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Blood Oxidant and Antioxidant Status in Rats Feeding with Insect-infested Wheat Flour

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Abstract: It has long been known that frequent oral exposure to insect- infested flour run an increased risk of many diseases, including cancer, kidney failure etc in experimental animals. The susceptibility to all of these diseases may be a consequence of superimposed oxidative stress due to lack of defensive mechanisms. In concern with this hypothesis, the present study was carried out to assess the influence of feeding insect-infested wheat flour on the oxidant and antioxidant status in rats. Wheat flour samples were infested with *Tribolium confusum* for eight weeks then introduced immediately in feeding the rats for ten weeks. During the feeding period, blood samples were drawl for hematological studies periodically after each two weeks. The obtained data revealed that activities of antioxidant enzymes GSH-Px and GSH-R were found lower in the erythrocytes of rats feeding insect-infest wheat flour. By ten weeks of feeding the infested flour, the lowering ratios in these enzyme activities were 9.27 and 32.09% respectively. The GSH/GSSG ratio in plasma, which provide a sensitive index of whole body oxidative stress, were lower significantly and recorded 5.29 ±1.35. Plasma levels of antioxidant vitamin (vitamins A, C and E) were also significantly lower in plasma of rats feeding insect-infested wheat flour for ten weeks of feeding the infested flour and the lowering ratios for these vitamins level were 38.25, 37.49 and 43.21%, respectively. Additionally, levels of oxidants in plasma, TBARS, nitrite (NO_2) and nitrate (NO_3), were higher by the ratio of 124.92, 188.97 and 178.35%, respectively. Results suggested that enzymatic antioxidant defence system of erythrocytes was depressed and the erythrocytes were exposed to oxidant stress due to insect-infested flour feeding. Increased plasma TBARS, NO_2 and NO_3 levels indicated that not only erythrocytes but also some other tissues and cells might be exposed to the radical stress by insect-infested flour. Feeding insect-infested flour also caused significant changes in the levels of antioxidant vitamins partly protected erythrocytes against such harmful effects of feeding on infested flour by scavenging free radical species and by activating or inducing antioxidant enzymes.

Key words: Wheat flour, infested, insect, feeding, blood, oxidant, antioxidant

Introduction

Flour beetle *Tribolium confusum*, is represent a specie of insects belong to the very large family, Tenebrionidae, of which more than ten thousand species are known. It is a pest of stored cereals and products made from them. It has been spread all over the world including Egypt and is the commonest beetle found in bran, flour, rice, ground-nuts and other stored products. The life history of *Tribolium confusum* is shown and summarized in Fig 1. The white oval eggs (a) are laid singly or in small batches in the flour (400 - 500 eggs during life history) and they hatch in about a fourth night into elongate, yellow larvae with six legs (b). The larvae's are transferred and formed pupas (C), which are hatched to gave the adults (d). The adult is reddish brown and slightly less than a quarter of an inch in length. It could be lived for one year or more and there may be five generations a year. The period of generation is in the range 5-7 weeks under the optimal conditions i.e temperature, 25°C and humidity, 60%. Therefore, it can increase rapidly in numbers if it once infests a store or pantry. (Skaife, 1978).

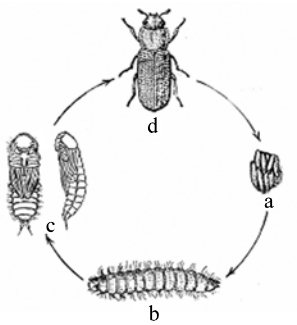

Fig 1: The life history of confused flours beetle *Tribolium confusum*

It has long been known that frequent oral exposure to insect- infested flour run an increased risk of many disease including cancer and kidney failure. For example, the force feeding flour infested with *Tribolium sp.* and biscuits made of this flour, induced the formation of hepatocellular carcinomas in 22% of the experimental toad

(*Bufo regularis*) and also induced liver, spleen and breast tumors in 35.2% of the experimental Swiss albino mice (El-Mofty *et al*., 1988, 1989 and 1992). Also, severe malfunctions were observed in the liver and kidney of rat as fed insect-infested wheat flour for 4 weeks (Abd El-Hameed, 2001). Therefore, much research has been devoted to identifying and characterizing the compounds responsible for the all of these adverse effects resulted from insect–infested flour. Our previous studies and others indicated that almost of these compounds include, polycyclic aromatic hydrocarbons (PAH) and their derivatives, quinones, uric acids, fat oxidation and its oxidative rancidity products, malonaldehyde (MDA), trans fatty acids etc. (Pagani *et al.,* 1994; Ghaedian and Wehling, 1996; Nasr, 1998; Elhassaneen and Tawfik, 2000 and Abd El-Hameed, 2001).

One of the most useful theories to explain some mechanistic aspects of these chemical mutagenesis and carcinogenesis is the theory of toxification, i.e. the formation of reactive metabolites by enzymes and the covalent linkage of these activated intermediates with cellular macromolecules to initiate the carcinogenic process (Varanasi, 1989). Although the intermediate pathway of toxification for PAH has received wide acceptance, many other pathways and mechanisms have been suggested. For example, chemical toxification via the free-radical pathway has received much attention with long time ago. In this regard, studies from the laboratory of Ts'o are interesting, they propose that toxic quinones of PAH i.e., secreted by the defensive gland of flour beetles owe their activity to oxidation-reduction cycles involving quinone, hydroquinone and molecular oxygen (Ladisch *et al*., 1967 and Lorentzen and Ts'o, 1977). The reactive reduced oxygen radicals and semiquinone radicals formed during these cycles have been shown to be implicated in the pathogenesis of many diseases, including coronary heart diseases and cancer (Halliwell, 1987).

The primary defense protecting the biological systems against the potentially harmful effect of free radicals is provided by antioxidants. Therefore, in the present study, we investigated whether oral exposure to insect-invested wheat flour can be affected in the blood oxidants and antioxidants status of rats.

Materials and Methods

Materials and instruments

Flour samples: Fresh wheat flour samples (commercial Red-American wheat, 72% extraction) were obtained from the Gharbia Mills Company, Holding Company for Rice and Flour Mills (HCRFM), Ministry of Supplies, Egypt and stored immediately on the refrigerator at 0 °C until using in infestation experiments.

Insects: Specimens of *Choetospila elegans* Westw. (Hymenoptera: Pteromalidae), used for biological monitoring, were obtained from Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Flour beetles *Tribolium confusum* (Tenebrionidae: Coleoptera), were collected from the infested wheat present in open Shouns proper to the Holding Company for Rice and Flour Mills (HCRFM), Ministry of Supplies, Egypt.

Rats: Four to 5 week-old male albino rats of *Sprague Dawley*, weighing 140 ± 17 gm (Males sex) were obtained from Helwan Research Station, Vaccines Association, Ministry of Health and Populations, Cairo, Egypt were housed in an environmentally controlled animal facility operating on a 12 h dark/light cycle at 24-26°C.

HPLC system: Throughout this study a SP Thermo Separation Products Liquid Chromatograph (Thermo Separation products, San Jose, CA, USA) was used with a pump Consta Metvic 4100, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The columns used (Alltech, Baltimore, USA) were a reversed-phase water Adsorbosil C_{18} (5 μM, 100 mm × 4.6 mm I.d.) for vitamin C normal Ultrasphere Si (5 μM, 250 mm × 4.6 mm I.d.) for vitamins A and E and a reversed-phase water Spherosorb ODC-2 (3μM; 150 mm × 4.6 mm I.d.,) for GSH and GSSG analysis.

Biological experiments

Flour infestation treatment: To ensure that the flour samples were not already infested and contained no residual pesticides, biological monitoring was first carried out such as mentioned by Domenichini *et al.* (1994). For residue testing, ten specimens of *C. elegans* were placed in petri dishes containing samples of the flours to be used in the experiments. Almost all the Hymenoptera were survived over a period of two weeks. For insects residue investigation, five samples of flour to be used in the experiments (100 gm per each) were placed in petri dishes and incubated at 25°C with 60% humidity. Over a period of five weeks, all of these dishes were examined by using lens (20 X) and were free against insect's residue.

Thirty-five 2 kg samples of wheat flour were placed in 5 liters plastic jars covered with 0.5 mm nylon netting. Larvae of *Tribolium confusum* were introduced into the thirty jars (100 adult per each) and five jars, not infested, were kept as control. The jars were kept during the infestation period at 25°C, 60% relative humidity and 12 h light in 24 h. After eight weeks of infestation, all jars were sieved to recover the insects and then used in preparing the diet for rats feeding.

Rats feeding: The experiments were conducted in accordance with the provisions of the "Guide for Care and Use of Laboratory animals" NIH (1985). Sixty-four animals were distributed in 15 cages (4 rats per cage) and the rest 4 rates were used as a zero time animals. The cages of animals were divided into three major groups (5 cages per each) and fed with different diets as follow: the first group (untreated control negative) was fed with the basal diet consists of casein 10%, corn oil 10%, vitamin mixture 1%, salts mixture 4% and starch up to 100%; the second group (untreated control positive) was fed with the basal diet after substituted the starch by the un-infested wheat flour and the last group (treated) was fed with basal diet after substituted the starch by the infested wheat flour. The vitamin and salt mixture were prepared according to Campbell (1963) and Hegested *et al.* (1941), respectively. After each two weeks of feeding, one cage from each group was drawl for hematological studies and the experiments were terminated after 10 weeks.

Hematological analysis

Blood sample preparation: Blood samples were withdrawn from rats into glass centrifuge tubes, in which there was oxalate solution (1.34%) as anticoagulant. After centrifugation at 3000 rpm for 10 min., plasma was draw off and used for the analysis of blood lipid parameters and vitamins. Erythrocyte residue were washed with three successive portions of sodium chloride solution (0.9%) and then haemolysed with deionised water for 30 min. Haemolysate was then centrifuged at 3000 rpm for 30 min. and the supernatant fractions was transferred to a clean test tube and for the analysis of antioxidant enzymes Stroev and Makarova (1989).

Antioxidants: GSH-Px, GSH-Rd activities were measured as described by Splittgerber and Tappel (1979) and ICSH (1979), respectively. While Glutathione (GSH) and oxidized glutathione (GSSG) were measured by HPLC chromatography such as described by McFarris and Reed (1987).

Vitamins: All vitamins (A, C and E) were extracted according to the methods (Epler *et al.*, 1993; Moeslinger *et al.*, 1994 and Hung *et al.*, 1980; respectively). The chromatographic conditions for Vit. C were flow rate, 1 ml min^{-1}; detection, UV absorption at 254 nm, volume of injection, 20 μl; temperature, room temperature and mobile phase composition was an isocratic system of 100% methanol while in Vit. A and E were flow rate, 1.5 ml min^{-1}; detection, UV absorption at 265 nm, volume of injection, 20 μl; temperature, room temperature; and the mobile phase composition was an isocratic system of isopropanol : hexane (1:99).

Oxidants: TBARS and nitrite/nitrate levels were measured as described by Stroev and Makarova (1989) and Misko *et al.* (1993), respectively.

Results

Changes in antioxidant enzyme activities (Mean±SD) in erythrocytes and glutathione fractions level in plasma of rats were fed wheat flour infested with *Tribolium confusum* were given in Table 1. As seen from such data, GSH-Px and GSH-R activities were significantly lower in the erythrocytes of rats feeding insect-infest wheat flour. By ten weeks of feeding the infested flour, the lowering ratios in these enzyme activities were 9.27 and 32.09% respectively. The GSH/GSSG ratio in plasma, which provide a sensitive index of whole body oxidative stress, were lower significantly and recorded 5.29±1.35 after ten weeks of feeding with the infested flour. According to Di Giulio (1991), in the healthy cell, the ratios of GSH/GSSG is typically very high (>10).

Plasma levels of vitamins A, C and E were significantly lower in plasma of rats feeding insect-infest wheat flour (Table 2). By ten weeks of feeding the infested flour, the lowering ratios in these vitamins level were 38.25, 37.49 and 43.21, respectively.

Oxidants level, TBARS and nitric oxides (nitrite, NO_2 and nitrate, NO_3), in plasma of rats were fed wheat flour infested were given in Table 3. Extremely higher concentrations of these oxidants were established as a concomitant reduces in enzymatic and nonenzymatic antioxidants. By ten weeks of feeding the infested flour, the increasing ratios in TBARS, NO_2/NO_3 and NO_2 levels were 124.92, 194.51and 178.35, respectively.

Intra correlation analyses of data indicated that positive correlation's were observed between the GSH fractions and vitamins as a consequence of feeding the insect-infested flour (Table 4). The oxidants (TBARS and nitric oxides) recorded negative correlation's with GSH/GSSG ratios (Table 5). Also, the most important correlation's established were between all oxidants and antioxidant vitamins, which were negative for all relations (Table 6).

Discussion

It has long been known that frequent oral exposure to insect- infested flour run an increased risk of many diseases including cancer and kidney failure (El-Mofty *et al.*, 1988; 1989 and 1992 and Abd El-Hameed, 2001). Therefore, much research has been devoted to identifying and characterizing the compounds responsible for the all of these adverse effects resulted from insect–infested flour. Our previous studies and others indicated that almost of these compounds include, polycyclic aromatic hydrocarbons (PAH) and their derivatives, quinones, uric

Table 1: Changes in antioxidant enzyme activities (Mean ±SD) in erythrocytes and glutathione fraction levels in plasma of rats were fed wheat flour infested with *Tribolium confusum*

Feeding Period (Week)		GSH-Px (IU g^{-1} Hb)	GSH-R (IU g^{-1} Hb)	Glutathione fractions: GSH (μmol L^{-1})	GSS G (μmol L^{-1})	GSH/GSSG ratio
Group I	0	34.73±2.45	13.26±1.80	8.35±0.58	0.653±0.039	12.84±1.50
	2	34.64±1.35	13.20±0.93	7.72±0.47	0.662±0.020	11.67±0.71
	4	34.48±0.51	13.17±0.80	8.16±1.02	0.667±0.010	12.23±1.65
	6	34.57±1.18	12.61±1.11	7.55±0.63	0.622±0.057	12.17±0.96
	8	34.64±1.03	12.87±0.25	7.70±0.28	0.707±0.044	10.90±0.36
	10	34.59±1.63	12.84±1.61	8.04±0.64	0.678±0.030	11.85±0.54
Group II	0	34.73±2.45	13.26±1.80	8.35±0.58	0.653±0.039	12.84±1.50
	2	34.38±1.14	12.72±1.00	7.37±0.12	0.747±0.078	9.95±1.03
	4	34.07±0.44	12.14±1.18	7.32±0.16	0.790±0.070	9.33±0.97
	6	34.36±0.46	12.48±0.37	6.85±0.80	0.727±0.065	9.47±1.33
	8	34.03±2.59	12.21±1.66	7.34±0.04	0.700±0.011	10.48±0.21
	10	33.96±0.88	12.36±0.52	7.03±0.52	0.753±0.054	9.38±1.07
Group III	0	34.73±2.45	13.26±1.80	8.35±0.58	0.653±0.039	12.84±1.50
	2	33.69±1.58	11.20±1.37	6.66±0.48	0.799±0.031	8.35±0.81
	4	32.92±0.84	10.90±0.80	6.47±0.77	0.885±0.071	7.34±1.07
	6	32.37±0.98	9.77±1.50	5.97±0.30	0.919±0.083	6.53±0.39
	8	33.11±1.30	10.74±1.07	6.12±0.47	0.896±0.056	6.87±0.90
	10	31.01±2.49	9.01±1.24	5.21±0.37	1.03±0.260	5.29±1.35
Statistical analysis						
I-II		*	p<0.0148	p<0.0019	p<0.0001	p<0.0001
I-III		p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001
II-III		p<0.0008	p<0.0001	p<0.0001	p<0.0001	p<0.0001

* Non significant (P>0.05).

Table 2: Changes in antioxidant vitamin levels (Mean ±SD) in plasma of rats were fed wheat flour infested with *Tribolium confusum*

Feeding Period (Week)		Vitamin A (μ mol L^{-1})	Vitamin C (μ mol L^{-1})	Vitamin E (μ mol L^{-1})
Group I	0	1.26±0.14	46.99±1.46	22.51±2.39
	2	1.20±0.11	44.06±1.06	20.07±1.52
	4	1.22±0.07	40.85±4.23	20.48±0.94
	6	1.25±0.09	39.55±3.34	20.49±1.52
	8	1.18±0.05	45.46±3.86	18.50±2.95
	10	1.22±0.11	44.86±3.27	19.43±1.50
Group II	0	1.26±0.14	46.99±1.46	22.51±2.39
	2	1.08±0.05	40.85±1.94	18.81±1.17
	4	1.07±0.05	38.52±2.75	18.23±0.62
	6	1.00±0.11	41.42±2.76	16.61±2.13
	8	1.08±0.01	35.34±4.38	16.85±1.97
	10	1.06±0.02	38.32±3.00	17.79±0.45
Group III	0	1.26±0.14	46.99±1.46	22.51±2.39
	2	0.96±0.05	34.99±3.09	16.42±1.94
	4	0.90±0.06	32.36±3.51	16.25±1.07
	6	0.85±0.02	29.46±3.65	14.02±1.90
	8	0.84±0.07	26.86±3.70	15.30±2.18
	10	0.78±0.06	26.69±2.41	14.07±1.40
Statistical analysis				
I-II		p<0.0001	p<0.0004	p<0.0005
I-III		p<0.0001	p<0.0001	p<0.0001
II-III		p<0.0001	p<0.0001	p<0.0003

Group I: Untreated control negative, Group II: Untreated control positive, Group III: Treated group with infested flour

acids, fat oxidation and its oxidative rancidity products (ORP), malonaldehyde (MDA), *trans*-fatty acids etc (Pagani *et al.*, 1994; Ghaedian and Wehling, 1996; Nasr, 1998; Elhassaneen and Tawfik , 2000 and Abd El-Hameed, 2001).

Exposure to almost of these compounds including are associated with the development of cancer in mammals,

Table 3: Changes in oxidant levels (Mean ±SD) in plasma of rats were fed wheat flour infested with *Tribolium confusum*

Feeding Period (week)		TBARS (nmol ml^{-1})	NO^2 (nmol L^{-1})	NO_2/NO_3 (nmol L^{-1})
Group I	0	0.90±0.05	2.42±0.53	4.55±0.94
	2	0.98±0.05	2.94±0.55	4.95±0.54
	4	0.99±0.03	2.38±0.29	3.97±1.73
	6	0.88±0.15	2.83±0.72	4.73±0.57
	8	1.00±0.01	2.49±0.39	5.12±0.77
	10	0.97±0.11	3.10±1.01	5.12±0.54
Group II	0	0.90±0.05	2.42±0.53	4.55±0.94
	2	1.12±0.09	3.16±0.35	6.50±1.84
	4	1.13±0.08	2.86±0.35	6.94±1.53
	6	0.99±0.11	2.88±0.22	7.12±1.21
	8	1.13±0.02	3.25±0.57	6.00±0.59
	10	1.19±0.06	3.44±0.60	6.92±1.20
Group III	0	0.90±0.05	2.42±0.53	4.55±0.94
	2	1.28±0.31	3.69±0.52	11.61±2.35
	4	1.34±0.28	4.67±1.28	11.49±1.13
	6	1.64±0.14	4.46±0.47	14.90±2.49
	8	1.66±0.24	6.07±1.24	10.78±1.71
	10	2.02±0.08	6.75±1.30	13.40±0.97
Statistical analysis				
I–II		p<0.0001	*	p<0.0001
I–III		p<0.0001	p<0.0001	p<0.0001
II–III		p<0.0001	p<0.0001	p<0.0001

* non significant (p>0.05)

Table 4: Intra correlation analysis between antioxidant enzyme activities, glutathione fractions level and plasma vitamins level of the groups

Parameters	Group I	Group II	Group III
GSH-Px/Vit A	n.c.	n.c.	n.c.
GSH-Px/Vit C	n.c.	n.c.	n.c.
GSH-Px/Vit E	n.c.	-0.531	n.c.
GSH-R /Vit A	0.449	n.c.	0.535
GSH-R /Vit C	n.c.	0.483	n.c.
GSH-R /Vit E	n.c.	n.c.	0.540
GSH/Vit A	n.c.	0.746	0.816
GSH/Vit C	n.c.	n.c.	0.593
GSH/Vit E	n.c.	n.c.	0.764
GSSG/Vit A	n.c.	n.c.	n.c.
GSSG/Vit C	0.585	n.c.	n.c.
GSSG/Vit E	n.c.	n.c.	n.c.
(GSH/GSSG) ratio/Vit A	n.c.	0.585	0.745
(GSH/GSSG) ratio/Vit C	-0.571	n.c.	0.555
(GSH/GSSG) ratio/Vit E	n.c.	n.c.	0.592

Table 5: Intra correlation analysis between antioxidant enzyme activities, glutathione fractions level and oxidants levels of the groups

Parameters	Group I	Group II	Group III
GSH-Px/TBARS	n.c.	n.c.	n.c.
GSH-Px/ NO_2	n.c.	n.c.	n.c.
GSH-Px/ (NO_2/NO_3)	n.c.	n.c.	n.c.
GSH-R /TBARS	n.c.	n.c.	-0.536
GSH-R / NO_2	n.c.	n.c.	n.c.
GSH-R /(NO_2/NO_3)	-0.491	n.c.	n.c.
GSH/TBARS	n.c.	n.c.	-0.816
GSH/ NO_2	n.c.	n.c.	-0.543
GSH/ (NO_2/NO_3)	n.c.	-0.469	-0.659
GSSG/TBARS	0.745	n.c.	0.485
GSSG/ NO_2	n.c.	n.c.	n.c.
GSSG/ (NO_2/NO_3)	n.c.	n.c.	n.c.
(GSH/GSSG) ratio/TBARS	n.c.	n.c.	-0.767
(GSH/GSSG) ratio/ NO_2	n.c.	n.c.	-0.557
(GSH/GSSG) ratio/(NO_2/NO_3)	n.c.	-0.482	-0.549

Group I: Untreated control negative, Group II: Untreated control positive, Group III: Treated group with infested flour
n.c.: no correlation (p>0.05)

Table 6: Intra correlation analysis between antioxidant vitamins and oxidants level of the groups

Parameters	Group I	Group II	Group III
Vit A/TBARS	-0.671	n.c.	-0.865
Vit A/ NO_2	-0.620	n.c.	-0.770
Vit A (NO_2/NO_3)	n.c.	-0.710	-0.613
Vit C/TBARS	n.c.	-0.449	-0.622
Vit C/ NO_2	n.c.	n.c.	-0.547
Vit C/(NO_2/NO_3)	n.c.	n.c.	-0.500
Vit E/TBARS	-0.506	n.c.	-0.620
Vit E/ NO_2	n.c.	n.c.	-0.530
Vit E/ (NO_2/NO_3)	n.c.	n.c.	-0.574

Group I: Untreated control negative, Group II: Untreated control positive, Group III: Treated group with infested flour
n.c.: no correlation ($p>0.05$)

rodent and fish (Harvey, 1985; Plakunov *et al.*, 1987 and Elhassaneen, 1996). They induced the carcinogenic effects through the theory of toxification, i.e. the formation of reactive metabolites by enzymes and the covalent linkage of these activated intermediates with cellular macromolecules to initiate the carcinogenic process (Varanasi, 1989 and Elhassaneen, 1996). Also, some of these compounds in particular quinones induced several cytotoxic effects in experimental animals which precede cell death including disruption of intracellular calcium homeostasis, cell membrane integrity and mitochondria as well as lysosome dysfunction's. The mechanisms of toxicity of quinones act by redox cycling involving quinone, hydroquinone and molecular oxygen (Orrenius, 1985). As a result of redox cycling, a number of reactive oxygen species (ROS) are formed including $O_2^{\cdot}$, H_2O_2, HO and $^{1/2}O_2$ (Kappus and Sies, 1981). H_2O_2 may be rapidly detoxified either by catalase or by selenoprotein glutathione (GSH) peroxidase, which simultaneously oxidizes GSH to GSSG (Heffner and Repine, 1989). Also, reduced glutathione (GSH) may play an important role in detoxification process throw severe as a nonenzymatic scavenger of oxyradicals (Halliwell and Gutteridge, 1985). Therefore, these enzymatic and nonenzymatic antioxidants with others severe as a defense system to guard the cell against the toxic effects of ROS.

We found significant reduced in GSH-Px and GSH-R activities in rat erythrocytes as well as GSH levels in plasma as a consequence of feeding insect-infested wheat flour. A fall in GSH observed generally accompanied by a concomitant rise in GSSG and decreased in the ratio of GSH/GSSG. We think that different compounds found in insect-infested wheat flour such quinones that causes a depression on the antioxidant defense potential of erythrocytes and plasma. Our think was supported by the work of Gant *et al.* (1988) who found that following exposure of hepatocytes to various quinones lead to a rapid fall in GSH. Additionally, Di Giulio (1991) mentioned that plasma GSSG concentration to provide a sensitive index of whole body oxidative stress in the rat. Increased fluxes of oxyradicals might be decreased in the GSH/GSSG ratio, due either to direct radical scavenging or to increased peroxidase activity.

On the other side, the reducing in antioxidant defense potential of erythrocytes was contrary with decreasing in antioxidant vitamins in rat plasma as a consequence of feeding insect-infested wheat flour (Table 2). These vitamins include A, E and C, considered important antioxidants through acting as singlet oxygen ($^{1/2}O_2$) quenches, trap of peroxyl radicals and inhibit free radical reactions (Truscott, 1990; Stahl and Sies, 1993 and Jialal *et al.*, 1995). With these studies as a background, decreasing in rat serum vitamins in the present study could be attributed to their consumption in scavenge, quench and trap different ROS found and generated as a consequence of feeding insect-infested wheat flour.

Accompanied by a concomitant reduce in enzymatic and nonenzymatic antioxidants, high concentrations of different oxidants include TBARS and nitric oxides (NO_2 and NO_3) as established in the present study in rats feeding of insect-infested wheat flour (Table 3). In our opinion, if there were no change in the antioxidant defense system of rat feeding ingested insect-infested wheat flour, it would be difficult to observe high concentrations of TBARS and nitric oxides. High levels of malondialdehyde, one of the most important compounds in TBARS, in the plasma of patients were associated with rather low levels of beta-carotene (Lepage *et al.*, 1996). In some model systems, a combination of α-tocopherol and β-carotene interacts synergistically to inhibit lipid peroxidation subsequently increased TBARS (Bohm *et al.*, 1997). On the other side, increased in nitric oxides level as a consequence of increased in nitric oxide synthase can react with hemoglobin to form iron-nitrosyl adducts and with the amino and thiol groups of protein to produce nitrosylated species (Manahan, 1989). The excess production of nitric oxides has been implicated in the pathogenesis and tissue destruction of a growing number of immunological and inflammatory diseases including septic shock, arthritis, graft rejection and diabetes (Jacob *et al.*, 1992).

In the intra correlation analyses, important differences were found as a consequence of feeding the insect-infested flour, for example, the positive correlation's between the GSH fractions and vitamins (Table 4). Also, the most important correlation's established were between the oxidants (TBARS and nitric oxides) and antioxidant vitamins as well as the GSH/GSSG ratios, which were negative for all relations (Tables 5,6). This might be an importance because it indicated that the factor(s) leading to oxidant stress and peroxidation in the cells might also cause lowering in the levels of plasma vitamins and GSH/GSSG ratios.

It could be concluded that the infestation of wheat flour with insects leads to the formation of ROS and some compounds, which act by redox cycling forming a number of ROS. This is confirmed by the results of this study, which showed that feeding of insect-infested wheat flour causes reduction in the enzymatic antioxidant defense potential of erythrocytes and nonenzymatic antioxidant in plasma. It was accompanied by a concomitant high concentration of different oxidants in plasma including TBARS and nitric oxides.

References

Abd El-Hameed, D.A., 2001. Toxic and carcinogenic effects for some bakery products processed from unproper storage wheat flour. M.Sc. Thesis, Fac. of Home Econo, Minufiya University, Shebin El-Kom, Egypt.

Bohm, F., R. Edge, E.J. land, D.J. MvGarvey and T.G. Truscott, 1997. Carotenoids enhance vitamin E antioxidant efficiency. J.Am. Chem. Soc., 119: 621-622.

Campbell, J.A., 1963. Methodology of Protein Evaluation. RGA., New York.

Di Giulio, R.T., 1991. Indices of oxidative stress as biomarkers for environmental contamination. Aquatic toxicology and risk assessment: 14th volume, ASTM STP 1124, M.A. Mayes and M.G. Barron, Eds., American Society for Testing and maerials, Philadelphia, pp: 15-31.

Domenichini, M.G., M. Pagani and D. Foglazza, 1994. Infestations by *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) on durum wheat and their influence on the rheological characteristics of the semolina. Proceedings of the 6th International Working Conference on stored- protection, 17-23 April, Canberra, Australia, 2: 689 - 694.

Elhassaneen, Y.A., 1996. Biochemical and technological studies on pollution of fish with pesticides and polycyclic aromatic hydrocarbons. Ph.D., Dept. of Agric. Biochemistry, Fac. of Agric. Mansoura University, Mansoura, Egypt.

Elhassaneen, Y.A. and L.M.Tawfik, 2000. Identifying some toxic, carcinogenic and mutagenic compounds in wheat flour infested with insects. Home Economics-Sixth scientific Conference (Home Econo. and Future Prospects, 23–24 April, 2000), Faculty of Home Economics. Helwan University, Egypt.

El-Mofty, M.M., V.V. Khudoley, S.A. Sakr and N.G. Fathala, 1992. Flour infested with *Tribolium castaneum*, biscuits made of this flour and 1,4-benzoquinone induce neoplastic lesions in Swiss Albino mice. Nutrition and Cancer, 17: 97-104.

El-Mofty, M.M., S.I. Osman, S.A. Sakr and B.A. Toulan, 1988. Carcinogenicity of flour infested with *Tribolium castaneum* in *Bufo regularis*. Oncology, 45: 65 - 67.

El-Mofty, M.M., S.A. Sakr, S.I. Osman and B.A.Toulan, 1989. Carcinogenic effect of biscuits made of flour infested with *Tribolium castaneum* in *Bufo regularis*. Oncology, 46: 63-65.

Epler K.S., R.G. Zeigler and N.E. Craft, 1993. Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. J Chromatog., 619: 37–48.

Gant, T.W., Rao, D.N., R.P. Mason and G.M. Cohen, 1988. Redox cycling and sulfahydryl arylation: Their relative importance in the mechanism of quinone cytotoxicity to isolated hepatocytes. Chem. Biol. Interact., 65: 157-163.

Ghaedian, A.R. and R.L. Wehling, 1996. Stability of uric acid used as an indicator of insect Contamination during extrusion of wheat flour. Cereal Chem., 73: 625–627.

Halliwell, B.,1987. Oxidants and human disease: Some new concepts. FASEB. J., 1: 358-364.

Halliwell, B. and J.M. Gutteridge, 1985. Free radicals in biology and medicine, Clarendon Press, Oxford.

Harvey, R.G., 1985. Polycyclic hydrocarbons and carcinogenesis. ACS Symp. Ser. 283, American Chemical Society, Washington, D.C., USA.

Heffner, J.E. and J.E. Repine, 1989. Pulmonary strategies of antioxidant defence. Am. J. Respir. Dis., 140: 531-554.

Hegested, D.M., R.C. Mills, C.A. Elvehjem and E.B.Hart, 1941. Choline in nutrition of chicks. J. Biol. Chem., 138: 459–470.

Hung, S.S., Y.C. Cho and S.J. Slinger, 1980. Hight performance liquid chromatographic determination of alpha-tocopherol in fish liver, J. Assoc. Off. Anal. Chem., 63: 889-893.

ICSH., 1979. Recommended methods for red cell enzyme analysis. Br. J. Haem., 35: 331-340.

Jacob, T.D., M.K. Morrell., S. Manzi, J.B. Ochoa, V. Verdile, A.O. Udekwu, S.A. Berceli, R.L. Simmons and A.B. Peitzman, 1992. Nitric oxide: Implications for drug research, pp: 28, IBC, South Natick, MA.

Jialal, I., C.J. Fuller and B.A. Huet, 1995. The effect of alpha-tocopherol supplementation on LDL oxidation: A dose-response study. Artherioscler. Thromb. Vasc. Biol., 15: 190-198.

Kappus, H. and H. Sies, 1981. Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid perioxidation. Experimentia, 37: 1233-1241.

Ladisch, R.K., S.K. Ladisch and P.M. Howe, 1967. Quinoid secretions in grain and flour beetles. Nature Lond., 215: 939 - 940.

Lepage, G., J. Champagne, N. Ronco, A. Lamarre, I. Osberg, R.J. Sokol and C.C. Roy, 1996. Supplementation with carotenoids corrects increased lipid peroxidation in children with cystic fibrosis. Am. J. Clin. Nutr., 64: 87-93.

Lorentzen, R. and P. Ts'o, 1977. Benzo(*a*)pyrenedione/ benzo(*a*)pyrenediol oxidation/reduction couples and the generation of reactive reduced molecular oxygen. Biochem., 16: 1467-1476.

Manahan, S.E., 1989. Toxicological chemistry: A guide to toxic substances in chemistry, PCR press, New york.

McFarris, M.W. and D.J. Reed, 1987. HPLC of thiols and disulfides:dinitrophenol derivatives. Methods Enzymol., 143: 101–109.

Misko, T., R. Schilling, D. Salvemini, W. Moore and M. Currie, 1993. A Fluorometric assay for the measurement of nitrite in Biological samples. Anal. Biochem., 214: 11-16.

Moeslinger, T., M. Brunner and G. Spieckermann, 1994. Spectrophotometric determination of dehydroascorbic acid in biological samples. Anal. Biochem., 221: 290-296.

Nasr, W.M., 1998. Nutritional, chemical and rheological studies on flour and its products infested with insects. MSc. Thesis, Fac. of Home Econo. Minufiya University, Shebin El-Kom, Egypt.

NIH, 1985. Guide for Care and Use of Laboratory animals. NIH publication 88-23, Washington DC.

Orrenius, S., 1985. Biochemical mechanism of cytotoxicity. Trends Pharmacol. Sci., 29: 1393–1401.

Pagani, M., D. Fogliazza, C. Cademartiri and A. Pietri, 1994. Esgosterol content in flour from tender wheat infested by *Tribolium confusum* du Val and *Ephestia Huhniella* Zeller. J. Appl. Ent., 117: 318-320.

Plakunov, I., T.A. Smolarek, L.D. Fischer, J.C. Wiley and W.M. Baird, 1987. Separation by ion-pair high-performance liquid chromatography of the glucuronid, sulfate and glutathione conjugates formed from benzo(a)pyrene in cell cultures rodents, fish and humans. Carcinogenesis, 8: 59-66.

Skaife, S.H., 1978. African insect life. Longmans Green and Co., London.

Splittgerber, A.G. and A.L. Tappel, 1979. Inhibition of glutatione peroxidase by cadimium and other metal ions. Arch. Biochem. and Biophys.,197: 534-542.

Stahl, W. and H. Sies, 1993. Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. Ann. N.Y. Acad. Sci., 691: 10-19.

Stroev , E.A. and V.G. Makarova, 1989. Laboratory Manual in Biochemistry, Mir Publishers Moscow, USSR.

Truscott, T.G., 1990. The photophysics and photochemistry of the carotenoids. J. Photochem. Photobiol. Biol., 6: 359-371.

Varanasi, U., 1989. Metabolism of polycyclic aromatic hydrocarbons in aquatic environment. CRC Press, Inc., Boca Raton Florida.