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Medical Drawbacks Post α- Cypermethrin Pesticide Occupational Exposure

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Abstract: ***A cross-sectional study was performed to find out the medical drawbacks including oxidative stress, immunological and genotoxic health hazards among Alfa cypermethrin pesticide (CYP) exposed production workers. The study is including 200 workers divided into 3 groups according to the level of exposure. Highly exposed group (50), moderately exposed group (50) and unexposed group (100). All workers were subjected to detailed laboratory investigation for gene p53 mutations, immunological parameters as CD3%, CD4% and CD8% in addition to peripheral blood total leukocytic and platelet counts were measured. Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione Peroxidase (GLx) were measured. Air samples were collected with a High Volume Small Surface Sampler for measurement of cypermethrin level.***

Keywords: Alfa-Cypermetherin, immunological, groups

1. Introduction

Alfa Cypermethrin (CYP) is a widely used synthetic pyrethroid II insecticide worldwide. It is mainly used in agriculture and home pest control [1]. Occupational exposure to CYP may occur during preparation, mixing and transportation of the product. It may also occur during application of the product in farms for pest control [2]. CYP can be absorbed through inhalation, ingestion or skin contact. Accordingly, strict safety precautions and work hygiene should be enforced to control health hazards [3]. However, this is extremely difficult in absence of a known permissible exposure level for CYP [4]. According to WHO safety report, CYP has been classified as grade II or moderate toxicity chemical [1]. Acute symptoms may occur especially with poor work conditions like skin rashes, conjunctivitis or respiratory irritation [5]. Researches confirmed that CYP is probably carcinogenic agent that may lead to either tumor initiation or promotion in mammals [5]. Genotoxic effect of CYP has been also reported[5] . Recent studies found that cypermethrin itself could form DNA adducts and when metabolized through the cytochrome P450 microsomal system, it can lead to DNA cross links and oxidative stress. [6, 7]. However, the exact mechanism of CYP genotoxicity is still not well known. In addition, [8] have studied the impact of the CYP chronic exposure on the occurrence of oxidative stress in experimental rat brain and blood. They marked reduction of Glutathione (GSH) and lipid peroxidation [8]. Reproductive effects of CYP were investigated in many experimental researches that concluded possible disturbance and direct effect of CYP on the reproductive system [9]. Egypt is considered as one of the highest countries using pesticides. More than 8600 tons of pesticides are available in the Egyptian market per year with 148 different including pyrethroids [10]. In spite of this high availability and usage of pesticides which is associated with high occupational and non-occupational exposure, few epidemiological researches studying health effects of pyrethroids, and in particular CYP, are available. Accordingly, the current study may be essential to highlight the health effects associated with long-term exposure to CYP products among working population regarding the oxidative stress, immunological and genotoxic effects of CYP.

2. Material and Methods

Study Design and timing

A comparative cross-sectional study was conducted from July 2016 to March 2017.

Study population and Setting

The study included workers at different departments of a CYP production factory in Egypt. Of a mean age of 37+10.7 years

Sampling size and sample plan

Sample size was calculated according to the following formula [11].

$N = t^2 \times p (1-p) / m^2$

t = confidence level at 95% (standard value of 1.96)
p = estimated prevalence of pesticides induced hazards
m = margin of error at 5% (standard value of 0.05)

Accordingly, the total sample was estimated as 200 workers divided into 3 groups according to their level of exposure. Group 1 (Highly exposed): this group included 50 workers working in the raw materials weighing and production departments. They were highly exposed to Alfa CYP and not exposed to any other pesticide or solvent.

Group 2 (Moderately exposed): this group included 50 workers working in the packaging and storage departments. They had moderate exposure to Alfa CYP and not exposed to any other pesticide or solvent.

Group 3 (unexposed): this group included 100 workers working in administrative departments.

They were not exposed to Alfa CYP or any other pesticide or solvent. All workers were pooled in a common frame by ID number and simple random selection was computed to allocate workers to be included.

Study Methods

1) An interviewed questionnaire was used to collect data about socio-demographic data, smoking and occupational history, use of protective equipment and relevant medical history of all participating workers.
2) Environmental monitoring of the residual indoor air CYP concentrations. Air samples were collected with a High Volume Small Surface Sampler (HVS3)from different random sites covering all facility areas:
   - Area I: Raw materials weighing and production departments
   - Area II: Packaging and storage departments

Sampling method: Collected dust particles containing CYP residues were manually sieved to obtain the fine fractions (<150 µm) which is more likely to adhere to human skin, followed by ultrasonication extraction. Finally, the concentrated extracts were analyzed with an electron impact gas chromatography mass spectrometer in the multiple ion detection modes (Phenomenex company ZB-35 column, 30m × 0.25mm ID, 0.25µm film) with temperatures adjusted from 130- 340°C at 6°C/min.

Sample analysis

Working range standards were prepared by diluting stock solutions with desorbing solution. Stock and dilute standards were stored in a freezer. The 13-mm glass fiber filter and the 270-mg sampling section of the tube were transferred to a 4-ml vial. The first foam plug and the 140-mg backup section were placed in a separate vial. The instrument was adjusted according to the following program: injector temperature; 25°C, detector temperature; 300°C, column temperature; 230°C, Hydrogen flow rate; 11:1, and the injection volume was 1 ug . Detector response was measured using electronic integration. A calibration curve was constructed by plotting concentration of analyte per mL versus response of standard concentration (ug/ml) of cypermethrin samples with prepared analytical standards over a range of concentrations. The pg/ml of cypermethrin in both sections of each sample was determined and blanked from the calibration curve. Cypermethrin found on the backup section was added to the amount found on the front section. Blank corrections were performed before adding the results together.

3. Evaluation of Oxidative Stress

Blood samples were collected from all participating workers for determining the enzymatic activities of the superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx). Collected blood samples were centrifuged after clotting at 3000 rpm for 15 minutes using a cooling centrifuge to separate the corresponding sera samples[12].

A. Determining the enzymatic activities of SOD: The collected sera samples were divided and transferred into small vials. The PhenazineMetasulphate (PMs) was diluted 100 times immediately with distilled water before use (0.1ml + 9.9ml distilled water). A sera sample was also diluted to give an inhibition percent between 30 and 60. Test tubes were prepared for each serum sample vial by adding 1ml of buffer (pH 8.5) to 0.1ml of Nitroblue tetrazolium and 0.1ml of Nicotinamide Adenine Dinucleotide (NADH) , 0.05ml of each serum sample was dispensed to the corresponded test tube and well mixed using vortex (Fisher scientific –USA) . The reaction was initiated by added 0.01ml of PMs to each test tube. A control tube was included. Part of each test tube either the samples or control was poured into 1cm path length cuvette and the increase in absorbance was measured using the UV spectrophotometer [12].

b. Determining the enzymatic activities of CAT: The chromogen–buffer was prepared by mixing 100mM/L of Phosphate buffer, pH 7.0 with 1mM/L of detergent) using vortex machine and H2O2 was diluted 1000 times with distilled water immediately before use (10ul + 10 ml distilled water) . 0.5ml of the prepared chromogen buffer was added to the sample test tube and well mixed using mechanical vortex. The sample test tube was incubated (Jouan- France) at 25°C for exactly 1 minute. Add 0.1ml of catalase inhibitor to the sample test tube then mix 0.5ml of peroxidase enzyme using mechanical vortex. The sample test tube was incubated at 37°C for 10 minutes. Finally, the sample test content was poured into 1 cm path length cuvette to be read using the UV spectrophotometer [13].

c. Determining the enzymatic activities of GSH: GSH was measured using ELISA method; 270µl ice-cold 5% MPA was added to the serum sample tube and mechanical vortex was used briefly (dilution factor = 4) , centrifuged at 1000 x g at 4°C for 10 minutes. 50µL of the supernatant was add to 700 µl assay buffer in a new micro - centrifuge tube (dilution factor = 15, to make the final dilution factor = 60). The diluted extract was placed on ice until use and the GSH sample (50 µL) was thawed and mixed immediately. 350µl ice cold 5% MPA was added to the micro-centrifuge tube and mechanical vortex was used briefly (dilution factor = 8) then centrifuged at 1000 x g at 4°C for 10 minutes. Then 25µl of the supernatant was added to 1.5ml assay buffer in a new micro-centrifuge tube (dilution factor = 61, to make the final dilution factor = 488). The diluted extract was placed on ice until use. The standard curve was prepared by labeling dilution tubes and dispensing the indicated volumes of the assay buffer.

d. Determining the enzymatic activities of GPx: GPx enzymatic activity was determined using ELISA method. Assay reagents was prepared by adding 500µl assay phosphate buffer to glutathione peroxidase positive control tube, then mixed well by mechanical vortex and kept it into ice. The caliber was mixed with 188µl of distilled water (equivalent to 6mM NADPH) and 10µl of the diluted caliber were transferred into wells of a clear flat-bottom 96-well plate, then 190µl of assay phosphate buffer were added to all wells. 10µl of sample serum and 10µl of reconstituted GPx positive control were then transferred into separate wells of the 96-well plate, including a background control that only contain 10µl assay buffer. Enough working reagents for samples and controls wells were prepared by mixing 85µl assay buffer, 2µl glutathione, 2µl 35mM NADPH and 8µl of

glutathione reductase enzyme for each well. Using a multi-channel pipette, 100µl of 0.35mM H2O2 reagent were add to all samples and control wells [14].

4. Detection of p53 gene mutations: Detection of p53 gene mutations was carried out on three main steps

Step 1 (DNA extraction from blood): 200µl of EDTA-blood samples were well mixed with 800µl of cell lyses buffer in a sterile test tube. The lysed EDTA-blood samples were incubated in ice bath for 10 minutes. The mixture in the sterile test tube was centrifuged at 5000 rpm for 15 minutes by using of a cooling centrifuge (Jouan Ki-22, France) . 600µl of nuclei lyses buffer (NLB) were dispensed to the mixture in the sterile test tube. The sediment (the lysed cells) was re-suspended. 2.16 µl of 20% Sodium Dodecyl Sulfate (SDS) and 5µl of proteinase-K (PK) were added to the lysed cells in the test tube and were incubated for 1 hour at 55°C. After incubation, 200µl of sodium chloride was dispensed to the test tube content and were mixed using electric vortex for 15 seconds. All test tubes content were centrifuged at 5000rpm for 15 minutes at 4°C. The supernatant was transferred into a new sterile test tubes at which a double volume of 10% ethanol was dispended and well mixed with the supernatant. The new mixture was centrifuged at 10000 rpm for 20 minutes at 4°C. The resulted pellets were washed with 70% ethanol and then centrifuged at 9500rpm for 5 minutes at 4°C. The washed pellets were dried after discarding of the supernatant and resuspended in 50µl of Tris-EDTA (TE).

Step 2 (Polymerase chain reaction (PCR) technique): 1µl of each specific primer sequences for p53 tumor suppressor gene were mixed well with 1µl of isolated DNA (treated pellets) from the last step in PCR-tubes, then the volume was completed to 20µl of water for injection (WFI).The thermal cycler was adjusted according to special program parameters; the incubation condition was at 94°C for 10 minutes to fix the linearity of DNA and numbers of cycles were 30 cycles. Each cycle included three steps; denaturation step (94°C for 30 seconds) at which the DNA base pairs were broken and the single stranded DNA was released to act as a template, annealing step (55°C for 30 seconds) at which the primers were hyperdized one to each complementary strand of the DNA so that each primer was attached to its complementary templates. The last step was anextension step (72°C for 30 seconds) at which DNA was synthesized by Taq-polymerase. At the same time another DNA synthesis (de-novo synthesis) was accelerated to a maximal velocity by using a Taq-polymerase and the specified nucleotides. During this de-novo DNA synthesis, another DNA strand was created complementary to the original DNA strand.

Step 3 (Single stranded conformational polymorphism (SSCP) staining): The target of this step was detecting the amplified fragment of the DNA by using 2% agarose gel electrophoresis. This step was performed in 2 stages; loading and running stage and silver staining of the gel**.**

Finally, evaluation of p53 gene exons mutations was done by comparing the resulted scanned bands with certain standard known bands submitted with the primers sequences for p53 tumor suppressor gene, the evaluation was available to judge on the exons (5a, 5b, 6, 7, 8) if there were possibilities for mutations occurrence [15].

5. Hematological Parameters

a) Total leukocyte count (TLC):
20µl of EDTA-blood samples were mixed well with 0.38ml of lymphocytes diluting buffer in a test tube for 2 minutes using a mechanical vortex. Diluted EDTA-blood samples were dispensed into the chambers of the hemocytometer slide with its cover glass in position. The diluted blood samples were left for 2 minutes to settle. The white blood cells were counted in the four large squares on both sides of the hemocytometer slide chamber by using binocular microscope and the counting was based on area measured and the dilution factor used [16].

b) Platelets count
20µl of EDTA-blood samples were mixed well with 0.38ml of platelets diluting buffer in a test tube for 2 minutes using a mechanical vortex. The diluted EDTA-blood samples were dispensed into the hemocytometer chambers and covered with a Petri dish for 10 to 20 minutes before examination to allow the platelets to be settled. A piece of wet cotton was left in the Petri dish to prevent evaporation. The platelets were counted by using a binocular microscope in the large squares of 1mm of the hemocytometer slide. Platelets counting were applied in many squares to reach granular structure.
6. Immune phenotyping assessments of CD3, CD4 and CD8 using flowcytometry technique 50µl of EDTA-blood samples containing up to 10000 cells were mixed with specific monoclonal antibodies in a test tube. This mixture was shacked gently, and incubated at dark place for 30 minutes. 1–2ml of erythrocytes lysing reagent was added to each test tube and was mixed gently. The mixture included lysed erythrocytes was centrifuged at 1500rpm for 5 minutes using a cooling centrifuge. The supernatant was aspirated leaving about 50µl of solution. 3ml of the phosphate buffer saline was added to the remaining solution and the new mixture was shacked gently and centrifuged at 1500rpm for 5 minutes. The supernatant was aspirated leaving about 50µl of the solution. At the same time, there was another test tube (control tube) without monoclonal antibodies adjusted in order to obtain basic histogram showing the main cells population and to adjust the auto-fluorescence region. The argon laser (488nm) of the flowcytometry machine; Fluorescence activated cell sorter (FACS Caliber immunocytometry systems) was warmed up for 30 minutes before use and a full alignment method was performed using the standard flow-check alignment fluorospheres for adjusting forward scatter. Other tubes were introduced to the machine, at which about 10000 cells were passed in front of the laser for each case. From these cells, only the lymphocytes were passed in front of the laser for each case. From these cells, only the lymphocytes were selectively gated for immunophenotyping analysis of the studied markers CD3, CD4 and CD8 either total (%) or its absolute

count (cells/cmm) according to their forward angle scatter (FSC) and side angle light scatter (SSC) [17].

6. Ethical Considerations

Written consent was signed by workers after explanation of the aim of the study and confidentialityof information was guaranteed.

7. Statistical Analysis

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 16). Description of quantitative variables as mean, SD and description of qualitative variables as number and percentage, chi-square test was used to compare qualitative variables between groups. One way ANOVA-test was used to compare quantitative variable having more than two groups with post hoc test LSD (least significant difference) [18].

8. Results

Illustrated data regarding the socio-demographic profile of working group was summarized in [table 1] recording a non-significant difference between the studied groups regarding their age, gender, residence, body mass index (BMI), smoking habit, working duration and use of PPE (p-value ≥ 0.05).

In the mean time, the mean residual air CYP concentrations in area-I (high exposure area) and area-II (moderate exposure area) were 1.3±0.5ug/m^3 and 0.8±0.40 ug/m^3 respectively [table 2]. Group 1 had lower CD4/CD8 ratio compared to the unexposed group with statistically significant difference (p<0.04). On the other hand, there was no statistically significant difference (P≥0.05) between the studied groups as regard CD3%, CD4%, CD8%, TLC and platelet count [table 3]. Regarding gene mutation; exon 5a was detected only among highly exposed group (24%). Moreover, exon 6 and 7 were detected among moderately and highly exposed groups and not detected in unexposed group with significant difference between them (p≤0.0001) [table 4]. As regard antioxidants; SOD, CAT, GSH and GPx were significantly higher among unexposed group compared to moderately exposed group and highly exposed group, which had the lowest levels (p≤0.001) [table 5]. Correlation between the measured immunological and hematological parameters and the age of exposed workers and their working duration revealed that there was negative correlation between age and SOD (p≤0.01). On the other hand there were no statistically significant correlation between age and other laboratory findings. Results also revealed that the duration of work was negatively correlated to SOD, CAT, GSH and GPx (p ≤0.01) [table 6]. Concerning the relation between age of exposed workers and their duration of work versus P53 gene mutation, there were no statistically significant differences neither in the age of exposed workers nor in their duration of work between workers with or without exon expression [table 7]. Furthermore, there were no statistically significant differences in antioxidant levels and other immunological and hematological parameters of the exposed groups in relation to their gender or smoking habit [table 8].

Table 1: Comparison between the studied groups as regard socio-demographic and occupational data

Variables	Workers' groups#			p-value*
	Group1 N=50	Group2 N=50	Group3 N=100	
Age Mean ±SD	38±8.6	37±7.3	36.7±7.9	0.37
Gender Male Female	 42(84%) 8(16%)	 43(86%) 7(14%)	 90(90%) 10(10%)	0.53
Residence GreaterCairo OutsideCairo	 48(96%) 2(4%)	 50(100%) 0	 97(97%) 3(3%)	0.39
Smoking Mean±SD	36(72%)	40(80%)	75(75%)	0.39
BMI Mean±SD	28±4.3	29.1±5	28.5±3.9	0.56
UseofPPE No Yes	 31(62%) 19(38%)	 26(52%) 24(48%)	 60(60%) 40(40%)	0.54
Work duration (yrs.) Mean ±SD	9.3±3	8.1±4	8.9±4.3	0.23

#Group1: highly exposed, group2: moderately exposed, group3: unexposed

*the difference is significantat p-value≤0.05

Table 2: Comparative evaluation of residual air cypermethrin concentration (ug/m3) in different work place areas

Areas	Residual Air CYP Conc. Mean+SD	Range	
		Min	Max
Area-I	1.3+ 0.5	0.2	2.2
Area–II	0.8+0.4	<LOD*	2

* LOD (Limit of Detection) ≤ 0.01

Table 3: Comparison between the studied groups as regard laboratory data

Variables	workers 'groups#			p	LSD^	Reference ranges	
	Group 1 N=50	Group2 N=50	Group3 N=100			Male	Females
Platelets count/cmm	343 ± 72	358± 62	329 ± 43	0.23			
TLC (cell/cmm)	6.2 ± 2	6.5 ± 1.4	5.1 ± 1.6	0.14			
CD3%	54± 6.8	66.8±1.3	65.2±10.5	0.35		46-78.9	57.8-82.7
CD4%	34 ± 11	42± 8.9	41.3 ± 7	0.66		24.1-50.7	
CD8%	25.2± 7	27.3± 7	23± 7.3	0.41		18-42.7	19.5-44.6
CD4/CD8%	1.2 ± 0.3	1.4± 0. 2	2 ± 0.7	0.002*	-G1versus G3 (p=0.04)* -G1 versusG2 (p=0.23) -G2 versusG3 (p=0.14)	0.65-2.49	

#Group 1: highly exposed, group 2: moderately exposed, group3: unexposed
^LSD=least significant difference
*the difference is significant at p-value≤0.05

Table 4: Comparison between the studied groups as regards P53 gene mutationalexons

Variables	Workers'Groups#			P
	Group1 N=50	Group2 N=50	Group3 N=100	
Exon-5a	12(24%)	0	0	0.0001*
Exon-5b	0	0	0	-
Exon-6	3(6%)	8(16%)	0	0.0001*
Exon-7	3(6%)	8(16%)	0	0.0001*
Exon-8	0	0	0	-

Data is described as frequency and %

#Group1: highly exposed, group2: moderately exposed, group 3: unexposed
*the difference is significant at p-value≤0.05

Table 5: Comparison between the studied groups as regard antioxidant parameters and CYP

Variables	Workers'Groups			p	LSD^
	Group1 N=50	Group2 N=50	Group3 N=50		
SOD (U/ml)	62.1 ± 17	75± 3	116± 18.2	0.0001*	-G1. VersusG3 (p=0.0001)
					-G2 versusG3 (p=0.001)
					-G1 versusG2 (p=0.03)
CATU/L)	17.2±8.8	24± 11	33.7± 8.4	0.0001*	-G1 versusG3 (p=0.0002)
					-G2 versusG3 p=0.03)
					-G1versusG2 (p=0.13)
GSH (µM)	2.25± 0.19	2.5± 0.2	2.8± 0.09	0.0001*	-G1 versusG3 (p=0.03)
					-G2 versusG3 p=0.11)
					-G1 versusG2 (p=0.39)
GPx (U/L)	0.78± 0.21	1.08± 0.17	1.24 +0.12	0.002*	-G1 versusG3 (p=0.002)
					-G2 versusG3 (p=0.20)
					-G1 versusG2 (p=0.03)

Data is described as Mean±SD
#Group1:highly exposed, group 2: moderately exposed, group3: unexposed
*the difference is significant at p-value≤0.05
^LSD=least significant difference

Table 6: Correlation between working years and age versus laboratory. Parameters among exposed groups "n=100"

Variables	Workingyears		Age	
	r	P	r	P
SOD(U/ml)	-0.77	0.0001*	-0.45	0.01*
CAT(U/L)	-0.68	0.0001*	-0.11	0.45
GSH(µM)	-0.48	0.0001*	-0.09	0.67
GPx(U/L)	-0.44	0.001*	0.02	0.81
Platelets(count/cmm)	0.18	0.22	0.04	0.85
TLC(cell/cmm)	0.02	0.55	0.10	0.41
CD3%	-0.09	0.60	0.11	0.35
CD8%	0.12	0.33	-0.18	0.40
CD4%	0.13	0.21	0.01	0.84
CD4/CD8%	0.10	0.38	0.19	0.12

*thedifferenceissignificantatp-value≤0.05

Table7: Relation between P53 genemutationalexons versus age and working years among exposed group "n=100"

Variables	Variables					
	Working years Mean±SD		p	Age Mean ±SD		p
Exon-5a						
No	8.2	2	0.30	38.3	8	0.49
Yes	8.5	3.1		37.4	7.3	
Exon-6						
No	8.4	3.2	0.72	37.1	6.5	0.32
Yes	8.7	3		38	7	
Exon-7						
No	8.3	2.9	0.12	37.5	7	0.27
Yes	9	3		38.2	5.5	

Data is described as frequency and%
#Group1:highly exposed, group 2: moderately exposed, group3:unexposed
*the difference is significant at p-value≤0.05

Table 8: Relation between smoking and gender versus laboratory parameters among exposed groups"n=100"

Variables	Smoking			Gender		
	no	yes	P	Male	Female	P
SOD(U/ml)	69.1±10	67.8±12.6	0.78	70±18	68.3±11.9	0.45
CAT(U/L)	19.2±8.1	17.5±8.5	0.80	18.4±9.1	20.1±9.6	0.31
GSH(µM)	2.1+0.6	2.4±0.7	0.13	2.7±0.9	2.09±0.5	0.40
GPx(U/L)	1±0.2	0.79±0.3	0.26	0.78+0.3	0.80±0.4	0.83
Platelets (count/cmm)	348±80	361±76	0.87	351±70	355±69	0.89
TLC (cell/cmm)	6.2±2	6±1.3	0.76	6.3±1.5	6.4±1.1	0.68
CD3%	62±10	54±7.3	0.22	61±9.3	59±7.5	0.77
CD8%	36±8	34.5±10	0.90	39±7	40.1±8.2	0.73
CD4%	24.6±8	23±6.7	0.82	24.1±5	25.1±7	0.86
Cd4/CD8%	1.4±0.4	1.2±0.3	0.56	1.3±0.4	1.2±0.2	0.47

Data is described as Mean±SD
*the difference is significant at p-value≤0.05

9. Discussion

Cypermethrin, as a pyrethroids derivative, has been widely used for agricultural and domestic purposes. However, a limited number of studies referred to its acute or chronic health effects on humans. The majority of available researches were either in vivo or in vitro animal studies performed. In the current work, study groups were classified based on the occupational exposures to CYP. Except for the level of exposure, no significant difference between the study groups as regard different socio-demographic and occupational characteristics. Monitoring of the residual air content of CYP in different departments of the factory was

investigated was the base for dividing the workers according to their level of exposure.

The mean residual air CYP concentration was higher in Raw materials weighing and production areas than in Packaging and storage areas. Lack of available information on the permissible CYP exposure level of workplace did not allow for judgment of the safe level [19, 20]. Accordingly, workers were divided into highly, moderately and unexposed groups. The highly exposed group had lower CD4/CD8 compared to unexposed group is in accordance to [21] recording that exposed workers had lower level of CD4% and CD4/CD8% compared to other less or not exposed groups. Although the reference range for normal value was not exceeded in both studies but the possibility of immune system imbalance was in place with prolonged exposure or higher levels of exposures may occur. Other studies investigated the harmful effect of pesticides on the immune system as a European wide study which described minimal changes of the immune markers as detected in the current study [22]. However, this study was not studying specifically CYP but included other pesticides as well. As regard gene mutation, the current study revealed that CYP affected tumor suppressor gene; P53 inducing gene mutation as shown in exon 5a extended to involve in which nitrogenous base was replaced with adenine, which was more frequent among highly exposed group compared to the moderately exposed and unexposed groups. Moreover, exons 6 and 7 mutations were more frequent among moderately exposed group compared to the other two groups in which cytosine was replaced by adenine base. These results are consistent with **Hewehy** and his coworkers, their study included 4 groups according to CYP exposure. It was concluded that genotoxic effects of CYP was not dose exposure dependent especially in household and environmental exposures. The difference between both studies was in the site of mutation [21]. Cypermethrin-induced DNA damage was investigated also in big list of in vitro studies on human extracted elements like sperm or lymphocytes. Marked DNA damage at different exons was detected among exposed sample in one of the in vitro studies on human spermatozoa. Dramatic improvement was also observed after treatment using vitamin C and E accordingly use of antioxidant supplementation was recommended for exposed workers [23]. Although, this work had different methodology than the current study, it could support the same hypothesis of genotoxic possibility of CYP alone even in case of doses exposure

Other researchers found in vitro and in vivo gene mutations, one of these researches tested Alfa CYP mixture with another pesticide on human peripheral lymphocyte and concluded that mitotic index of lymphocytes was inhibited compared to controls [24]. Another in vitro human study detected DNA damage in workers' lymphocytes after adding Alfa CYP at 200µg dose [25]. Different mechanisms of possible carcinogenicity and chronic effect of pyrethrin's exist, one of which is oxidative stress. The current study concluded that SOD, CAT, GSH and GPx antioxidants were higher among unexposed group compared to the highly exposed workers indicating the presence of oxidative stress in the latter group represented as low level of antioxidant enzymes. These findings are in agreement with the results of [26]and her coworkers studied the toxic effects of pyrethroids, including CYP, on some hormonal profile and biochemical markers among workers in an insecticide production plant. Oxidative stress and lipid peroxidation were evaluated in their study and significant decline of the total antioxidants among the exposed group compared to the controls was detected [26]**.** Moreover, a study on rats, draw the attention on the possibility of occurrence of chronic diseases as Parkinsonism as a result of oxidative stress caused by CYP exposure [27]. The current study investigated the effect of socio-demographic and occupational factors on oxidative stress, genotoxicity and immunological disturbances among exposed workers. It was found that antioxidant levels reversibly related to the exposure duration. Moreover, SOD level was found to be lower in older workers. These findings confirm that longer duration of exposure to CYP may have a direct effect on increasing the oxidative stress among exposed workers. Other factors as gender, smoking, use of Personal Protective Equipment (PPE) during work, residence and BMI were not significantly related to oxidative stress, genotoxicity or immunological disturbances among exposed workers. decreased by increasing the duration of work. These findings are consistent with the results of [26] and coworkers who studied the chronic toxic effects of synthetic pyrethroids among workers in pyrethroids Insecticides Company in Egypt. Their results showed that there were no statistically significant differences between the studied groups as regard general factors and occupational factors [26]

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