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Genoprotective Role of Purslane Methanol Extract Against Somatic Mutations Induced by Bifenthrin, a Third Generation Prethyroid Insecticide

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ABSTRACT
In this study, in vitro and in vivo genotoxic effects of Bifenthrin (BIF), an important insecticide used in agricultural production, storage and processing, were investigated. The genoprotective properties of purslane (*Portulaca oleracea* L.) against the genotoxic effects of BIF were also determined by using the methanol (POmet) extract of this plant. In vivo experiments were performed with somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. In in vitro studies, human peripheral blood cultures were prepared and different concentrations of BIF were applied to lymphocyte cells in accordance with the procedure of both the micronucleus (MN) and sister chromatid exchange (SCE) assay. The results obtained from all applied tests showed that BIF is genotoxic and induces chromosomal mutations. Later, another experiment was conducted and it was determined that the genotoxic effects of BIF were reduced with POmet (1:1 v/v). This result, which was observed in all in vivo and in vitro tests, shows that purslane plant is a potent radical scavenger. Due to the healing properties of POmet, gas chromatography-mass spectrometry (GS-MS) method was used to determine the components in its content. Some of the components found in the highest ratio in this extract are γ-sitosterol (21.86%), 13-docosenamide, (13.30%), palmitic acid (12.85%), stigmasteol (6.64%), campesterol (5.69%), linoleicacid (5.46%) and 2-methyl-1-hexadecanol (3.88%).

Keywords: *Drosophila melanogaster*, *Portulaca oleracea*, Household insecticides, Human peripheral blood cultures, Gas chromotography-mass spectrometry

1. Introduction

In cases where agricultural products cannot be protected from diseases and pests, it becomes difficult to obtain healthy and sufficient food. Today, about 20% of the world's grain production is lost in the pre-harvest and post-harvest stages (Durmuşoğlu et al. 2010). One of the most important causes of these losses is pests that infect agricultural products and reduce yield. Pesticides are still used as the most effective method to fight with pests (Singh et al. 2020).

Pesticides are chemicals that are used to reduce the devastating effects of live forms on human and animals and on crops such as insects, rodents, wild weeds and fungi to damage inflicted or reduced nutritional value for the food resources production, storage and consumption (Meister 1999; Pazır & Turan 2017). The pesticides group most widely used against pests are insecticides. Insecticides, which are a sub-group of pesticides used in many areas and providing control of harmful organisms, are chemical compounds used in agricultural production, in the storage of products and at homes for the purpose of killing harmful insects or preventing their reproduction. Insecticides are of great help in controlling harmful insects, which can increase product loss up to 100%. However, since insecticides are not specific, they affect not only target organisms but also non-target organisms (Sayılı & Akman 1994; Özyurt et al. 2018). The unconscious use of insecticides has been shown to cause the destruction of beneficial organisms and endanger genetic diversity (Güngör 2003).

Wild plant and animal populations are decreased as a result of intensive use of insecticides in agriculture. Therefore, it was found to be endangered and started destruction of the beneficial organisms and genetic diversity. Insecticides are poisonous compounds that are purposely released into the environment for the specific purpose of killing insects. The broad use of insecticides represents a potential risk to humans and the environment (Cantelli-Forti et al. 1993; Tiryaki et al. 2010). 30% of the world synthetic insecticides consist of pyrethroids and they are often preferred because despite being highly toxic to the target organisms, they are less toxic to birds and mammals (Mazmancı et al. 2008). Pyrethroid is an organic compound similar

to natural pyrethrins formed by pyrethrum flowers (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids are now the bulk of commercial house hold insecticides (Robert 2002; Dev 2017).

Bifenthrin (BIF), a member of the synthetic pyrethroid family of pesticides, is a third generation insecticide used extensively in agricultural production. This group of pyrethroids is not found naturally and is more resistant to light and exhibits higher toxic activity (Mokrey & Hoagland 1989). BIF, with a half-life of approximately 7 days to 8 months, is insoluble in water or very slightly soluble, leaving a lot of residues in the soil (EXTOXNET 1996). In our country and in the world, they are used especially against aphids, fire ants, lice, fleas, spiders, ticks and flies, against ornamental plants, hops, raspberry, corn and cotton pests, as well as in homes, workplaces and schools (EPA 2010).

DNA disruption and oxidative stress play an important role in numerous cancers and pathological disorders, including carcinogenesis and aging (Soltani et al. 2009; Jacobsen-Pereira et al. 2018). Numerous experiments have shown that plant-derived natural compounds demonstrate defensive behaviors against genotoxicity caused by oxidative stress (Plazar et al. 2008; El-Nekeety et al. 2017; Rahmouni et al. 2018). Fruits and vegetables include many types of phytochemicals with antioxidant, anti-mutagenic and anti-carcinogenic properties (Arora et al. 2002; Shahidi & Ambigaipalan 2015; Janet al. 2018). Despite the development of medical science in a tremendous way in the 20th century, plants are still used in traditional medicine (Jain et al. 2007; Izquierdo-Vega et al. 2017).

Purslane (*Portulaca oleracea* L.) is a wide spread wild edible plant with green leaves and is used as a medicinal plant. It is used as salad, vegetable and medicinal plants in all parts of the world. Purslane contains more omega-3 fatty acids (α-linolenic acid in particular) than any other leafy vegetable plant. Since it is used to cure many diseases, the World Health Organisation named the plant “Global Panacea”, which means “good for every disease” (Kızılet & Uysal 2018).

In this study, the genoprotective of methanol extracts of purslane (POmet) against the possible genotoxic effects of BIF were investigated. BIF is a powerful insecticide used against insects, their eggs and larvae, especially in agricultural areas and warehouses where agricultural products are stored. However, this insecticide is also used against insects in crowded environments such as homes, schools and workplaces where people live. In this case, humans as well as insects are exposed to insecticides in both agricultural and living areas. In this study, it was aimed to determine the genotoxic effects of BIF in both *Drosophila melanogaster*, an invertebrate insect species, and humans. The genotoxic effects of BIF were determined in Drosophila by in vivo Somatic Mutation and Recombination Test (SMART). In vitro Sister Chromatid Exchange Test (SCE) and Micronucleus Test were also used to determine the genotoxic effects of BIF in humans. POmet was applied to resolve the genotoxic effects of BIF in the highest application group of all three testing techniques. Additionally, the chemical contents of the methanol extracts of *Portulaca oleracea* was also defined by gas chromatography-mass spectrometry (GS-MS) method.

2. Material and Methods

2.1. Chemicals

The Bifenthrin (CAS No:82657-04-3, state powder purity 96%), methanol (CAS No:67-56-1),dimethyl sulfoxide (CAS No:67-68-5), ethyl methane-sulfonate (CAS No:62-50-0), 5–bromo–2–deoxyuridine (CAS No: 59-14-3), potassium chloride (CAS No: 7447-40-7), giemsa (CAS No: 51811-82-6), bisbenzimide H 33342 (CAS No:23491-52-3), sodium citrate (CAS No: 6132-04-3), sodium chloride (CAS No:7647-14-5), acetic acid (CAS No: 64-19-7),chromosome medium (CAS No: F 5023), colchicine (CAS No: L 6221) and cytochalasin-B (CAS No: 14930-96-2) were purchased from the Sigma-Aldrich Company while Drosophila Instant Medium has been acquired from the Carolina Biological Supplies Company.

2.2. Preparation of the methanol extracts of Portulaca oleracea L.

Purslane plant, which was determined to be used as an antigenotoxic agent in the experiments, was collected from the vicinity of Hasancık village in Adıyaman province and from an altitude of 600-900 meters. Methanol extract (POmet) was prepared with all of the above-ground organs of the purslane plant, such as stem, leaves and flowers, which were collected in its natural environment, during the flowering period and from lands far from agriculture (Uysal et al. 2015). POmet extract was dissolved with DMSO in the course of applications.

2.3. GC-MS System and conditions

Chromatographic analyzes were conducted on the Agilent 7820 A gas chromatography system. Various temperature programs have been studied for the GC-MS process. Components determined according to POmet spectrum. GS-MS analysis of POmet according to Kızılet et al. (2019).

2.4. Experimental animals and laboratory condition

The selected two Drosophila strains (mwh and flr^3) were used in a previous study for somatic mutation and recombination test

(SMART) (Kızılet & Uysal 2019). The flies were kept according to Uysal et al. (2006) laboratory condition.

2.5. Somatic mutation and recombination test (SMART)

The method for somatic mutation and recombination test (SMART) was determined by Graf et al. (1984). According to this protocol, it was adapted to our laboratory conditions and applied. For this purpose, 4, 5, 6 and 7 ppm BIF application groups have been created and highest (7 ppm) BIF concentration was tested antigenotoxically with 1% POmet. The data is analyzed in compliance with the multiple-decision protocol of Frei &Würgler (1995).

2.6. Donors for peripheral blood assays

All donors were determined according to Kızılet et al. (2019). Permission for the study was sought from the Erzurum Provincial Training and Research Hospital Local Ethics Committee (Number: 37732058-53/2467/BEAH KAEK 2015/9-67) and the rules of the committee were observed during the inquiry. Documented informed consent was received from all patients who engaged in the study.

2.7. *Sister chromatid exchange (SCE) assay*

To determine the genotoxic effects of BIF at different concentrations (50, 100, 250 and 500 ppm) and equal concentration of 500 ppm BIF + POmet by SCE, 1–2.5 mL of human peripheral blood was added to 5 mL chromosome media. Different sets were prepared for each concentration and each donor. 5–bromo–2–deoxyuridine (BrdU) was added to all experiment sets at a final concentration of 10-4M. All substances added to the tubes were sterilized to prevent contamination during the experimental stage. The experimental sets were incubated for 72 hours at 37 °C in dark incubators. At 70 hours of the experiment, colchicine was added to the medium at a final concentration of 0.5 µg/mL to stop the mitosis at the metaphase stage. At the end of the 72 hours incubation, the tubes were centrifuged and removed the supernatants. Hypotonic solution (0.075 M KCl) was added onto pellet and incubated for 30 minutes in dark incubator at 37 °C. At the end of the time, the supernatant was removed after centrifugation. The remaining pellet was washed with a fixative consisting of a methanol/acetic acid (3:1 v/v) mixture. This procedure was repeated three times.

Peripheral blood smears were then prepared from the remaining pellet and allowed to dry in the dark for 3 days. These preparations were stained according to Rooney & Czepulkowski 1986 fluorescence plus Giemsa method. For this purpose, preparations were wetted with 0.5 µg/mL bisBenzimide (Hoechst 33342) for 20 minutes. Wet preparations were incubated for 1 hour under 366 nm UV. The preparations were then kept in a 2 X SSC (1:1 v/v 0.03 M sodium citrate/0.03 M sodium chloride) solution in a 65 °C water bath for 1 hour. Finally, the preparations were stained with 5% giemsa.

Preparations were examined at 1000 X magnification and sister chromatid changes were recorded on chromosomes in metaphases. The data obtained were analyzed with SPSS package program.

2.8. Micronucleus (MN) assay

Experimental setups for MN test were prepared by adding 5 mL chromosome medium to the test tubes containing 50, 100, 250 and 500 ppm BIF and equal concentrations of BIF+POmet (only for 500 ppm BIF). All test tubes were allowed to incubate for 72 hours. In the experimental procedure, unlike SCE, BrdU was not added to the tubes. All solutions added to the tubes were sterilized to prevent contamination. Cytochalasin-B was added to the tubes at a final concentration of 3 µg/mL at 48 hours of incubation (In order to have binucleated cells). The tubes were centrifuged at the end of the incubation and hypotonic fluid was applied to the pellet (0.075 M KCl) and incubated for 15 minutes at 37 °C. At the end of the period, the tubes were centrifuged and remaining pellets washed with fixative solution (1:3 v/v acetic acid-methanol) 3 times. Then, the supernatant was discarded and smear preparations were made from the pellet and stained with 4% giemsa. 1000 cells from each preparation were counted and investigated under a light microscope at 400 X magnification. The data obtained were analyzed with SPSS package program.

3. Results

3.1. SMART findings

The findings obtained from distilled water, dimethyl sulfoxide (DMSO), EMS, BIF and 7 ppm BIF+1% POmet application groups for the normal wings (mwh/flr^3) and serrate wings (mwh/TM3) phenotypes are shown in Table 1. As shown in Table 1, there were no significant differences between the values, which were obtained with distilled water and 1ppm DMSO applications for both normal and serrate wing phenotypes. When the BIF application groups (4, 5 and 6 ppm) were compared with the DMSO application group, inconclusive (i) results were observed for all spots, although increased mutation frequency in two phenotypes.

Table 1- Wing spot test data obtained after exposure of BIF and BIF + POmet

Compound concentration (ppm)	*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 frequency (CIF)*
		No	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	*No*	*Fr.*	*D*	
Normal wings (mwh/flr³)																	
Distilled water	80	8	(0.10)		1	(0.01)		0	(0.00)		9	(0.11)		9	(0.11)		0.46
DMSO	80	9	(0.11)	i	1	(0.01)	i	0	(0.00)	i	10	(0.13)	i	10	(0.13)	i	0.51
EMS	80	29	(0.36)	+	11	(0.14)	+	3	(0.04)	i	39	(0.49)	+	43	(0.54)	+	2.00
4 BIF	80	11	(0.14)	i	0	(0.00)	-	0	(0.00)	-	11	(0.14)	i	11	(0.14)	i	0.56
5 BIF	80	12	(0.15)	i	1	(0.01)	i	0	(0.00)	-	13	(0.16)	i	13	(0.16)	i	0.66
6 BIF	80	18	(0.23)	i	0	(0.00)	-	0	(0.00)	-	18	(0.23)	i	18	(0.23)	i	0.92
7 BIF	80	21	(0.26)	+	0	(0.00)	-	0	(0.00)	-	21	(0.26)	+	21	(0.26)	+	1.07
7BIF+%1POmet	80	12	(0.15)	i	0	(0.00)	-	0	(0.00)	-	12	(0.15)	i	12	(0.15)	i	0.61
Serrate wings (mwh/TM3)																	
Distilled water	80	7	(0.09)		0	(0.00)					7	(0.09)		7	(0.09)		0.35
DMSO	80	7	(0.09)	i	0	(0.00)	i	Balancer			7	(0.09)	i	7	(0.09)	i	0.35
EMS	80	19	(0.24)	-	10	(0.13)	+	chromosome			29	(0.36)	+	29	(0.36)	+	1.49
4 BIF	80	9	(0.11)	i	0	(0.00)	-	TM3 does			9	(0.11)	i	9	(0.11)	i	0.46
5 BIF	80	9	(0.11)	i	0	(0.00)	-	not carry			9	(0.11)	i	9	(0.11)	i	0.46
6 BIF	80	10	(0.13)	i	0	(0.00)	-	the flr3			10	(0.13)	i	10	(0.13)	i	0.51
7 BIF	80	11	(0.14)	i	0	(0.00)	-	mutation.			11	(0.14)	i	11	(0.14)	i	0.56
7BIF+%1POmet	80	9	(0.11)	i	0	(0.00)	-				9	(0.11)	i	9	(0.11)	i	0.46

No: number of clones; Fr: frequency; D: statistical diagnosis +: positive; -: negative; i: inconclusive; m:multiplication factor

However, positive (+) results were observed in the highest BIF application group (7 ppm) due to the increase in the number of mutant clones for the normal wing phenotype ($P<0.05$). The clone induction frequency (CIF) was also calculated according to the values obtained in this study. While the CIF value for mwh/flr^3 genotype (normal wing phenotype) in the application of 7 ppm BIF was 1.07, this value for the distilled water and DMSO control groups was measured as 0.46 and 0.51 (Table 1).

In addition, as shown in Table 1, 1% POmet application reduced the frequency of mutations in all spots. Important variations were found between the values obtained with 7 ppm BIF and 7 ppm BIF+1% POmet applications for both normal and serrate wing phenotypes ($P<0.05$). The CIF calculated as 1.07 for mwh/flr^3 genotype (normal wing phenotype) in the 7 ppm BIF application was found to be 0.61 for 7 ppm+1% POmet application group. In the mwh/TM3 genotype (serrate wing phenotype), the CIF values were found to be 0.46 and 0.56 for the same application groups (Table 1). The decrease in CIF values in both normal and serrate wing phenotype was found statistically significant in 7 ppm+1% POmet application group.

3.2. SCE findings

The SCE values, as a result of the application to human peripheral lymphocyte cells at 50, 100, 250 and 500 ppm BIF concentrations, were detected as 4.13±0.01, 4.62±0.01, 6.50±0.03 and 7.05±0.02 respectively. The results were statistically significant ($P<0.05$). These values were determined at 3.60±0.02 for distilled water, 3.70±0.01 for negative control group DMSO and 25.96±0.02 for EMS (positive control group) (Table 2). The difference between positive control group and negative control groups are statistically significant ($P<0.05$). While the replication index (RI) values were accounted, these values decreased in all the application groups. The SCE value of BIF + POmet application at the rate of 1:1 to determine the therapeutic effect of purslane has decreased from 7.05±0.02 to 3.80±0.01 (Table 2). According to these values obtained from BIF + POmet application, the decrease observed for SCE was statistically significant ($P<0.05$).

Table 2- Statistical significance of SCE induction after exposure to four concentrations of BIF and BIF + POmet

Application Groups	*Concentration*	*RI*	*SCE/cell (Average)*	*Min.-Max. SCE*
Distilled Water	-	2.41±0.03	3.60±0.02	1-11
DMSO	%2	2.24±0.07	3.70±0.01	1-10
EMS	2mM	2.25±0.09	25.96±0.02	8-36
Bifenthrin (ppm)	50	2.33±0.08	4.13±0.01*	1-9
	100	2.01±0.07*	4.62±0.01*	1-13
	250	1.95±0.06*	6.50±0.03*	1-11
	500	1.90±0.04*	7.05±0.02*	4-13
BIF + POmet	1:1	2.25±0.11**	3.80±0.01**	1-10

*: Statistical difference is significant according to DMSO at the 0.05 level, **: Statistical difference is significant according to 500 ppm BIF at the 0.05 level.

3.3. MN findings

MN frequencies measured after exposure to varying concentrations (50, 100, 250 and 500 ppm) of BIF in human peripheral lymphocyte cells were detected as 1.100±0.73, 1.475±0.85, 1.850±0.44 and 2.050±0.68, respectively. These values were determined at 0.700±0.38 for distilled water, 0.825±0.65 for DMSO and 3.175±1.40 for EMS (Table 3). The results were statistically significant between the application and all control groups (P<0.05). In addition, as shown in Table 3 NBI decreased in all BIF application groups. NBI was found to be 1.54±0.17 in the DMSO negative control group. In the highest BIF application group, this value decreased to 1.25±0.18 (P<0.05).

In the BIF + POmet application, the MN frequency decreased from 2.050±0.68 to 0.875±0.72 (P<0.05). In this application NBI value increased from 1.25±0.18 to 1.56±0.19 (P<0.05).

Table 3- Statistical significance of MN induction after exposure to four concentrations of BIF and BIF + POmet

Application Groups	*Concentration*	*Investigated of binucleated cells*	*Number of MN within binucleat*			*MN frequency±S.E.*	*Nuclear division index (NDI)±S.E.*
			(1)	*(2)*	*(3)*		
Distilled water	-	4000	28	-	-	0.700±0.38	1.52±0.15
DMSO	%2	4000	33	-	-	0.825±0.65	1.54±0.17
EMS	2mM	4000	88	12	5	3.175±1.40	1.48±0.18
Bifenthrin (ppm)	50	4000	38	3	-	1.100±0.73	1.55±0.13
	100	4000	51	4	-	1.475±0.85*	1.46±0.21
	250	4000	66	4	-	1.850±0.44*	1.38±0.20*
	500	4000	71	4	1	2.050±0.68*	1.25±0.18*
BIF+POmet	1:1	4000	35	-	-	0.875±0.72**	1.56±0.19**

*: Statistical difference is significant according to DMSO at the 0.05 level, **: Statistical difference is significant according to 500 ppm BIF at the 0.05 level.

3.4. C-MS findings

In this study, the components found in P. oleracea were determined by GC/MS method and listed in Table 4. 23 components (99.305%) were identified for POmet. The most common compounds in POmet are γ-sitosterol (peak no. 22), 13-docosenamide (peak no. 13), (Z) and palmitic acid (peak no. 5). The total amount of these three compounds is 48.001%. Compounds such as phytol, linoleic acid, campesterol and stigmasterol were also found in different rates in the content of POmet at All of these compounds induce antigenotoxicity. Therefore, POmet can be considered as a strong radical scavenger against BIF insecticide.

Table 4- Chemical compositions of the POmet

Peak number	*Retantion time(min)*	*Component*	*Molecular formula*	*Molecular weight (g/mol)*	*% ratio in total component*
1	16.137	2,4 dihydroxy-2,4,6-trimethylcyclohexylidene-acetic acid 8-lactone	C11H16O3	196.10	2.034
2	16.727	2-cis-9-octadecenloxyethanol	C20H40O2	312.30	0.863
3	16.856	Hexahydrofamecyl acetone	C18H36O	268.27	1.067
4	18.188	Palmitic acid methyl ester	C17H34O2	270.25	0.768
5	19.104	Palmitic acid	C16H32O2	256.24	12.848
6	21.420	Phytol	C20H40O	296.30	4.101
7	22.121	Linoleic acid	C18H32O2	280.24	5.455
8	29.612	Behenicalchol	C22H46O	326.35	2.008
9	30.026	Dipalmitin	C35H63O5	568.50	1.301
10	30.481	Diisoactyl phthalate	C24H38O4	390.27	2.352
11	31.281	Docosyl acetate	C24H48O2	368.36	2.745
12	32.264	2-methyl-1-hexadecanol	C17H36O	256.27	3.880
13	33.233	13-docosenamide,(Z)=(Erucylamide)	C22H43NO	337.33	13.297
14	33.423	2-bromo octadecanol	C18H35BrO	346.18	2.177
15	33.870	Methyl epoxystearate	C19H36O3	312.26	0.917
16	34.069	Dihyaroxanthin	C17H24O5	308.16	1.303
17	35.163	14-octadecenal	C18H34O	266.26	1.247
18	36.502	α-tocopherol	C29H50O2	430.38	1.245
19	37.714	Campesterol	C28H48O	400.37	5.692
20	38.113	Stigmasterol	C29H48O	412.37	6.642
21	38.245	Ethyl iso-allocholate	C26H44O5	436.31	2.157
22	38.949	γ-sitosterol	C29H50O	414.38	21.856
23	39.467	Olean-12-en-3-one	C30H48O	424.37	3.350
Total component percentage ratio					99.305

4. Discussion

Physical factors such as solar radiation, x-rays and a wide variety of chemicals can affect cellular DNA. Oxidative stress can cause damage to lipids, proteins, and nucleic acids, resulting in improvements in signal transduction path ways, gene expression, cell mutation, and cell death (Demirci et al. 2008; Popracet et al. 2017). Pesticides are a significant group of human-made hazardous chemicals. Their probable synergistic or antagonistic side effects on humans have not yet been thoroughly studied (Demsia et al. 2007). Over the three decades since the launch of the first compounds with adequate photo-stability for agricultural use, synthetic pyrethroids have been valuable instruments for pest control in agriculture, public health and a wide range of household applications.

However, pyrethroids are not only neurotoxic to plants, but also to mammals (Soderlund et al. 2002; Costa 2015). Synthetic pyrethroids are neuropoisons that function on axons in peripheral and central nervous systems by interfering with sodium channels in mammals or insects (IPCS 1990). BIF is chemically classified as a pyrethroid. BIF interferes with the nervous system of the insect when it is eaten or touched (Miller & Salgado 1985; Yanget et al. 2018). It is more harmful to insects than it is to humans, since insects have lower body temperatures and smaller body sizes. Therefore, it can show toxic effects at high doses.

In this study, while 7 ppm BIF was showing genotoxic effects on *D. melanogaster*, upwards of 50 ppm BIF were showing genotoxic effects on human peripheral blood cells. In conclusion; the higher concentrations of BIF were caused by somatic mutation and chromosomal defects in this study. Our findings are in accordance with other research performed in a similar fashion.

BIF was determined reduce the motor activity in rats at high doses (Wolansky et al. 2007; Scollon et al. 2011). BIF alone is not harmful to rodent nerve cells at a concentration of 10-3 M. However, a house hold use substance containing BIF has been found to be neurotoxic at concentrations between 10-6 and 10-7 M. The house hold formulation of the BIF insecticide decreased the viability of rodent nerve cell cultures, while the BIF did not. Both the formulation and the active ingredient decreased the development of neuritis in vitro, although the effect of the formulation was more extreme (Tran et al. 2006). These findings indicate that inert ingredients would strengthen the developmental neurotoxic effects of BIF. According to Walker & Keith (1992) evidence of mutagenic effects from exposure to BIF are inconclusive. Studies of mouse white blood cells were positive for gene mutation. However, other tests of BIF's mutagenic effects, including the Ames test and experiments in liver rat bone marrow cells, were negative.

Sadowska-Woda (2010) has shown that BIF-induced oxidative stress induces increased lipid peroxidation and reduced antioxidant function in human peripheral blood. DNA disruption and oxidative stress play an important role in numerous cancers and pathological disorders, including carcinogenesis and aging (Soltani et al. 2009; Birch-Machin & Bowman 2016). There has lately been a great deal of interest in the anti-mutagenic and anti-carcinogenic ability of plant-derived compounds and natural food ingredients (Bhuvaneswari 2005; Xu et al. 2007; Shahidi 2009). The mechanism of defence of these structurally very diverse compounds may be multifactorial, since the anti-mutagenic behaviour of most of these chemicals is linked to their scavenging properties (Larson 1988). *P. oleracea* is a rich source of omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, and glutathione (Sharmaet et al. 2009; Gharneh & Hassandokht 2012; Naeem & Khan 2013). Purslane contains in large quantities l-norepinephrine (in fresh leaf 0.25%) that neurohormone of helpful to brain fatigue. It has the highest omega-3 fatty acid content of all leafy vegetables (Kumlay et al. 2010). Antioxidant and antimutagenic functions of extracts of *P. oleracea* have been previously demonstrated and are proposed to be linked to their constituents, such as A, B1, B2, C, niasinamid, nicotinic acid, α-tocopherol, β-carotene, β-alanin, β-cyanine, magnesium, calcium, potassium, iron, omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, flavonoids, ascorbic acid and glutathione (Simopoulos 2004).

In our study was determined that POmet, which was used in the experiment, inhibited somatic mutation in *D. melanogaster* and decreased the SCE and MN rate in human peripheral lymphocytes. Our result is supported by similar studies of purslane. Water extract of purslane significantly inhibited the DNA breakage (Behravan et al. 2011) and it's leaves stems and roots showed very strong antioxidant at in vivo and in vitro experiments on rabbits (Yu et al. 2007). Yen et al. (2001) have demonstrated that *P. oleracea* extract has antimutagenic activity against 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) as a mutagenagent.

In POmet sample (Table 4), 99.305% of the total extract was identified, predominating γ-sitosterol (21.856%), 13-docosenamide, (Z) (13.297%), palmitic acid (12.848%), stigmasteol (6.642%) campesterol (5.692%), linoleic acid (5.455%) and 2-methyl-1-hexadecanol (3.880%).

β-sitosterol and γ-sitosterol are most widely found sterols in the plant. It has also been documented that the volume and function of components of the extrinsic apoptic pathway in human lung and breast adenocarcinoma cells can be impaired by γ-sitosterol (Balamurugan et al. 2011). γ-sitosterol and 13-docosenamide, (Z) also shows high antimicrobial activity (Kanimozhi & Bai 2012; Rukshana et al. 2017). Palmitic acid is the most common saturated fatty acid present in animals and plants. It is the first synthesized fatty acid in the formation of fatty acids in living things and the longer fatty acids are produced from it.

Palmitic acid, like other fatty acids, is not free in nature. Reduces hydrogen peroxide formation (Aydın 2009). Stigmasterol and campesterol are a group of phytosterols. Stigmasterol is an unsaturated plant sterol. It is also useful for the treatment of many tumours, including ovarian, lung, breast and colon cancers. It also has strong antioxidant, hypoglycaemic and thyroid inhibiting effects (Panda et al. 2009). Linoleic acid is an unsaturated essential fatty acid. Linoleic acid exhibits potent antioxidant activity as opposed to α-tocopherol (vitamin E), a known antioxidant (Ha et al. 1990).

Consequently, the higher concentration of BIF caused somatic mutation and chromosomal defects in this study. As a result of our study, the increase in mutations in SMART, and SCE and MN in in vitro tests, respectively, can be assumed as a marker of damage in genetic material. As seen above in similar studies, this genetic damage is caused by oxidative stress. Our results and previously conducted studies indicate that the effect of natural components of purslane is radically scavenging on the genotoxicagent BIF. Additionally, the repair of this damage by POmet indicates that purslane can be a strong antigenotoxic agent. This scavenger effect is due to the high concentration on the content of the compound in POmet.

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