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RESEARCH ARTICLE

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ROLE OF ELLAGIC ACID AGAINST CADMIUM-INDUCED GENOTOXCITY IN *DROSOPHILA MELANOGASTER*

ABSTRACT:

Cadmium (Cd) is a highly toxic and carcinogenic environmental pollutant. Ellagic acid (EA), a plant phenol presents in various human foods, has been reported to have both anti-mutagenic and anti-carcinogenic potential. The present study was conducted to investigate whether EA could play any protective role against genotoxicity induced by Cd in *Drosophila melanogaster*. Five doses of Cd: 0 (control), 1/2, 1, 2 and 4 folds of the allowable concentration in drinking water, were applied to measure some fitness parameters such as viability, developmental time and body size (thorax and wing lengths). Moreover, protein banding patterns and esterase isozymes activities were studied as biochemical effects of Cd pollutant at first, third and sixth generations of treatments. The Cd effects were studied either alone or combined with 0.07 mg/ml of EA for all Cd doses. At all Cd concentrations, viability and body size significantly decreased except for body size only at the lowest concentration (1/2 fold) compared to control experiment. Meanwhile, developmental time did not affect at any Cd concentrations, as compared with control. Biochemical studies revealed that total protein bands decreased, while esterase isozymes activities increased as the Cd concentrations increased. When EA was used in combination with Cd, viability and body size were modulated at the lowest and the recommended doses, while developmental time significantly decreased at the recommended and the highest doses, as compared with control. Moreover, biochemical studies revealed several changes in the number of protein bands and esterase activities than those in Cd alone. These results assume that EA has a good role in minimizing the toxic effects of Cd.

KEY WORDS:

Drosophila melanogaster, cadmium, ellagic acid, genotoxicity, fitness components, biochemical effects

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INTRODUCTION:

Cadmium (Cd) is an environmental pollutant and is considered as one of the toxic heavy metals, which is widely used in many industries. Cadmium toxicity has been widely studied as genotoxic (Rozgaj *et al.*, 2002; Lutzen *et al.*, 2004) and carcinogenic (Schwerdtle *et al.*, 2010) for human beings and can cause damaging effects to humans and animals even at very low concentrations. Within many organisms, the major ligands for Cd are small metal binding proteins known as metallothionein 'MT' (Knapen *et al.*, 2005).

Ellagic acid (EA) is a dimer of gallic acid, which is a naturally occurring polyphenolic found in numerous fruits and vegetables including raspberries, strawberries, cranberries, walnuts, pecans, pomegranates and other plant foods. EA has anti-mutagenic and anti-carcinogenic properties (Ammar *et al.*, 2007). It has also anti-proliferative and antioxidant properties in a number of *in vitro* and small animal models (Seeram *et al.*, 2005). EA has the ability to prevent the binding of carcinogens to DNA and strengthens connective tissues. Furthermore, it can inhibit mutation induction within the cellular DNA (Mertens-Talcott *et al.*, 2003).

Drosophila fly has been used successfully as a genetic indicator organism to investigate environmental mutagens due to its numerous advantages for research in mutagensis. *Drosophila melanogaster* provides one of the main factors in this respect due to its metabolizing system which is similar to that of mammals (Vogel and Nivard, 1993). The present study was conducted to throw light on the genotoxic effects of Cd as an ecological stressor on some fitness component traits in *D. melanogaster*. Moreover, the effect of Cd on gene expression of total protein as well as esterase isozymes was also studied. Another attempt for this study was to elucidate whether the natural phenolic compound; EA, could antagonize Cd-genotoxicity.

MATERIAL AND METHODS:

The present investigation is carried out in the *Drosophila* Laboratory, Department of

Genetics, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

Experimental flies:

The experiment was conducted using wild type flies of a natural population of *D. melanogaster* collected from the Experimental Farm of Faculty of Agriculture, Kafrelsheikh University, Egypt. Flies were maintained at 25±1°C on the standard *Drosophila* medium (cornmeal, agar, molas, yeast and anti-fungal agent; propunic acid).

Chemicals:

Cadmium was used in the form of cadmium chloride (purity, 99%) supplied by Universal Fine Chemicals, PVT, LTD, INDIA. Five concentrations were used: Zero (control), 0.0025 (Cd_1), 0.005 (Cd_2), 0.01 (Cd_3) and 0.02 (Cd_4) mg/L. These doses represent 0, 1/2, 1, 2, and 4 folds of the allowable concentrations in drinking water according to the health standard set by the United States Environmental Protection Agency (EPA).

Ellagic acid (95% purity) was purchased from Sigma (E2250-1G). It was used at a single concentration of 0.07 mg/ml which represents half of that found in pomegranate juice (POM Wonderful LLC, Los Angeles, CA, USA), which was commercially available for human consumption.

Experimental procedure:

The experiment consisted of two treatments: The first treatment received Cd alone in its five doses, while the second one received 0.07 mg/ml of EA combined with each of the Cd doses. Each treatment was tested in four replicates for each concentration.

One hundred third instar larvae (72-h old) were put into 250 ml culture bottles containing 50 ml of treated food medium and kept at 25±1°C. Larvae were allowed to develop into adults. Newly emerged flies were allowed to lay eggs for two days and then the parents were removed to obtain the F_1's. One hundred third instar larvae were then transferred to new bottles to be the parents for the next generation. The same procedure was carried out every generation until the sixth one. Sample flies were obtained only at the first, third and sixth generations for assessments.

Traits studied:

Two life history traits; viability and developmental time, were estimated, they were measured for each replicate. Emerging flies were collected and counted every 12-h until the end of the emergence (the emergence was considered finished when no flies had emerged for 72-h). Viability was calculated as the percentage of emerged adults to initial number of treated larvae (100 larvae). Larva to adult developmental time was estimated as the mean time in days from the mid-point of the oviposition period to the recorded time of emergence.

For size-related traits, 20 females were randomly chosen from each bottle and measured for thorax and wing lengths as indicators of adult body size. Thorax length was measured as the distance between the anterior margin of the thorax and the posterior tip of the scutellum from the dorsal view. Wing length was measured along the fourth longitudinal vein as the sum of two partial lengths, i.e., from the base of the fourth longitudinal vein to the posterior cross-vein and from the posterior cross-vein to the tip of the vein (Prevosti, 1955). Measurements were made with an ocular micrometer inserted into a 10x ocular lens in combination with a 3.2x objective. One micrometer unit equals 0.01 mm.

Electrophoretic analysis:

Alterations in protein banding patterns as well as in number and activities of esterase isozymes bands were detected as biochemical effects of Cd alone or combined with EA at the first, third and sixth generations of treatments.

Total extracted proteins were separated using 7.5% SDS-polyacrylamide gel electrophoresis according to Laemmli (1970). Banding patterns were detected using coomassie brilliant blue-R250. All of the separated bands molecular weights (M.W.) were determined against the standard M.W. marker (66, 45, 36, 29, 24, 20.1, and 14.2 kDa).

Esterase isozymes were detected using 7.5% polyacrylamide gel electrophoresis according to the method of Davis (1964). Esterase bands were detected on the gel as described by Vallejos (1983) using α and β-naphthyl acetate as substrate and subsequent colour development with fast blue RR salts.

Statistical analysis:

Data for the life history and size-related traits were analyzed for each treatment separately using ANOVA. Measurements of developmental time (days) were log transformed and data on viability (proportions) were arcsine transformed, prior to the ANOVA.

The significance of the differences between means of the two treatments (Cd alone or in combination with EA) for concentrations and generations was observed using *Student's t*-test.

RESULTS AND DISCUSSION:

Life history traits:

Data in table 1 show that there was a negative relationship between viability and the Cd doses, without taking into account among-generations differences, being 70.58% for control experiment, which exhibited the

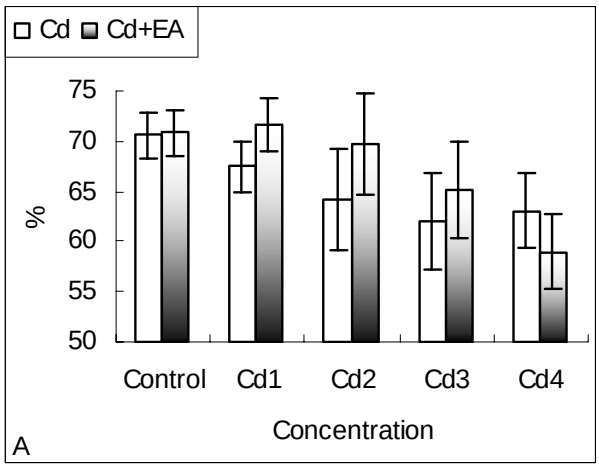
highest value, compared with all other Cd doses. Thus, the presence of Cd in medium reduced viability and therefore reduced fitness. Another trend appeared when EA was used in combination with Cd, since viability was modulated at the lowest and recommended doses (71.65 and 69.67%) which did not differ from control (70.83%). Thus, it was obvious that EA decreased the harmful effect of Cd in the combined treatment than those raised on Cd alone. Another interesting trend was clearly noticed, since the viability of flies reared either on Cd alone or with EA decreased as generations increased only until the 3rd generation, although this decline did not differ significantly between the 1st and 3rd generations; being 60.50 and 57.10% for Cd alone and 63.30 and 59.60% for EA + Cd, respectively. While the viability value increased significantly at the 6th generation for both treatments (78.83 and 78.77%, respectively). This result agreed with those of Otomo and Reinecke (2010) for *Eisenia fetida*, the authors reported that increased tolerance could develop after long-term exposure to a sub-lethal concentration of Cd.

Table 1. Means of larva-adult viability (%) for *D. melanogaster* flies treated with either Cd alone or in combination with EA.

	Gen. \ Conc.	Control	Cd_1	Cd_2	Cd_3	Cd_4	Mean
Cd	F_1	67.75 ± 3.79	62.25 ± 4.37	61.00 ± 3.58	59.75 ± 5.14	51.75 ± 4.66	60.50 ± 0.02 b
	F_3	67.75 ± 2.72	64.75 ± 1.93	54.00 ± 3.19	48.50 ± 1.04	50.50 ± 2.90	57.10 ± 0.01 b
	F_6	76.25 ± 2.96	75.33 ± 3.64	77.75 ± 9.35	77.81 ± 2.81	87.00 ± 4.15	78.83 ± 0.02 a
	Mean	70.58 ± 0.02 a	67.44 ± 0.01 ab	64.25 ± 0.04 ab	62.02 ± 0.02 b	63.08 ± 0.02 b	65.48 ± 0.01
EA +Cd	F_1	67.00 ± 2.74	64.25 ± 2.43	66.75 ± 4.13	69.00 ± 3.67	49.50 ± 2.33	63.30 ± 0.01 b
	F_3	67.00 ± 4.02	63.75 ± 2.17	58.75 ± 3.90	50.50 ± 4.52	58.00 ± 5.07	59.60 ± 0.02 b
	F_6	78.50 ± 4.11	86.94 ± 3.30	83.51 ±7.18	75.67 ± 9.48	69.25 ± 4.72	78.77 ± 0.03 a
	Mean	70.83 ± 0.01a	71.65 ± 0.02 a	69.67 ± 0.03 a	65.06 ± 0.04 ab	58.92 ± 0.02 b	67.22 ± 0.01

- Values with similar alphabetical letter in the same row or column of each treatment do not significantly differ from each other, using LSD test at 0.05

The comparison between the two treatments (according to *t*-test) revealed that the viability increased at all doses when EA was mixed, except for the highest dose (Cd_4), although the degree of increase did not statistically vary (Fig. 1A). On the other hand, figure 1B shows that there were no significant differences between treatments of Cd alone or in combination with EA at the three tested generations.



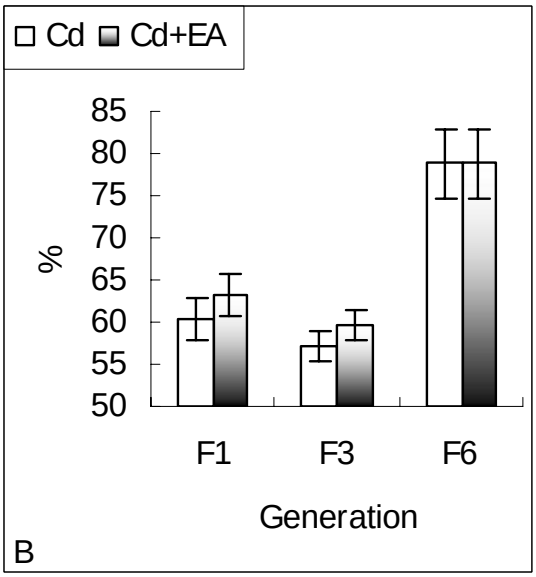


Fig. 1. Comparison between the effects of Cd alone or in combination with EA on viability of *D. melanogaster* flies according to *t*-test at the different concentrations (A) and generations (B). Data presented as the means ± SE

Developmental time of *D. melanogaster* flies was determined and shown in table 2. Data show no significant effects of all Cd doses when used alone as compared with control (11.81 days). This phenomenon was consistent with that reported by Michaud and Grant (2003); they showed that the exposure to copper sulfate did not affect developmental time of the two ladybeetles (*Curinus coeruleus* and *Harmonia axyridis*). Nevertheless, the same treatment had significantly longer developmental time for the ladybeetle (*Olla v-nigrum*). This result was not in agreement with those reported by Yi *et al*. (2001); they showed that Cd-contaminated mediums slowed fly development. When EA was combined with Cd, the lowest value of developmental time (11.85 days) was detected following the treatment with the recommended dose (Cd_2), and then it increased again as concentration increased being 12.21 and 12.23 days after treatment with Cd_3 and Cd_4, respectively.

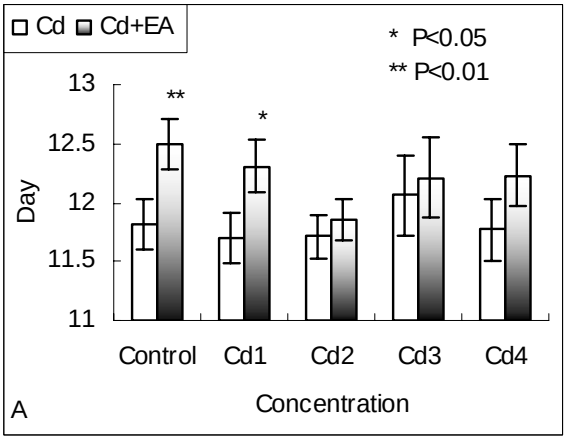
Table 2. Means of developmental time (days) for *D. melanogaster* flies treated with either Cd alone or in combination with EA.

Gen.	Conc.	Control	Cd_1	Cd_2	Cd_3	Cd_4	Mean
Cd	F_1	11.98 ± 0.05	12.48 ± 0.43	12.26 ± 0.34	12.24 ± 0.23	12.42 ± 0.30	12.27 ± 0.11a
	F_3	11.25 ± 0.19	11.21 ± 0.19	11.68 ± 0.37	11.83 ± 0.19	11.48 ± 0.14	11.49 ± 0.09 b
	F_6	12.21 ± 0.15	11.43 ± 0.15	11.20 ± 0.05	12.10 ± 0.22	11.42 ± 0.34	11.67 ± 0.09 b
	Mean	11.81 ± 0.07 a	11.70 ± 0.17 a	11.71 ± 0.17 a	12.06 ± 0.12 a	11.77 ± 0.12 a	11.81 ± 0.06
EA+Cd	F_1	12.74 ± 0.32	12.34 ± 0.37	12.20 ± 0.24	12.78 ± 0.61	12.47 ± 0.38	12.50 ± 0.10 a
	F_3	12.48 ± 0.31	12.45 ± 0.12	12.22 ± 0.23	12.84 ± 0.27	12.71 ± 0.26	12.54 ± 0.07 a
	F_6	12.24 ± 0.10	12.13 ± 0.10	11.13 ± 0.09	11.00 ± 0.00	11.49 ± 0.13	11.60 ± 0.04 b
	Mean	12.49 ± 0.11 a	12.31 ± 0.13 a	11.85 ± 0.11 b	12.21 ± 0.18 ab	12.23 ± 0.10ab	12.21 ± 0.06

- Values with similar alphabetical letter in the same row or column of each treatment do not significantly differ from each other, using LSD test at 0.05

Data also showed that the developmental time significantly decreased at the 3rd and 6th generations (11.49 and 11.67 days, respectively) as compared with the 1st generation (12.27 days) when flies were reared on Cd alone. Moreover, when EA was added, a significant decrease was obtained only at the 6th generation (11.60 days). This means that EA modulate the effect of Cd on the 3rd generation.

In general, flies reared on combined medium had longer developmental time than those on Cd alone. While, this was statistically significant (according to *t*-test) only for control and the lowest dose; 1/2 fold (P<0.01 and 0.05, respectively) as shown in figure 2A. This was also clear when comparing between the two treatments for generations (Fig. 2B), since developmental time differed significantly only at the 3rd generation (P<0.01).



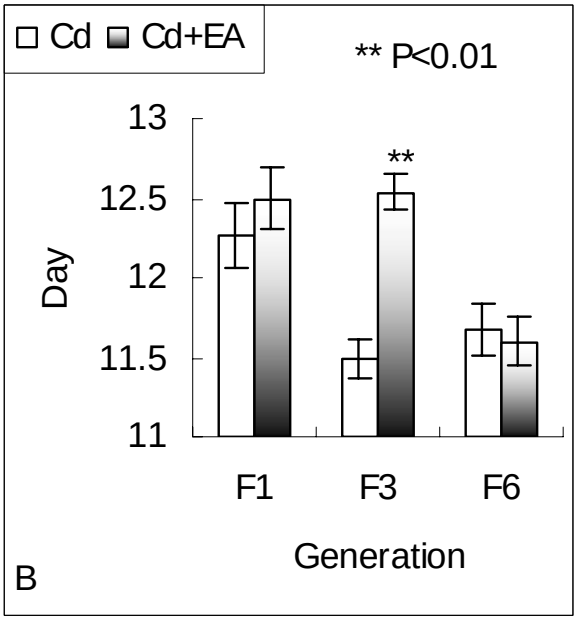


Fig. 2. Comparison between the effects of Cd alone or in combination with EA on developmental time of *D. melanogaster* flies according to *t*-test at the different concentrations (A) and generations (B). Data presented as the means ± SE

Size-related traits:

Data in table 3 show that the means of thorax and wing lengths did not differ significantly for *D. melanogaster* flies reared on Cd_1 medium as compared to control (P<0.05) for both treatments. Similar result for thorax length trait was obtained in the combined medium only for Cd_2. In general, it was clear that the recommended dose and the highest doses (Cd_3 and Cd_4), either in Cd alone or in co-treatment with EA, significantly decreased the thorax and wing lengths of *D. melanogaster* flies as concentration increased, without taking into account among-generations differences. This result is further confirmed by the results obtained in case of the smaller mean body size, which was attributed as a common stress effect in *Drosophila* (Hurtado *et al.*, 1997; Imasheva *et al.*, 1999).

For generations, thorax length had the highest values at the sixth generation for both treatments (32.52 and 32.95 micrometer unit for Cd alone and EA+Cd, respectively), while the first and third generations did not show significant differences; being 30.75 and 30.73-micrometer unit for Cd alone and 30.97 and 30.91 micrometer unit for combined treatment, respectively. On the other hand, wing length increased significantly as generation increased. These results indicated that the tolerance in *D. melanogaster* increased after long-term exposure to stressor.

Table 3. Means of thorax length (TL) and wing length (WL) traits for *D. melanogaster* flies treated with either Cd alone or in combination with EA. Measurements of length are in micrometer unities

treatment	Gen. \ Conc.		Control	Cd_1	Cd_2	Cd_3	Cd_4	Mean
Cd	F_1	TL	32.50 ± 0.10	31.29 ± 0.16	30.05 ± 0.19	30.32 ± 0.17	29.59 ± 0.09	30.75 ± 0.07 b
		WL	64.08 ± 0.15	62.04 ± 0.25	60.51 ± 0.32	60.94 ± 0.29	59.75 ± 0.17	61.46 ± 0.12 c
	F_3	TL	31.06 ± 0.08	30.56 ± 0.18	30.62 ± 0.12	30.80 ± 0.15	30.59 ± 0.08	30.73 ± 0.06 b
		WL	62.28 ± 0.14	61.86 ± 0.31	61.75 ± 0.19	62.00 ± 0.26	62.26 ± 0.10	62.03 ± 0.10 b
	F_6	TL	31.61 ± 0.10	33.24 ± 0.12	33.53 ± 0.13	32.16 ± 0.12	32.09 ± 0.07	32.52 ± 0.06 a
		WL	62.75 ± 0.14	65.49 ± 0.19	65.04 ± 0.16	63.13 ± 0.18	63.63 ± 0.15	64.00 ± 0.09 a
	Mean	TL	31.73 ± 0.07 a	31.70 ± 0.09 a	31.40 ± 0.09 b	31.10 ± 0.10 c	30.75 ± 0.07 d	31.33 ± 0.04
		WL	63.03 ± 0.11 a	63.13 ± 0.12 a	62.43 ± 0.14 b	62.02 ± 0.16 c	61.88 ± 0.11 c	62.50 ± 0.06
EA +Cd	F_1	TL	31.82 ± 0.12	31.27 ± 0.12	31.25 ± 0.15	30.23 ± 0.11	30.26 ± 0.17	30.97 ± 0.07 b
		WL	62.66 ± 0.24	62.26 ± 0.21	62.45 ± 0.25	60.94 ± 0.22	60.34 ± 0.27	61.73 ± 0.12 c
	F_3	TL	31.45 ± 0.13	31.20 ± 0.11	31.30 ± 0.15	30.56 ± 0.13	30.04 ± 0.15	30.91 ± 0.05 b
		WL	62.97 ± 0.23	63.11 ± 0.17	62.30 ± 0.29	61.70 ± 0.21	61.14 ± 0.25	62.24 ± 0.09 b
	F_6	TL	32.35 ± 0.08	33.16 ± 0.10	33.15 ± 0.08	33.85 ± 0.09	32.24 ± 0.11	32.95 ± 0.05 a
		WL	64.90 ± 0.06	64.98 ± 0.14	64.76 ± 0.16	65.61 ± 0.13	63.76 ± 0.24	64.80 ± 0.09 a
	Mean	TL	31.88 ± 0.07 a	31.88 ± 0.06 a	31.90 ± 0.07 a	31.55 ± 0.07 b	30.85 ± 0.09 c	31.61 ± 0.03
		WL	63.51 ± 0.12 a	63.45 ± 0.12 a	63.17 ± 0.12 ab	62.75 ± 0.13 b	61.75 ± 0.16 c	62.93 ± 0.06

- Values with similar alphabetical letter in the same row or column of each treatment do not significantly differ from each other, using LSD test at 0.05

Figure 3 illustrates the comparison between the two treatments; Cd alone or combined with EA. It was clear that both body size traits increased in almost all Cd doses and at all generations when EA was mixed than when Cd was alone. According to *t-test*, this difference was highly significant (P<0.01) for Cd_2 and Cd_3 concentrations for both thorax and wing lengths, while it was significant (P<0.05) in control for wing length trait (Fig. 3 A&C). For generations, a highly significant differences (P<0.01) for both traits was observed at the 6th generation and only significantly (P<0.05) for thorax length at the1st and 3rd generations (Fig. 3B&D). This means that EA had a good role in the improvement of body size as fitness component.

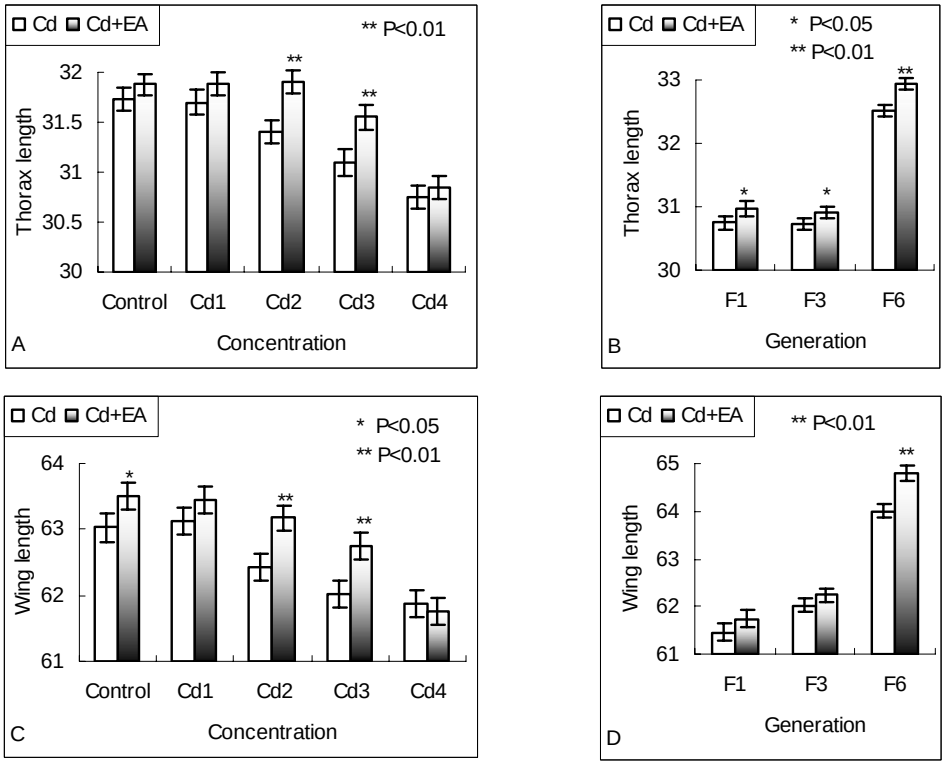
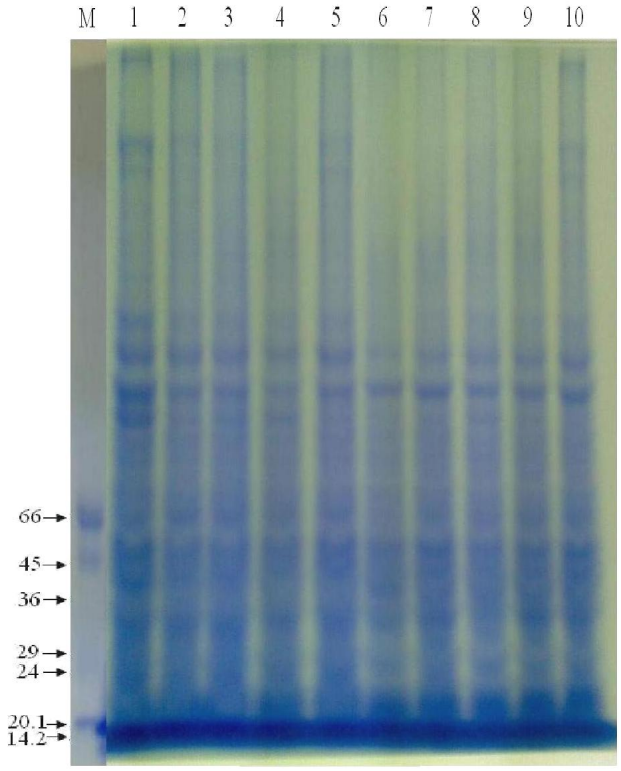


Fig. 3. Comparison between the effects of Cd alone or in combination with EA on thorax and wing lengths of *D. melanogaster* flies according to *t*-test at the different concentrations (A&C) and generations (B&D). Data presented as the means ± SE. Measurements of length are in micrometer unities

Protein Electrophoresis:

Data in figure 4 and table 4 show that all Cd doses showed equal or decreased number in protein bands separated from *D. melanogaster* flies as compared to control (20, 21, and 27 protein bands for the 1st, 3rd and 6th generations, respectively).



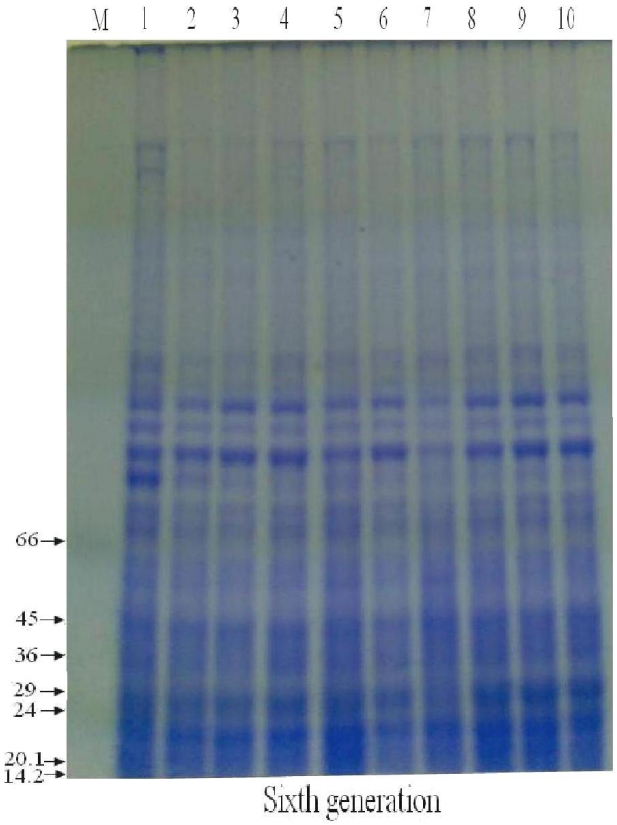


Fig. 4. Protein banding patterns of *D. melanogaster* flies after treatment with different concentrations of Cd alone or in combination with EA at the 1st, 3rd and 6th generations. Lanes:- M: marker; 1: control; 2: Cd_1; 3: Cd_2; 4: Cd_3; 5: Cd_4; 6: EA; 7: EA+Cd_1; 8: EA+Cd_2; 9: EA+Cd_3; 10: EA+Cd_4.

Table 4. Total number of protein bands for *D. melanogaster* flies treated with either Cd alone or combined with EA at the three generations (1, 3, and 6)

Gen. \ Conc.	Control	Cd_1	Cd_2	Cd_3	Cd_4	EA	EA+ Cd_1	EA+ Cd_2	EA+ Cd_3	EA+ Cd_4
F_1	20	19	19	16	18	13	15	15	17	20
F_3	21	21	21	16	15	20	21	21	21	20
F_6	27	26	26	26	26	24	26	28	28	28

Results revealed also that exposure to all Cd doses at the 1st generation led to a decrease in the number of bands. The highest decline was in Cd_3 concentration, where only 16 bands were observed. For the third generation, hazard effect of Cd was observed in the highest concentrations (Cd_3 and Cd_4) resulting in a decrease in band numbers (16 and 15 bands, respectively) as compared to control (21 bands), with no clear differences between the two lowest doses of Cd for both generations (1 and 3). In addition, results for the 6th generation demonstrated the presence

of 26 protein bands at all tested Cd doses, thus only one protein band disappeared as compared to control. The appearance of a definite decrease in protein band numbers in Cd treatment agreed well with the results of Surosz and Palinska (2005), they reported that the synthesis of protein was repressed under heavy metals tested (Hg, Pb, Cu, and Ni). Moreover, studies of Novo *et al.* (2000) and Labra *et al.* (2006) indicated that the treated test organisms with chemicals and heavy metals responded to stress by synthesizing a new set of proteins.

With long exposure period (6^{th} generations), no clear differences among Cd doses and control were detected. This means that treated flies may be highly resistant (less affected) to the applied Cd doses. This was in agreement with the results of viability and body size, which were increased in the 6^{th} generation. This was consistent with Rae *et al*. (1999), who reported that excessive metal accumulation could saturate protective metal-binding cytosolic proteins (e.g. metallothionein), which have an important role in the detoxification of essential and nonessential heavy metals by binding and sequestering metal ions, thus keeping the concentration of free metal exceedingly low.

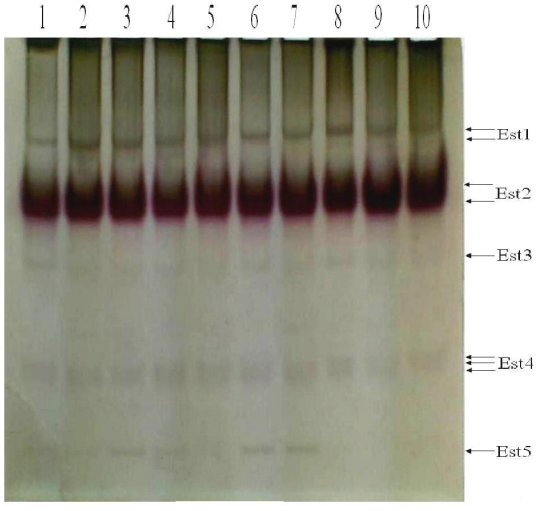
On the other hand, when EA was used, there was a decrease in the number of bands as compared to control, showing 13, 20 and 24 protein bands for EA alone at the 1^{st}, 3^{rd} and 6^{th} generations, respectively. While, using of EA in mixture with Cd caused increase in protein bands in comparison with EA alone, except for Cd_4 at the 3^{rd} generation, which showed 20 bands as EA alone. In addition, it was observed that number of bands increased as generations increased. This might be because flies may be highly resistant as generation progress.

The interaction between Cd and EA resulted in a complete elimination of four protein bands when EA was mixed with either Cd_1 or Cd_2, at the 1^{st} generation (15 bands), compared to Cd alone (19 bands). On the other hand, this interaction resulted in creation of new bands for the highest doses (Cd_3 and $Cd_{4)}$ at the 1^{st} and 3^{rd} generations and for the mixture of EA with Cd_2, Cd_3 and Cd_4 at the 6^{th} generation as compared to Cd alone. The foregoing data showed that EA reacts differently against Cd treatment. These responses could be attributed to the reaction against Cd, as well as its doses. These findings were consistently with the results of Barch *et al.* (1996) who reported that EA is a bifunctional modulator that can bind some toxins directly, rendering them non-toxic, and can directly bind and protect DNA. Lavid *et al.* (2001) found that infact polyphenols (tannins, gallic acid and tannic acid) could detoxify Cd in water lily.

Furthermore, the changes in protein intensity between Cd doses and control were observed. Most of the protein bands gradually became weaker with increasing Cd concentration, particularly at the 1^{st} and 3^{rd} generations. This was in agreement with Bhardwaj *et al.* (2009) who observed that soluble protein content decreased as concentration of metals increased as compared to control plants. While many of the subunit bands became lighter, Melnichuk *et al.* (1982) reported that protein content under heavy metal influence might be affected due to: the enhanced protein hydrolysis resulting in decreased concentration of soluble proteins or the protein synthesis became reduced under all stress conditions. On the other hand, there was an increase in the intensity of some bands as EA treatment. This may indicate that the organism can express some special proteins in response to EA by activating specific groups of genes, which may translate specific proteins.

Isozymes polymorphism:

Results illustrated in figure 5 show the presence of five zones of esterase activities; Est.1, Est.2, Est.3, Est.4, and Est.5. It was evident from zymogram that, at the first generation; common 6 out of 9 bands were observed under both Cd as well as combined treatment with EA. Meanwhile the results of zone Est.1 and Est.4 showed new isozyme bands; the 2^{nd} band for Est.1 appeared in all Cd doses and the 3^{rd} one for Est.4 appeared in the highest Cd doses (Cd_3 and Cd_4) as compared to control. On the other hand, the band of Est.5 at the highest concentration (Cd_4) disappeared as compared to control. In contrast, in combined treatments, Est.1 (the 2^{nd} band) disappeared from EA+Cd_1 as compared to Cd_1 alone (the same as control), while Est.5 disappeared from the Cd_2 and Cd_3 as compared to Cd alone treatments.



First generation

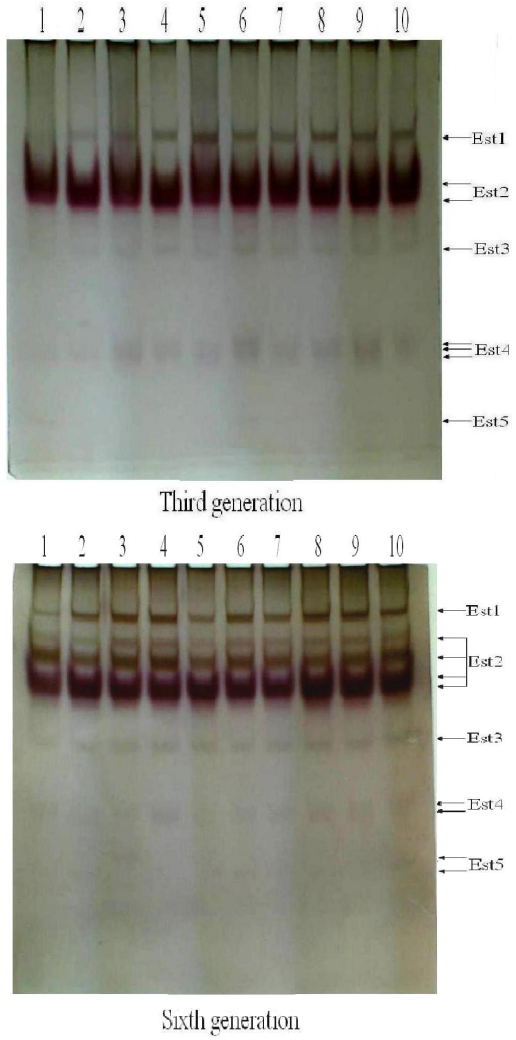


Fig. 5. Esterase isozyme patterns of *D. melanogaster* flies after treatment with different concentrations of Cd alone or in combination with EA at the 1st, 3rd and 6th generations. Lanes:- 1: control; 2: Cd_1; 3: Cd_2; 4: Cd_3; 5: Cd_4; 6: EA; 7: EA+Cd_1; 8: EA+Cd_2; 9: EA+Cd_3; 10: EA+Cd_4

Results of esterase polymorphism at the 3rd generation showed that 6 out of 8 esterase isozyme bands were observed in both treatments at all doses. While zone Est.4 showed new band; the third band, which appeared at Cd_2 and Cd_4 doses and at all combined doses; except for Cd_4, as compared to control. The band of Est.5 disappeared from all Cd doses; except Cd_1, while it disappeared only in the co-treatment between EA and Cd_4 as compared to control. Each of Est.1, and Est.3 bands exhibited a clear increase in activity with Cd doses as compared to control.

The esterase isozymes activity at the 6th generation was also investigated. Ten bands were detected; eight from them were common. An additional band appeared in Est.5 zone than those in the first and third generations; the first band was observed in Cd_2 and the second one in Cd_1 when Cd was used alone. Moreover, the second band of Est.5 appeared at EA alone and EA+Cd4. On the other hand, most of the bands of the Est.1 and Est.2 gradually became stronger as the Cd doses increased, except for Cd_4 (the highest dose), which decreased. Bhardwaj *et al*. (2009) have observed similar observations; they found that esterase activity increased slightly as the concentration of heavy metal increased. The increase of esterase activity at low concentrations of Cd treatments may be due to its role in detoxification mechanisms for heavy metal or due to the *de novo* protein synthesis under conditions of metal stress (Kranthi *et al.*, 2001). On the other hand, the reduction in enzyme activity at the high level of Cd may be due to the interaction of metal with functional sulphydryl (SH)-groups of the enzyme (Prasad and Prasad, 1987). Furthermore, when *Drosophila* flies were treated with combination of Cd and EA, Est.1 and Est.2 zones became stronger than in Cd treatment alone, especially at the three highest doses. These results revealed that the number and activities of some bands decreased while others increased at the 1st, 3rd and 6th generations for the combined treatments as compared to Cd alone. The reduction of esterase bands intensity or the disappearance of some bands might be because EA acts on the nucleic acid level, in which transcriptional, posttranscriptional, translational, or other inhibition processes might be involved.

Based on all the previous results, it could be concluded that *D. melanogaster* flies were strongly affected by Cd exposure, especially at the highest concentrations studied. In addition, Cd has accumulated toxic effect up to the 3rd generation on the fitness parameters and gene expression of total protein as well as esterase isozymes. Afterwards, the same effects were declined at the 6th generation. In addition, EA was considered as an effective anti-toxic agent against the toxic activity of Cd, although these impacts were much smaller in some traits. On the other hand, the studied dose of EA (0.07 mg/ml) itself did not cause any hazardous genetic effect on this system. Thus, it might be possible that the same application might cause the same effects on human being.

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الدور الوقائي لحامض الايلاجيك ضد السمية الوراثية للكادميوم في حشرة الدروسوفيلا ميلانوجاستر

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يعتبر الكادميوم ملوث بيئي عالي السمية ومسرطن. بينما حامض الايلاجيك وهو أحد الفينولات النباتية الموجودة في عديد من الأغذية التي يتناولها الإنسان فانه يعتبر مادة مضادة لكلا من المواد المطفرة والمسرطنة. ولقد اجريت هذه الدراسة بهدف تحديد التأثير الوقائي لحامض الايلاجيك ضد السمية الوراثية للكادميوم في حشرة *الدروسوفيلا ميلانوجاستر*. تناول البحث دراسة تأثير خمسة تركيزات مختلفة من الكادميوم وهى صفر، 0,5، 1، 2، 4 أضعاف التركيز الموصي به في ماء الشرب وتم أخذ بعض القياسات الخاصة بالمواءمة مثل الحيوية، طول فترة التطور، وحجم الجسم (متمثلا في طول الصدر والجناح). بالإضافة إلى دراسة التأثيرات البيوكيميائية للكادميوم على البروتين الكلي والمشابهات الإنزيمية لإنزيم الاستيريز. تم أخذ عينات الحشرات للدراسة عند كل من الجيل الأول والثالث والسادس من المعاملة. تم استخدام الكادميوم في الدراسة اما منفردا أو ممزوجا مع التركيز 0.07 ملليجرام/ملليلتر من حامض الايلاجيك. أظهرت الدراسة أن صفة الحيوية وحجم الجسم قد انخفضت معنويا عند كل تركيزات الكادميوم المستخدمة فيما عدا التركيز المنخفض (بالنسبة لحجم الجسم) فانه لم يختلف معنويا عن المعاملة الضابطة، بينما لم تتأثر صفة طول فترة التطور عند أي من تركيزات الكادميوم. أظهرت الدراسات البيوكيميائية أن زيادة التركيز المستخدم يؤدي إلى الانخفاض في عدد حزم البروتين المنفصلة وزيادة في نشاط إنزيم الاستيريز. من ناحية أخرى أدي استخدام حامض الايلاجيك ممزوجا مع التركيزات المنخفضة من الكادميوم (نصف الجرعة أو الجرعة الموصي بها) الي تعديل في كلا من صفة الحيوية وحجم الجسم، بينما انخفض طول فترة التطور معنويا عند الجرعة الموصي بها والجرعات الأعلى, مقارنة بالمعاملة الضابطة. ولقد ظهرت التأثيرات البيوكيميائية من خلال إحداث تغيرات في كثافة وعدد حزم البروتين المنفصلة وأيضا نشاط إنزيم الاستيريز مقارنة بجرعات الكادميوم المنفردة. يتضح من هذه النتائج دور حامض الايلاجيك في تقليل التأثيرات السامة للكادميوم.