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Selenium Protective Activity Against Aflatoxin B_1 Adverse Affects on *Drosophila melanogaster*

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ABSTRACT

The aim of this work was to investigate the effects of AFB_1 and AFB_1+Se^{4+} on various developmental stages of Drosophila melanogaster were investigated. Both different concentrations of AFB_1 and Se^{4+} applied with AFB_1 were fed during the flies developmental period (egg, larva and pupae). When F_1 progeny of control and application groups were compared with each other, AFB_1 was found to have extending the process of metamorphosis and decreasing the total number of offsprings. But, these negative effects were inhibited with selenium treatment at different concentrations (4.0 and 8.0 ppm). These results suggested that selenium could effectively inhibit AFB_1-induced abnormalities of the developmental stages of D.melanogaster.

Key words: Selenium, Aflatoxin B_1, Developmental stages, Protective effect, *D. melanogaster*

INTRODUCTION

Aflatoxins are a group of naturally occurring, highly toxic mycotoxins that contain a characteristic dihydrobisfuran moiety in their molecular structures.These fungal metabolites are produced by specific strains of *Aspergillus flavus* and *Aspergillus parasiticus*, which are commonly found as contaminants of a variety of foodstuffs.The most common form of aflatoxins, aflatoxin B_1 (AFB_1), has been shown to be a potent mutagen ,hepatocarcinogen and teratogen in several species of experimental animals (Wogan, 1973; Garner and Martin, 1979; Garner,1980; Busby and Wogan, 1985).

These adverse biological effects of AFB_1 are manifested after its metabolic activation and subsequent interaction with cellular macromolecules (Wogan, 1973; Garner and Martin, 1979; Garner, 1980; Essigman et al., 1982). Several factors naturally present in foods of common consumption have been shown to modify these critical reaction of AFB_1, i.e. its microsomal activation and interaction with DNA (Bhattacharya et al., 1984). These include vitamin, trace metals, fatty acids, flavonoids, phenolic acids and other compounds. It is expected that these substances will also counteract the adverse biological effects of AFB_1. Several studies have demonstrated that certain selenium dietary provide a protective effect against AFB_1 toxicity in several animal species. Newberne and Conner (1974) first reported that selenium supplemantion up to a dietary level 1.00 ppm progressively reduced the acute toxicity of AFB_1 in rat, while greater levels enhanced the observed mortality. Studies that have been initiated to test this concept recorded that selenium and certain trace metals have exceptional ability to modify microsome mediated mutagenic activation of AFB_1 in *Salmonella typhimurium* strains TA100 and TA98 (Francis et al., 1988).

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Selenium is an essential micronutrient in the mammalian diet and deficiency of this trace element can cause a variety of severe pathological conditions (Diplock, 1981). There is increasing evidence that selenium can act as an anticarcinogen and inhibit tumor inititation and progression (Horvath and Ip,1983; Darodo et al., 1985; Milner, 1985). Epidemiological studies have revealed an inverse relation between cancer risk and dietary intake or geographic levels of selenium content. Studies in laboratory animals suggested that selenium was also effective in inhibiting AFB_1 induced hepatocarcinogenesis (Baldwin and Parker, 1987; Yu et al., 1988; Lei et al., 1990). This protective effect was confirmed in several later studies with swine and turkeys (Burguera et al., 1983; Davila et al., 1983). In view of the demonstrated protective effect of selenium against the acute toxicity of AFB_1 and the postulated anticarcinogenic effect of selenium studies were conducted by Chen and associates to investigate the possible influence of selenium and vitamin E on certain aspects of the metabolism of AFB_1 in rats and chicks (Chen et al., 1982_a; Chen et al., 1982_b). Their results suggested that combined vitamin E-selenium deficiency enhanced aflatoxin binding to hepatic DNA and RNA in rat.

Howewer, the protective effect of selenium against AFB_1 induced teratogenic effects and influence on some development stages of *Drosophila melonogaster* has not been elucidated. The main aim of present study was to see whether selenium has any protective effect against the adverse effects of AFB_1 on *Drosophila melonogaster*.

MATERIALS AND METHODS

The flies used in experiments were Oregon-R wild type (w.t.) strain of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). This stock had been maintained for many years in culture vials and was, therefore, highly inbred with little genetic variation. The experimental medium was a yeast-agar-sugar medium (Standard Drosophila Medium:SDM). Flies were grown and aged in culture bottles containing SDM. All experiments were carried out at 25°C and 40-60% relative humidity.

The tested substance, crystalline aflatoxin B_1 (AFB_1, Acros Organics, No:227340100, New Jersey, USA), was dissolved in a 10% solution of dimethyl sulfoxide (DMSO, Sigma-Aldrich Laborchemikalies GmbH). A sodium selenite solution (4.0 and 8.0 ppm) was prepared by adding of Na_2SeO_3 (Sigma Chemical Co., St. Louis, MO) to distilled water. In our experiments, parental generations of *D.melanogaster* were treated with various concentrations of both AFB_1 (0.2, 0.5 and 0.8 ppm AFB_1/ ml SDM) and sodium selenite (4.0 and 8.0 ppm Se^{4+}/ ml SDM). To test the effects of the AFB_1 and AFB_1+Se^{4+} on the growth of *D.melanogaster*, this species was cultivated in 9 different media, shown in Table 1. All the females used in the experiments were virgins. The flies with the same age were used for experiments and seven pairs were mated. Then, the developmental stages were followed daily. Offsprings were counted everyday from the first day of eclosion and phenotypic abnormalites of F_1 individuals examined under microscope were observed. Statistical analysis of data was done using Duncan's one-way range test.

Table 1 - The composition of different media used for this study

	Composition			
Medium	**SDM (ml)**	**DMSO (ml/100ml)**	**AFB_1 (ppm)**	**Selenium (ppm)**
Control (C)	100	__	__	__
I (C+DMSO)	100	10	__	__
II	100	__	0.2	__
III	100	__	0.5	__
IV	100	__	0.8	__
V	100	__	0.2	4.0
VI	100	__	0.2	8.0
VII	100	__	0.5	4.0
VIII	100	__	0.5	8.0
IX	100	__	0.8	4.0
X	100	__	0.8	8.0

RESULTS AND DISCUSSION

The effects of the AFB_1 and AFB_1+Se^{4+} on the development stages of *D. melanogaster* are shown in Table 2. In the laboratory at 25±1°C, the life cycle (egg-adult) was 9 days (Uysal and Bahçeci, 1995; Uysal et al., 2002). In control (C), C+DMSO (Medium I) and all experimental groups laid eggs were observed at the second day of mating.In control and Medium I, first adult was emerged from the pupa at 9^{th} days of mating. But in the presence of 0.2, 0.5 and 0.8 ppm of AFB_1 (Medium II, III and IV), various development stages were retarded. At these concentrations of AFB_1, first offsprings of F_1 progeny were seen at 10^{th},11^{th} and 13^{th} days, respectively (Table 2).

Table 2 - Occurrence of egg- adult developmental stages after application of different doses of AFB_1 and AFB_1+ Se^{4+}

	Days of stages at control and applications (ppm)										
Developmental stages	**C**	**I**	**II**	**V**	**VI**	**III**	**VII**	**VIII**	**IV**	**IX**	**X**
Mating	1	1	1	1	1	1	1	1	1	1	1
Egg	2	2	2	2	2	2	2	2	2	2	2
1^{th} instar larvae	3	3	4	3	3	4	3	3	5	3	3
2^{nd} instar larvae	4	4	5	4	4	6	4	4	7	4	4
3^{rd} instar larvae	5	5	6	5	5	7	5	5	9	5	5
Prepupa	6	6	7	6	6	8	6	6	10	6	6
Pupa	7	7	8	7	7	10	7	7	11	7	7
Adult	9	9	10	9	9	11	9	9	13	9	9

On the other hand, one of the most interesting results obtained was that the developmental stages of *D. melanogaster* were observed at the same days with control (C) with shown that Se^{4+} applications (Medium. V-X). Table 2, both 4.0 and 8.0 ppm Se applications prevented the negative effects of AFB_1 on developmental stages and these stages were completed within their normal developmental phases (9 days).

Similar findings have been observed in previous studies also. For example, Lalor et al., (1976) found that growth on AFB_1 media of *D.melanogaster* caused significant increases in egg-to- adult developmental time. Larval and pupal toxic effects caused by AFB_1 have also been demonstrated by various authors in *Musca domestica* (Beard and Walton, 1971) and *D.melanogaster* (Kirk et al., 1971). Besides, according to Chinnici et al.,(1979), second and third larvae of strain A-9 showed significant mortality rates when grown at 0.88 ppm AFB_1. At the 0.88 ppm concentration, both A-9 and A-11 strains showed significant mortality rates for first instar larvae, but the A-9 larvae died at higher rates than the A-11 larvae. Similar results were also obtained after the application of mycotoxins such as rubratoxin B, patulin and diacetoxyscirpenol (Reis, 1975).

The total number of offsprings (for F1 progeny) of aflatoxin B_1 treated *D.melanogaster* were affected by all application groups (Medium II, III and IV) of toxin (Table 3). The maximum inhibition effect was produced with higher toxin concentration (0.8 ppm/ ml, Medium IV). As seen in Table 3 too, the difference between the number of the offsprings of control (2305) and control+DMSO (Medium I, 2218) was statistically unimportant ($P> 0.05$). But, as 2305 F_1 individuals were in control group, the number of offsprings decreased in the application groups (Medium II, III and IV), depending on the dosage increase. Statistical analysis showed that this decreasing was significant ($P< 0.01$).

In our unpublished study, besides the above mentioned dosages of AFB_1, three different dosages have also been examined (1.1, 1.4 and 1.7 ppm/ ml), but, no offsprings were obtained. Furthermore, metamorphosis stopped in 1^{st}, 2^{nd} and 3^{rd} instar larvae according to the increasing concentrations and was not completed.

For many researchers, the most important reason of the decrease of offsprings in *D. melanogaster* exposed AFB_1 was the decrease of fertility, both in male and female (Matsumura and Knight, 1967; Chinnici et al., 1976). Similar effects have also been observed in different animal groups such as mice, rat and mouse (Bashandy et al., 1994; Nair and Verma, 2000; Verma and Nair, 2001).

Table 3 - The effect of different concentrations of selenium together with AFB_1 on the total number of F_1 individuals and formation of malformed individuals (%)

Medium	F_1 progeny	Malformed individuals	%
C	2305	9	0.39
I	2218	7	0.32
II	1120**	73	65.1
V	1623*	52	32.04
VI	1761*	20	11.36
III	924**	75	81.1
VII	1536*	50	32.55
VIII	1602*	16	0.99
IV	39**	3	76.92
IX	1062*	36	33.8
X	1130*	10	0.88

Duncan's one-way range test: * $P<0.05$; ** $P<0.01$

Besides, the extreme sensitivity of larvae and pupae and also their mortality in high concentrations (1.4 ppm) were one of the most important reasons of the decrease in the number of offsprings (Kirk et al., 1971; Llwellyn and Chinnici, 1978).

Furthermore, when F_1 individuals were examined as phenotypic, malformed individuals were also observed and malformation was concentrated mainly on wing, leg and thorax and neither the formation of extremities nor their lackness have been found. While the rate of the malformed individuals in the control was 0.39%, they changed between 65.17-81.16% in Medium II, III and IV, respectively. This ratio of change was statistically important ($P<0.01$). Depending on the increase of concentration in Medium II, III and IV, both the number of individuals decreased and malformation increased (Table 3).

In previous studies, similar results have also been obtained. For example, teratogenic effects of AFB_1 were observed on fetuses of hamster, mice, rat and cow by Aleksandrowicz and Smyk (1973). These teratogenic effects were also found as follows: decreases in body size (Chinnici et al., 1976) and wing length (Lalor et al., 1976), formation of tumor on different body parts (Sidorov et al., 2001), hepatocarcinogen effects at vertebrates (Pier, 1981). These findings are in accordance with our results. According to our data, the most important reason for the formation of phenotypic abnormalities was delaying of metamorphosis, because AFB_1 caused some faults during transcription of developmental genes and defects in homeotic genes which affected the final condition of imaginal discs (Wallace et al., 1991). It has also been reported that AFB_1 is metabolically activated by the microsomal mixed-function monooxygenase system to the 8, 9-epoxide, which readily binds to the nucleophilic sites in DNA to form DNA adducts (Stark,1986). AFB_1 preferentially attacks guanine residues in DNA and the major adduct for is 8,9- dihydro-9-hydroxy-(N7-guanyl) AFB_1, which accounts for over 90% of the total adducts (Essigmann et al., 1982). The formation of AFB_1- DNA adducts in the target cell gives rise to promutagenic sites in DNA.

The addition of different concentrations of Se^{4+} to aflatoxin containing media (Medium V-X), was found to be interesting for the increase of F_1 individuals and the decrease of the rate of malformation. The individuals number belonging to the F_1 was 1120 and the rate of malformed individuals was 65.17 % in the medium containing 0.2 ppm AFB_1 (Medium II). In the media containing 4.0 and 8.0 ppm Se^{4+} (Medium V and VI), F_1 individuals were 1623 and 1761, respectively. Furthermore, the number of malformation decreased from 65.17% to 11.36%. Similarly, while the number of F_1 individuals was 924 in the medium of 0.5 ppm AFB_1 (Medium III), this number was 1536 and 1602 in Se^{4+} added media (Medium VII and VIII). The rate of malformed individuals dropped from 81.16 to 0.99%. The antagonistic effect of Se^{4+} was clearly observed in 0.8 ppm AFB_1 (Medium IV, Table 3).

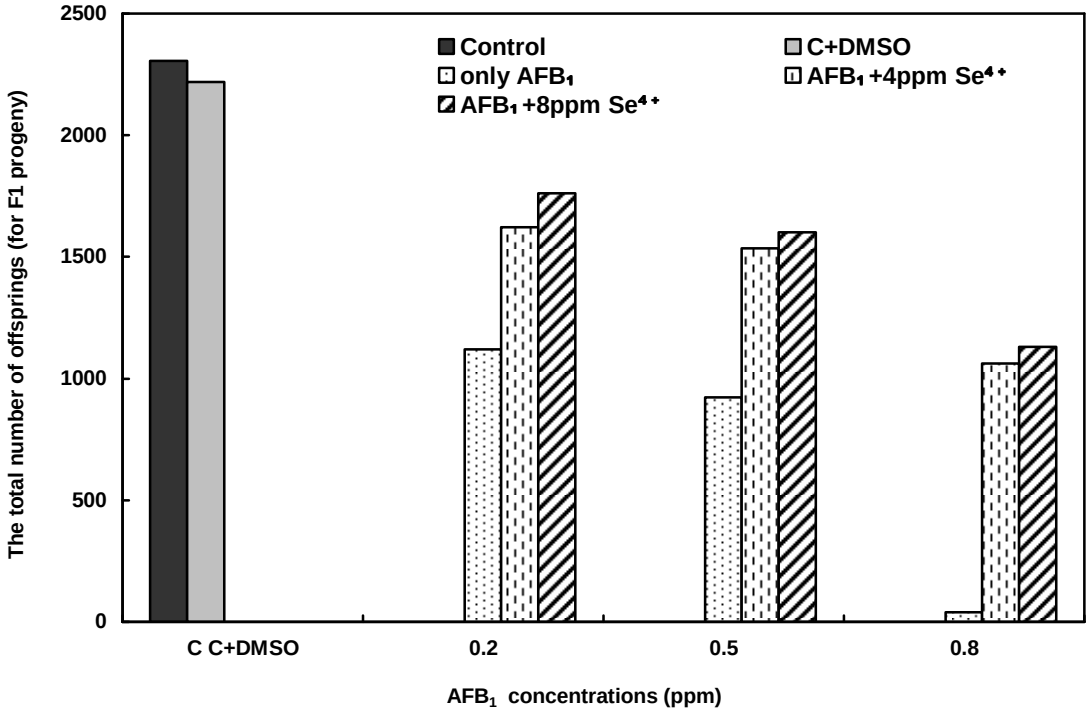

Figure 1 - Comparison of the total number of offsprings obtained from *D.melanogaster* treated with different concentrations of AFB_1 and AFB_1+Se^{4+}.

As only 39 individuals were counted at the 0.8 ppm AFB_1, the individuals number with the increasing Se^{4+} concentrations was found to be important. This was statistically important ($P<0.05$), (Fig. 1).

Although protective effects of selenium on teratogenic disorders has not been identified, some studies demonstrated that it has protective effect against the acute toxicity of AFB_1 and carcinogenesis in animal species (Chen et al., 1982a).Carcinogen-induced cellular oxidative damage and its role in the cytotoxicity and carcinogenesis have attracted much attention (Imlay and Linn,1988; Farber et al., 1990). Oxidative damage usually refers to the impairment of the function of cellular components, e.g., enzymes, nucleic acids, membranes and proteins, by reactive oxygen species such as superoxide radicals (O_2^-), hydroxyl free radical (OH), and hydrogen peroxide (H_2O_2). Oxidative damage causes cellular damage, in particular ageing and some irreversible pathologic processes such as cancer (Gracy et al., 1999). Therefore, it could be possible that AFB_1 also contributed to the genotoxic damage. The identification and function of free radicals have increased the interest in substances with antioxidant properties such as selenium, vitamin A, β karotene etc. Selenium has antimutagenic and anticarcinogenic activity. Previous studies on the protective effects of antioxidants such as selenium and vitamin A against the cytotoxicity and genotoxicity of AFB_1 mostly focused on the metabolism and detoxification of AFB_1 or the formation of AFB_1-DNA adduct (Chen et al., 1982b; Mandel et al., 1987; Decoudu et al., 1992). It was also suggested that the protective effect of selenium could be mediated through a cellular mechanism related to glutathione detoxification pathways (Shen et al., 1994). Glutathione has been shown to play an important role in the detoxification of AFB_1 through conjugating reactions catalyzed by glutathione S transferase. Selenium is closely involved in metabolic pathways of glutathione, being an essential element in the selenoprotein glutathione peroxidase (GPX). Because selenium is a component part of glutathione peroxidase, it is possible that selenium neutralizaties free radicals present in cell following the hydrogen peroxide treatment that induces DNA damage (Bronzetti et al., 2001).

Our results have also showed that selenium has a protective effect on the development disorders of *D. melanogaster*. It could be concluded that more investigations should be carried on in order to understand whether or not the mechanism of the effect of selenium on *D. melanogaster* is as shown in the present experiments.

RESUMO

O objetivo deste trabalho foi investigar os efeitos da AFB_1 e AFB_1 + Se ^{4} em vários estágios de desenvolvimento da *Drosophila melanogaster*. Ambos diferentes concentrações da ABF_1 e Se^{4+} aplicado com AFB_1 foram alimentados durante a fase de desenvolvimento da mosca (ovo, larva e pulpa). Quando a progenese F_1 do controle e aplicações foram comparadas com outros grupos, ABF_1 ampliou o processo de metamórfose e na redução do número total de ovos. Porém esses efeitos negativos foram inibidos com o tratamento com selênio em diferentes concentrações (4.0 e 8.0 ppm). Esses resultados sugerem que o selênio pode efetivamente inibir AFB_1 que induz anomalias nos estágios do desenvolvimento da *Drosophila melanogaster*

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