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EFFECTS OF A PORFIRAZIN DERIVATIVE ON SOME DEVELOPMENTAL CHARACTERISTICS OF *DROSOPHILA MELANOGASTER*

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In this study, the effects of a porphyrazine derivative, [octakis (2-trimethylammoniumethyl thio) porphyrazinatocobalt] octaiodide (CoPzq), were explored on the developmental characteristics, sex ratio and egg fertility of *Drosophila melanogaster*. CoPzq was preferred in the study due to its probability to be used as a chemotherapic agent in medicine. As a result of the application of CoPzq, a phenotypic abnormality was revealed within the individuals of F_1 and F_2 generation of *Drosophila*. Although individuals of F_2 generation did not directly come into contact with the chemical, the rate of abnormality is high. An increase was observed within both generations in terms of the egg fertility. Besides, a deviation emerged in the sex-ratio in some concentrations.

Keywords: Porphyrazine – *Drosophila melanogaster* – toxic effect – sex ratio – egg fertility

INTRODUCTION

For the treatment of cancerous cells, photodynamic therapy (PDT) has become a commonly recognized procedure. A candidate for PDT preferred to have a high molar absorbance that accumulates preferentially in tumours and exhibits a high singlet oxygen quantum yield [31]. As potential imaging/therapeutic agents, porphyrin derivatives in which the meso (CH) groups are replaced with nitrogen atoms for linking the pyrrole rings show extreme promise [34]. According to current data, porphyrazines are attractive agents with their spectral properties and strong photosensitizing activities [6]. Thus, the photophysical, photochemical and photobiological properties of porphyrin type photosensitizers have been studied, intensively [30]. However, no research has been found about the effects of these molecules on living organisms. For this reason; investigation of the effects of CoPzq on a living organism such as *Drosophila melanogaster* that will enlighten the metabolic activities in mammals plays vital importance. The synthesis and the characterization of CoPzq molecule has been already shown previously in literature [26, 14].

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Drosophila melanogaster with its genetic properties, reproductions and developments is a well-known organism. It has nearly all the metabolic activation enzymes present in mammals. 75% of human genetic diseases shows a clear homology with this organism. It is obvious that any material which can effect *Drosophila* DNA can create an effect on mammalian and human DNA, too [4, 27]. Therefore, trying an organic compound which carries the possibility of being used as a chemotherapic agent primarily on *Drosophila* shall be helpful in terms of seeing its effects on the living ones. In this study, the effects of CoPzq on egg fertility, sex-ratio and morphological properties of *Drosophila melanogaster* was investigated.

MATERIALS AND METHODS

An isogenized line of wild-type strain Oregon R of *Drosophila melanogaster* was used in all studies. All studies were conducted on standard *Drosophila melanogaster* medium (agar, yeast, cornmeal, sucrose, and propionic acide). The cultures used were kept in a refrigerated incubator set to 25 ± 1 °C. The CoPzq was applied to culture medium by means of nutrition. CoPzq solutions were added to medium in experimental group, and no application was made to control group. Since the effect of CoPzq on an organism was searched for the first time, cadmium chloride ($CdCl_2$) which is a heavy metal with a well known toxicity was used as the positive control group in order to fully investigate the toxicity rate of CoPzq on the organism [18].

The lethal concentration value (LC_{50}) of a molecule which used in toxicology investigations has to be set [1]. Thus, living percentage experiments were done between 100 ppm and 20.000 ppm concentrations. In these concentrations, 20 female and 20 male individuals were mated in the culture mediums each contains 1 ml of CoPzq solution. After 24 h, the dead and alive individuals were counted. After many pretesting assays, the experiment concentration was determined as 1500 ppm.

1 ml of CoPzq solution was mixed with 500 ml of culture medium and added to culture media of the experimental group. Morphologies of adult individuals of F_1 and F_2 generations were checked according to the sex [15, 20, 21, 22, 32, 33]. F_2 generation was obtained from individuals that were exposed to toxic chemicals in F_1 generation. The culture medium of the newly formed group of F_2 did not contain any chemicals. Previouslyi in order to investigate the effect of chemicals on egg fertility, 20 virgin female and 20 male individuals were transferred to two different culture mediums, which contain CoPzq and $CdCl_2$ solutions, respectively. These individuals were kept at culture mediums for 5 days. Randomly chosen 10 female species from these individuals were transferred to seperate standart culture mediums which do not contain any chemicals. They were incubated for one day in order to egg laid and then the adult flies were removed. The eggs in culture mediums were counted under a dissection microscope [9].

Statistical comparison of the data was conducted first between the control group and the experiment group and then between the experiment groups themselves. For a better understanding of the comparison on the tables, experimental groups are abbre-

viated as G1, G2, G3 starting from the control group and these abbreviations are used on the tables.

For the statistical evaluation of data, z-test which is the comparison of the contrast between two avarage rates was applied. Minitab for Windows ver. 13.0 statistics software was used in calculations.

$$Z = \frac{(p_1 - p_2)}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}};\ q_i = 1 - p_i$$

p_1, p_2, q_1, q_2: rates between the compared groups
n: the number of samples in the compared groups

RESULTS

Statistics and results related to the effect of the substances applied on egg fertility in *Drosophila* are given in Tables 1 and Fig. 1. In the experimental group where $CdCl_2$ is applied, both total number of eggs and average daily number of eggs come out lower compared to the control group. The same situtation is observed also on F_2 generation. However, CoPzq applied experimental group on both F_1 and F_2 generations had higher average daily number of eggs and the total number of eggs than the control group and this difference is found to be statistically meaningful. CoPzq increased egg fertility on both generations on *Drosophila.*

Table 1
Effect of CoPzq on egg numbers

Generation	Group	The number of observation day	Minimum number of observed eggs	Maximum number of observed eggs	Total number of eggs	Avarage daily number of eggs
F_1	Control group	10	124	249	2,277	228
	CoPzq group	10	126	323	2,882	288
	$CdCl_2$ group	10	30	92	742	74
F_2	Control group	10	238	260	2,468	247
	CoPzq group	10	208	290	2,773	277
	$CdCl_2$ group	10	141	233	2,205	221

Table 2
The effect of CoPzq on the egg fertility

Generation	Group	N	X	S_x	z	Sd	P
F_1	Control group	10	227.70	37.47	–2.760	18	0.013[a]
	CoPzq group	10	288.20	58.33			
	Control group	10	227.70	37.47	11.579	18	0.000[b]
	$CdCl_2$ group	10	74.20	18.80			
	$CdCl_2$ group	10	74.20	18.80	–11.042	18	0.000[b]
	CoPzq group	10	288.20	58.33			
F_2	Control group	10	246.80	8.95	–3.707	18	0.002[a]
	CoPzq group	10	277.30	24.43			
	Control group	10	246.80	8.95	2.826	18	0.011[a]
	$CdCl_2$ group	10	220.50	28.03			
	$CdCl_2$ group	10	220.50	28.03	4.830	18	0.000[a]
	CoPzq group	10	277.30	24.43			

[a] $p<0.05$; [b] $p<0.001$.

In the second part of the research, the effect of CoPzq on the sex ratio was investigated. The data related to the effect of CoPzq on the sex ratio are given in Table 3 and Fig. 2. Sex ratio did not differ from the control group for both generations within the experimental groups to which CoPzq was applied. But a difference was observed in the positive control group (females 1218, males 1029). The sex ratio difference in this group was found to be statistically significant. This statistical significance on the sex ratio of the positive control group is concluded not to be the effect of applied CoPzq.

In another part of the study, the morphologies of individuals that completed their developments within the medium that contains the solution of CoPzq were analyzed (Tables 4–6, Fig. 3**).** In some individuals, phenotypic abnormalities were observed. According to the frequency of occurence, the main abnormalities that were displayed are wing, leg, thorax, and abdomen abnormalities**.** Abnormalities observed in wings, being in left or right wing or in both wings, are curly wing, tassel shaped wing tip, lance shaped wing, athrophy in wing, and wing stuck to the abdomen. The abnor-

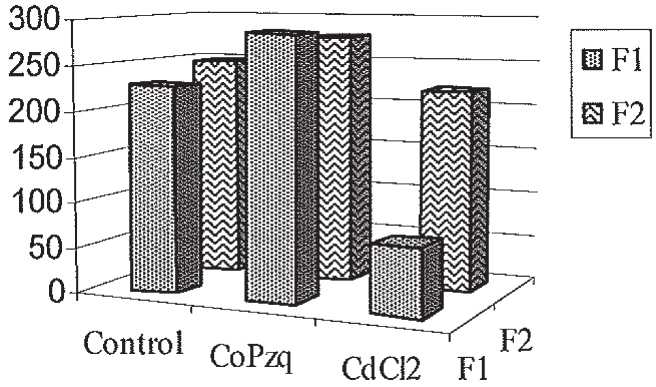


Fig. 1. Effect of CoPzq and $CdCl_2$ on the daily egg fertility. CoPzq causes a positive effect in the daily average egg fertility. The egg fertility on F_1 and F_2 generations where CoPzq applied are higher than that of control group. Especially, in F_1 generation, the difference between the CoPzq applied group and both control group and positive control group ($CdCl_2$) is significant

Table 3
The effect of CoPzq on the sex ratio

Generation	Ppm	Number of female individuals	Z score Female	P Score Female	Number of male individuals	Z score Male	P Score Male
F_1	Control (G1)	1239	(G1-G2) 0.176	0.430	1148	(G1-G2) –0.176	0.430
	CoPzq (G2)	1536	(G2-G3) –1.822[a]	0.034	1437	(G2-G3) 1.568	0.058
	$CdCl_2$ (G3)	1218	(G1-G3) –1.568	0.058	1029	(G1-G3) 1.822	0.034[a]
F_2	Control (G4)	1774	(G4-G5) –0.414	0.339	1783	(G4-G5) 0.414	0.339
	CoPzq (G5)	1594	(G5-G6) 1.031	0.151	1570	(G5-G6) –1.031	0.151
	$CdCl_2$ (G6)	1266	(G4-G6) 0.666	0.252	1317	(G4-G7) –0.666	0.252

[a] $p < 0.05$.

Table 4
The effect of CoPzq on the morphological properties

Generation	Ppm	Number of normal individuals	Number of abnormal individuals	Percentage of abnormal individuals	Z score	P Score
F_1	Control (G1)	2341	46	1.9649	(G1-G2) –3.512	0.000[b]
	CoPzq (G2)	2870	103	3.5888	(G2-G3) –10.980	0.000[b]
	$CdCl_2$ (G3)	1982	265	13.3703	(G1-G3) –10.980	0.000[b]
F_2	Control (G4)	3497	60	1.7157	(G4-G5) –10.778	0.000[b]
	CoPzq (G5)	2938	226	7.6923	(G5-G6) –1.662	0.0483[a]
	$CdCl_2$ (G6)	2368	215	9.0793	(G4-G6) –11.348	0.000[b]

[a] $p<0.05$; [b] $p<0.001$.

malities seen in legs were seen mostly in third pair and rarely in the second pair of legs. These abnormalities are structural abnormalities observed in different parts of the leg. For instance, abnormalities such as pyknosis in the femur, decrease in the segment number in tibia as well as the shortening of tibia were observed all together or separately. In F_1 generation; the abnormality rate which was 1.96% within the control group increased to 3.58% within the experimental group of CoPzq. The dif-

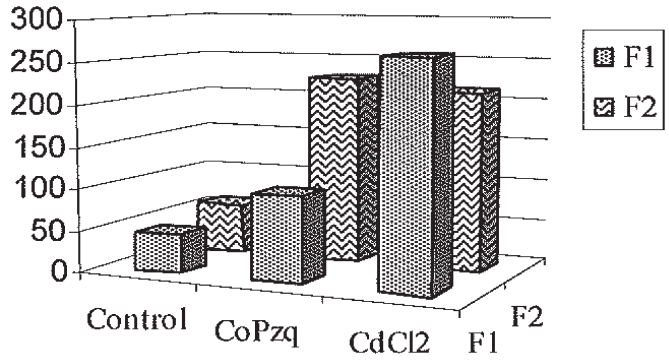


Fig. 2. Effect of CoPzq and $CdCl_2$ on the abnormality rate. The correlation between the abnormal individual rate and the toxicity can be clearly observed in the figure. In fact, the effect of $CdCl_2$, which is used as the positive control group, on the abnormality rate in both generations is high. On CoPzq applied group; abnormality rate is high on F_1 generation compared to the control group and this ratio is very close to the $CdCl_2$ applied group in F_2 generation

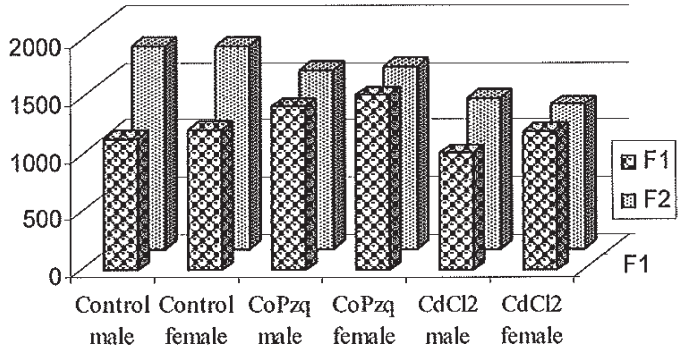


Fig. 3. Effect of CoPzq and $CdCl_2$ on the sex ratio. The figure clearly shows that in all groups (CoPzq or $CdCl_2$ applied and control group) the sex ratio on both F_1 and F_2 generations are close to each other, and thus sex ratio is not changed

ference in between was statistically found significant. On the other hand, the abnormality rate (13.37%) on the positive control group where $CdCl_2$ was applied was considerably high compared to the experimental group (3.58%) and this result was statistically meaningful.

Similar results were, also, observed in F_2 generation. The abnormality rate within the experimental group in F_2 generation (7.69%) that contained the solution of CoPzq is higher than the rate within the control group (1.71%) and F_1 generation (3.58%) (Table 4). Similarly, in F_2 generation of the $CdCl_2$ applied experimental group, abnormality rate (9.07%) is found higher than the control group (1.71%). Individuals of the

Table 5
The ratio of abnormality on the female individuals

Generation	Ppm	Number of normal female individuals	Number of abnormal female individuals	Percentage of abnormal individuals	Z score	P Score
F_1	Control (G1)	1204	35	2.8248	(G1-G2) –1.673	0.047[a]
	CoPzq (G2)	1475	61	3.9713	(G2-G3) –9.598	0.000[b]
	$CdCl_2$ (G3)	1037	181	17.4541	(G1-G3) –10.721	0.000[b]
F_2	Control (G4)	1725	49	2.7621	(G4-G5) –8.139	0.000[b]
	CoPzq (G5)	1442	152	9.5357	(G5-G6) –1.524	0.063
	$CdCl_2$ (G6)	1123	143	11.2954	(G4-G6) –8.788	0.000[b]

[a] $p<0.05$; [b] $p<0.001$.

Table 6
The ratio of abnormality on male individuals

Generation	Ppm	Number of normal male individuals	Number of abnormal male individuals	Percentage of abnormal individuals	Z score	P Score
F_1	Control (G1)	1137	11	0.9581	(G1-G2) –3.712	0.000[a]
	CoPzq (G2)	1395	42	2.9227	(G2-G3) –5.446	0.000[a]
	$CdCl_2$ (G3)	945	84	8.1632	(G1-G3) –5.446	0.000[a]
F_2	Control (G5)	1772	11	0.6169	(G5-G6) –7.236	0.000[a]
	CoPzq (G6)	1496	74	4.7133	(G6-G7) –0.915	0.180
	$CdCl_2$ (G7)	1245	72	5.4669	(G5-G7) –7.424	0.000[a]

[a] $p < 0.001$.

F_2 generation were produced through the transfer of the individuals from the F_1 generation exposed to the substance to the normal culture medium. Although the individuals of F_2 generation have never come into contact with the substance, the fact that phenotypic abnormality rate is higher than that of the F_1 generation is an interesting result. This fact may be due to the hereditary mutations.

The effect of porphyrazine on the morphological structure was also separately analyzed in terms of female and male individuals (Table 5 and Table 6). For the CoPzq applied experimental group in F_1 generation, the abnormality rate in female individuals (%3.97) is high, whereas this ratio is lower in the males (%2.92). On the other hand, individual numbers in the females (1475) are higher than in males (1395). The same situation is valid for the positive control group ($CdCl_2$). However, although abnormality rate in female individuals on F_2 generation is still high, adolescent individual numbers in males are high (eventhough it is meaningles in terms of the statiscal manner). Thus, it can be thought that female individuals are more successful than the male ones in completing their developments even with a phenotypic malformation. In other words, male individuals are more ineffective in completing their developments, and therefore they are much more sensitive. Increase of the individual number on males in the F_2 generation may indicate that male individuals are more likely to resist than the females.

A part of the study covers the comparison of the numbers of adolescent individuals in F_1 and F_2 generation. Porphyrazine increased the number of individuals in F_1 generation and this difference is statistically significant (Table 7). This increase experienced due to CoPzq was not observed in the positive control group, eventhough the individual number of this group was found to be lower than the that of the control

Table 7
Comparison of adult individual number

Generation	Applied Compound	Number of total individuals	Z Score	P score
F_1	Control (G1)	2387	(G1-G2) 0.5	0.000[a]
	CoPzq (G2)	2973	(G2-G3) 0.5	0.000[a]
	$CdCl_2$ (G3)	2247	(G1-G3) 0.5	0.000[a]
F_2	Control (G4)	3557	(G4-G5) 0.5	0.000[a]
	CoPzq (G5)	3164	(G5-G6) 0.5	0.000[a]
	$CdCl_2$ (G6)	2583	(G4-G6) 0.5	0.000[a]

[a] $p < 0.001$.

group. The increase of the number of adolescent individuals in F_1 generation could be due to the increase of the egg fertility. In the F_2 generation, adolescent individual number was found to be lower in both CoPzq experimental group and positive control group when compared to the control group. In other words, individual number increased in the F_1 generation that directly contacted with CoPzq in the culture medium. Therefore, an increase in the egg fertility of the F_1 generation may explain the increase of the adolescent individual number. Also, although the egg fertility is high in the F_2 generation (Table 1), adolescent individual number is low (Table 7). It can be stated that toxic effect in the F_2 generation was more dramatical than in the F_1 generation in terms of completing the development.

DISCUSSION

At the beginning of the is work, LC_{50} value of CoPzq solution was investigated. Eventhough it was tried on very high concentrations (20,000 ppm), did not cause any lethal effect on *Drosophila*. No study, in which the lethality or toxic effects of CoPzq or the porphyrazine group of which it is a member is searched, was encountered. However, porphyrazines form structural hybrid known as phthalocyanines (Pc). Thus, the research results related with the toxicity of phthalocyanines may also be used as references. For instance, Cu-Pc sulfonate, a phthalocyanine, did not induce any toxic effects when given to mice, rabbits, cats and dogs up to 100 mg/kg [38]. Also, on another work, two commercial Cu-Pc sulfonates at concentrations of 5000 ppm were nontoxic to protozoans, small crustaceans, roundworm, small fish and injected to rab-

bit and rat [23]. Similar results were found on another research where, after 19 months, both mice and their offsprings displayed no effects of ilness from five daily injections of uranyl tetrasulfonated Pc 5000 mg/kg in total [37]. The results of these works support that no death was observed during the lethal concentration experiments even at high concentrations such as 20.000 ppm.

CoPzq causing an increase in the daily average egg fertility and total egg fertility in *Drosophila melanogaster* was an unexpected and interesting result (Figure 1). This probably caused an increase also in the total number of adolescent individuals in F_1 generation as a result of this effect. Considering all these data, it can be said that, toxic effect has not been observed on egg fertility in female individuals because the exposure of females was limited to only one day period (Table 1 and 2). In another study that supports this result, the effect of malathion which is an insect was investigated and the egg number laid by female individuals of *Pimpla turinellae* L. was found to be higher than that of the control group [24)]. In another supporting study where the effects of spray formulation of cypermethrin on *Euoniticellus intermedrus* was investigated, no remarkable difference between the experimental and control groups in the life of adults and larvae, egg production, fertility and fecundity was observed [16]. In another research, starvation was applied to the female individuals as the source of the environmental stress and it was observed that the number of the ovarioles increased. Moreover, egg fertility was found to be higher in the female individuals selected from starvation-resistant lines than that of the control group after the tenth day of exposure to starvation. But, this increase is not found meaningful in terms of the statistical manner. As a result, maternal starvation resulted in progeny with a greater number of ovaioles in both selected and unselected lines. In the same study, it was declared that maternal starvation resulted in an increase in progeny ovariole number indicating an integral relationship between maternal food deprivation and the state of the ovary in the next generation. In this study, a greater number of ovarioles was associated with reduced early age fecundity and resistance to a specific stressor, starvation. Starvation and ovariole number in progeny are apparently genetically and phenotipically linked, but the functional basis for this association is not yet understood [36]. In a different research, exposure of fertile females to methoprene resulted in an increase in female reproduction and increased susceptibility to oxidative stress and starvation [28].

According to these studies that support our findings, it can be thought that a low-level toxic effect triggers the stress and female individuals increase their ovulation rate to get over the stress factors. Reproductive potential of the insects is under the influence of a series of behaviors and physiological events occuring as a consequence of the joint operation of the nerve and endocrine system. It was stated that there was no accurate explanation of the molecular cause for the positive effects of insecticides on insect reproduction potential [24]. However, it is argued that insecticides of low sublethal dose stimulate the neuroendocrine system and this can cause an increase in the egg number leading to the secretion of the juvenile hormone much more than normal. In fact, a balance between the levels of juvenile hormone (JH) and 20-hydroxy ecdysone (20HE) that comes up due to the neurotransmitter dopamine is vital for oogenesis. JH also plays a key role in regulating egg-laying behavior under adverse conditions

such as starvation and heat-stress [19, 3]. *Drosophila melanogaster* can itself regulate the number of eggs on condition that it feed on different nutrients. This regulation of egg numbers is suggested to be made in order not to endanger insect generation [25]. As a matter of fact, lethality was not observed in our study even at high substance dosage of 20,000 pm. Our application doses were far below the potential LC_{50} value and this might have increased the egg fertility by stimulating the neuro-endocrine system.

The phenotypes of the F_1 generation completing their developments in the culture medium that contained the substance as well as that of the F_2 generation completing their developments in the normal culture medium were examined. Porphyrazine caused phenotypic abnormalities (wing, leg, thorax and abdomen) on F_1 and F_2 generations on *Drosophila melanogaster* (Table 4). These abnormalities that were mainly observed on the wings and legs were much higher in female individuals than in male ones (Table 5 and 6). A high rate of phenotypic abnormalities in the F_1 generation can be considered normal since the organisms completed their egg and larval stages in a culture medium with substance (Figure 2). Although DNA, the inheritance material in living being, is quite well preserved and has a stable structure, it can be damaged by the enviromental effects or by itself in time. The modification in the molecular structure of DNA can cause considerable problems for the living being. Mutagenic materials can not only create changes in the living being at the chromosomal level but also can cause point mutations at gene level. While numerical chromosome aberrations can be easily observed cytologically, point mutations can only be understood when there is a change in phenotype [35]. In another study, the effects of the sulphated phtalocyanines on the development of the chick embryo were examined, a formal disorder was observed at the back of the body. In the same study, the developmental disorders of the axial organs related to the back side of the body, mesonephros and legs were also observed microscopically [29]. In another work, it was found that ClAl-Pc sulfonate has a very low toxicity when administered in mice and a dose of 100 mg/kg had no effect on it. But, a dose of 200 mg/kg caused %7 phenotypic abnormallities [37].

There are some other studies about *Drosophila* where abnormalities caused by the application of substances other than phthalocyanines were observed. For instance; cypermethrin [10], diazinon [11], arsenic and chromic compounds [13] and malathion [2], were applied on *Drosophila* with similar methods and, abnormalities faced with the phthalocyanines were similarly observed. These researches support the phenotypic abnormallities observed in our work. The cause of the cypermethrin's potential cytotoxic, cytogenotoxic and allergic effects is accepted to be due to the covalent bonds that these compounds or metabolites establish with endogenic macromolecules. As a matter of fact, it was shown that cismehtrin and bioresmethrin connected to the cytoplasmic membrane, endoplasmic reticulum, nucleus and RNA. This situation was confirmed partially in vitro [8]. Covalent bonds that are observed in between the CoPzq (or metabolites) and the homeotic genes (or proteins) are responsible for the regulation of these genes and they might have caused morphological abnormalities as a result of developmental disorders.

Phenotypic abnormalities observed in large numbers and in various ways during our studies are thought to be due to the genotoxic or mutagenic effects of these chemicals especially on the developmental genes. A deletion mutation in the Antennapedia complex of *Drosophila melanogaster* causes both dominant and recessive loss-of-function phenotypes. This mutation is also associated with dominant thoracic defects [5]. Limbs and legs take their roots from the thorax segments. Phenotypic abnormalities, on the other hand, are commonly observed on limbs and legs. These frequently-seen abnormalities could have been formed as a result of such homeotic gene mutations. Because *Drosophila melanogaster* genome [17] was analyzed for the phenotypes observed in the research and phenotype descriptions of the resulting individuals with abnormal phenotype were not observed. This supports the theory that mutations occur through the developmental genes.

The abnormality rate in the F_2 generation, however, is found higher than that of F_1 generation (Figure 2). None of the individuals of the F_2 generation got into direct contact with the substance and they completed their developments in the normal culture medium. Therefore, the high rate of abnormal phenotypes is an unexpected result. There was no difference between the culture mediums of the experiment groups and the control group in terms of the conditions of the F_2 generation but the abnormality rate was relatively high compared to the control group (Table 4–6). Therefore, it can be claimed that CoPzq and $CdCl_2$ cause hereditary mutations. As a result of the chemical application on both F_1 and F_2 generations, the abnormality ratio was observed higher in female individuals than in male ones (Table 5 and 6). However, when these tables were analyzed, it was seen that the number of female individuals were higher than that of the male individuals on F_1 generation. This may be due to the fact that male ones are not as succesfull as female ones in terms of completing their developments. In addition, the reason why the abnormality rate is high in female individuals may be connected with the state of females to be stronger against the toxic effect and overcome through phenotypic malformation.

In the other studies that we conducted, a high phenotypic abnormality rate in the F_2 generation as a result of the substance application was also observed. Abnormality was found to be high in F_2 generation through the application of diazinon to *Drosophila*. This abnormality observation was attributed to the genetic mutations [11]. When cypermethrin was applied, abnormality rate of the F_2 generation was found to be lower than that of the control group [10]. These abnormalities observed in the F_2 generation could lead to genetic mutations by establishing bonds with the DNA molecule or through the replacement of base pairs. Similar to this situation, when both sodium arsenic and chromium (III) chloride ($Cr(Cl)_3$) were applied to *Drosophila* together, it was observed that abnormality rate of the arsenic compund in the F_2 generation was high at low $Cr(CI)_3$ concentrations [13].

The effect of CoPzq on the sex ratio was also examined. CoPzq has not changed the sex ratio on both F_1 and F_2 generations, but a deviation was observed in favor of the female individuals in the F_1 generation of the positive control group (Figure 3). In a similar experiment, cypermethrin [10] and diazinon [11] were applied to *Drosophila* in the same manner and it was observed that they did not cause a devia-

tion on sex ratio. However, the deviation observed in favor of the female individuals in the positive control group has been supported by the other studies. When sodium arsenite and $Cr(Cl)_3$ were applied to *Drosophila*, male individual number decreased and a deviation on the sex ratio was observed in favor of the female individuals [12]. This result that we obtained only in the positive control group can be the indication of the male individuals' being sensitive. This deviation as the decrease of the number of male individuals could show that male individuals can not complete their development. This decrease in the development rate of male individuals in *Drosophila* because of the substance application, might be due to the fact that recessive mutation is seen in females with double X chromosomes and that recessive mutation could easily come out in males [7].

Adolescent individual number increased in the F_1 generation that was treated with CoPzq (Table 7). This result may be due to the increase in egg fertility (Table 1–2). However, adolescent individual number was observed to be low in the F_2 generation despite its high egg fertility (Table 7). Similar results were observed when sodium arsenite and $Cr(Cl)_3$ were applied to *Drosophila* [13]. When this situation is considered together with the fact that F_2 generation has a high abnormality rate, it can be attributed to the fact that some mutations hindered the development.

Assessing all these results together, it could be thought that CoPzq encourages fertility. Consequently, when CoPzq was applied to *Drosophila melanogaster* during the adolescence period, it caused a high level of phenotypic abnormality in F_1 and F_2 generation. But it increased the egg fertility in both generations. It also caused an increase in the number of adolescent individuals but it did not cause any change in the sex ratio. Whether the molecule in question has the feature of encouraging the fertility or not needs to be studied further. Its usibility also needs to be investigated more with other studies.

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