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Health Effects of Environmental Pollutants on Workers in The Libyan Plastic Factories, Part A: Based on Biochemical Analysis

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الأثار الصحية للملوثات البيئية على العاملين في مصانع البلاستيك الليبية
الجزء أ: بناءً على التحليل الكيميائي الحيوي

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Abstract

This study aimed to disclose the reasons for the health damage and heritable genetic mutations among workers in plastic manufactories due to negative environmental impacts. Moreover, this study may constitute an initial database expressing the negative impact on the environment in the plastics factories that may have a negative impact on workers. To study the effects of long-term exposure to plastic and its solvent vapors during plastic manifesting on the blood protein contents, blood samples were withdrawn from volunteers working in a plastics factory in Kasr Alakhyar City, North-west Libya. Six different samples were collected from Clarkes exposed to plastic vapor for different periods as follows, control (did not expose to plastic vapor), one-year exposure, three years exposure, six years exposure, seven years exposure, and twelve years exposure. Blood samples were collected and subjected to protein electrophoresis and isoenzymes analysis for esterase and peroxidase. The results of protein electrophoresis indicated that a total number of 14 protein bands were resolved on polyacrylamide gel electrophoresis ranging from 16.5 KDa to 105 KDa. Five bands out of the resulted from 14 bands were considered common bands with molecular weights of 105, 98, 85, 63, and 16.5 KDa. (i.e. these bands were presented in all the six blood samples under investigation). The results also showed that too many changes were recorded in the protein banding patterns between the control volunteer and those who were exposed to plastic and its solvents vapor for different exposure periods. These changes in proteins suggested that a modification in gene excretion occurred as a response to the different exposure times to plastic during manifesting. Also, in esterase and peroxidase isozymes changes in the banding patterns between the control volunteer and those who were exposed to plastic and its solvents vapor for different exposure periods. These changes in proteins suggested that a modification in gene excretion occurred in the genes conferring these isozymes as a response to the different exposure periods to plastic during manifesting.

Keywords: *Electrophoresis, Genetic mutations, Plastic Factory, Protein.*

الملخص

تهدف هذه الدراسة إلى الكشف عن الأسباب التي تؤدي إلى أضرار صحية وراثية مثل الطفرات الوراثية بين العاملين في المصانع البلاستيكية بسبب الآثار البيئية السلبية. وعلاوة على ذلك، يمكن أن تشكل هذه الدراسة قاعدة بيانات أولية عن التأثير السلبي على البيئة في مصانع البلاستيك التي قد يكون لها أثر سلبي على العمال. تم دراسة آثار التعرض على المدى الطويل لأبخرة البلاستيك والمذيبات الداخلة في تصنيعها والذي يظهر على محتويات البروتين في الدم، حيث تم سحب عينات دم من المتطوعين الذين يعملون في مصنع البلاستيك في مدينة قصر الأخيار، ليبيا. تم جمع ست عينات مختلفة خمسة منهم من عاملين بالمصنع ممن يتعرضوا لبخار البلاستيك لفترات مختلفة على النحو التالي، الكنترول (لم تتعرض لبخار البلاستيك)، التعرض

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لسنة واحدة، ثلاث سنوات من التعرض، التعرض ست سنوات، سبع سنوات واثنتي عشرة سنة من التعرض للأبخرة. كما تم جمع عينات الدم وإجراء تحليل التفريد الكهربائي للبروتين وتحليل isoenzymes للإستريز والبيروكسيديز. أشارت نتائج التفريد الكهربائي للبروتين أن العدد الإجمالي للحزم 14 حزمة من البروتين تم حلها على مادة الأكريلاميد، تراوح الوزن الجزيئي للحزم من 16.5 كيلو دالتون إلى 105 كيلو دالتون. واعتبرت خمس حزم من 14 الناتجة حزم مشتركة بالأوزان الجزيئية 105، 98، 85، 63 و 16.5 كيلو دالتون. كانت جميع هذه الحزم موجودة في جميع عينات الدم ستة قيد الدراسة. كما أظهرت النتائج أن العديد من التغييرات على السلوك التحزمي للبروتين قد حدثت بين المتطوعين (الكنترول وأولئك الذين يتعرضون للمواد البلاستيكية وبخار المذيبات لفترات تعريض مختلفة). دلت هذه التغييرات في البروتينات على حدوث تعديلا في التعبير الجيني للجينات التي تتحكم في إنتاج هذه الحزم البروتينية كنتيجة للتعرض لأبخرة البلاستيك و مذيباته لفترات مختلفة الطول. أيضا، في المشابهات الإنزيمية لإنزيمات الاستريز والبيروكسيديز أظهرت النتائج حدوث تغيرات في أنماط الحزم بين المتطوع الكنترول وأولئك الذين يتعرضون للمواد البلاستيكية وبخار المذيبات لها لفترات تعريض مختلفة. دلت هذه التغييرات في البروتينات التي حدثت على تعديلا في التعبير الجيني وقعت في جينات التي تحكم هذه المشابهات الانزيمية كاستجابة لفترات التعرض المختلفة لأبخرة البلاستيك ومذيباته.

الكلمات الدالة: الهجرة الكهربائية، تغيرات جينية، مصنع البلاستيك، البروتين.

1. Introduction

Live and working near plastic mills, hundreds of thousands of people worldwide. Integrated plastic goods manufacture chemical waste such as plastics and other polymers which contain compounds that can cause genetic damage (Williams *et al.*, 1990). Chemical mutagens that contaminate atmospheric and ecosystems cause a human and wildlife genetic risk. Nesting near steel mills on the Great Lakes, Herring gulls (*Larus argentatus*) showed higher germline mutation rates at minisatellite DNA loci than those at rural sites and increased mutation frequency with colony proximity to integrated plastic mills. It was believed that inhaled airborne contaminants released from plastic factories, such as polycyclic aromatic compounds, are largely responsible for mutation induction; nevertheless, as contributing factors, contaminants in the aquatic food web and variations in disease and nutritional quality among gull colonies could not be excluded. Therefore, it was not possible to determine the role of air pollution in the development of germline mutations and the threat to people living near plastic mills.

Use in situ exposed sentinel laboratory animals is an effective research approach to determining air pollution hazards as it blends monitored elements of laboratory studies with direct exposure to rates of environmental pollution. Compare germ line ESTR mutation rates in laboratory mice exposed to ambient air at an industrial site near integrated plastic mills with those exposed at a rural reference site, with the goal of evaluating industrial air pollution inhalation as a route of chemical mutagen exposure (Larsen and Nielsen, 2012).

This study aimed to disclose the reasons for the health damage and heritable genetic mutations among workers in plastic manufactories due to negative environmental impacts. Moreover, this study may constitute an initial database express the negative impact to the environment in the plastics factories that may harm workers.

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2. Materials and Methods

To study the effects of long term exposure to plastic and its solvents vapors during plastic manifesting on the blood protein contents, blood samples were withdrawn using 10ml syringe from volunteers working in a plastics factory in Kasr Alakhyar city, North-west Libya. Six different samples were collected from Clarkes exposed to plastic vapor for different periods as follow, control (did not exposed to plastic vapor), one-year exposure, three years exposure, six years exposure, seven years exposure and twelve years exposure.

Blood samples were collected in polypropylene tubes coated with EDTA to avoid the agglutination of blood and prepared for protein electrophoresis and isoenzymes analysis.

2.1. Assessment of Genotoxicity on The Protein Level

The aforementioned samples of the volunteers' blood were collected and subjected to biochemical analysis using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein electrophoresis and isozymes electrophoresis. The SDS-PAGE included extraction of soluble protein.

2.1.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) used according to Laemmli (1970) method of for the aforementioned six blood samples.

2.1.2. Water-Soluble Protein Extraction

Extraction of water- soluble proteins was performed as the following:

Five ml of each blood sample was mixed in pre-chilled mortars with 10 ml of water-soluble extraction buffer and pestles.

Samples were transferred to Eppendorf tubes and incubated at 4 ^{o}C in the refrigerator overnight, then centrifuged for 20 minutes at 12,000 rpm at 4 ^{o}C, supernatant containing water-soluble proteins fraction were transferred to clean tubes and stored at -20 ^{o}C .

2.1.3. Water-Soluble Proteins Extraction Buffer (0X)

Tris (1 M pH 8.0)	6.0 ml
EDTA (0.25 M)	800 µl
H_2O (dd) up to	100 ml

2.1.4. Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed as follow:

a) Resolving gel

Acrylamide	30 g
Bis- acrylamide	0.8 g

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H_2O (dd) up to	100 ml

b) Stacking gel

Acrylamide	30 g
Bis- acrylamide	1.0 g
H_2O (dd) up to	100 ml

2.1.5. Buffers

a) Resolving gel buffer (pH 8.4)

Tris	18.15 g
HCl (1 N)	3.5 ml
H_2O (dd) up to	100 ml

b) Stacking gel buffer (Tris-HCl 1M pH 6.8)

Tris (adjust pH to 6.8 using HCl)	12.11 g
H_2O (dd) up to	100 ml

c) Running buffer

Tris	15.14 g
glycine	72.07 g
%10SDS	50 ml
H_2O (dd) up to	5 l

2.1.6. Gel Preparation

Vertical slabs (18x16 cm) gel electrophoresis apparatus was used manufactured in Biorad. Glass spacers 1.5 mm were used.

a) Resolving gel (12.5% Acrylamide gel)

Acrylamide stock	31.65 ml
Tris (pH 8.4)	16.25 ml
H_2O (dd)	16.1 ml

This solution was filtered, and then the following ingredients were added:

SDS (10%)	750 µl
Ammonium persulfate (10%), freshly prepared	500 µl
TEMED	100 µl

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Gels were poured simultaneously to a height of 1.5 cm below the bottom of the comb. Gels were overlaid with isopropanol and left to polymerize. Then isopropanol was removed before the stacking gel was poured.

b) Stacking gel

Acrylamide stock (for stacking gel)	2.66 ml
Tris (pH 6.8)	2.56 ml
H2O (dd)	14.70 ml

This solution was filtered, and then the following were added:

SDS (10%)	250 µl
Ammonium persulfate (10%), freshly prepared	100 µl
TEMED	40 µl

This stacking gel solution was quickly poured off over the two resolving gels, and then combs were used. Gels were left to polymerize.

2.1.7. Sample Preparation

A volume of 50 µl extracted protein for each sample was added to equal volume of loading buffer (2x for the water-soluble fraction and 1x for the water-no soluble fraction), 10 µl ß-Mercaptoethanol was added to each sample. Samples were boiled for 10 min in a water bath, then 10 µl bromophenol blue was added for each sample. 25 µg protein of each sample was loaded .

2.1.8. Gel running and Staining

Four liters of pre-cold run buffer were poured into the running tank; cooler circulator was used during the run to keep the gels cold. Gels were run at 100 vol. for 15 min, and then the voltage was raised up to 250 vol. till the samples reached one inch from the bottom of the gel. Gels were removed from the apparatus and placed in tries for staining. Gels were covered in the tries with staining solution. Gels were gently agitated overnight.

2.1.9. Staining Solution

Staining solution consisted of;

Commassie brilliant blue-R250	1.0 g
Methanol	455 ml
Acetic acid	90 ml
H_2O (dd)	455 ml

After removing the staining solution, gels were covered with a distaining solution which consisted of ك

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Methanol	700 ml
Acetic acid	200 ml
H_2O (dd)	3.5 l

Gel was gently agitated for 1hr in the distaining solution. The distaining solution was changed several times until it becomes clear when removed from the tray and the gels background becomes clear and bands were clear enough for a photograph .

Banding patterns of each sample were recorded as (+) for the present band and (-) for the absent ones. Also, the bands' intensities were recorded and classified as (+) for faint bands, (++) for moderate-intensity and (+++) for heavy or dark bands.

2.2. Isozymes Electrophoresis

The volunteers' blood samples from the six exposure periods were collected and subjected for biochemical analysis using polyacrylamide gel electrophoresis (PAGE) protein electrophoresis for isozymes electrophoresis. The PAGE included-water soluble protein extraction. Homogeneous (native) polyacrylamide gel electrophoresis (PAGE) was performed according to Stegmann *et al.* (1983).

2.2.1. Peroxidase Isozymes

Peroxidase isozymes were performed and its banding patterns were developed from water-soluble protein extracts of the six samples according to Larsen and Benson (1970), using the following developer solution:

Benzidine-dihydrochloride	16 mg
Glacial acetic acid	1 ml
Hydrogen peroxide (H_2O_2)	0.5 ml
H_2O	100 ml

The reaction was stopped by using 200 ml of tap water.

Banding patterns of each sample were recorded as (+) for the present band and (-) for the absent ones. Also, the bands' intensities were recorded and classified as (+) for faint bands, (++) for moderate-intensity and (+++) for heavy or dark bands.

2.2.2. Esterase Isozymes

Esterase isozymes were performed and its banding patterns were developed using water soluble protein extracts of the six aforementioned blood samples according to Scandalios (1984) and using the following developer solution:

Sodium phosphate buffer (pH 6.2, 0.1 M)	100 ml
α-naphthyl acetate	50 mg
Fast blue RR salt	100 mg

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The reaction was stopped by using 200 ml of tap water.

Banding patterns of each sample were recorded as (+) for the present band and (-) for the absent ones. Also, the bands' intensities were recorded and classified as (+) for faint bands, (++) for moderate-intensity and (+++) for heavy or dark bands.

3. Results and Discussion

Results of the Protein electrophoresis and isoenzymes analysis are presented the assessment of genotoxicity on the protein level. On this level sodium dedocayl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two isozymes, peroxidase and esterase were analyzed. Their banding patterns were recorded and used to assess the genotoxicity of plastic and solvents vapors on the base of the variations in the banding patterns among the six blood samples in the aforementioned techniques.

3.1. Sodium Dedocayl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Total water-soluble proteins were analyzed electrophoretically using sodium dedocayl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein banding patterns of the six blood samples under investigation were developed and recorded as described previously. The results are shown in Figure (1) and presented in Table (1). The results indicated that a total number of 14 protein bands were resolved on poly acrylamide gel electrophoresis ranged from 16.5 KDa to 105 KDa. Five bands out of the resulted 14 bands were considered as common bands with molecular weights of 105, 98, 85, 63 and 16.5 KDa. (i.e. these bands were presented in all the six blood samples under investigation).

The results also showed that the band with molecular weight 79 KDa was absent in control (a volunteer never exposed to plastic or its solvents vapor) but it present in all blood samples from volunteers exposed for different periods to plastic or it solvents vapors, suggesting that this newly synthesized protein was produced in the blood as response for the exposure to plastic or its solvents vapor during plastic manifesting.

Another three protein bands with molecular weights of 74, 49, and 23 KDa were absent in control (a volunteer never exposed to plastic or its solvent5s vapor) and all samples which extracting from volunteers spent a period of time for 7 years or less exposed for plastic or its solvents vapors but it present only in blood samples from volunteer exposed for 12 years (the longest investigated exposure period) to plastic or it solvents vapors, suggesting that these bands could be induced after this long period exposure.

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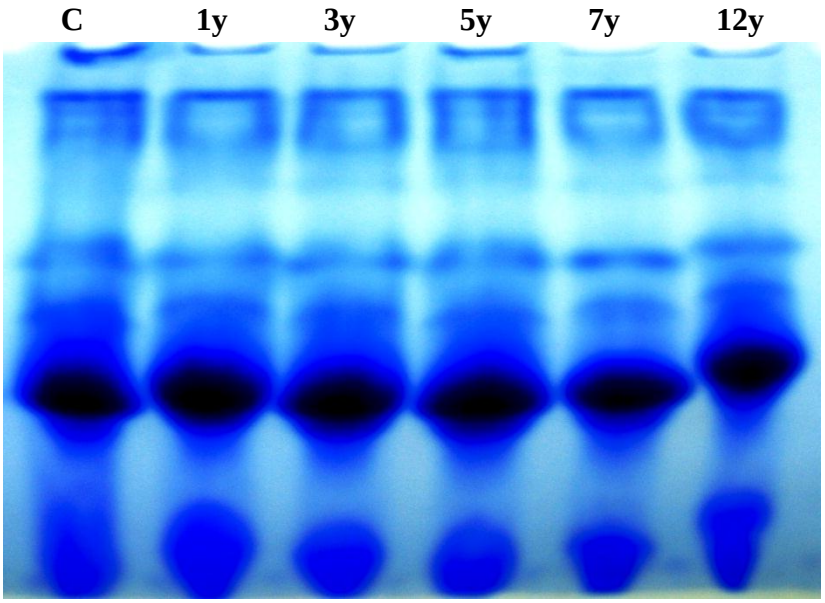

Figure 1. The banding patterns on polyacrylamide gel electrophoresis for the extracted water-soluble proteins from six volunteers blood of exposed to plastic and its solvents for different periods.

Table 1. The banding patterns on polyacrylamide gel electrophoresis for the extracted water-soluble proteins from six volunteers blood exposed to plastic and its solvents for different periods*

Molecular weight (KDa)	**Control**	**1 year**	**3 years**	**6 years**	**7 years**	**12 years**
105	++++	++++	++++	++++	++++	++++
98	++	++	++	++	++	+++
85	+++	+++	+++	+++	+++	++++
78	-	++	+++	+++	+++	++++
71	-	-	-	-	-	++++
63	+++	-	-	-	-	-
57	+++	+++	+++	+++	+++	++++
52	-	-	-	-	+++	+++
49	-	-	-	-	-	++++
42	++++	++++	++++	++++	++++	-
32	+++	-	-	++	-	-
23	-	-	-	-	-	+++
16.5	++++	++++	++++	++++	++++	++++

* Represented signs as; (+) for present and (-) for absent bands, and the number of + representing the bands intensities.

One more bandwidth molecular weight of 52 KDa was absent in control (a volunteer never exposed to plastic or its solvent5s vapor) and all samples which extracting from volunteers spent a period of time for 6 years or less exposed for plastic or its solvents vapors but it present only in blood samples from volunteers exposed for 7 and 12 years (the two longest investigated exposure periods) to plastic or it solvents vapors, suggesting that these bands could be induced after this long period exposure.

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Moreover, there were two bands with molecular weights of 72 and 32 KDa which presented only in control only but they were absent in all of the blood samples from the volunteers who exposed to plastic or its solvents vapors. It possible to conclude that the absence of these two bands referred to the exposure to the vapors .

Finally, there were two bands with molecular weights of 57, and 42 KDa which presented in control and all the exposed volunteers except the 12 years exposure where it was absent in the 12 years exposure. It possible to the absence of these two bands referred to the exposure for a very long time to the vapors.

3.2. Isozymes Electrophoresis

Isozymes (also referred to as isoenzymes) are enzymes that differ in the sequence of amino acids but catalyze the same chemical reaction. Such enzymes usually show different kinetic parameters (e.g. different Km values) or different regulatory characteristics. The presence of isozymes enables metabolism to be fine-tuned to meet the specific needs of a given tissue or stage of development Isozymes (or isoenzymes) in biochemistry are enzyme isoforms (closely related variants). In many cases, homologous genes that have diverged over time are coded for them. While allozymes are enzymes from different alleles of the same gene and isozymes are enzymes from different genes which process or catalyze the same reaction, the two terms are generally used interchangeably.

Isozymes usually result from gene duplication, but can also result from polyploidization or hybridization of nucleic acid. If the new variant's function remains identical to the original over an evolutionary period, then it is possible that one or the other will be lost as mutations occur, resulting in a pseudo-gene. However, if the mutations do not necessarily stop the enzyme from functioning but instead alter either its function or gene expression pattern, then both variants can be preferred by natural selection and specialize in different functions. They can be articulated, for example, at various developmental stages or in different tissues.

Allozymes can be the result of point mutations or insertion-deletion (indel) events affecting the gene's DNA coding sequence. There are three things that can happen to a new allozyme, like any other new mutation:

- The new allele is most likely to be non-functional in which case it is likely to result in low fitness and will be eliminated by natural selection from the population.
- Instead, if, for instance, the modified amino acid residue is in a relatively unimportant part of the enzyme far from the active site, the mutation may be strictly neutral and susceptible to genetic drift.
- In rare cases, the mutation can lead to an enzyme that is more effective and capable of catalyzing a slightly different chemical reaction, in which case the mutation may lead to an increase in fitness and benefit natural selection.

3.3. Peroxidase Isozymes

Peroxidases are a broad enzyme family usually catalyzing a type reaction:

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$$ROOR' + \text{electron donor } (2\ e^-) + 2H^+ \rightarrow ROH + R'OH \quad \ldots\ldots\ldots (1)$$

The optimal substrate for many of these enzymes is hydrogen peroxide, but others with organic hydroperoxides such as lipid peroxides are more active. Peroxidases in their active sites may contain a heme cofactor, or residues of redox-active cysteine or selenocysteine.

The nature of the electron donor depends heavily on the enzyme's structure. For example, as donors of electrons and acceptors, horseradish peroxidase can use a variety of organic compounds. Horseradish peroxidase has an active site that is accessible and many compounds can enter the reaction site.

The compounds that donate electrons are very unique to an enzyme such as cytochrome c peroxidase because there is a tightly closed active site. Although the exact mechanisms have yet to be established, peroxidases are known to play a role in raising the defenses of a plant against pathogens (Zhang *et al.*, 2015). Peroxidases are sometimes used as a marker of histology. Cytochrome c peroxidase is used in cytochrome oxidase as a soluble, easily purified template. The family of glutathione peroxidase is made up of eight known human isoforms. Glutathione peroxidases are involved with both hydrogen peroxide and organic hydroperoxide substrates as an electron donor. It has been shown that Gpx1, Gpx2, Gpx3 and Gpx4 are selenium-containing enzymes, while Gpx6 is a selenoprotein in humans with rodent homologs derived cysteine. Amyloid beta was shown to have peroxidase activity when bound to heme. Haloperoxidases are a common class of peroxidoses. This group can form reactive species of halogen and, as a result, natural substances of organohalogen. Many protein sequences for peroxidase can be found in the Peroxidase database.

Obtained results are shown in Figure (2) and presented in Table (2), the zymogram with eight different peroxidase isozymes were detected using the substrate and developer solution specific for peroxidase, these eight bands suggested that there are eight alleles conferring peroxidase isozymes in human. The eight peroxidase isozymes were exhibited with different intensities among the six blood samples under investigation. On the other hand, relative mobility was presented between these peroxidase isozymes. The relative mobility reflecting different sizes of peroxidase molecules. Peroxidase 1 and peroxidase 3 were absent only in the control sample but they presented with different intensities in all the exposed volunteers. These results suggested that peroxidase 1 and peroxidase 3 were positively regulated as response for the exposure to plastic or its solvent vapors.

On the contrary, peroxidase 2 and peroxidase 4 were presented with a very dark intensity only in the control blood sample but they were absent in all the blood samples of the exposed volunteers. These results suggested that peroxidase 2 and peroxidase 4 were negatively regulated as response for the exposure to plastic or its solvent vapors.

Furthermore, peroxidase isozyme 6 was present in blood samples collected from volunteers exposed for 3 years or more to plastic or its solvents vapors, but it was absent in both blood samples of control and the blood sample of the volunteer exposed for one year to

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the vapor. These results suggested that peroxidase 6 was positively regulated as a response for 3 years or more exposure to plastic or its solvent vapors.

Finally, peroxidase isozymes 5 and 8 were present in all the blood samples reflecting that there was no effect for the exposure to plastic or its solvent vapors on the regulation of these two peroxidase isozymes .

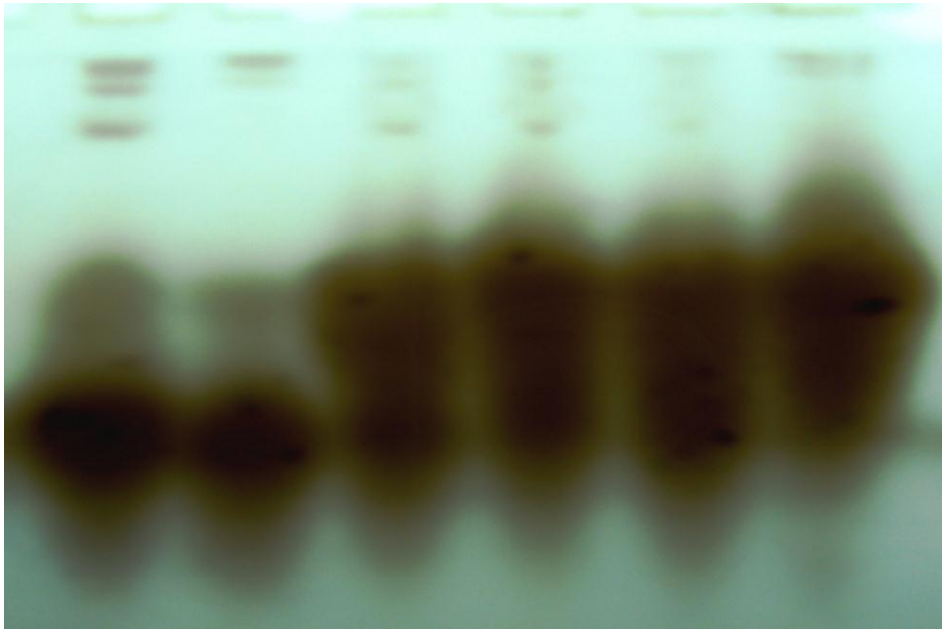

Figure 2. The Zymogram of peroxidases as detected using electrophoresis analysis for extraction from six blood samples of control and volunteers exposed for different times to plastic or its solvents vapors during plastic manifesting

Table 2. The Zymogram of peroxidases as detected using electrophoresis analysis for extraction from six blood samples of control and volunteers exposed for different times to plastic or its solvents vapors during plastic manifesting*.

Peroxidase	Control	1 year	3 years	6 years	7 years	12 years
Perox 1	-	+++	++	++	++	+++
Perox 2	++++	-	-	-	-	-
Perox 3	-	+	++	++	++	+++
Perox 4	++++	-	-	-	-	-
Perox 5	++++	+	+++	+++	+++	++
Perox 6	-	-	+++	+++	+++	++++
Perox 7	++	++	++++	++++	++++	++++
Perox 8	++++	++++	++++	++++	++++	++++

* Represented signs as; (+) for present and (-) for absent bands and the number of + representing the bands' intensities.

3.4. Esterases Isozymes

An esterase is an enzyme of hydrolysis that separates esters into an acid and an alcohol in a chemical reaction called hydrolysis with water. There are a wide range of different

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esterases that vary in their specificity of substrates, their structure of proteins, and their biological function.

The results are shown in Figure (3), and Table (3), the represented zymogram with five different esterase isozymes were detected using the substrate and developer solution specific for esterase, these five bands suggested that there are five alleles or loci conferring esterase isozymes in these six blood samples which were withdrawn from the volunteers under investigation. The five esterase isozymes were exhibited with different intensities between the six volunteers under investigation. On the other hand, relative mobility was presented between these esterase isozymes. The relative mobility reflecting different sizes of esterase molecules. Esterases 1 and 2 were presented in all the six blood samples under investigation with similar intensities (the darkest intensity), for that, they considered as common bands indicating no effects of plastic or its solvents vapors during manifesting on these two esterase isozymes.

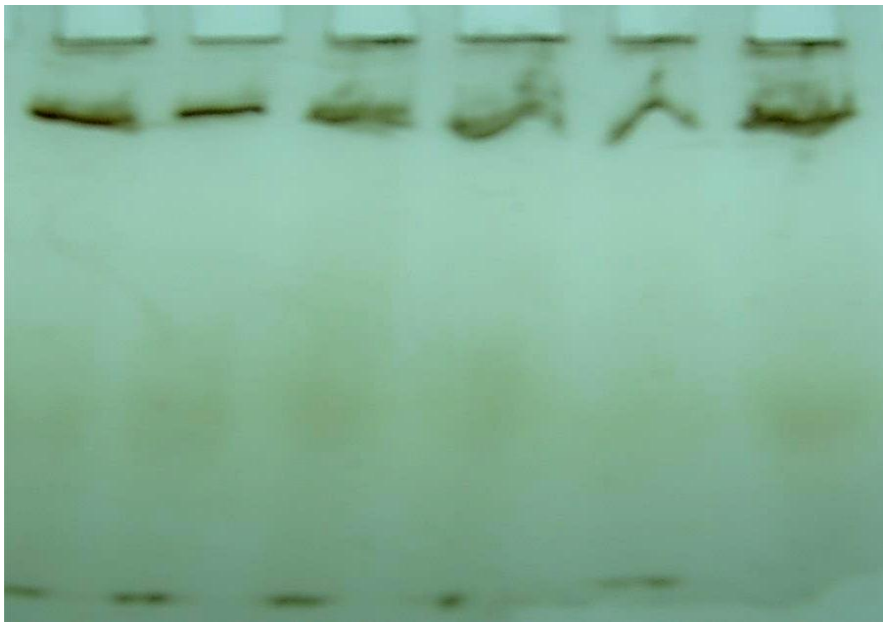

Figure 3. The Zymogram of Esterases as detected using electrophoresis analysis for extraction from six blood samples of control and volunteers exposed for different times to plastic or its solvents vapors during plastic manifesting

The results indicated also, that esterase isozyme 3 was presented only in the control, one-year exposure and 3 years exposure blood samples. On the other hand, it was absent in the blood samples of 6, 7, and 12 years of exposure volunteers. This banding pattern indicated that a negative effect for the exposure to plastic or its solvents vapors for a period of time longer than 3 years was occurred in esterase isozyme 3. Esterase 4 was presented in all the six blood samples of the volunteers under investigation with different intensities.

Esterase isozyme 5 was presented in all samples except the sample which withdrawn from the volunteer exposed to the vapors for a period of time of 12 years. This result indicated that the

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negative effect resulted from the exposure to plastic or its solvents vapors during plastic manifesting occurred after a long time of exposure, longer than 7 years exposure.

Table 3. The Zymogram of Esterases as detected using electrophoresis analysis for extraction from six blood samples of control and volunteers exposed for different times to plastic or its solvents vapors during plastic manifesting

Esterase	control	1 year	3 years	6 years	7 years	12 years
Est 1	++++	++++	++++	++++	++++	++++
Est 2	++++	++++	++++	++++	++++	++++
Est 3	++	+	+	-	-	-
Est 4	++	++	++	+++	+++	+++
Est 5	+++	+++	+++	+++	+++	-

* Represented signs as; (+) for present and (-) for absent bands and the number of + representing the bands' intensities.

These results could be explained by the findings of (Dolinoy *et al.*, 2007), which stated that epigenetic effect bioaccumulation after exposure to BPA (plastic) could be reversed/ eliminated. Differential DNA methylation after exposure of gestating female rats to a mixture of BPA and phthalate was also confirmed to be transgenerational, but the synergistic effect of both BPA and phthalate remains to be determined.

The increasing evidence suggests that epigenetics has the potential to develop biological markers to predict that chemicals would put exposed subjects at risk and which individuals would be more susceptible to disease. t is still important to note that to better understand the nature of epigenetic changes and the health effects of toxic influences on these disease-associated epigenetic modifications, the mechanisms by which environmental toxicants modulate the epigenetic landscape of individual cells have yet to be elucidated. Better-defined mechanisms can lead to better detection of the toxic potential of environmental chemicals such as BPA and phthalates and allow more tailored and effective strategies for disease prevention.

For human studies, it will be important to use laboratory methods with increased accuracy, tolerance and coverage to detect epigenetic changes as early as possible and well in advance of disease diagnosis. Now new technologies available to allow the global study of epigenetic changes, which can provide insight into the degree and patterns of changes between normal human and diseased tissues. It is necessary to consider suitable in vitro models. In this sense, human embryonic stem cells can be extremely useful in enhancing understanding of epigenetic effects on human growth, health and disease, as the creation of in vitro embryonic bodies is very similar to the early stage of embryogenesis (Li *et al.*, 2006; and Chen *et al.*, 2011).

Also, Manikkam *et al.* (2012) confirmed that the transient exposure of female rats to a plastic mixture (BPA and phthalates) during the embryonic sex determination cycle was demonstrated to encourage transgenerational female early-onset puberty (F3 generation) and

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decrease the pool size of primordial ovarian follicles. Spermatogenic cell apoptosis was also transgenerationally affected, and all exposed lineage males found differential DNA methylation of the F3 generation sperm promoter regions.

4. Conclusion

This study aimed to clarify the dangerous effects of different periods of exposure to plastic and its solvents vapors during plastic manifesting in the form of the changes in the blood protein contents and the modification in the genomic materials. To reach this target, blood samples were withdrawn from volunteers working in a plastics factory. Six different samples were collected from Clarkes exposed to plastic vapor and their solvents vapors for different periods as follow, control (did not exposed to plastic vapor), six months exposure, one-year exposure, three years exposure, six years exposure and twelve years exposure. The results of protein electrophoresis indicated that a total number of 14 protein bands were resolved on polyacrylamide gel electrophoresis ranged from 16.5 KDa to 105 KDa. Five bands out of the resulted 14 bands were considered as common bands with molecular weights of 105, 98, 85, 63 and 16.5 KDa (i.e. these bands were presented in all the six blood samples under investigation). The results also showed that too many changes were recorded on the protein banding patterns between the control volunteer and those who exposed to plastic and its solvents vapor for different exposure periods. These changes in proteins suggested that a modification in gene excretion was occurred as a response for the different exposure time to plastic during manifesting. Also, in esterase and peroxidase isozymes changes in the banding patterns between the control volunteer and those who exposed to plastic and its solvents vapor for different exposure periods. These changes in proteins suggested that a modification in gene excretion was occurred in the genes conferring these isozymes as response for the different exposure periods to plastic during manifesting.

References

Chen B.Z., Yu S.L., Singh S., Kao L.P., Tsai Z.Y., Yang P.C., Chen B.H., and Li S.S.L. (2011). Identification of microRNAs expressed highly in pancreatic islet-like cell clusters differentiated from human embryonic stem cells. *Cell Biol. Int.*; 35: 29–37.

Dolinoy D.C., Huang D., and Jirtle R.L. (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl. Acad. Sci. USA*; 104: 13056–13061.

Laemmli U.F. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature*; 227: 680-685.

Larsen S.T., and Nielsen G.D. (2012). Acute airway irritation of methyl format in mice. *Arch Toxicol*.; 86(2): 285-292.

Larsen A.G., and Benson W.C. (1970). Variety-specific variants of oxidative enzymes from soybean. *Crop Sci.*; 10: 493-495.

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

Li S.S.L., Liu Y.H., Tseng C.N., Chung T.L., Lee T.Y., and Singh S. (2006). Characterization and gene expression profiling of five new human embryonic stem cell lines derived in Taiwan. *Stem Cells Dev*.; 15: 532–555.

Manikkam M., Guerrero-Bosagna C., Tracey R., Haque M.M., and Skinner M.K. (2012). Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PLoS One*; 7: 93-107.

Scandalios J.C. (1984). Tissue-specific isozymes variation in maize. *J. Heredity*; 55: 281-285

Stegemann H., Burgenneister W., Krogerrecklenfort E. (1983). *PAGE-manual 1983*. Braunschweig, FRG: Institut fflr Biochemie, Biologische Bundesanstalt, Messeweg 11, 38104 Braunschweig.

Williams J.G., Kubelik A.R., Livak K.J., Rafalski J.A., and Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, 18(22): 6531-6535.

Zhang L., Zhang H.G., and Pang Q.Y. (2015). Physiological evaluation of the responses of Larix olgensis families to drought stress and proteomic analysis of the superior family. *Genet. Mol. Res*;14: 15577-15586.