INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.47*

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 577 - 587

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

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

LAMIA A. M. AL-MASHHEDY

Chemistry Department, Babylon University, Babel, Iraq

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±5.2 years compared with 40 person working in an office business aged 35±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.

LAMIA A. M. AL-MASHHEDY
Chemistry Department, Babylon University, Babel, Iraq

***Corresponding author**

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 [1,2,3]. 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 [4,5,6]. 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 [7,8]. The human skin and gastrointestinal tract harbor a complex and diverse microbiota comprised of at least several thousand species [9,10]. 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[11,12,13]. Free radicals and ROS are unwanted products of aerobic metabolism and are formed endogenously [14]. 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 [15]. Cellular antioxidant defenses control the levels of endogenous ROS under normal conditions [14]. 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’’ [16,17]. 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 [14]. Lipid peroxidation that is caused by oxidative damage may inactivate cellular compounds which may lead to the development of various disorders, including aging [18]. 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 ($O2^{-\cdot}$) into less-toxic H2O2 and O2, while CAT reduces H2O2 to non-toxic H2O and O2 [19,20,21]. Hence, the activities of these enzymes can be served as early indicators of exposure to contaminants that cause oxidative stress [2]. 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[22]. 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 [23].

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 [24] (Lunec, J, 1990). Vitamin E was measured using the methods described by Toro et al. [25]. α- tocopherol reacts with α,ά – dipyridyl to produce a complex, which has λmax 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 [26]. The activity of SOD is determined by adding 75mM of tris-HCl buffer, 30 mM of EDTA and 2mM of pyrogallol to 50 µL of serum. An increase in absorbance was recorded at 420nm for 30 min. The activity of enzyme is expressed as U\L of serum[27]. Catalase was assayed calorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed \min\ml of serum described by Sinha[28]. 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 ± 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 stress15,. The balance between prooxidant endogenous and exogenous free radicals (such as chemicals, drugs andpollutants) 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[29]. 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 (µmole/l), vitE (mg/dl) and GSH (µmole/l) levels for textile workers and controls

	Workers	Control	P value
	N=45	N=40	
	Mean ±SD		
MDA(µmole/l)	2.58±0.9	1.43±1.02	0.000
Vit. E(mg/dl)	0.35±0.1	1.2±0.4	0.00
GSH(µmole/l)	5.04±1.5	21.3±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 ±SD		
CAT U/ml	32.37 ± 8.0	27.96 ± 5.2	0.00
SOD U/L	443.13 ± 149.5	350.4 ± 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[30]. Cerniglia *etal*.,[31] 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*.,[32] 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_2O_2 could be produced during the metabolism of nitrosamines and increase oxidative stress [13]. 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[17]. 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 [29]. In the present study, the increased activities of serum enzymes ALT and AST have been detected in exposure of textile azo dyes. Sweengey *etal.,* [33] and Siraki *etal.,* [34] 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 $O_2^{\bullet -}$ which scavenging with SOD to produce H_2O_2 , the substrate for catalase enzyme [35,36], which decomposed to water and oxygen, the estimation of CAT activity could be considered a biomarker for oxidant-antioxidant status[37,38]. 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[39,40]. Jadhav *etal.,*[13] 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 *etal.,* [41] 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, $NO^{\bullet}$ can react rapidly with superoxide anion($O_2^{\bullet -}$) to produce the potent oxidant peroxynitrite($ONOO^{--}$), which is very reactive species initiating oxidation and nitration [42]. Bartesaghi *etal*. 2004) .The elevated of peroxynitrite levels could be the essential cause to depletion of vitamin E concentration because α- tocopherol was regarded as a defense substrate against peroxynitrite attack, and lead to increasing of lipid peroxidation[43].

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 ±SD		
Cholesterol(mg/dl)	254.2 ± 57.2	±20.4 194.3	0.09
TG(mg/dl)	236.5 ± 86.9	145.9± 20.5	0.00
LDL(mg/dl)	145.93 ± 28.8	± 10.5 121.3	0.05
HDL(mg/dl)	40.45 ± 9.8	2.650.45 ±	No sign
vLDL(mg/dl)	62.46 ± 29.2	4.5 ±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[44,29]. 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 [45] 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 [46,47]. 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) [48,49,50,51]. Other less tangible environmental factors, such as air pollution may also have an adverse relationship with lipid levels[52,53,54,]. Free fatty may be released from adipose tissue caused by exposure to the pollutant such as cigarette smoking [55,56]. 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[57].

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 ±SD		
ALT(U/L)	50.93 ± 8.7	3.5±30.5	No sign
AST(U/L)	43.35 ± 6.17	±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.* [58] 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*., [59] 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*. [59] 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*., [60] 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 [29]. 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) [20,2]. Shakoori *etal.*,[61] 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 [29]. 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 riiodothyronine (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) [62].

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.* [63] 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" [63]. Videlal *etal.*, [64], showed that T3 calorigensis in rat involves a higher rate of O_2 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 [62] except kupffer cells which undergo hyperplasia and hypertrophy, was found to tigger the redox regulation of gene expression [64]. 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.

REFERENCES

1. Bae, J.S.,Freeman,H.S.,2007."Aquatic toxicity evaluation of new direct dyes to the Daphnia magna". DyesPigments73,81–85.
2. Li-Ying, Shi Jia-Qi, Juan R. Qu, Feng M-B., Fu Liu , Wang M., Wang Zun-Yao "Toxicity assessment on three direct dyes(D-BLL,D-GLN,D-3RNL)using oxidative stress bio-assay and quantum parameter calculation" *Ecotoxicology and Environmental Safety*. 2012; 86: 132–140
3. Farah M.D, Gisele A.R, Elisa R.A., Juliano C.C., Maria V.B.and Danielle P.D., "Textile dyes: Dyeing process and environmental impact" chapter 6, "Textile technology" Eco-Friendly textile Dyeing and finishing" edited by Melih G., 2013, Publish with InTech
4. Chung KT. "The signiWcance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes". *Mutat Res.* 1983; 114:269–281
5. Chen H, Hopper SL, Cerniglia CE. "Biochemical and molecular characterization of an azoreductase from Staphylococcus aureus, a tetrameric NADPH-dependent flavoprotein." *Microbiology* 2005;151:1433–41.

6. Xu H., Heinze Th. M. , Paine D.D. , Cerniglia C. E. a, Chen H, "Sudan azo dyes and Para Red degradation by prevalent bacteria of the human gastrointestinal tract." *Anaerobe* .2010; 16:114–119.
7. Osano O, Oladimeji AA, Kraak MH, Admiraal W. "Teratogenic effects of amitraz, 2,4-dimethylaniline, and paraquat on developing frog (Xenopus) embryos." *Arch Environ Contam Toxicol* 2002;43:42–9.
8. Chen H, Wang RF, Cerniglia CE. "Molecular cloning, overexpression, purification, and characterization of an aerobic FMN-dependent azoreductase from Enterococcus faecalis." *Protein Expr Purif*. 2004;34:302–10.
9. Gao Z, Tseng CH, Pei Z, Blaser MJ. "Molecular analysis of human forearm superficial skin bacterial biota." *Proc Natl Acad Sci U S A* 2007;104:2927–32.
10. Eckburg P.B., Bik E.M., Bernstein C.N, Purdom E., Dethlefsen L., Sargent M., Gill S.R., Nelson K.E., Relman D.A. " Diversity of the human intestinal microbial flora." *Science* 2005; 308:1635–8.
11. Ferraz, E. R. A., Umbuzeiro, G. A. G., Caloto-Oliveira, A., Chequer, F. M. D., Zanoni, M. V. B., Dorta, D.J., and Oliveira, D. P." Differential toxicity of Disperse Red 1 and Disperse Red 13 in the Ames test, HepG2 cytotoxicity assay, and Daphnia acute toxicity test." *Environmental Toxicology*. a, 2011; 26, 489–497.
12. Ferraz, E.R.A.; Grando M.D., Oliveira D. P. "The azo dye Disperse Orange 1 induces DNA damage and cytotoxic effects but does not cause ecotoxic effects in *Daphnia similis* and *Vibrio fischeri". Journal of Hazardous Materials*, b, 2011; 192(2), 628-633.
13. Jadhav S. B., Phugare S. S., Patil P. S., Jadhav J. P. "Biochemical degradation pathway of textile dye Remazol red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies International" *Biodeterioration & Biodegradation*. 2011; 65: 733-743
14. Sahu SC. "Dual role of organosulfur compounds in foods." *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2002; 20(1):61–76
15. Sies H, Stahl W, Sevanian A . "Nutritional, dietary and postprandial oxidative stress." *J Nutr* 2005; 135(5):959–972
16. Demirkol, O., Zhang, X., Ercal, N. "Oxidative effects of Tartrazine (CAS No. 1934-21-0) and New Coccin (CAS No. 2611-82-7) azo dyes on CHO cells." *Journal of Consumer Protection and Food Safety.* 2012; 7: 229-236.
17. Halliwell, B. "Oxidants and human diseases." *FASEB. J.* 1987; 1:441-445.
18. Packer L, Ong ASH (1998) Biological oxidants and antioxidants: molecular mechanisms and health effects. AOCS Press, Champaign, p 372.
19. Mahmoud Hussein Hadwan, Lamia A. Almashhedy, Abdul Razzaq S. Alsalman: Precise method for the assessment of catalase-like activity in seminal fluids, International Journal of Pharma and Bio Sciences 4(1),949-954(2013).
20. Di Giulio R.T., Washburn P.C, Wenning RJ. "Biochemical responses in aquatic animals: a review of determinants of oxidative stress." *Environ. Toxicol. Chem*. 1989; 8: 1103–1123.
21. Bakan, N., Taysi, S., Yilmaz, O., Bakan, E., Kuskay, S., Uzun, N., Gundogdu, M., 2003. "Glutathione peroxidase, glutathione reductase, Cu–Zn superoxide dismutase activities, glutathione, nitric oxide, and malondialdehyde concentrations in serum of patients with chronic lymphocytic leukemia." *Clin. Chim. Acta* 338, 143–149.
22. Ozden S. , Catalgol B., Gezginci-Oktayoglu S., Arda-Pirincci P., Bolkent S., Alpertunga B., "Methiocarb-induced oxidative damage following subacute exposure and the protective effects of vitamin E and taurine in rats." *Food and Chemical Toxicology*. 2009; 47: 1676–1684.
23. Hedef DE. "Biochemistry of Hormones". *A Case oriented approach 2012.*
24. Lunec, J. Free radicals: their involvement in disease processes. *Ann. Clinc. Biochem*. 1990; 27:173-182

25. Toro, G., Acherman, P.G., "Practical clinical chemistry," 1st edition, little, Brown and company, 1975; pp:222-223.
26. Ellman G.L. "Tissue sulfhydryl groups." *Arch. Biochem. Biophys*. 1959; 82: 70.
27. Marklund S., Marklund C. " Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase". *Eru.J. Biochem* 1974; 47: 469-474.
28. Sinha KA. "Colorimetric assay of catalase" *Anal. Biochem* 1972; 47(2): 389-394.
29. Valavanidis, A., Vlahoganni, T., Dassenakis, M., Scoullos, M. "Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants." Ecotoxicol Environ Saf. 2006; 64(2): 178-89.
30. Amin K.A. Abdel Hameid H. Abd- Elsttar A.H. "Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats." *Food and Chemical Toxicology*. 2010; 48:2994-99.
31. Cerniglia, C.E., Zhuo, Z., Manning, B.W., Federle, T.W., Heflich, R.H. "Mutagenic activation of the benzidine-based dye Direct Black 38 by human intestinal microflora." *Mutat. Res*. 1986;175 (1):11–16.
32. Li L, Gao HW, Ren JR, Chen L, Li YC, Zhao JF, et al. "Binding of Sudan II and IV to lecithin liposomes and E. coli membranes: insights into the toxicity of hydrophobic azo dyes." *BMC Struct Biol* 2007;7:16.
33. Sweeney, A.E., Chipman, J.K., Forsythe, S.J. "Evidence for direct-acting oxidative genotoxicity by reduction products of azo dyes". *Environ. Health Perspect*. 1994; 102 (6), 119–122.
34. Siraki, A.G., Chan, T.S., Galati, G., Teng, S., O'Brien, P.J. "N-oxidation of aromatic amines by intracellular oxidases." *Drug Metab. Rev*. 2002; 34 (3), 549–564.
35. McCord, J.M., Fridovich, I., 1969. "Superoxide dismutase. An enzymic function for erithrocuprein (hemocuprein)." *J. Biol. Chem*. 244, 6049–6055
36. Frank S. ,Kämffer H.,Podda M., Kaufmann R., and Pfeils-Chifter P. "Identification of copper/zinc superoxide dismutase as a nitric oxide regulated gene in human(Ha CaT) keratinocytes. Implication for keratinocyte proliferation." *Biochem. J.* 2000; 346:719-728.
37. Aebi, H., 1984. "Catalase in vitro". *Method. Enzymol*. 105, 121–126.
38. Cross C.E., Van der vliet A. O., Neill C.A., Louie S., and Halliwell, B. "Oxidants, antioxidants and respiratory tract lining fluids." *Environ. Health perspect*. 1994,102(10):185-191.
39. Schützendübel, A., Polle, A. "Plant responses to abiotic stresses: heavy metalinduced oxidative stress and protection by mycorrhization." *Journal of Experimental Botany*. 2002; 53 (372):1351-1365.
40. Jeng, H.A., Pang, C.H., Iwara, N., Chang-Chien, G., Lin, W., Huang, C., Ho, C., Wu, M. "Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration." *Occupational and Environmental Medicine*. doi:10.1136/oem.2010.055020.
41. Phugare S.S., Kalyani D.C., Patil A.V., Jadhav J.P. "Textile dye degradation by bacterial consortium and subsequent toxicological analysis of dye and dye metabolites using cytotoxicity, genotoxicity and oxidative stress studies." *Journal of Hazardous Materials*. 2011; 186:713-723.
42. Bartesaghi, S., Trujillo,M., Denicola,A., Folkes, L., Wardman, P. and Radi, R. 2004. "Reactions of desferrioxamine with peroxynitrite-derived carbonate and nitrogen dioxide radicals" *Free Radic. Biol. and Med*.3 6(4): 471-83.
43. Hoeldtke R. D., Bryner K. D., McNeill D. R., Hobbs G. R., Riggs J. E., Warehime S. S., Christie I., Ganser G., and Van Dyke K. "Nitrosative Stress, Uric Acid, and Peripheral Nerve Function in Early Type 1 Diabetes" *Diabetes* 2002;.51:2817-2825;

44. Turley, S.D., Dietschy, J.M. "Sterol absorption by the small intestine." *Curr. Opin. Lipidol*. 2003; 14 (3), 233–240.
45. Costanza MC, Cayanis E, Ross B.M., Flaherty M. S., Alvin G. B., Das K., and Morabia A. "Relative contributions of genes, environment, and interactions to blood lipid concentrations in a general adult" *Am J population Epidemiol*. 2005; 161: 714–24
46. Kris-Etherton PM, Krummel D., Russell ME. et al. "The effect of diet on plasma lipids, lipoproteins, and coronary heart disease". *J Am Diet Assoc* 1988;88:1373–400.
47. Schaefer EJ. "Lipoproteins, nutrition, and heart disease." *Am J Clin Nutr* 2002;75:191–212.
48. Sergeev AV. and Carpenter DO. "Hospitalization rates for coronary heart disease in relation to residence near areas contaminated with persistent organic pollutants and other pollutants." *Environ Health Perspect* 2005; 113: 756–61.
49. Lee DH., Lee IK., Porta M., Steffes M., Jacobs DR Jr. "Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002." *Diabetologia* 2007;50: 1841–51.
50. Lee DH., Steffes MW., Sjodin A., Jones RS., Needham LL., Jacobs DR Jr. "Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes." *PLoS One* 2011;6:e15977.
51. Lee DH., Steffes MW., Sjodin A., Jones RS., Needham LL., Jacobs DR Jr. "Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study." *Environ Health Perspect* 2010;118: 1235–42.
52. Park SK, Son HK, Lee SK et al." Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among non-diabetic adults." *J Prev Med Public Health* 2010;43:1–8.
53. Brook RD, Rajagopalan S, Pope CA III , Brook J. R. , Bhatnagar A., V. Diez-Roux A.V., Holguin F., Hong Y., Luepker R. V., Mittleman M. A., Peters A., Siscovick D., Smith Jr S.C., Whitsel L., Kaufman J.D. "Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association." *Circulation* 2010;121: 2331–78.
54. Brischetto C.S., Connor W.E., Connor S.L., Matarazzo JD. "Plasma lipid and lipoprotein profiles of cigarette smokers from randomly selected families: enhancement of hyperlipidemia and depression of high-density lipoprotein." *Am J Cardiol.* 1983;1:52(7):675–680.
55. Meenakshisundaram R., Rajendiran Ch., and Thirumalaikolundusubramanian P. "Lipid and lipoprotein profiles among middle aged male smokers: a study from southern India." *Tob Induc Dis.* 2010;8(1): 11-23.
56. Wendy Y Craig, Glenn E Palomaki, James E Haddow. "Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data." *BrAied_J* 1989;298:784-800.
57. Abdel-Rahim, E.A., Ahmed, F.A., El-Desoky, G.E., Ramadan, M.E. "Biochemical role of some natural and synthetic food colourants on liver function of rats. "*Minia. J. Agric. Res. Dev.*1987; 9 (3), 11–17.
58. Mekkawy, H.A., Ali, M.O., El-Zawahry, A.M. "Toxic effect of synthetic and natural food dyes on renal and hepatic functions in rats." *Toxicol. Lett*.1998; 95 (1), 155.
59. Helal, G.E., Zaahkouk, A.M., Mekkawy, A.H. "Effect of some food colorants (synthetic and natural products) of Young Albino rats." *Egypt. J. Hosp. Med*. 2000; 1, 103–113.
60. Shakoori, A.R., Khan, M.T., Hac, R., Ali, S.S. "Haematological and biochemical changes induced in rabbit blood by chronic doses of thioactamide." *Pak. J. Zool*. 1987;19 (3), 273–282.
61. Schall R.F.Jr, Fraser A.S., Hansen H.W.,Kern C.W., and Teneso H.J. " A sensitivity manual enzyme immunoassay

for thyroxine." *Clin.Chem*. 1978, 24(10):1801-06

62. Prescott E, Netterstrom B, Faber J, Hegedus L, Suadicani P, Christensen JM. "Effect of occupational exposure to cobalt blue dyes on the thyroid volume and function of female plate painters." *Scand J Work Environ Health* 1992;18(2):101-104
63. Tapia G., Pepper I., Smok G., Videla L.A.,"Kupffer cells function in Thyroid hormone induced liver oxidative stress." *Free Radic. Res*. 1997; 26:267-79.
64. Videla L. A., Fernandez V., Tapia G., Varela P. " Thyroid hormone calorigenesis and mitochondrial redox signaling upregulation of gene expression." *Font. Biosci*.,2007; 12: 1220-28.