinoma cells by the MTT method. Pycnogenol has been shown to induce CP cytotoxicity via combined treatment on HepG2 cells. In a study conducted by Nanau & Neuman (2013), it was reported that VPA has many risk factors with its toxic effect as a result of high doses, and long-term, and multiple drug use. Minagawa *et al.* (1996) determined a 53% presence ratio of psychiatric disorders in cancer patients and found that 42% of them achieved delirium and dementia. Psychiatric disorders were determined at a high level in advanced cancer patients (Rashid *et al.* 2021). Toxic effects that may develop during the use of VPA alone or with antipsychotics or antineoplastics have also been demonstrated in different studies (Zhuo *et al.* 2019, Duarte *et al.* 2021).

Paul *et al.* (2010) researched MC and observed a reduction in chromosome breakage, indicating its potential to decrease the genotoxic activity using RAPD and SCAR. The genotoxic/antigenotoxic potential of many plants used for therapeutic purposes in the literature has been evaluated with SMART. A study demonstrated that *Artemisia herba-alba* exerted a significant and potent antimutagenic activity in the wing and eye spot of *D. melanogaster* (Amkiss *et al.* 2021). The genoprotective effect of extracts belonging to the aboveground parts of the MC at concentrations of 2.5, 5, 10, and 20 mg/mL against 0.125 mg/mL doxorubicin (DXR) was investigated by the somatic mutation and recombination tests in *D. melanogaster*. It was found that the aqueous extracts of the aerial parts of MC used against the DXR mutation showed a healing effect depending on the concentration increase (Guterres *et al.* 2015). In the present study, it was observed that the extracts from MC fruits and seeds were effective in reducing the genotoxic effects caused by CP and VPA. When the results obtained from the evaluation of plant extracts with somatic mutation and recombination test in *D. melanogaster* and the reliability of the test are examined, it is seen that the test technique we selected for our study is the right choice. It is known that MC used in our study has properties such as anti-inflammatory, antioxidant, antibacterial, antiseptic, antiviral, analgesic, antifungal, and anticancer, and has a positive effect on other malignant diseases, pain, diabetes, and allergies (Anilakumar *et al.* 2015, Li *et al.* 2020, Poovitha & Parani 2020, Khalid *et al.* 2021a, b). Aqueous extract from MC fruits has been shown to significantly decrease neuroinflammation, thereby improving the associated neurodegenerative diseases (Nerurkar *et al.* 2011). MC, a versatile vegetable with numerous health benefits, holds significant promise as both a functional food and a valuable medicinal resource. Among its components, peel extract exhibits the highest chemopreventive potential, followed by seed and fruit extracts (Li *et al.* 2020). In this study, it is thought that the antigenotoxic effect of MC extracts, which have gained an important place in the ethnobotanical field, is due to the potential effects of the plant's phenolic compounds.

In the experimental conditions described, the extracts of MC exhibit antigenotoxic activity against the direct-acting mutagen EMS in the *Drosophila* wing spot test. This activity leads to a reduction in the loss of heterozygosity induced by CP and VPA. The present results can be supported by other genotoxicity tests to clarify the toxic effect mechanism caused by CP and VPA. In addition, this study will be useful in drawing attention to the use of these and similar substances that are of great concern to human health.

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