KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

ASSESSMENT OF LIVER AND KIDNEY FUNCTION, AND LIPID PROFILE OF NTONSO TRADITIONAL TEXTILE DYERS

BY:

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DECLARATION

I hereby declare that this academic work is my original work towards the Bachelor of Science degree in Biochemistry and Biotechnology and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree at Kwame Nkrumah University of Science and Technology or any other educational institution, except where due acknowledgment has been made in the text.

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(Supervisor)	(Signature)	(Date)

DEDICATION

To Ma and Pa for exceling in the nearly impossible task of raising me

ACKNOWLEDGEMENT

First and foremost, I am grateful to God for good health and His inspiration. I am also very grateful for the unflinching support and love of my siblings and my parents.

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ABSTRACT

Due to the eclectic use and demand of textiles, the traditional textile industry resorts to using metal-containing synthetic dyes in addition to natural dyes. A plethora of evidence show that heavy metals, particularly, the transition metals play a role in the oxidative deterioration of biological molecules. The purpose of the research was to evaluate the effects of exposure to textile dyes on the liver function, kidney function, and lipid profile of workers of the Ntonso traditional textile dyeing industry. The study was a case-control study which involved 50 dyers and 50 non-dyers as a control group. Serum obtained from the dyers and non-dyer control groups were analysed for liver function (measured albumin, globulin, total protein, ALP, ALT, AST, and GGT levels), kidney function (measured creatinine, blood urea nitrogen, potassium, sodium, and chloride ion levels), and lipid profile (measured HDL, LDL, VLDL, total cholesterol, and triglycerides) with a fully automated biochemistry analyser. Using SPSS 20, the means of the various parameters was compared between the dyers and the non-dyer control group. A significant decrease was observed in the levels of albumin ($p=0.001$), globulin ($p=0.005$), and total protein ($p=0.000$) indicating an impairment in the protein synthetic function of the liver. There was also a significant increase in the serum levels of creatinine ($p=0.013$), and chloride ($p=0.025$) implying a deterioration in the glomerular filtration rate of the kidney.

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LIST OF ABBREVIATIONS

ALP	-	Alkaline Phosphatase
AST	-	Aspartate transaminase
ALT	-	Alanine transaminase
CR	-	Cardiovascular risk
GGT	-	Gamma-glutamyltransferase
HDL	-	High density lipoprotein
LDH	-	Lactate dehydrogenase
LDL	-	Low density lipoprotein
VLDL	-	Very low density lipoprotein

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Textiles play a pivotal role in all Ghanaian communities. Ghanaians wear different shades of colour and brightness on all sorts of events – from weddings to funerals. In 2004, the Ghana government introduced the National Friday Wear programme and that craze has not died out since showing the country's ever-increasing love for its traditionally made textiles (Asmah *et al*., (2015). The population's growing interest and the increasing competition from the foreign textile market challenges local textile manufacturers to incorporate new decorative patterns and colours into their textiles. Depending on the type of textile, these aesthetic patterns are drawn, printed, woven, or dyed on fabrics (Baumann and Fletcher, 1966). This dissertation focuses on the repercussions of textile dyeing on traditional textile dyers.

Dyes are substances that add colour to the substrate to which it is applied. Dyes have been used for a very long time. The first historically recorded use of dye dates back 4000 years when indigo – a natural dye with a blue colour – was found in the wrappings of Egyptian mummies. Ntonso is a reputable textile dyeing town in Ghana and like ancient Egyptians, textile dyers in Ntonso use naturally extracted dyes. For example; the "kuntunkuni" dye is extracted from the bark of *Rhodognaphalon brevicuspehis* (locally known as kuntunkuni) (Asmah *et al*., 2015). Due to the aforementioned eclectic use and demand of textiles, the traditional textile industry resorts to using synthetic dyes in addition to the

natural dyes. However, the use of synthetic dyes comes with the dangers associated with some environmentally harmful components like heavy metals (Verma, 2008). There is not a clear-cut definition of what a heavy metal is but density is usually a good defining factor. They are, therefore, commonly defined as metals that have specific density of more than $5g/cm^3$ (Jarup, 2003). Most heavy metals are known to be vital part in normal biological functioning. Heavy metal like copper, iron, zinc, and manganese take part in the controlling of various metabolic and signalling pathways. Their chemical coordination and redox properties have given them the benefit of circumventing control mechanism such as compartmentalization, homeostasis, and transport (Jaishankar *et al*., 2014). They interact with protein site other than the ones designated for them by displacing other metals from their natural binding sites (Demirbag *et al*., 2012). The resulting heavy metal induced toxicity is a well recorded in literature and this includes oxidative stress. Oxidative stress is the condition when there is excessive production of free radicals and reactive metabolites than the body's antioxidants systems can eliminate (García-Niño and Pedraza-Chaverri, 2014). This instability leads to DNA, proteins, and lipid damages that underlie liver diseases, kidney diseases, and lipid abnormalities (Flora *et al*., 2008).

1.2 Problem Statement

Textile dyes, like many dyes, contain heavy metals (Verma, 2008) which have been implicated in oxidative stress resulting in a wide range of biological damages (Flora *et al*., 2008). At Ntonso, a town in Ashanti region where most of Ghana's traditional textile manufacture is outsourced, textile dyers are constantly exposed to such dyes. Ignorant of western industrial ethics, dyers do not have regulations to protect themselves from these

hazardous dyes. There are several studies that implicates exposure to dyes – mostly by inhalation – in skin and respiratory diseases (Ahmed *et al*., 2009). However, there is limited attention given to the effects of exposure to dyes on the liver function, kidney function, and lipid profile of traditional textile industry workers.

1.3 Main Objective

The goal is to evaluate the effect of exposure to textile dyes on the biochemical profile of workers at the Ntonso traditional textile dyeing industry.

1.4 Specific Objectives

- To assess the liver function of Ntonso traditional textile dyers
- To assess the kidney function of Ntonso traditional textile dyers
- To assess the lipid panel profile of Ntonso traditional textile dyers
- To assess the cardiovascular risk of Ntonso traditional textile dyers

1.5 Justification

The outcome of this study would shed more light on the effects prolonged exposure to heavy metals have on the liver function, kidney function, and lipid profile of traditional textile dyers.

The study would also encourage the implementation of hazard prevention and control regulations in the traditional textile dyeing community which are presently non-existent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Dyes

Dyes, or dyestuffs, are organic or inorganic substances that are used to impart colour on a substrate – in the context of textile dyeing, the substrate used is a fabric (Baumann & Fletcher, 1966). Dyes can be applied to fabric either by dispersion, absorption, or by chemical reactions (Verma, 2008). Dyeing is different from painting. In painting, colour pigments just adhere to a substrate but dyes become a part of the substrate upon which it is applied. There are several ways to classify dyes. Based on their sources, they can be classified into natural and synthetic dyes (Baumann and Fletcher, 1966).

2.2.1 Natural dyes

Natural dyes are dyes extracted from natural sources. Natural sources include plant, animal, and mineral sources. They have been used by humans for a very long time (Samanta and Agarwal, 2009). In fact, Shani – meaning the scarlet dye in Hebrew – is mentioned in the Bible as substance that gives cloths a red colour. The use of natural dyes is, however, currently dwindling. An example of a natural dye is the Tyrian purple dye. It is a reddish-purple dye secreted by some species in the family of sea snails called *Muricidae* (McGovern and Michel, 1985).

2.2.2 Synthetic dyes

Synthetic dyes are derived from organic or inorganic compounds. They can further be grouped into 14 categories with respect to their general chemical characteristics. These categories are: acid, direct, azoic, vat, basic, oxidation, reactive, sulphur, organic pigment,

developed, mordant, optical, solvent, and dispersed dyes (Benkhaya *et al*., 2017). Some dyes are synthesized in the form of a metal complex. These metal complex dyes have features that improve their dyeing characteristics. Metal complex dyes have better penetration characteristics, light-fastness, and more water soluble (Radulescu-Grad *et al*., 2015).

Table 2. 1:Metals found in different types of dye

DYE TYPE	**METALS PRESENT**
Acid dyes	Copper, lead, chrome, zinc, cobalt
Basic dyes	Copper, lead, zinc, chromium
Direct dyes	Copper, zinc, lead, chromium
Mordant dyes	Chromium
Pre-metallized	Copper, cobalt, chromium
Reactive dyes	Copper, lead, chromium

2.2 Heavy metals and oxidative stress

Heavy metals are a broad range of metals defined as having a specific activity of more than $5g/cm^3$ (Jarup, 2003). Although heavy metals have the infamous reputation of causing biological deterioration, most of them are essential in the maintenance of various physiological functions in living organisms. For example; zinc is a vital cofactor of transcription factors that regulate gene expression. While some heavy metals have no biological function and are toxic even in the slightest of amounts, most of them, like the aforementioned zinc, are only lethal beyond a certain threshold. The chemical coordination and redox characteristics of heavy metals give them the benefit to evade homeostatic control mechanisms (Jaishankar *et al*., 2014).

Fenton in 1894 described the interaction between $Fe^{2+}$and H_2O_2 – a reaction that would later be called Fenton reaction. The eponymous reaction was found to produce hydroxyl radicals. Among the various heavy metals, especially the transition metals, play a major role in the Fenton reaction and a similar reaction called the Haber-Weiss reaction all of which produce reactive oxidative species (ROS) – superoxide, hydrogen peroxide, hydroperoxyl radical, and hydroxyl radical (*Figure 2.1*). The continuous production of ROS due to the continuous exposure to heavy metals results in concomitant oxidative damage to cellular components and alter cellular functions. Reactive oxidative species are responsible for lipid peroxidation, disruption of calcium homeostasis, depletion of

sulfhydryl, and DNA damage (*Figure 2.2*) (Stohs and Bagchi, 1995). Damages to these biological components in the damages major body organs like the liver and kidney.

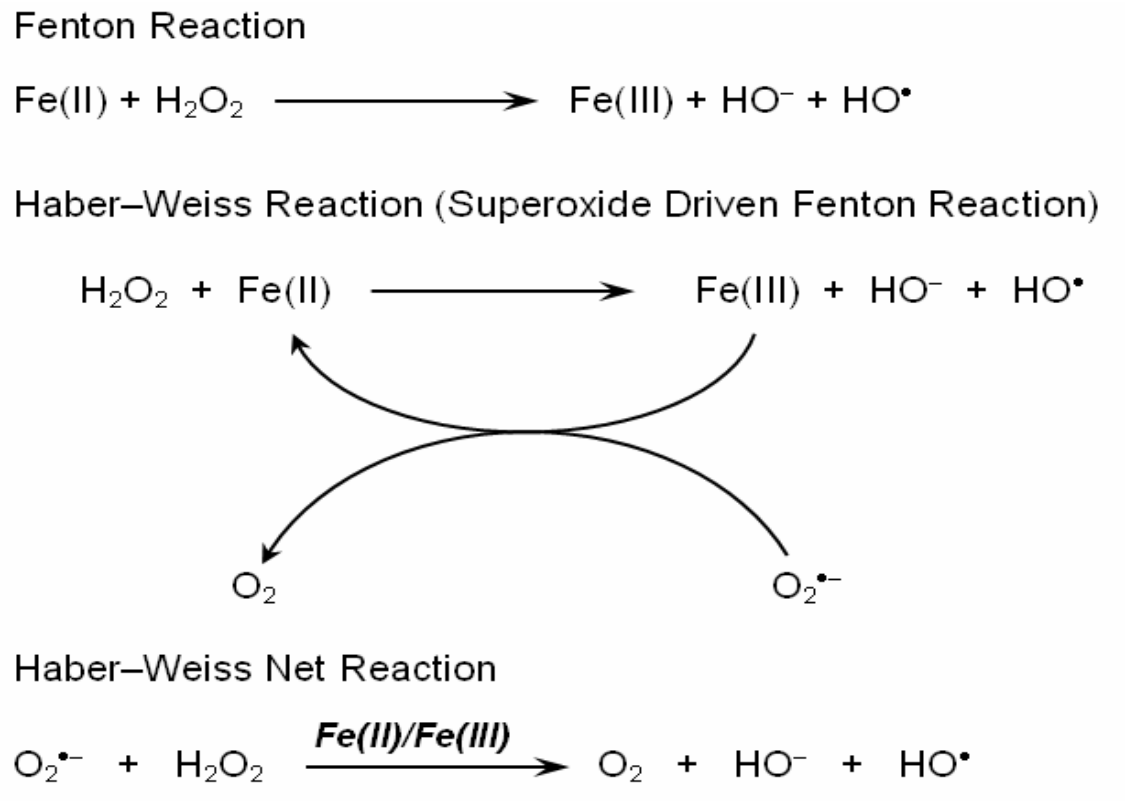


Figure 2. 1:Mechanism of ROS production through Fenton and Haber-Weiss reactions

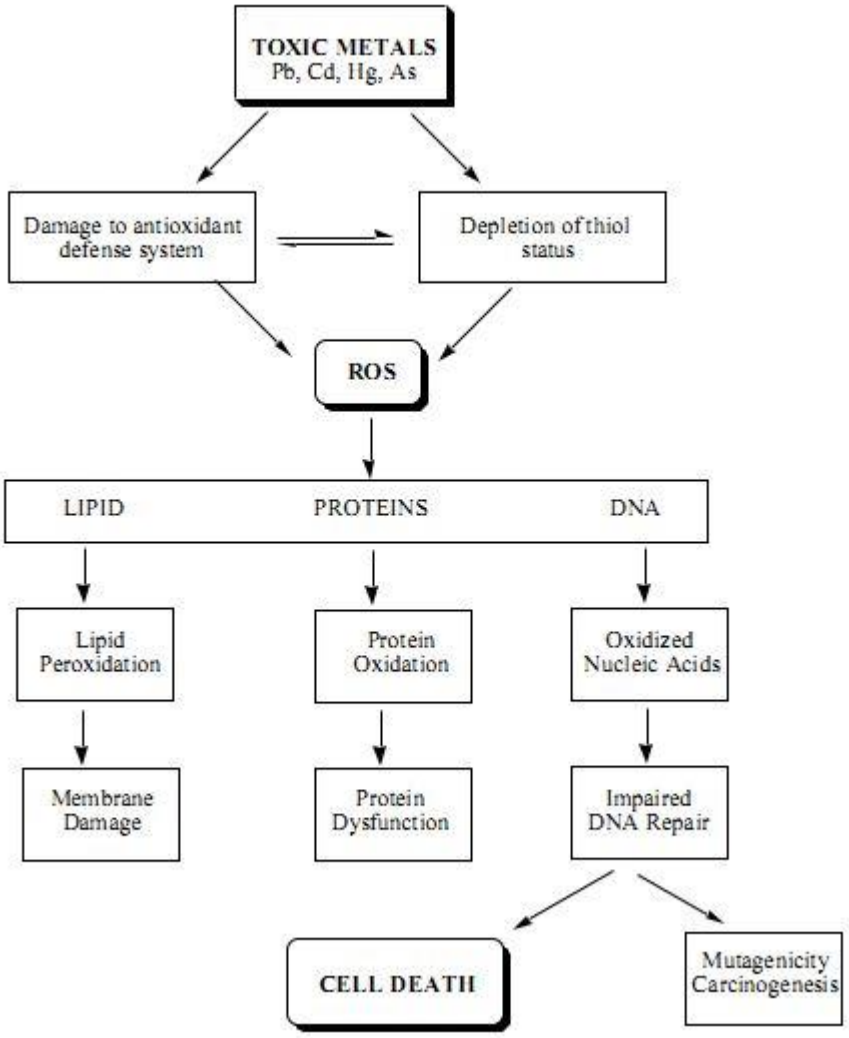


Figure 2. 2:Mechanism of metal-induced oxidative stress

Despite the acknowledgement that heavy metals have lethal long-lasting health effects, heavy metal exposure is on the rise in many parts of the world, especially in developing countries.

2.3 Effects of Heavy metal-induced Oxidative Stress on the Liver and Kidney

2.3.1 Liver

The liver is a reddish four-lobed organ positioned beneath the diaphragm in the epigastric and hypochondriac region of the abdomen. It is the largest internal organ weighing about 1.3kg. The liver is responsible for the production of bile, synthesis of blood proteins, removal of toxic substances, and the synthesis, storage, and release of glycogen (Van De Graff, 2002). The liver is a major organ susceptible to attack of reactive oxidative species (Sanchez-Valle *et al*., 2012). Parenchymal cells of the liver mainly are susceptible to oxidative stress induced injuries. Hepatic stellate cells, Kupffer cells, and endothelial cells of the liver are more exposed to reactive oxidative species (ROS) (Sakaguchi *et al*., 2011). In Kupffer cells, the cytokine, TNF-α production is induced by oxidative stress which consequently increases inflammation and apoptosis of hepatocytes (Cichoz-Lach and Michalak, 2014). Hepatic stellate cells, on the other hand, are activated. Activation of the hepatic stellate cells is characterized by proliferation, chemotaxis, and contractility. The amount of stored vitamin A in the liver also increases upon stellate cell activation (Stanciu *et al*., 2002). The liver, under normal circumstances, has a complex antioxidant system that eliminates ROS as shown in Figure 2.3 (Li *et al*., 2015). Figure 2.4 shows the various ways oxidative stress causes liver injury. When ROS levels are excessive, however, the balance in ROS production and their elimination by the antioxidant system is disturbed resulting in oxidative stress which plays a decisive role in liver diseases (Li *et al*., 2014). Oxidative stress triggers liver damage inducing irreversible alterations in proteins, DNA, and lipids (Flora *et al*., 2008). Oxidative stress also modulates normal biological pathways like gene

transcription, protein expression, cell apoptosis, and hepatic stellate cell activation (Singal, et al., 2011).

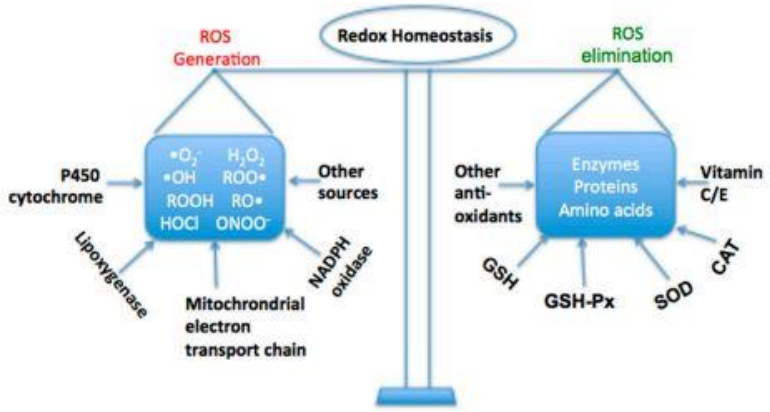


Figure 2. 3:Redox Homeostasis in the Liver

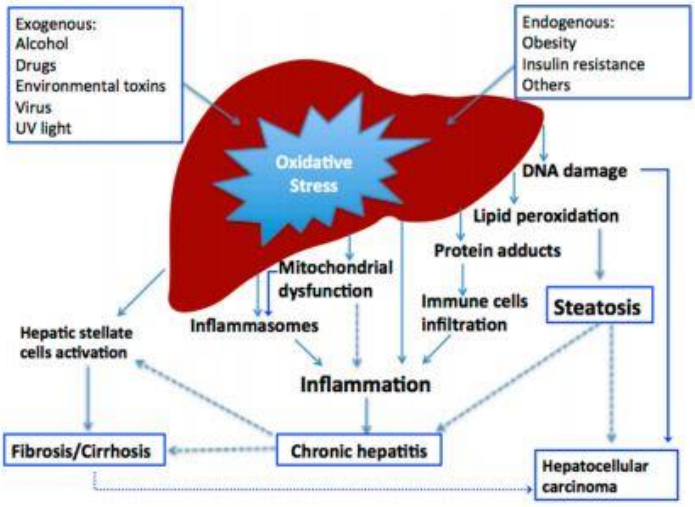


Figure 2. 4:Mechanism of oxidative stress induced by various factors in liver diseases

2.3.1.1 Liver Function Tests

The liver is a very important organ involved in several biochemical functions. Due to its wide range of functions, there is no one test to assess its function. On account of this, a battery on non-invasive tests collectively called liver function tests is employed in times when liver function needs to be evaluated (Daniel and Marshall, 1999). These tests measure the quantities of some chemicals and enzymes in the blood. The substances that are usually tested for include; bilirubin, albumin, alkaline phosphate, alanine aminotransferase, aspartate aminotransferase, and γ glutamyltransferase (Thapa and Anuj, 2007).

2.3.1.1.1 Bilirubin

Bilirubin is a yellow end product of catabolic break down of haem in vertebrates. It is what give bruises their yellow colour as well as the yellowish complexion of jaundiced individuals (Friedman *et al*., 2003). Bilirubin is readily broken down by light hence it is important to keep serum to be tested away from light (Thapa and Anuj, 2007). It was classified by the Dutch physician Abraham Hijmans van der Bergh into direct and indirect bilirubin based on their conjugation with glucuronic acid. Direct bilirubin or conjugated bilirubin is bound to glucuronic acid by the enzyme glucoronyl transferase. Indirect bilirubin, on the other hand, travels through the blood aided by albumin, unbound to glucuronic acid (Friedman *et al*., 2003).

Bilirubin is estimated with the diazo method. This method is based on the reaction between bilirubin and Ehrlich's diazo reagent to form a pink coloured compound called azobilirubin. The diazo method, however, has proven to be less accurate in detecting

bilirubin at low levels (Rosalki and Mcintyre, 1999). Fortunately, there is a contemporary more accurate method based on the alkaline methanolysis of bilirubin and an accompanying chloroform extraction of bilirubin methyl esters. The esters of bilirubin are subsequently separated by chromatography and their concentrations determined spectrophotometrically at 430nm (Daniel and Marshall, 1999).

2.3.1.1.2 Albumin

Most of serum proteins originates from the liver. The parenchymal cells of the liver are responsible for the synthesis of albumin, globulins, fibrinogen and other coagulation factors (Thapa and Anuj, 2007). Albumin is the most vital serum protein synthesized by the liver hence it is a useful marker of hepatic damage (Rosalki and Mcintyre, 1999). Albumin levels in the serum are low in patients with liver diseases like cirrhosis. Its levels in an average adult is estimated to be 3.5 – 4.5 g/dL and its levels at any point in time depicts its rate of synthesis and turnover (Mizuno *et al*., 1996).

2.3.1.1.3 Aminotransferases

Aminotransferases, also known as transaminases, are enzymes that catalyse the transfer of an amino group (NH_2) of an amino acid to a keto acid. The aminotransferases measured in a liver function test are; aspartate aminotransferases (AST) and alanine aminotransferases (ALT) (Thapa and Anuj, 2007). The former and latter transfer the amino group of aspartate and alanine respectively to alpha ketoglutaric acid. While ALT is found only in the cytosol of the liver, AST is found in the kidneys, brain, heart, skeletal muscles, and the liver (Boyde and Latner, 1961). Unlike ALT, AST is found both in the cytosol and the mitochondrion of the liver although the AST found in the cytosol and

mitochondrion are isozymes hence distinct in function (Green and Flamm, 2002). ALT and AST are markers of hepatocellular necrosis. Significant increases in their levels in the blood are recorded after hepatic tissue necrosis. On account of this, assaying ALT and AST is advocated in hepatic issues (Nalpas *et al*., 1986).

2.3.1.1.4 Alkaline Phosphatase (ALP)

Alkaline phosphatases are zinc metalloenzymes present in nearly all body tissues. They possess serine at their active centres. Alkaline phosphatase release phosphates (Rosalki and Mcintyre, 1999). In the liver, it is found in the microvilli of the bile canaliculi – a tube that collects the bile secreted by the liver – and the sinusoidal surface of the liver cells. Levels of alkaline phosphates vary with ages and gender. It is higher in males than females; higher in childhood but decreases during middle age and increase again in old age (Gordon, 1993). An increase in the levels of ALP is a biomarker of cholestasis – a disorder in which the flows of bile from the liver to the digestive tract is obstructed (Thapa and Anuj, 2007).

2.3.1.1.5 $^{\gamma}$ Glutamyltransferase

$^{\gamma}$ Glutamyltransferase is a membrane bound glycoprotein responsible for the transfer of gamma glutamyl groups to other amino acids (Daniel & Marshall, 1999). It is found in the liver in large quantities. Its levels are higher in infants up to 1 year but the levels increase again in old age. Men have higher levels than women (Rosen & Keefe, 2000).

Table 2. 2: Normal reference ranges of substances tested for in liver function tests (Thapa and Anuj, 2007)

TEST SUBSTANCE	NORMAL RANGE
Bilirubin	0 – 1 mg/dL
Albumin	3.5 – 4.5 g/dL
Aspartate aminotransferase	10 – 40 U/L
Alanine aminotransferase	10 – 55 U/L
Alkaline Phosphatase	45 – 115 U/L
γ Glutamyltransferase	0 – 30 U/L

2.3.2 Kidney

The kidney is a retroperitoneal bean-shaped reddish-brown organ (Van De Graff, 2002). It regulates blood ionic composition, blood pH, blood volume and pressure, maintain blood osmolarity, produce hormones, regulate blood glucose level, and excrete waste and foreign substances (Tortora and Derrikson, 2014). Kidney dysfunction is often associated with oxidative stress due to the increased levels of oxidative stress biomarkers in patients with kidney failure (Dounoousi, et al., 2006). There is also an established inverse correlation between different markers of oxidative markers and glomerular filtration rate (Terawaki *et al*., 2004). Also, increase in oxidative stress biomarkers has been linked to longer durations of dialysis therapy (Ferretti *et al*., 2008).

ROS targets podocytes – cells in the glomerular capsule of the kidneys that wrap and capillaries of the glomerulus (Ogura and Shimosawa, 2014). Injuries in the podocytes underlie renal conditions like proteinuria and glomerulosclerosis (Kris, 2012).

2.3.2.1 Kidney Function Tests

Kidney function tests are a collective of tests used to assess the health of the kidneys. Kidney function decreases with age hence age must be considered when interpreting renal function test values. Since renal function cannot be quantitatively determined with serum testing, the concentration of serum creatinine and blood urea nitrogen – substances excreted by the kidneys – are used to assess kidney function (Finco and Duncan, 1976).

2.3.2.1.1 Creatinine

Creatinine is a product of the breakdown of creatinine phosphate, a high energy compound in the muscle. Since creatinine is easily excreted by the kidney unchanged and can be easily be detected, it is an important marker of renal health (Taylor, 1989).

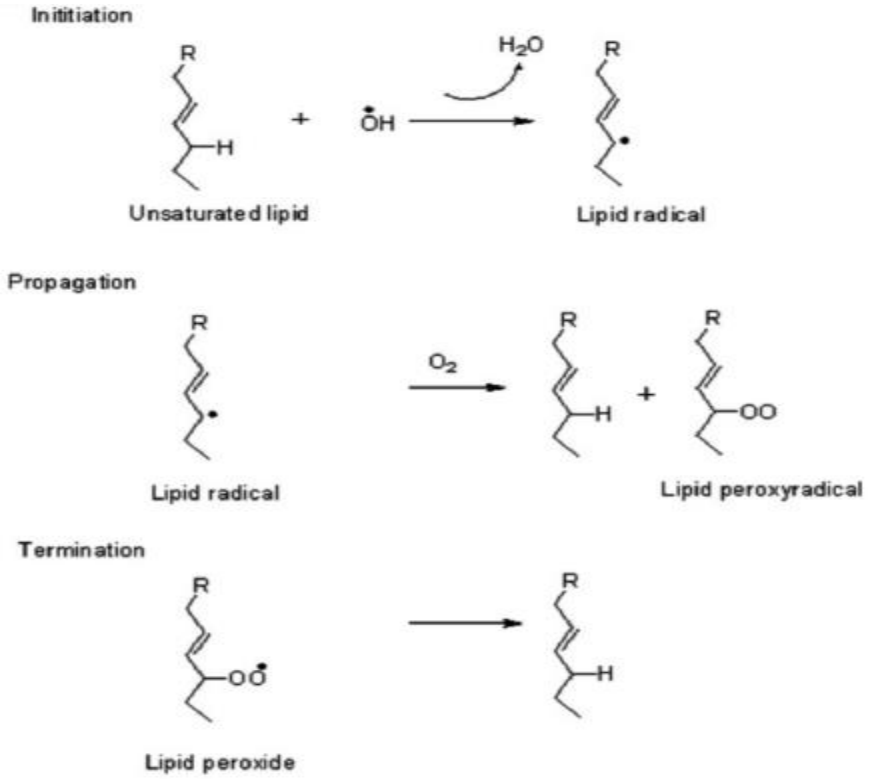
2.3.2.1.2 Blood Urea Nitrogen

Urea is produced in the urea cycle as a waste product of protein digestion. Blood urea nitrogen is an assay that measures the amount of urea in the blood. Levels of urea measured can further be an indication of kidney health (Lyman, 1986) .

2.3.3 Lipid profile

Phospholipids, triglycerides, cholesterol, and cholesterol esters are major lipids in the body. Since lipids are insoluble in water, their transport in the blood requires a carrier. Fatty acids are carried by the serum albumin. The steroids are carried by steroid carrier

proteins. The rest of the lipids and the lipid soluble vitamins (except vitamin A which is carried by retinol binding retinol) are transported in large complexes called lipoproteins. Aforementioned LDL, HDL, and VLDL are lipoproteins. Lipoproteins consist of a core of lipids surrounded by a protein coat. Lipoproteins are classified according to their weight. The more protein shell a lipoprotein has relative to its lipid content, the "heavier" it is. HDL has the highest protein to lipid ratio (Garrett and Grisham, 2010). When there is excess ROS in the body, these lipids are oxidized by three different mechanisms; free radical mediated chain oxidation, non- free radical chain oxidation, non-enzymatic oxidation, and enzymatic oxidation. The polyunsaturated fatty acid side chains cholesterol esters and triglycerides in lipoproteins are very susceptible to oxidation by free radical species, especially hydroxyl radicals. This vulnerability results from the low energy needed by free radicals and reactive oxygen species to abstract electrons between two adjacent double bonds. Hydrogen abstraction creates a lipid radical that reacts nearly instantaneously with any molecular oxygen present in the environment. The resulting lipid peroxide radical can then propagate the radical reaction by abstracting hydrogens from neighbouring lipids or can react with itself to create many secondary peroxidation products (Figure 2.5) (Moore and Roberts, 1998). There are two broad classes of secondary peroxidation products that is relevant to atherogenesis – oxidized lipids and reactive aldehydes that exert their effects by modifying proteins and other macromolecules.



The reactive aldehydes include malondialdehyde and 4-hydroxynonenal which are used as biomarkers for oxidative stress. Clinical, genetic, and pharmacological investigations have implicated elevated levels of LDL in the pathogenesis of atherosclerosis, the leading cause of death in industrialized communities. However, LDL fails to show the proposed atherogenic effects in its native form in vitro indicating it must be modified to promote

Figure 2. 5:Mechanism of lipid peroxidation

atherosclerotic pathogenesis (Witztum and Steinberg, 1991). The prevailing theory suggests that oxidative damage to LDL is a critical mechanism for rendering LDL atherogenic (Brown and Goldstein, 1992). Macrophages collect the oxidized LDL (oxLDL) initiating a wide range of bioactivities that may drive development of atherosclerotic lesions (Kita *et al*., 2001).

2.3.3.1 Lipid Profile Test

Lipids cover a wide range of biomolecules, including fats and oils as well as the steroids, with the defining feature of being insoluble in water (Nelson and Cox, 2004). Lipids are vital to the human body. Apart from the fact that fats and oils provide more energy more than any other biomolecule, lipids like steroids play major roles in the biosynthesis of some hormones. Waxes are used as protective coating in the human ear. The cell membrane of the cell is made of phospholipids (Garrett and Grisham, 2010). Like other biomolecules, there is a range that lipids have to fall in for a healthy body system. The lipid profile is a group of blood tests that serves as a screening tool for abnormalities in lipids. The results of the lipid profile can help in identification and diagnosis of certain genetic diseases and determine risks for cardiovascular diseases, pancreatitis, and other diseases. The lipid profile usually tests for high-density lipoprotein (HDL), triglycerides, and total cholesterol. With these tests, one could calculate other parameters like low-density lipoprotein cholesterol to HDL ratio. The Friedewald formula, stated below, for example is used to calculate the LDL concentration in blood (Friedwald, et al., 1972).

$$LDL = TOTAL\ CHOLESTEROL - HDL - (\frac{TRIACYLGLCERIDES}{5})$$

The Friedewald formula, however, has its shortcomings. The Friedewald formula is not valid when chylomicrons (lipoproteins with smaller density than LDLs), or when triglycerides are over 400 mg/dl.

CHAPTER THREE

3.0 METHOD

3.1 Study Design

The study was a case-control design. It involved local textile dyers who were exposed to textile dyes and individuals who were not textile dyers.

3.2 Study Site

The study was carried out at Ntonso. It is a town in the Kwabre East District of the Ashanti Region, Ghana. It has an estimated population of 7,500 (Population and housing census, 2010). Most of the natives are in the traditional textile industries. The textile manufacturers also supply the textile to other towns and regions in Ghana. Other natives find means of livelihood through farming and trade.

3.3 Eligibility Criteria

3.3.1 Inclusion Criteria

Participants of the study were healthy men and women aged 18 and above with no chronic disorders. The test participants had been working as textile dyers for at least 5 years. The control group consisted of men and women aged 18 and above who did not work with dyes of any kind.

3.3.2 Exclusion Criteria

Children, pregnant women, and the elderly were excluded from the study. Sick individuals and individuals diagnosed with chronic diseases like diabetes and cancer were also be excluded from the study.

3.4 Recruitment

The study site – Ntonso – was scouted to find the native local dyers. Participants were briefed on the objectives of the study. A signed consent was also obtained from the participants.

Natives of the study sites were invited to voluntarily participate in the study. Consent was sought from volunteers after the study had been explained to them.

3.5 Questionnaire administration

Questionnaires were administered to participants. The questionnaires sought information on gender, age, health status, and types of dyes they use.

3.6 Sample Collection

Venous blood of 3ml volume was drawn from each participant into a vacutainer containing anticoagulants (EDTA, sodium citrate, or heparin). The participants were advised to fast for 12 hours prior. The clot was subsequently removed by centrifuging the blood sample at 3000g for 10 minutes in a refrigerated centrifuge. The serum – supernatant formed – was then transferred into a 1.5 ml Eppendorf tube. The serum would be subjected to liver function tests, kidney function tests, and lipid profile tests at Tamale teaching hospital

3.7 Biochemical Analysis

The liver function, kidney function, and lipid profile parameters were carried out by a fully automated biochemistry analyser. An automated analyser measures the desired parameters with little to no human assistance. It operates based on the continuous flow

analysis. The sample is injected into a flowing solution passing through a tubing system. The sample mixes with a reagent to develop colour that is used to determine the sample concentration. Results are retrieved in a print-out form. The following parameters were measured:

- Bilirubin, albumin, ALT, AST, GGT, and ALP to test for liver function
- Creatinine, blood urea nitrogen, potassium, sodium, and chloride ion to test for kidney function
- HDL, LDL, VLDL, total cholesterol, and triglycerides to test for lipid profile.
- Cardiovascular risk was calculated

3.8 Statistical Analysis

The results of the tests were expressed as a mean ± SME. Using SPSS at a p-value of 0.05, parametric data were analysed with the student's t-test while non-parametric data were analysed with the Mann-Whitney test. Pearson's correlation was used to determine the relationship between the measured parameters and duration each dyer has been

3.9 Ethical Approval

Ethical approval was obtained at the KNUST school of medical sciences from the Committee for Human Research Publications and Ethics (CHRPE).

CHAPTER 4

4.0 RESULTS

In this study, 43 of the sample have been currently tested. Out of the 43, 26 of them are dyers while 17 are non-dyers. The sample consist of mostly females above 45 years. (table 4.1)

Table 4. 1: Demographic segmentation of sample

AGE (YEARS)	DYERS		NON-DYERS		TOTAL
	MALES	FEMALES	MALES	FEMALES	
18-30	3(7%)	3(7%)	1(2.3%)	1(2.3%)	8(18.6%)
31-45	0	5(11.6%)	1(2.3%)	6(14%)	12(27.9)
46-60	2(4.6%)	12(27.9)	0	9(20.9)	23(53.5)
TOTAL	5(11.6%)	20(46.5%)	2(4.6%)	16(37.2%)	43(100%)

4.1 Liver Function

In comparison, the means of the serum concentration of albumin, total protein, and globulin were significantly lower in the exposed sample than the respective concentrations in the non-dyers group ($p<0.05$). The serum activities of ALP, ALT, and GGT of the dyers sample were, however, not significantly different in the compared to

that of the non-dyers sample. AST activity was significantly higher in the serum of the non-dyers sample compared to the respective activity in the dyers sample.

Table 4. 2: Biochemical parameters of liver function of exposed and non-dyer groups

PARAMETER	DYERS (N=26)	NON-DYERS (N=17)	p-value	REFERENCE RANGE
Albumin (g/L)	41.16 ± 0.45	43.95 ± 0.7	0.001	35-50
Total protein (g/L)	71.55 ± 0.81	78.56 ± 1.13	0.000	60-80
Globulin (g/L)	29.38 ± 1.25	34.62 ± 1.28	0.005	25-35
ALP (U/L)	122.38 ± 7.65	108.18 ± 7.24	0.185	45-115
ALT (U/L)	6.09 ± 0.85	7.84 ± 2.13	0.479	7-55
AST (U/L)	10.89 ± 0.91	16.78 ± 2.38	0.003	8-48
Bilirubin (µmol/L)	6.10 ± 0.74	5.46 ± 0.47	0.719	3-22
GGT (U/L)	32.37± 3.90	32.01 ±2.37	0.585	9-48
ALB/GLO	1.68 ± 0.31	1.31 ± 0.06	0.253	

Table 4. 3: Correlation of duration of exposure to dyes and liver function parameters

PARAMETER	r	p-value
Albumin	-0.24	0.907
Total protein	-0.177	0.381
Globulin	0.14	0.494
ALP	0.168	0.411

ALT	-0.083	0.687
AST	-0.065	0.752
Bilirubin	0.176	0.391
GGT	0.170	0.406
ALB/GLO	-0.283	0.161

r= correlation co-efficient

4.2 Renal Function

There was a significant increase in the creatinine and chloride ion levels in serum of the dyers compared to that of the non-dyers groups. There was no significant difference in the sodium, blood urea nitrogen, and potassium between the dyers and non-dyers samples

Table 4. 4: Biochemical parameters of renal function of dyers and non-dyers groups

PARAMETER (mmol/L)	**DYERS (N=26)**	**NON-DYERS (N=17)**	**p-value**	**REFERENCE RANGE**
CRE	107.00 ± 6.17	84.72 ± 3.95	0.013	60-110
UREA	4.68 ± 0.18	4.24 ± 0.16	0.070	2.9-8.2
Na	133.79 ± 0.68	135.93 ± 0.62	0.250	135-145
K	4.25 ± 0.15	4.47 ± 0.25	0.456	0.3-4.8
Cl	94.89 ± 0.64	91.88 ± 1.09	0.025	96-106

Table 4. 5: Correlation of duration of exposure to dyes and renal function

PARAMETER	r	p-value
CRE	-0.067	0.736
UREA	0.470	0.82
Na	0.141	0.473
K	0.191	0.350
Cl	0.222	0.275

r= correlation co-efficient

4.3 Lipid Profile Panel

There was no significant difference between the total cholesterol, LDL, HDL, VLDL, and, triglycerides in the serum of the dyers and that of the non-dyers group ($p<0.05$).

Table 4. 6: Lipid Profile Panel of dyers and non-dyers groups

PARAMETER	DYERS (N=26)	NON-DYERS (N=17)	p-value	REFERENCE RANGE
T. CHOL(mmol/L)	4.66 ± 0.19	4.86 ± 0.23	0.500	>5.2
LDL (mmol/L)	2.97 ± 0.16	2.91 ± 1.99	0.837	2.6-3.3
HDL (mmol/L)	1.27 ± 0.07	1.39 ± 0.06	0.227	0.1-1.7

VLDL (mmol/L)	0.43 ± 0.02	0.4 ± 0.03	0.392	1-1.5
TRIG (mmol/L)	2.11 ± 0.09	2.00 ± 0.13	0.449	1.7-2.2

Table 4. 7: Correlation of duration of exposure to dyes and lipid profile

PARAMETER	**r**	**p-value**
T. CHOL	0.139	0.497
LDL	0.071	0.659
HDL	0.189	0.354
VLDL	-0.162	0.430
TRIG	-0.117	0.570

r= correlation co-efficient; T. CHOL = total cholesterol

4.4 Cardiovascular Risk

There was no significant difference in LDH and CR between the serum of the dyers and the non-dyers group ($p<0.05$).

Table 4. 8: Cardiovascular risk of dyers and non-dyers groups

PARAMETER	**DYERS**	**NON-DYERS**	**p-value**	**REFERENCE RANGE**

LDH (U/L)	170.27 ± 7.75	222.21 ± 21.52	0.112	122-222
CR (mg/dL)	3.88 ± 0.22	3.44 ± 0.16	0.114	1-3

Table 4. 9: Correlation of duration of exposure to dyes and cardiovascular risk

Parameter	**r**	**p-value**
LDH	-0.103	0.615
CR	-0.039	0.849

r= correlation co-efficient

CHAPTER 5

5.0 DISCUSSION

This study was conducted to determine the effect of exposure of dyes has on the liver function, renal function, renal function, lipid profile panel, and the cardiovascular risk of dyers at Ntonso. Currently, 43 samples have been tested, 60.5% (26) of whom are dyers and 39.5% (17) are a control group of non-dyers. There were 7 males (16.3%), 5 of whom were dyers and 2 non-dyers. Out of 36 females (83.7%), 21 were dyers and 15 were non-dyers.

5.1 Liver Function

The liver's function is vital. It is involved in a number of synthetic, excretory, and detoxification reactions. These vital functions were assessed in this study relative to exposure of textile dyes to dyers. Such assessment can give insight into the state of the liver with respect to exposure to textile dyes.

The normal trend observed in liver function damage is decrease in serum concentration of serum proteins (albumin, globulin) – as a biomarker of a compromised synthetic ability of the liver, increase in serum activities of ALT, AST – as markers of hepatocellular damage, and an increase in serum bilirubin concentration – as a marker of decreased excretory function (Kale *et al*., 2001).

The recorded significant decrease in serum concentration of albumin globulin, and total protein is consistent with this trend implying a marred synthetic function of liver (Table 4.2). The liver is the chief site for the synthesis of the serum proteins hence a malfunction or interruption of protein synthesis occur as a consequence of impaired liver function. This finding is consistent with that of Oluwatosin *et al.* (2007) who observed the inhibition of protein synthesis in individuals exposed to vat dyes. Albeit significant, the differences that was observed between the mean serum concentration of proteins in the dyers groups and the respective serum concentration in the non-dyers group fall within the normal reference range. This testifies to the liver's large functional capacity and its resilience. Significant decrease in serum proteins will not be evident except in severe hepatic conditions. Even in some liver conditions like cirrhosis, the protein levels can remain normal (Johnston, 1999).

The mean ALP of the dyers was higher than in the non-dyers groups. However, the difference was not significant ($p=0.185$). Higher ALP and bilirubin values are usually consistent with cholestasis liver diseases. Cholestatic liver diseases (both intra- and extrahepatic) results in the synthesis of ALP and its subsequent regurgitation into the blood (Thapa and Anuj, 2007).

In a similar study conducted by Singh (2003), a significant increase in transaminase (AST, ALP) was reported. This increase was attributed to the deterioration of membrane integrity by reactive oxygen species due to an increase in lipid peroxidation and an accompanying decrease in glutathione and ascorbic acid content resulting in a leakage of the transaminases within cells. The difference in mean concentration of transaminases in this study did not fit this pattern, however. While the difference in ALT levels was not

significant (p=0.479), there was a significant decrease in serum AST activity in the dyers group (p=0.003). This decrease in AST levels can only be explained by a mild reduction in its synthesis.

The insignificant difference in serum bilirubin levels is evident that the excretory functions of the liver is intact hence the absence of cholestasis.

5.2 Renal Function

Creatinine is produced from the breakdown of creatinine and phosphocreatine. Majority of creatinine is produced in the muscle and transported to the kidney for excretion by glomerular filtration. Blood urea nitrogen – a non-protein waste product is also transported to the kidney for excretion. Therefore, a rise in creatinine levels in the blood reflects a defective glomerular filtration system hence an impaired kidney.

In this study, there was elevated levels of creatinine and blood urea nitrogen in the dyers compared to the non-dyers group (Table 4.4). However, the elevation in blood urea nitrogen was insignificant (p=0.07). The significant increase in creatinine levels in the dyers implies a less efficient glomerular filtration in the dyers.

The electrolyte panel of a renal function test is used to detect electrolyte or acid-base imbalances. The electrolytes assessed in this study were sodium, potassium, and chloride. Although there was no significant difference in the mean serum concentration of sodium and potassium, there was a significant elevation of chloride levels in the dyers in comparison to the non-dyers group. Abnormal chloride levels alone unaccompanied by abnormal sodium levels may imply a serious underlying metabolic disorder like metabolic acidosis.

5.3 Lipid Profile Panel

There was no statistically significant difference between the mean lipid profile parameters – LDL, total cholesterol, HDL, and VLDL of the dyers and non-dyers groups (Table 4.6).

However, there was a discernible pattern in these parameters. The dyers showed a relatively high mean concentration of the atherogenic lipoproteins (LDL and VLDL) while the non-dyers groups showed relatively high levels of the antiatherogenic lipoprotein, HDL. Also, the cardiovascular risk was higher in the dyers.

5.4 Cardiovascular Risk

Lactate dehydrogenase (LDH) – the enzyme that catalyzes the conversion of lactic acid to pyruvate and its reverse reaction – is found in all living cells. Consequently, when tissues are injured, they release lactate dehydrogenase. In a study conducted by Buckner *et al.* (2016), there was evidence depicting the diagnostic value of LDH levels implicating LDH as biomarker for cardiovascular risk.

In this study, there was no significant difference between the mean LDH of the dyers and the non-dyers group ($p=0.112$, Mann-Whitney). Also there was no significant difference in the CR score of the dyers and non-dyers ($p=0.114$, Mann-Whitney). Hence it is unlikely any cardiovascular disease any participant of this study suffers is on account of exposure to textile dyes.

5.5 Correlation of duration of exposure to dyes and the various biochemical parameters

There was no significant correlation between the duration dyers have been working with dyes and the various liver (Table 4.3), renal (Table 4.5), lipid profile parameters (Table 4.7), and lactate dehydrogenase levels (Table 4.9). This is not consistent with a similar study by Oluwatosin *et al*. (2007). This can, however, be explained by the assumption that the ages of the dyers were complementary to their respective duration in the dyeing business.

CHAPTER 6

6.0 CONCLUSION

Textile dyes have been implicated to be the cause of liver dysfunction, renal dysfunction, and abnormalities in the human body's lipid profile. This study set out to test the meaning of this implication in the health of dyers in the dyeing town of Ntonso.

The study showed that the protein synthetic functions of the livers and the glomerular filtration system of the kidneys were compromised in dyers of Ntonso indicating exposure to heavy metal-containing dyes are involved with the aforementioned pathology.

RECOMMENDATIONS

1. A similar study should be run with an animal sample so as to assess histopathology of the liver and kidney.
2. Further studies should include antioxidant assays.
3. Oxidative stress biomarkers like malondialdehyde levels in the serum should be determined in future studies.

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