



## TOXICITY ASSESSMENT OF TEXTILE DYES VIA OXIDATIVE STRESS HYPOTHESIS FOR IRAQI TEXTILE WORKERS

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### ABSTRACT

Textile dyes are the most important environment-polluting agents and particularly working environment, so the workers are vulnerable to many diseases, especially cancer, such as skin and lung cancer. Many of the workers are occupation exposed to dye, but little is known for their risk and chemical influence of the dyes on their health, for that the present study was taken up to assess the azo dyes toxicity, risk and knowledge about the health hazard of these dyes by determination of oxidant and antioxidant status of Iraqi textile workers in Hilla city. The mean age of 45 textile workers was  $37 \pm 5.2$  years compared with 40 person working in an office business aged  $35 \pm 2.5$  years. When enquired about whether dyes to affect body organ(s), all the workers agreed that dye(s) will affect skin, but they are not aware that dyes could affect other parts of the body for that, the present study included evaluation of lipid peroxidation via determining of the main end product malondialdehyde (MDA) as an oxidant and Vitamin E and reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), as an antioxidant as well as assessment of Lipid profile (cholesterol, triglyceride TG, high-density lipoprotein HDL, low-density lipoprotein LDL and very low-density lipoprotein vLDL) beside the evaluation of liver enzyme activity (AST and ALT) rather than determination of thyroid hormone T4 and T3 in an attempt to estimate the effects of dyes on various tissues inside the body.

**KEYWORDS:** Textile azo dyes, oxidative stress, lipid peroxidation, lipid profile, liver enzymes, thyroid hormone.



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## INTRODUCTION

Textile dyes are widely used in the world today and textile industry plays an important role in the nation's economy. Direct dyes are the most popular class of dyes owing to easy application, wide color range, and available at modest cost. Most direct dyes have diazo and triazo structures<sup>1,2,3</sup>. Azo dyes are the main constituents of such pollution because of their wide applicability and usages, and therefore, these are present extremely in textile industrial effluents. Mammalian and microbial enzyme systems have been reported to degrade azo dyes. Azo dyes that may be activated via direct oxidation of the azo linkage to highly reactive electrophilic diazonium salts<sup>4,5,6</sup>. In the mammalian liver, including man azo compounds are enzymatically cleaved by cytosolic and microsomal enzymes to the corresponding amines. Some aromatic amines can be metabolically activated to DNA binding intermediates that are mutagenic and carcinogenic to the human hepatic cell by azo-reductase. It is a non-specific enzyme, it is found in various micro-organism (such as in intestinal bacteria) that catalyze a nicotinamide adenine dinucleotide phosphate NAD(P)H dependent reductions<sup>7,8</sup>. The human skin and gastrointestinal tract harbor a complex and diverse microbiota comprised of at least several thousand species<sup>9,10</sup>. The microbiota also play roles in the degradation of azo dyes, In many cases the products formed after the degradation of the parent azo dye molecule are more toxic. These products are mainly in aromatic amine form. Azo dyes have been shown to be mutagenic to the human hepatic cell with azo reduction which being the most important reaction related to toxicity and mutagenicity<sup>11,12,13</sup>. Free radicals and ROS are unwanted products of aerobic metabolism and are formed endogenously<sup>14</sup>. In addition, they are produced exogenously by chemicals, drugs, and pollutants. Free radicals and ROS lead to biochemical and physiological lesions that may result in cell death from oxidative damages to lipids, proteins, and DNA<sup>15</sup>. Cellular antioxidant defenses control the levels of endogenous ROS under normal conditions<sup>14</sup>. If the balance between free radicals and antioxidants is disrupted due to either

overproduction of free radicals or decreased antioxidant defense, or both, this can result in a pathological condition called "oxidative stress"<sup>16,17</sup>. Oxidative stress causes oxidation of DNA, membrane lipids, and proteins. Cellular proteins, particularly sulfur-containing proteins, can easily be oxidized. This then results in inactivation of the enzymes<sup>14</sup>. Lipid peroxidation that is caused by oxidative damage may inactivate cellular compounds which may lead to the development of various disorders, including aging<sup>18</sup>. The antioxidants, SOD and the associated CAT enzyme comprise a first line of defense against oxidative stress. They can protect organisms from oxidative damage by partial remediation of ROS. SOD, the first line of defense against oxygen-derived free radicals, catalyzes superoxide anion radical ( $O_2^{\cdot-}$ ) into less-toxic  $H_2O_2$  and  $O_2$ , while CAT reduces  $H_2O_2$  to non-toxic  $H_2O$  and  $O_2$ <sup>19,20,21</sup>. Hence, the activities of these enzymes can be served as early indicators of exposure to contaminants that cause oxidative stress<sup>2</sup>. To verify the presence of oxidative imbalance more accurately, we measured the activities of SOD and CAT in parallel with MDA levels, which reflect the state of lipid peroxidation of the membranes. Vitamin E is the most important lipophilic antioxidant and exists mainly in the cell membranes, thus helping to maintain membrane stability. Vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, the resulting antioxidant radical is known to be unreactive<sup>22</sup>. Thyroid hormones (T3 and T4) are the primary regulators of human metabolism, growth, and stimulate protein synthesis. They secreted by the thyroid gland, it's one of the largest and most sensitive endocrine glands in the body these hormones stimulate oxidative respiration in most cells in the body and helping the body's basal metabolic rate<sup>23</sup>.

### ***The aims of the present study are***

(i) to evaluate the effect of inhalation and daily deal of textile azo dyes on oxidative stress via determination of lipid peroxidation, vit E, GSH

and antioxidant enzyme systems such as SOD, Cat for textile workers. (ii) to investigate the lipid profile including cholesterol, TG, HDL, LDL and vLDL to determine whether influence dyes in lipid metabolism. (iii) Measurement the hepatic enzymes GOT and GPT to clarify the effect of dyes on hepatotoxicity and to discuss their influence on protein metabolism. (v) To see if the thyroid hormone for textile workers affected by dye exposure compared with people who work somewhat without exposure to chemicals as if the administrative their work.

## MATERIALS AND METHODS

### *Patients and controls*

The study included the collection of samples from textile workers (male) in Hilla city, whom worked in the plant periods of time ranging from 5-20 years and their ages between 22-40 years compared to control group, their work was not direct exposure to any kind of chemicals and was there is a link between them in terms of age and sex. The study has also included a questionnaire about the followings: 1 – Age, 2 - duration of working, 3 - smoking and duration of smoking (excluded all the smoking workers), 4 – length, 5 –Weight, 6 - The presence of certain diseases such as high-pressure, diabetes, and other diseases as well as skin diseases. The assessment of lipid peroxidation in serum was determined by the colorimetric thiobarbituric acid (TBA) method. Under the acidity and heat condition of the reaction the lipid peroxides break down to form malondialdehyde(MDA) which complexes with the spectrophotometrically at 532 nm<sup>24</sup> (Lunec, J, 1990). Vitamin E was measured using the methods described by Toro et al.<sup>25</sup>.  $\alpha$ -tocopherol reacts with  $\alpha,\alpha$  – dipyrindyl to produce a complex, which has  $\lambda_{max}$  at 520 nm, vit E concentration was expressed as mg/ dl. Glutathione is determined by a modified procedure utilizing Ellman's reagents, the principles of this method is based on reduced of 5,5-dithiobis(2-nitro benzoic acid by sulfhydryl group of GSH to yellow compound, the absorbance is measured at 412nm<sup>26</sup>. The activity of SOD is determined by adding 75mM of tris-HCl buffer, 30 mM of EDTA and 2mM of pyrogallol to 50  $\mu$ L of serum. An increase in absorbance was

recorded at 420nm for 30 min. The activity of enzyme is expressed as U/L of serum<sup>27</sup>. Catalase was assayed calorimetrically at 620nm and expressed as  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> consumed /min/ml of serum described by Sinha<sup>28</sup>. Total cholesterol and triacylglycerol and HDL, LDL, v LDL determine by using BioMaghreb, France Kits. Thyroid Hormones (Tri-iodo-thyroid (T3) AND Thyroxin (T4)) Hormone: were assayed by using Monobind ELISA kits (Monobind Inc, USA).

### *Statistical analysis*

All results are expressed as a mean  $\pm$  SD (standard deviation), compared between patients and controls were performed by the student's t- test. Person's correlations were used to determine the relationship between parameters studied. A value of  $p \leq 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

In this study, the blood samples were collect from persons who don't suffer from any type of diseases except the skin diseases, and we found that most workers have different skin diseases, this observation encouragement to investigate the parameters in the study and to correlate the results with the influence of dyes on the worker's body. In the past years, mammalian species were used as models for the study of oxidative stress parameters caused by environmental pollutants to explain the mechanisms of cellular oxidative damage and to study the effects of some environmental pollutants with oxidative potential through the permanent exposure concentrations 29. This research introduced knowledge in the understanding of such oxidative processes in biological systems. This knowledge extends to specific applications in human being working in textile dyes and exposure to many toxic compounds like azo dye compound. Previous studies were mainly focused on the dye decolorization, genotoxicity, reproductive toxicity, as well as the toxicity to the biodegradation. However, this research studied the sensitive antioxidant defense system of textile workers. To fill the gap concerning the potential oxidative stress toxicity of dyes to the cells or organs and the relevant indicators have been investigated.

### 1. Effect of textile azo dyes on oxidant and antioxidant status

The potential of oxygen free radicals and other reactive oxygen species (ROS) to oxidative damage of cellular components and cell death, called oxidative stress<sup>15</sup>. The balance between prooxidant endogenous and exogenous free radicals (such as chemicals, drugs and pollutants) and antioxidant defenses in biological systems can be used to assess the toxic effects of oxidative stress induced by different chemical pollutants. The role of these

oxidant- antioxidant systems and their changes can be of great attention in environmental toxicology studies<sup>29</sup>. The present study shows a significant increase of MDA as an oxidant biomarker, while the levels of vit E and GSH as antioxidant were decreased significantly for textile workers compared to control group, these results illustrated in Table 1. As well as Table 2 show a significant increase of SOD and CAT activities for textile workers compared to control group.

**Table 1**  
**MDA (  $\mu\text{mole/l}$ ), vitE (mg/dl) and GSH (  $\mu\text{mole/l}$ ) levels for textile workers and controls**

	Workers	Control	P value
	N=45	N=40	
	Mean $\pm$ SD		
MDA( $\mu\text{mole/l}$ )	2.58 $\pm$ 0.9	1.43 $\pm$ 1.02	0.000
Vit. E( mg/dl)	0.35 $\pm$ 0.1	1.2 $\pm$ 0.4	0.00
GSH( $\mu\text{mole/l}$ )	5.04 $\pm$ 1.5	21.3 $\pm$ 2.5	000.0

**Table 2**  
**SOD (U/L) and CAT(U/ml ) activities for textile workers and controls**

	Workers	Control	P value
	N=45	N=40	
	Mean $\pm$ SD		
CAT U/ml	32.37 $\pm$ 8.0	27.96 $\pm$ 5.2	0.00
SOD U/L	443.13 $\pm$ 149.5	350.4 $\pm$ 131.3	0.00

These results may be explained by suggesting that the various azo dyes and their products are toxigenic and mutagenic which characteristic to many aromatic amines but rather through a mechanism involving oxygen radicals and superoxide free radical was produced by the azo dyes after reduction by intestinal bacteria<sup>30</sup>. Cerniglia *etal.*,<sup>31</sup> demonstrate that many intestinal bacteria are able to reduce the azo bond to produce aromatic amines and some of which are known carcinogens, have been found in the urine of dyestuff workers and test animals following administration of azo dyes. As well as Li *etal.*,<sup>32</sup> demonstrates that azo and hydroxyl group of sudan dyes are capable of forming hydrogen bonds with polar head group of membrane phospholipid. On the other hand the azo dyes metabolized into aromatic amines can generate reactive oxygen species as part of their metabolism (NOS) by interaction of these amines groups with intercellular nitrite or nitrate. The reactive

oxygen species (ROS) are chemically reactive molecules containing superoxide anion, hydroxyl radical and H<sub>2</sub>O<sub>2</sub> could be produced during the metabolism of nitrosamines and increase oxidative stress<sup>13</sup>. Under certain environmental conditions oxidative stress due to the increasing of ROS formation and decreased of the antioxidant defense mechanism of the cells including vit.E and GSH began to consumed to prevent the cell death by these toxic radicals so their levels in the tissue homogenate were decreased specially at higher doses of ROS, on the other hand MDA level was increased as a product of lipid peroxidation occurred by the ROS action on lipids of cellular membranes. Biological membranes are particularly prone to the ROS effect, the peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and disruption of membrane integrity and function, which is implicated in serious pathological changes<sup>17</sup>. Reactive oxygen species play an important

role in pathological changes in the liver, the increasing of free radical production is able to cause auto-oxidation of the hepatic cells, resulting in marked hepatic lesions<sup>29</sup>. In the present study, the increased activities of serum enzymes ALT and AST have been detected in exposure of textile azo dyes. Sweeney *et al.*,<sup>33</sup> and Siraki *et al.*,<sup>34</sup> found that incubation of hepatocytes with aromatic amines caused a decrease in the mitochondrial membrane potential before cytotoxicity ensured, hepatocyte GSH was also depleted by all aryl amines tested and extensive GSH oxidation occurred with o-anisidine and aminofluorene. On the other hand, the increasing of CAT and SOD activities are more indicative for oxidative stress and elevated of ROS especially  $O_2^{\cdot-}$  which scavenging with SOD to produce  $H_2O_2$ , the substrate for catalase enzyme<sup>35,36</sup>, which decomposed to water and oxygen, the estimation of CAT activity could be considered a biomarker for oxidant-antioxidant status<sup>37,38</sup>. Previous studies explained the environmental pollutant can induce the oxidative stress which can be accompanied with lipid peroxidation and depleted of antioxidant defense in plant and mammalian cells<sup>39,40</sup>. Jadhav *et al.*,<sup>13</sup> explain that textile

azo dyes may play a role in the generation of oxidative stress when examined the toxicity of azo dyes Remazol Red(RR) on the plants, while Phugare *et al.*,<sup>41</sup> demonstrate the formation of free radicals can lead to the lipid peroxidation reaction which are indices for oxidative stress. Nitric oxide radical has been identified as a biologically important molecule involved in a number of physiological processes,  $NO^{\cdot}$  can react rapidly with superoxide anion( $O_2^{\cdot-}$ ) to produce the potent oxidant peroxynitrite( $ONOO^-$ ), which is very reactive species initiating oxidation and nitration<sup>42</sup>. Bartesaghi *et al.* 2004). The elevated of peroxynitrite levels could be the essential cause to depletion of vitamin E concentration because  $\alpha$ -tocopherol was regarded as a defense substrate against peroxynitrite attack, and lead to increasing of lipid peroxidation<sup>43</sup>.

## 2. Effect of textile azo dyes on lipid profile levels

A non-significant increase of cholesterol levels, a significant increase of TG and LDL levels as well as a non-significant reduction of HDL levels for textile workers compared to control group were observed in Table 3.

**Table 3**  
**lipid profile levels for textile workers and controls**

	Workers	controls	P value
	N=45	N=40	
	Mean $\pm$ SD		
Cholesterol(mg/dl)	254.2 $\pm$ 57.2	$\pm$ 20.4 194.3	0.09
TG(mg/dl)	236.5 $\pm$ 86.9	145.9 $\pm$ 20.5	0.00
LDL(mg/dl)	145.93 $\pm$ 28.8	$\pm$ 10.5 121.3	0.05
HDL(mg/dl)	40.45 $\pm$ 9.8	2.650.45 $\pm$	No sign
vLDL(mg/dl)	62.46 $\pm$ 29.2	4.5 $\pm$ 32.6	0.01

Cholesterol is a soft waxy substance found among the lipids in the blood stream and in the body's cells. It is an important part of a healthy body because it is used to form cell membranes and to produce certain hormones. The total body content of cholesterol depends on the balance between the amount of cholesterol formed in the body plus that absorbed from the diet. Intestinal cholesterol absorption represents another major route for the entry of cholesterol into the body, and, thus, this source can influence the plasma

LDL-cholesterol concentration<sup>44,29</sup>. Serum lipid levels are risk factors for coronary heart disease (CHD), atherosclerosis and type II diabetes. Both genetic and environmental factors influence lipid level phenotypes. Lipid level variation can be influenced bygenetics<sup>45</sup>. On the other hand, environmental factors also play a role. For example, lifestyle factors such as physical exercise, smoking and diet have well-documented relationships with lipid levels<sup>46,47</sup>. There are a number of reports connecting lipid levels, cardiovascular

disease, type 2 diabetes and the metabolic syndrome with specific persistent pollutants, such as dioxins, organo chlorinated pesticides, dibenzofurans and polychlorinated biphenyls (PCBs)<sup>48,49,50,51</sup>. Other less tangible environmental factors, such as air pollution may also have an adverse relationship with lipid levels<sup>52,53,54</sup>. Free fatty may be released from adipose tissue caused by exposure to the pollutant such as cigarette smoking<sup>55,56</sup>. These fatty acids are a well-known stimulant of hepatic secretion of very low density lipoprotein and hence triglyceride as well as

high density lipoprotein concentrations vary inversely with very low density lipoprotein concentrations in serum. Complementary to this mechanism is the finding that free fatty acid also stimulates the hepatic synthesis and secretion of cholesterol<sup>57</sup>.

### 3. Effect of textile azo dyes on liver enzyme(ALT and AST) activities

The present study revealed that textile workers have a non-significant increase in serum ALT and AST when compared with control group as shown in Table 4.

**Table 4**  
**ALT and AST activities (U/L) for textile workers and controls**

	Workers	controls	P value
	N=45	N=40	
	Mean $\pm$ SD		
ALT(U/L)	50.93 $\pm$ 8.7	3.5 $\pm$ 30.5	No sign
AST(U/L)	43.35 $\pm$ 6.17	$\pm$ 3.826.4	No sign

These results may be due to tissues damage particularly in liver, kidney and heart caused by the toxic effect of these dyes, Abdel-Rahim *etal.*<sup>58</sup> found a significant increase in both serum AST and ALT of rats fed on brown food dye for three months, they attributed these changes in liver function to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood. Mekkawy *etal.*,<sup>59</sup> indicated that two doses of synthetic dyes (low or high doses) where tartrazine and carmoisine were among of them (ponceau, carmoisine, erythrosine, sunset yellow, tartrazine, fast green, indigotine, brilliant blue and brilliant black) showed a significant increase in serum AST, ALT, and alkaline phosphates activities. Mekkawy H.A. *etal.*<sup>59</sup> attributed these results to hepatocellular damage caused by the toxic effects of these synthetic dyes which indicated by vacuolation, swelling, necrosis and pyknosis of the liver cells Helal *etal.*,<sup>60</sup> found that oral administration of synthetic or natural colorants induced a marked increase in the serum AST and ALT level of all groups after 30 days of administrate. As well as the release of abnormally high levels of specific tissue enzymes into the blood stream is dependent on both the degree and the type of damage exerted by exposure to the toxic compound<sup>29</sup>.

Despite the fact that the available research to measure the aminotransferase and its relationship with the contaminated material a few but it is possible to interpret the results that have been reached in the search on the basis that inhalation of vapors dyes or frequent use in textile resulting contamination of bodies operating in this field with this type of chemical compounds and entry into the body, causing many changes, including the generation of free radicals, resulting in damage to the liver cells more than that one of the most important functions of the liver is metabolized xenobiotics, thus it was selected to conduct the research by measured the liver enzymes ALT and AST. Many xenobiotics released by industry entities may exert potentially adverse effects on aquatic organisms and elicit oxidative stress due to the generation of the cytotoxic reactive oxygen species (ROS)<sup>20,2</sup>. Shakoori *etal.*,<sup>61</sup> revealed that under pathological conditions the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediary metabolism, as a result of cellular damage, enzymes like AST, ALT, LDH and alkaline phosphatase reach out into the serum and hence their level indicates the type and extent of damage inflicted<sup>29</sup>. T4 and T3 concentration were nonsignificantly increased

in textile workers compared to control group as shown in Table 5. The thyroid hormone thyroxine(T4) and triiodothyronine (T3) are produced in response to stimulation of thyroid

stimulating hormone(TSH) and stored in the thyroid gland then circulate to the blood stream mostly bound with plasma protein, thyroxine binding globulin(TBG)<sup>62</sup>.

**Table 5**  
**Thyroid hormone (T4 and T3) levels for textile workers and controls**

	workers	control	P value
	N=45	N=40	
	Mean ±SD		
T4 (nmole/L)	94.64 ± 12.3	±6.886.5	No sign
T3(nmole/L)	1.45 ± 0.35	±0.21.09	No sign

It is well known that environmental chemicals can cause thyroid imbalance. However, the effects of chemicals on thyroid function have received little attention, and there is much controversy over their potential clinical impact, because few studies have been conducted in humans. This article reviews the literature on possible thyroid disruption in humans. Prescott *et al.*<sup>63</sup> demonstrated that long-term oral exposure to cobalt blue dyes can cause goiter and myxedema. The effect of industrial cobalt exposure on thyroid volume and function was determined for 61 female plate painters exposed to cobalt blue dyes in two Danish porcelain factories and 48 unexposed referents. These subjects also had increased levels of serum thyroxine (T4) and free thyroxine (FT4), unaltered serum thyroid stimulating hormone (TSH), and marginally reduced 3,5,3'-triiodothyronine (T3), whereas thyroid volume tended to be lower, and their

discussion of these results is Cobalt might inhibit the extrathyroidal 5'-deiodination of T4 into T3, which is of enzymatic nature, and create a "high T4-low T3 euthyroid state"<sup>63</sup>. Videla *et al.*,<sup>64</sup> showed that T3 calorogenesis in rat involves a higher rate of O<sub>2</sub> consumption in liver, with generation of ROS in hepatocyte and antioxidant depletion. This enhancement of the status of the oxidative stress of the liver, which is counted a mild redox alteration due to the deficiency of morphological changes occurrence in parenchymal cell of liver<sup>62</sup> except kupffer cells which undergo hyperplasia and hypertrophy, was found to trigger the redox regulation of gene expression<sup>64</sup>. The present study suggested that the increasing of T3 and T4 related to the exposure to dyes and this result indicates the damage of thyroid hormone or to the distribution of thyroid function.

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