KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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TREATMENT OF LEAD-POISONED RATS THROUGH ORAL ADMINISTRATION OF PALM OIL EXTRACTS

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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BY

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Philosophy degree 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 of the university, except where due acknowledgement has been made in the text.

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ABSTRACT

The palm fruit (*Elaies guineensis*) is the source of palmitic-oleic rich semi solid fat and fat-soluble minor components, made up of vitamin E (tocopherols and tocotrienols), carotenoids and phytosterols. This study was in two parts. Firstly, it examined the effects of palm oil fractionation methods on recovery and concentration of carotenes, tocopherols and tocotrienols which are known to have nutritional and medicinal properties, including antioxidant activities. Secondly, the study also investigated lead (Pb) poisoning effects on blood, liver and kidney functions of albino rats and the efficacy of treatment of the poisoning with crude palm oil and its various extracts. The palm fruits used were of the *Tenera* variety, obtained from Goaso in the Brong Ahafo Region. The crude palm oil contained 530 ppm carotenes, 1,040 ppm tocols and 4.6 % FFA. The extraction methods employed in the study were adsorption chromatography, saponification and bleaching. Examination of the palm oil extracts revealed that the unsaponifiable fraction contained 19,570 ppm carotene, 39,290 ppm Vitamin E (tocols) and 2.7% free fatty acids (FFA). The adsorption chromatographic isolated fraction contained 16,310 ppm carotenes, 19,870 ppm tocols and 3.3% FFA. The bleached fraction had a reduced level of carotenes and tocols with values of 209 ppm and 640 ppm respectively. A high FFA of 13.1 % was recorded. A 40-day oral administration of 2 g/L lead acetate daily, significantly decreased red blood cell count, haemoglobin level, haematocrit value and platelet count, compared with the control. In contrast, serum urea, and creatinine were significantly increased by the lead poisoning. Serum total protein decreased significantly in the treated rats. However, ALT and AST increased significantly. Crude palm oil and palm oil extracts, except the bleached fraction (0.5 ml/kg body weight/day) administered to lead poisoned rats significantly restored the normal blood, liver and renal functions. The unsaponifiable fraction and adsorption chromatographic-isolated fractions reversed such changes to near

control levels. The efficiency of these extracts in treating lead toxicity depended on their content of carotenes and tocols, which are biological antioxidants. The suggestion that oxidative stress is the mechanism of lead toxicity means that antioxidant action of palm oil and its extracts might play a role in the treatment of lead poisoning. Although orthodox medicines are widely used, natural products such as palm oil extracts hold great potential in lead poisoning treatment.

DEDICATION

This work is dedicated to my mother, Comfort Akua Tawiah, for all her support and encouragement in my academic pursuit.

ACKNOWLEDGEMENT

My ultimate gratitude goes to the Almighty God for His mercies, kindness and love to me from the beginning to the completion of this work. I express my deepest thanks to my supervisors, Dr Peter Twumasi and Dr Kwabena Nsiah, lecturers at the Department of Biochemistry and Biotechnology, KNUST, for their continuous guidance and support throughout this study. Without their advice, criticisms and directions, this work would not have been possible.

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALA	Aminolevulinic acid
ALAD	Aminolevulinic dehydratase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BLL	Blood lead level
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CPO	Crude palm oil
CTE	Creatinine
DAG	Diacylglycerols
DOBI	Deterioration of bleachability index
FFA	Free fatty acids
GSH	Reduced glutathione
GSSG	Oxidised glutathione
HGB	Haemoglobin
HPLC	High Performance Liquid Chromatography
MAG	Monoacylglycerols
MDA	Malondialdehyde
OSHA	Occupational Safety and Health Administration
PLT	Platelet
PORIM	Palm Oil Research Institute of Malaysia
RBC	Red blood cell
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAG	Triacylglycerol

CHAPTER ONE

1.0 INTRODUCTION

1.1 Research background

The oil palm, *Elaeis guineensis*, is believed to originate from West Africa. The commercial value of this crop lies mainly in its oil. The crop is unique in that it produces two types of oil. The fleshy mesocarp produces palm oil and the kernel produces palm kernel oil. Both are edible oils but with very different chemical composition, physical properties and applications (Poku, 2002).

Palm oil may be used in a variety of ways. The composition of palm oil, together with its natural consistency, appearance and pleasant smell make it an ideal ingredient in the development and production of a variety of edible oils, in particular, margarines and fats (Pantzaris, 1997). Palm oil is also ideal when making the following products; biscuits, cakes, sauces, fat substitutes when making condensed milk, powdered milk and ice-cream (Sambanthamurthi *et al*., 2000). Palm kernel oil is used in cream made from sugar, condensed milk and doughnut fillings. Also, palm kernel meal, a by-product of palm oil, is used in the production of concentrated foods and as a supplement in animal feed. (Kritchevsky, 2000). There are also a variety of non-edible uses of palm oil and palm kernel oil, such as soaps and detergents, printing ink, glue, biodiesel, candles, rubber processing, cosmetics, and as a drilling mud for the petroleum industry (Salmiah, 2000).

In addition to oils extracted from the oil palm fruit, other parts of the tree can be used in other industries. For example, leaf fibres and empty fruit bunches are used to produce chipboard and plywood. After plantations are cleared out, the trunks of old palms is used

to make furniture. The juice of the palm tree when tapped gives an alcoholic beverage called palm wine.

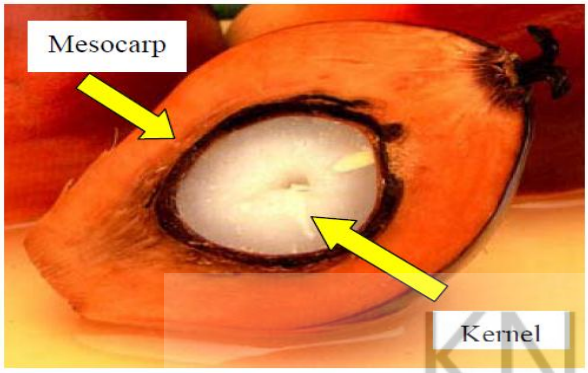


Figure 1.1 A cross-section of palm oil fruit showing the mesocarp and kernel of the fruit (Nor and Suria, 2000).

Although oil palm is native to Africa, most of the world's production comes from South-East Asia, in particular Malaysia and Indonesia. This has come about because traditional farmers in Africa did not embrace the high yielding variety early enough. Consumers complained that the palm oil produced from this variety was too fatty. This means that when the oil cools to ambient temperature it 'goes to sleep' or solidifies instead of remaining fluid and red (Poku, 2002). The oil did not have the right taste as oil or soup. Extension officers also failed to position the *Tenera* variety as high-yielding industrial purpose oil, as opposed to oil for home cooking. The negative perception on *Tenera* led to its slow adoption and the failure of Africa to maintain its lead in palm oil production (Hartley, 1988).

Indonesian crude palm oil production has increased over the years maintaining the country's position as the world's largest supplier of palm oil. Currently palm oil accounts for about 13% of the total world production of oils and fats and is projected to overtake soybean oil as the most important vegetable oil (Sundram *et al.*, 2001)

Table 1.1: Production levels of palm oil (metric tons) for 2010/11

Country	Production	Country	Production	Country	Production
1.Indonesia	25,400,000	**11.Costa Rica**	225,000	**21.Liberia**	42,000
2.Malaysia	18,700,000	**12.Guatemala**	197,000	**22.Peru**	40,000
3.Thailand	1,450,000	**13.Cameroon**	190,000	**23.Sierra Leone**	36,000
4.Colombia	885,000	**14.DR Congo**	185,000	**24.Benin**	35,000
5.Nigeria	850,000	**15.Ghana**	120,000	**25.Mexico**	27,000
6.Papua New Guinea	530,000	**16.Philippines**	70,000	**26.Dominican Republic**	22,000
7.Ecuador	500,000	**17.Venezuela**	70,000	**36.Togo**	7,000
8.Cote D'ivoire	300,000	**18.Angola**	58,000		
9.Brazil	275,000	**19.Guinea**	50,000		
10.Honduras	252,000	**20.India**	50,000		

Source: Qureshi, (2012).

Palm oil serves as the main dietary fat of many people in Ghana. It also serves as a source of livelihood for many workers who are on the plantation or involved in extraction and processing of the oil. The supply chain up to marketing provides thousands of jobs. Palm oil also serves as the main raw materials for local industries that produce soaps and margarines. It is refined to varying degrees and sold for cooking. However, the exact contribution of this important plant to the Ghanaian economy is not determined; estimation however indicates a substantial contribution.

The palm oil industry can be divided into four main sectors according to their activities, namely:

(i) Plantations – planting of the oil palm and harvesting of the fresh fruit bunches (FFB)

(ii) Palm oil mills – processing of the FFB into crude palm oil (CPO) and palm kernels

(iii) Palm kernel mills – extraction of the palm kernel oil

(iv) Palm oil and palm kernel processing – refining and fractionation, production of downstream products (Abdul, 2000).

In parts of West Africa and Brazil, unrefined palm oil containing carotenoids is a traditional food that is recognized for its medicinal aspects, including use as an ointment. However, unrefined palm oil is not widely available as a commercial product elsewhere. The main component of its carotenoids is β-carotene, which is a precursor to vitamin A. According to Choo and Ong (1991) palm oil, throughout history, has served as the primary source of dietary fat for countless people. Palm oil is regarded among many as essential in the diet for pregnant and nursing women in order to assure good health for the mother and child (Scrimshaw, 2000). Aside the nutritional properties, the healing properties of palm oil have been recognized for generations. Until the advent of modern medicine, red palm oil was the remedy of choice for nearly every illness in most parts of Africa. When someone got sick, drinking a cupful of palm oil was a remedy of choice. Even today, many people in the villages rely on this traditional age-old treatment technique (Kritchevsky, 2000).

Ancient civilizations depended greatly on local flora and fauna for their survival (Dossey, 2010). They would "experiment" with various berries, leaves, roots, animal parts or minerals to find out what effects they had. As a result, many crude drugs were observed by the local healer to have some medical purpose. Natural products have been the single most productive source of leads for the development of drugs (Sneader, 1996). In fact, most western medicines up until the 1920s were developed this way. Locally, palm oil is believed to have therapeutic effects towards different forms of poisoning, and it is widely

used in that regard (Hooper *et al*., 2001). It is therefore necessary to verify this claim with scientific data. Today, scientists are recognizing the value of red palm oil in the treatment and prevention of many diseases. There is an array of scientific presentation in this direction. Some of these studies are summarized below:

(1) Hornstra, (1988) in the Netherlands first demonstrated that palm oil has an anti-clotting effect, and is as antithrombotic as the highly unsaturated sunflower seed oil.

(2) A human study (Tomeo, 1995) showed that tocotrienols (from palm oil) supplementation can reduce re-stenosis of patients with carotid atherosclerosis. Tocopherol and its relative, tocotrienol in palm oil, inhibit human platelets from "sticking" to each other.

(3) A study conducted by Rand *et al*., (1988) showed that a palm oil diet either increases the production of prostacyclin which inhibits blood-clotting or decreases the formation of thromboxane which induces blood-clotting.

(4) A study by the Institute of Nutrition and Food Hygiene, Beijing, China compared the effects of palm oil, soybean oil, peanut oil, and animal lard (Zhang *et al*., 1997). Palm oil decreased total blood cholesterol and LDL cholesterol while increasing the level of HDL cholesterol. Soybean oil and peanut oil had no effect on blood cholesterol, but lard increased cholesterol levels. Among hypercholesterolaemic subjects, palm oil diets lowered the cholesterol levels. A similar cholesterol-lowering effect of palm oil was observed in 110 students in Malaysia (Marzuki *et al*., 1991). The study compared the effect of palm oil with that of soybean oil. Volunteers fed palm oil and soybean oil for five weeks, with a washout period in the sixth week, had comparable blood cholesterol levels.

1.2 Problem statement

Chelating agents have long been used in the pharmacological treatment of lead poisoning and other heavy metal poisoning, the management of which is still a problem, particularly in developing countries. These drugs produce significant and frequent side effects, depending on their mechanisms of action which may lead to minor complications such as headache, nausea and vomiting, rashes, anaemia, nasal congestions (Klaassen, 1990) and severe complications including renal toxicity progressing to nephrotic syndrome, have been described (Klaassen, 1990). Natural products used for treatments have proven to have minimal side effects and are also cost-effective. Thus, it is important to screen natural products to identify those that can treat conditions such as lead poisoning.

1.3 Main objective of the study

The main objective is to assess the efficacy of crude palm oil and palm oil extracts in the treatment of lead poisoning in rats.

1.4 Specific objectives of the study

Specific objectives are as follows:

1. To use three different methods (adsorption chromatography, saponification and bleaching) to fractionate palm oil.
2. To administer orally, sublethal dose of lead acetate to rats to find out the effects on the blood, kidney and the liver and treat rats using crude palm oil and palm oil extracts.

1.5 Justification

Natural products have inspired many developments in organic chemistry, leading to advances in synthetic methodologies and to the possibility of making analogues of original lead compound with improved pharmacological or pharmaceutical properties. A number of

drugs have therefore been derived from natural sources. Sunazuka, (2008) reported that, over a 100 natural product-derived compounds are currently undergoing clinical trials and at least 100 similar projects are in pre-clinical development. Most are derived from leads from plants and microbial sources. The drugs based on natural products are predominantly being studied for use in cancer or as anti-infectives, but many other therapeutic areas are being developed. It is anticipated that once the agent in palm oil with expected pharmacological or biological activity is identified, it could be used for pharmaceutical drug discovery and drug design.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Palm oil (*Elaeis guinensis*)

In 1434, a Portugese sailor, Gil Eannes first reported about oil palms (*Elaeis guineensis)* (Bockish, 1998). Today, they flourish mainly in the western part of Africa, Indonesia, and Malaysia and most recently in Brazil and Colombia. Oil palm tree (Figure 2.1), grows up to 20 metres in height and thrives best at temperature of 24-27^{o}C (Choo *et al*., 1997). The individual fruit, ranging from 6 to 20g, are made up of an outer skin (the exocarp), a pulp (mesocarp) containing the palm oil in a fibrous matrix, a central nut consisting of a shell (endocarp); and the kernel, which itself contains an oil, though quite different from palm oil and resembling coconut oil. Oil palm tree requires a humid climate. Cultivated oil palm carries fruit from their fourth year onward and could be harvested for 40-50 years (Bockish, 1998).

The wild oil palm groves of Central and West Africa consists mainly of a thick-shelled variety, with a thin mesocarp, called *Dura* (Maclellan, 1983). Breeding work, particularly crosses between *Dura* and a shell-less variety (*Pisifera*), have led to the development of a hybrid variety called *Tenera* which has much thicker mesocarp and thinner shell (Maclellan, 1983). All breeding and planting programmes now use this latter type (*Tenera*), the fruits of which have a much higher content of palm oil than the native *Dura*.

The extensive oil palm plantations in many countries in the tropics have been motivated by its high yield (Hartley, 1988). The oil palm gives the highest yield of oil per unit area compared to any other crop and produces two distinct oils - palm oil and palm kernel oil - both of which are important in world trade (Vickery and Vickery, 1979)

Modern high-yielding varieties, developed by breeding programmes, under ideal climatic conditions and good farm management, have been found to produce in excess of 20 tonnes of bunches/ha/yr, with palm oil in bunch content of 25 percent (Maclellan, 1983). This is equivalent to a yield of 5 tonnes oil/ha/yr (excluding the palm kernel oil), which far outstrips any other source of edible oil.

Figure 2.1 Palm oil tree (left) and palm oil fruit (right).

Figure 2.2 shows three varieties of palm oil, where clear differences in their fruit morphology are revealed. The *Dura* fruit form has a thick shell or endocarp surrounding the kernel whereas *Psifera* has no shell. The hybrid between the two, the *Tenera* fruit form has a shell of intermediate thickness and a surrounding of fibre strands in the mesocarp (Noor, 1995).

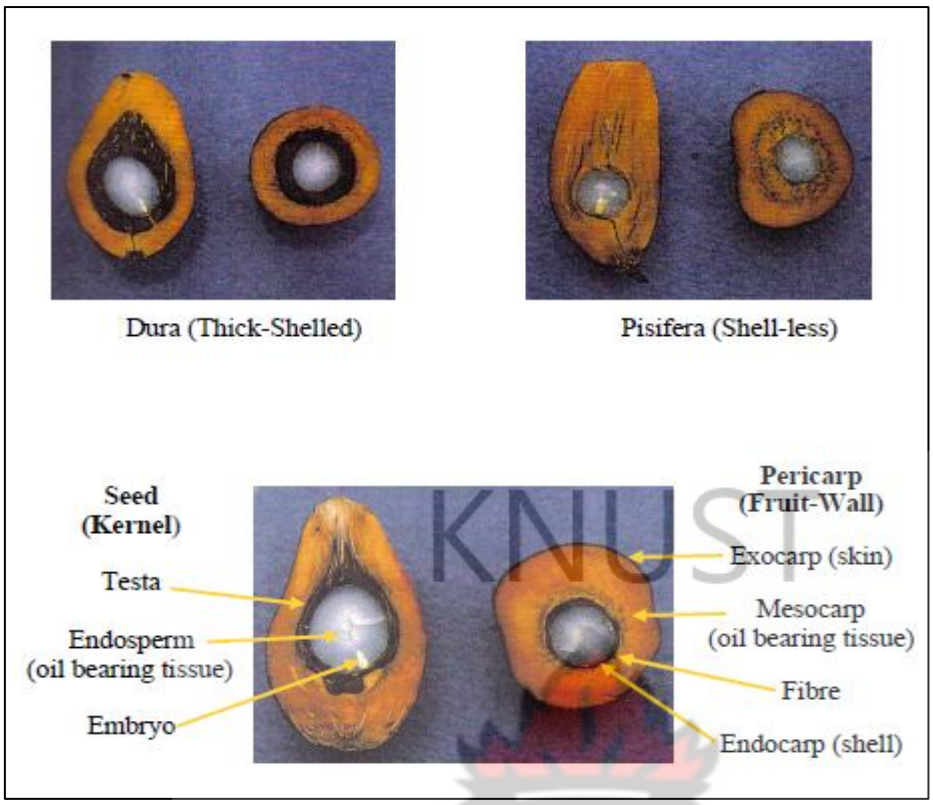


Figure 2.2 Fruits from *Dura, Pisifera* and *Tenera* varieties of palm tree (Noor, 1995).

2.1.1 Composition of Palm oil fruit

The mesorcarp accounts for about 60% of the total composition of palm oil fruit and crude palm oil is derived from this part (Bockish, 1998). Figure 2.3 below shows overall composition of palm oil.

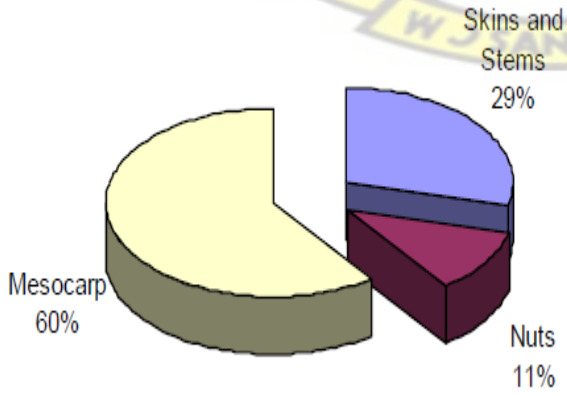


Figure 2.3 Composition of palm oil fruit (Bockish, 1998)

Figure 2.4 shows the composition of mesocarp, where the oil accounts for 39% of the overall composition. Crude palm oil (CPO) is obtained from the mesocarp part of palm oil fruit after undergoing several processes such as sterilization, stripping, extraction and purification.

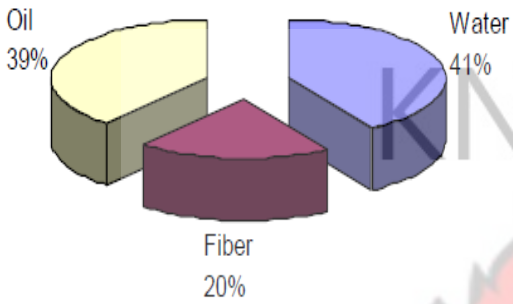


Figure 2.4 Composition of palm oil mesocarp (Bockish, 1998)

2.2 Crude palm oil

Crude palm oil (CPO) is the oil obtained from the mesocarp part of palm oil fruit. Shown in Figure 2.5 are the processes carried out on fresh fruit bunches (FFB) to produce CPO. The CPO produced, is further processed to yield either red or bleached cooking oil or detergents.

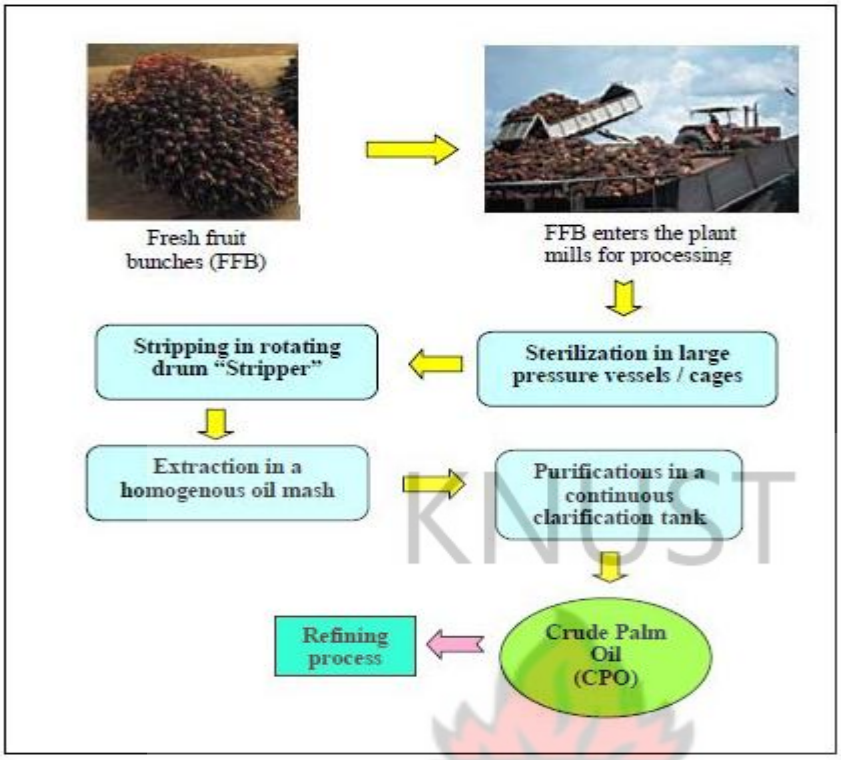


Figure 2.5 Flowchart for crude palm oil (CPO) production (Salmiah, 2000)

2.2.1 Composition and constituents of crude palm oil

Higuchi (1983) stated that crude vegetables oil commonly consists of desirable triglycerides, unsaponifiable matter, together with small amount of impurities. Most of the impurities contribute undesirable effects to the oil, for instance colour, flavour, odour, instability and foaming characteristics. These impurities should be removed by a purification step, in order to produce good quality of refined oil with minimal possible oil loss or damage to the oil and desirable contents, such as tocopherols and carotenes. The compositions of crude palm oil can be classified as a mixture of five main chemical groups (Abdul, 2000), shown in Table 2.1.

Table 2.1: General compositions of crude palm oil

Group	Chemicals present
Oil	• Triglyceride, diglyceride, monoglycride • Phospholipids, glycolipid and lipoprotein • Free fatty acids
Oxidised products	• Peroxides, aldehydes, ketones, furfurals (from sugars)
Non-oil (but oil-solubles)	• Carotene • Tocopherols • Squalene • Sterols
Impurities	• Metal particles • Metal ions • Metal complexes
Water- soluble	• Water (moisture) • Glycerol • Chlorophyll pigments • Phenols • Sugars (soluble carbohydrates)

(Source: Abdul, 2000)

2.2.2 Quality of crude palm oil

In general, quality of crude palm oil is dependent on the contents of free fatty acids, moisture, heavy metals, deterioration of bleachability index (DOBI), oxidized products and minor constituents such as phosphatides, carotenes and tocopherols (Formo *et al*., 1979).

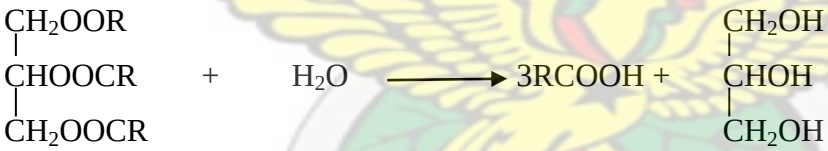
2.2.2.1 Free Fatty Acids (FFA)

The FFA content of crude palm oil is used as an index of oil quality by commercial oil refiners. Free fatty acids are formed when the bound fatty acids in triglyceride, diglyceride

and monoglyceride molecules are split either by chemical or enzymatic hydrolysis. According to Formo *et al*. (1979), the high moisture content of palm oil fruit enhances enzyme activity. Palm oil fruits are therefore susceptible to deterioration. Their lipolytic enzymes are so active that even under favourable conditions palm oil is seldomly produced with free fatty acid content less than 2%. Thus, crude palm oil with low FFA is an indication that the oil has been processed from fresh, unbruised fruits and carefully handled during production, storage and transportation. High FFA content must be avoided, as it will result in higher refining losses and possible bleachability problems, during refining (Formo *et al*., 1979).

2.2.2.2 Moisture

The miscibility of oils and water, under certain conditions will hydrolyze the triglycerides of oils to free fatty acids and glycerol (Formo *et al*., 1979).



Under practical conditions, the rate of hydrolyses of triglyceride into FFA is negligible at 0.1 % moisture content. Therefore as a quality control measure, crude palm oil should be stored with moisture content of 0.1% or below to prevent an increase in FFA and subsequent quality deterioration partly due to oxidation (Goh, 1991).

2.2.2.3 Heavy metals

Heavy metal content of oils such as iron (Fe) and copper (Cu) usually result from corrosion and/or mechanical wear of mills at refineries. These metals are prooxidant and thus, detrimental to the oil quality. They may be present as complexes surrounded by proteins, phospholipids and lipids or non-lipid carriers (Schwartz *et al*., 2000). Other sources of heavy metals in crude palm oil may include soil and fertilizers. They could also

result from storage tanks, transport tankers and pipelines. The use of stainless steel for certain mill machineries which are subjected to constant wear and tear helps to reduce metal contamination (Siew, 2000). Heavy metals catalyse decompositions of hydroperoxides to free radicals (Bechara, 1996). Iron increases the rate of peroxide formation, while copper accelerates the hydroperoxides destruction rate, thereby increasing the production of secondary oxidation products (Sambanthamurthi *et al*., 2000). Other metals reported in palm oil are manganese, cadmium and lead (Siew, 2000).

2.2.2.4 Deterioration of bleachability index (DOBI)

Deterioration of bleachability index is a parameter indicating the oxidative status of crude palm oil, its shelf life and refinability (Siew, 2000). It is affected by quality of oil palm fruits, ripeness of fruits, and post-harvest storage period of fruits and storage of palm oil (Siew, 2000). This index is also an indication of the bleachability of the crude palm oil, based on the amount of carotenes present in the crude oil and the amount of secondary oxidation products. A good easily bleached crude palm oil will have a DOBI of 4, while average quality crude will exhibit a DOBI of 2.5 to 3. Table 2.2 shows the refinability of CPO, according to DOBI values.

Table 2.2 Refinability of CPO according to DOBI values

DOBI	Grade
<1.7	Poor
1.8-2.3	Fair
2.4-2.9	Good
3.0-3.2	Very good
>3.3	Excellent

Source: (Siew, 1994)

2.2.2.5 Oxidization of palm oil

The formation of oxidized products will result in poor bleachability during refining and consequently leading to refined oils' poor keeping qualities (Hodgson and Watts, 2003).

Apart from this, any difference in oil is normally caused by the reaction of oxygen with double bonds in unsaturated fatty acids. Therefore, it is vital to prevent or minimize deterioration in crude oils by reducing aeration of the oil and gross contamination from iron or copper (Nor and Suria, 2000). The oxidation conditions can also be prevented by avoiding the oil from exposure to light, elevated temperature and the presence of pro-oxidants.

2.2.2.6 Minor constituents

Crude palm oil possesses 1% minor components; amongst them are carotenoids, tocopherols, tocotrienols and sterols (Choo *et al*., 1997). Although present in small quantities in the palm oil, these minor constituents, to a certain extent, affect the bleachability, stability and nutritional value of the palm oil (Kim *et al*., 2008). Phospholipids, which are complex esters that contain phosphorus, nitrogen bases, sugars and long-chain fatty acids, are the main culprits that need to be removed during degumming. This is achieved by coagulating them with phosphoric acid (Basiron and Sundaram, 1999).

2.3 Chemistry of palm oil

Like all oils, triglycerides (TGs) are the major constituents of palm oil, forming about 95% of palm oil (Choo *et al*., 1997). TG consists of glycerol molecule esterified with three fatty acids. During oil extraction from the mesocarp, the hydrophobic TGs attract other fat or oil soluble cellular components. These components form the minor components of palm oil such as phosphatides, sterols, pigments, tocopherols, tocotrienols and trace metals (Goh and Toh, 1988). Other components in palm oil are the metabolites in the biosynthesis of TGs and products from lipolytic activity. These include the monoacylglycerols (MAGs), diacylglycerols (DAGs) and free fatty acids (FFAs). Triacylglycerol or triglyceride

structures are shown below. Their differences lie in the types of fatty acids (RCOOH) attached to the glycerol backbone in positions 1, 2, and 3.

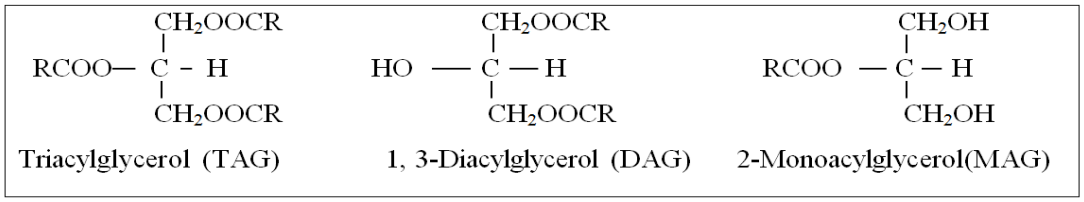


Figure 2.6 Some acylglycerides of palm oil.

Fatty acids are any of a class of aliphatic acids, such as palmitic (16:0), stearic (18:0) and oleic (18:1) in animal and vegetable fats and oils. The major fatty acids in palm oil are myristic (14:0), palmitic, stearic, oleic and linoleic (18:2) (Siew, 2000). A typical fatty acid composition of palm oil is presented in Tables 2.2 and 2.3. Palm oil has saturated and unsaturated fatty acids in approximately equal amounts. Most of the fatty acids are present as TAGs. The different positions and types of fatty acids esterified to the glycerol molecule produce a number of different TAGs. There are 7 to 10 % of saturated TAGs, predominantly tripalmitin. The fully unsaturated TAGs constitute 6 to 12 % (Kifli, 1981). The Sn-2 position has specificity for unsaturated fatty acids. Therefore, more than 85 % of the unsaturated fatty acids are located in the Sn-2 position of the glycerol molecule. The triacylglycerols in palm oil partially define most of the physical characteristics of the palm oil, such as melting point and crystallisation behaviour.

Table 2.3 Fatty acid composition of palm oil

Types of fatty acids	**Percentage composition of fatty acids**
Saturated acids	Palmitic (C16), 44.3 %, stearic (C18), 4.6 % and myristic (C14) 1.0 %
Mono unsaturated acids	Oleic (C18), 38.6 %
Poly unsaturated acids	Linolenic (C18), 10.6 %
Other	0.9 %

Source: David, (1999)

Table 2.4 Fatty acid composition of palm kernel oil

Saturated acids	Lauric (C12), 48.2 %, Myristic (C14), 16.2 %, Palmitic (C16), 8.4 %, Capric (C10) 3.3 %, Caprylic(C8) 3.3 %, Stearic (C18) 2.3 %
Monounsaturated acids	Oleic (C18) 15.3 %
Polyunsaturated acids	Linoleic (C18) 2.1 %
Other/Unknown	0.9 %

Source: David, (1999)

2.4 Phytonutrients of crude palm oil

Phytonutrients are plant-derived bioactive compounds that have a beneficial effect on the body (Steven, 2007). The phytonutrients of crude palm oil can be divided into two groups. The first group consists of fatty acid derivatives, such as partial glycerides (MAGs and DAGs), phosphatides, esters and sterols. The second group includes classes of compounds not related chemically to fatty acids. These are the hydrocarbons, aliphatic alcohols, free sterols, tocopherols, pigments and trace metals (Sambanthamurthi *et al*., 2000).

Most of the minor components found in the unsaponifiable fraction of palm oil are sterols, higher aliphatic alcohols, pigments and hydrocarbons. The other minor components, such as partial glycerides and phosphatides, are saponifiable by alkaline hydrolysis. The partial glycerides do not occur naturally in significant amounts, except in palm oil from damaged fruits (Chong and Jaais, 2001). Such oils would have undergone partial hydrolysis, resulting in the production of free fatty acids, water and the partial glycerides. Different isomers of MAGs and DAGs are found in palm oil. MAGs are more stable than their isomers. As in most vegetable oils, the DAGs (or 1, 3 DAGs) are the predominant DAGs in palm oil. Several minor nonglyceride compounds are found in palm oil. Table 2.5 gives the levels of these minor components in the oil. The nonglyceride fraction of palm oil consists of sterols, triterpene alcohols, tocopherols, phospholipids, chlorophylls,

carotenoids and volatile flavour components, such as aldehydes and ketones. Sterols are tetracyclic compounds with generally 27, 28 or 29 carbon atoms. They make up a sizeable portion of the unsaponifiable matter in oil. The total content of sterols in palm oil is about 0.03 % (Solomons, 2002).

Tocopherols and tocotrienols are fat-soluble vitamin E isomers and the major antioxidants of vegetable oils (Tan and Saleh, 1992). An essential nutrient for the body, vitamin E is made up of four types of tocopherols (alpha, beta, gamma, and delta) and four tocotrienols (alpha, beta, gamma, delta) (Brigelius- Flohé and Traber, 1999). Chemically, vitamin E is an antioxidant (Cerecetto and López, 2007). One model for the function of vitamin E in the body is that it protects cell membranes, active enzyme sites, and DNA from free radical damage (Ng *et al*., 2004). Tocotrienols are natural compounds found in select vegetable oils, wheat germ, barley, saw palmetto, and certain types of nuts and grains. This variant of vitamin E only occurs at very low levels in nature (Sen *et al*., 2007).

The pigmentation of palm fruits is related to their stage of maturity. Two classes of natural pigments occurring in crude palm oil are the carotenoids and the chlorophylls. Palm oil from young fruits contains more chlorophyll and less carotenoids than oil from mature or ripened fruits (Ng and Tan, 1999). The pigments in palm oil are involved in the mechanisms of autoxidation, photooxidation and antioxidation within the plant (Whittle *et al*., 1999). Carotenoids are highly unsaturated tetraterpenes biosynthesized from eight isoprene units. Their more favoured state is the all-*trans*. Carotenoids are divided into two main classes: carotenes, which are strictly polyene hydrocarbons, and xanthophylls, which contain oxygen. The oxygen in xanthophylls may be in the form of hydroxy (e.g. zeaxanthin and lutein), keto, epoxy or carboxyl groups (Gunstone and Norris, 1983). The simplest carotene is lycopene. Crude palm oil has a rich orange-red colour due its high content of carotene (700 – 800 ppm) (Sugawara *et al*., 1991). The major carotenoids in

palm oil are alpha and beta-carotene, which account for 90% of the total carotenoids (Bonnie and Choo, 2001). There are about 11 different carotenoids in crude palm oil. The major types and composition of carotenoids extracted from oils of different palm species were studied by Yap *et al*. (1997). They found 13 different types of carotenoids with the major isomer, carotene, accounting for the highest percentage of the total carotenoids. No significant difference in the types of carotenoids was found in the oils of *E. oleifera* and *E. guineensis*, and their hybrids and backcrosses to *E. guineensis*. The study also showed that *E. guineensis* contained a higher level of lycopene compared to *E. oleifera* and its hybrids with *E. guineensis.*

The fatty acid composition of palm oil (1:1 saturated to unsaturated fatty acids) is such that the oil is semi-solid at normal room temperature. This property and the oil melting range permit its use as a major component in margarine and shortening without hydrogenation (Nor and Suria, 2000). Thus, for most practical purposes, palm oil does not need hydrogenation. Nonetheless, the use of this process has been explored to maximise the utilisation of palm oil and its fractions in edible food products. Palm stearin is an excellent and economic starting material for certain food and non-food applications where fully hydrogenated fats are required (Nor and Suria, 2000). Cake shortenings made from palm oil products such as hydrogenated or interesterified palm oil, in combination with butterfat, produce cakes with better baking properties than cakes made with 100% butterfat (Sundram, 1997). Whereas the butterfat gave the cakes the desired buttery flavour, the palm products enhanced the baking performance (Yap *et al*., 1997).

Apart from palm oil and the fat-soluble minor constituents described above, the palm fruit contains large amounts of water-soluble phenolic compounds and flavonoids. These are basically extracted into the steriliser condensate and the palm oil milling effluent during the milling process (Goh, 1991). The sterilisation of palm fruits inactivates

polyphenoloxidases and retains the phenolics and flavonoids. These are water-soluble and demonstrated to have potent antioxidant properties (Sundram *et al*., 2001). A recent technology developed at the Malaysian Palm Oil Board uses an ecologically friendly process in which the constituent water added during the sterilisation of palm fruits is used to retain the compounds of interest for passage through a series of innovative separation techniques that isolate specific compounds of interest on the basis of their molecular weights. The final aqueous product can be further processed depending upon its intended applications.

Table 2.5 Minor components in crude palm oil

Minor Components	**Concentration (ppm)**
Carotenoids	500-700
Tocols	600-1000
Sterols	360-620
Phospholipids	5-130
Glycolipids	1000-3000
Triterpene alcohols	600-700
Methyl sterols	40-80
Squalene	200-500
Sesquiterpene and Diterpene	30
Aliphatic alcohols	100-200
Aliphatic hydrocarbons	50
Methyl esters	50

Source: Choo (1989)

2.5 Fractionation of crude palm oil

Crude palm oil is the world's richest source of natural plant carotenoids in terms of retinol (pro-vitamin A) equivalent (Choo, 1989). It contains about 15 to 300 times more retinol equivalent than carrots, green leafy vegetables, and tomatoes, all of which are considered to have significant pro-vitamin A activity (Tan, 1987). Methods to recover carotenoid from palm oil include saponification (Eckey, 1997), adsorption (Khoo, 1997), selective solvent extraction (Heidlas *et al*., 1998), transesterification, followed by distillation, and others

(Ooi *et al*., 1994). Only transesterification, followed by distillation has been further developed into a commercial-scale process.

2.5.1 Saponification

Triglycerides in crude palm oil can be saponified (hydrolyzed) in basic solution to give soap and glycerol. These products are water-soluble and by using a non-polar solvent, the unsaponifiables of palm oil can be recovered.

2.5.2 Adsorption

Carotenoids and tocols extraction by adsorption without a chemical conversion of palm oil has been reported by Ong and Boey (1980) and Mamuro *et al*., (1986). This method, however, can maintain an edible-oil quality and did not require converting CPO to methyl esters. A process of separating carotene from CPO by adsorption chromatography with a synthetic polymer adsorbent was developed by Goh and Toh (1988). However, this chromatographic process is still not commercially proven and may slow down the refining process if the process is to be introduced in the existing palm oil refining. Therefore, researchers have been trying to modify the process of carotene extraction from CPO by adsorption using synthetic adsorbent that could speed up carotene extraction process and at the same time maintain the edible oil quality of CPO.

2.5.3 Transesterification and molecular distillation

The solubility of a compound in a solvent depends on its molecular weight, polarity and solvent strength (Puah *et al*., 2007). Triglycerides in CPO have large molecular weights of 807-885 (Vicente *et al*., 2004). As a result, triglyceride has lower solubility in hexane. The carotenoids are transported together with triglycerides. The fatty acids methyl ester

(FAME) is more soluble than triglycerides. Transesterification process will convert the large molecules of triglycerides into smaller molecule of FAME, making it much easier to be separated (Puah *et al*., 2008). A process involving neutralization and transesterification of palm oil, followed by molecular distillation of the esters are used to recover valuable minor components from crude palm oil. Molecular distillation process represents a special type of vaporization at low pressures and low temperatures. This method makes it possible to separate and purify molecules which have high molecular weight and thermally sensitive, such as the vitamins. However, transestrification process converts the CPO irreversibly to methyl ester which is not edible and furthermore, changes the quality of oil (Puah *et al*., 2005). Further purification will be needed to produce crystalline carotenoids and tocols, safe for consumption.

2.5.4 Solvent extraction

Solvent extraction is a mass transfer operation in which palm oil is contacted with an immiscible or nearly immiscible solvent that exhibits preferential affinity or selectivity towards carotenoids and tocols in palm oil. Solvent extraction is one of the major processes of commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feedstock (Nagendran, 2000). Some extraction techniques involve partition between two immiscible liquids; others involve either continuous extractions or batch extractions. Because of environmental concerns, suitable solvents which exert less impact on the environment are more preferred nowadays. The solvent can be a vapour, supercritical fluid, or liquid and the sample can be a gas, liquid or solid.

Solvent extraction, which operates at normal condition, commonly used hexane as a solvent to extract carotenoids and vitamin E from crude palm oil (Latip *et al*., 2000).

However, hexane possess potential fire, health and environmental hazards (Choo *et al*., 1992). Short-chain alcohols, especially ethanol and isopropanol, have been proposed as alternative extraction solvents due to their greater safety and reduced probability of regulation (Ping and Gwendoline, 2006). Alcohols tend to extract more non-glyceride materials than hexane, due to their greater polarity. Typically, alcohol-extracted oils contain more phosphatides and unsaponifiable compounds (Lusas *et al*., 1991). Extraction is a process to separate a mixture into fractions or its constituents by suitable solvents. Organic solvents are partly toxic, inflammable and may cause explosion. Supercritical fluid has also been used for extraction since it can offer a highly selective process. High pressure technology is being used in Supercritical Fluid Extraction (SFE). Application of supercritical fluid extraction by using supercritical carbon dioxide (SC-CO_2) as solvent has advantages over other solvent extraction methods as carbon dioxide (CO_2) is non-hazardous and non-flammable (Watkins *et al*., 1994; Johnson and Lusas, 1983) when compared to highly flammable petroleum-based solvents, such as hexane or acetone. CO_2 is inert, non-toxic, non-flammable and recyclable, besides leaving no residue in the products.

2.5.5 Bleaching

The term bleaching refers to the treatment that is given to remove colour-producing substances and to further purify the fat or oil. The usual method of bleaching is by adsorption of the colour producing substances on an adsorbent material. There are a lot of adsorbent materials being used in vegetable oil industry; for example, acid-activated bleaching earth, natural bleaching earth, activated carbon and synthetic silicates. Acid-activated bleaching earth (fuller's earth) or clay, sometimes called bentonite, is the adsorbent material that has been used most extensively. This substance consists primarily

of hydrated aluminium silicate. Usually, bleaching earth does not remove all the colour-producing materials, much of which are actually removed by thermal destruction during the deodorization process. Activated carbon is also used as a bleaching adsorbent to a limited extent (David, 1999).

2.5.5.1 Types of bleaching methods

Three types of bleaching methods can be used in edible oil industry (Gunstone and Norris, 1983), namely:

2.5.5.1.1 Heat bleaching

Some pigments, such as the carotenes become colorless if heated sufficiently. However this leaves the pigment molecules in the oil and may have adverse effect on oil quality. According to Gunstone and Norris, (1983), if this oil comes into contact with air, coloured degradation products such as chroman-5,6-quinones from γ- tocopherol present, may be formed. These are very difficult to remove.

2.5.5.1.2 Chemical oxidation

Some pigments, for example carotenoids are made colourless or less coloured by oxidation. But such oxidation invariably affects the glycerides and destroys natural antioxidants. Consequently, it is never used for edible oil but restricted to oils for technical purposes, such as soap-making (Borner and Schneider, 1999).

2.5.5.1.3 Adsorption bleaching

Adsorption is the common method usually used for bleaching edible oil by using bleaching agents. Examples of bleaching agents are bleaching earths, activated carbon and silica gel. Bleaching agents normally posses a large surface that has a more or less specific affinity for pigment-type molecules, thus removing them from oil without damaging the oil itself (Rossi *et al*., 2003).

2.6 Toxicity of lead

2.6.1 Exposure to lead

Although lead occurs naturally, human activities have increased levels in the biosphere more than a thousand-fold in the past 300 years (Schwartz *et al*., 2000). Much of this occurred in the second half of the twentieth century due to use of lead compounds in petrol, a practice which has now ceased. Lead from a range of sources reaches humans via air, dusts, food and water. Once in the body, it circulates in the blood, while most is excreted, some can remain in the tissues, organs and bones for many years (Navas-Acien *et al*., 2007). Today, the most common source of lead exposure in communities not industrially exposed is restoration of homes, boats, cars and furniture coated with lead-based paints. Smokers and their children have higher blood lead levels than non-smokers and their children, respectively (Donovan, 1996).

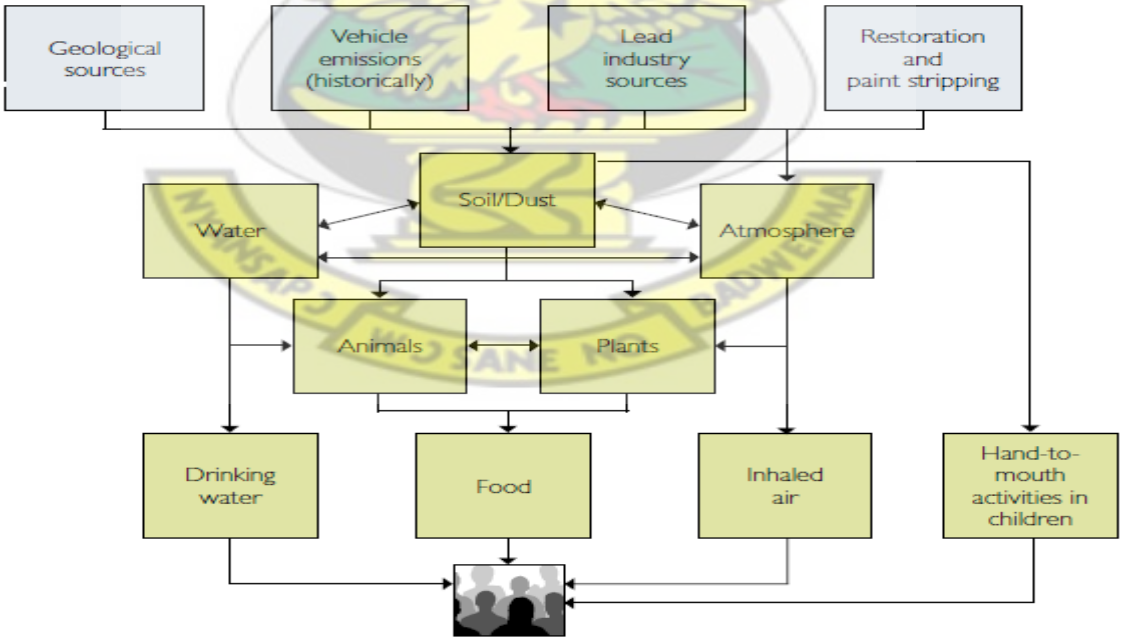


Figure 2.7 Principal Pathways of lead from the environment to humans

Source: Navas-Acien *et al*. (2007).

2.6.2 Absorption of lead

Lead is absorbed primarily through the respiratory and gastrointestinal systems, with the former being the more important route of entry in occupational exposures. Cutaneous absorption of inorganic lead is negligible. However, organic lead compounds, because of their lipid solubility, are readily absorbed through intact skin (Fischbein, 1992).

Respiratory lead absorption is primarily dependent on particle size; solubility, respiratory volume and physiological variation are less important factors. The percentage of inhaled lead reaching the bloodstream is estimated to be 30 to 40 percent (Saryan and Zenz, 1994).

Gastrointestinal absorption of lead is lower in adults than in children, with an estimated 10 to 15 percent of lead in an adult's diet absorbed gastrointestinally (Flora *et al*., 1989). The degree of lead absorption is increased considerably with fasting or in persons whose diet is deficient in calcium, iron, phosphorus or zinc. (Fischbein, 1992).

2.6.3 Distribution of lead

After lead is absorbed into the bloodstream, through either ingestion or inhalation, most of it is carried, bound, to erythrocytes. The freely diffusible plasma fraction is distributed extensively throughout tissues, reaching highest concentrations in bone, teeth, liver, lungs, kidneys, brain and spleen (Saryan and Zenz, 1994). Lead in blood has an estimated half-life of 35 days, in soft tissue 40 days and in bone 20 to 30 years (Grimsley and Adams-Mount, 1994). Inorganic lead does not undergo any metabolic transformation or digestion in the intestines, or detoxification in the liver (Fischbein, 1992). With chronic exposure over a long period of time, most absorbed lead ends up in bone. Lead, it appears, is substituted for calcium in the bone matrix. This is not known to cause any deleterious effect on bone itself. Bone storage likely acts as a ‘sink,’ protecting other organs, while

allowing chronic accumulation (Staessen *et al*., 2008). The lead that accumulates in the bone ultimately provides a source for remobilization and continued toxicity after exposure ceases (Keogh, 1992). The total body content of lead is called the body burden; in a steady state, about 90 percent of the body burden is bound to bone (Saryan and Zenz, 1994).

2.6.4 Excretion of lead

Although lead is excreted by several routes (including sweat and nails), only the renal and gastrointestinal pathways are of practical importance. In general, lead is excreted quite slowly from the body, with the biologic half-life estimated at 10 years (Schwartz *et al*., 2000). Since excretion is slow, accumulation in the body occurs easily (Saryan and Zenz, 1994)

2.7 Symptoms of lead poisoning

Low levels of exposure (up to 10 µg/dl) are associated with anaemia, headaches, general weakness, fatigue, learning disabilities, impaired development of the nervous system, and delayed growth, while greater exposure (70 µg/dl) causes symptoms such as decreased appetite, vomiting, abdominal pain, constipation and drowsiness. If blood lead levels exceed 70 µg/dl, coma, seizures, bizarre behaviour, impaired muscular coordination and even death can occur (Gurer *et al*., 1998).

2.7.1 Organ system dysfunction

Lead toxicity can be manifested clinically in multiple organs. Specific organ system dysfunction includes the central and peripheral nervous system, renal, haematologic, gastrointestinal and reproductive systems.

2.7.2 Haematologic effects of lead poisoning

Perhaps, the best-known and best-studied toxic effect of lead is the effect lead has on haem synthesis. Lead inhibits delta aminolevulinic acid dehydrase (delta-ALAD) and ferrochelatase (haem-synthetase). As a result, delta-ALAD cannot be converted into porphobilinogen nor can iron be incorporated into the protoporphyrin ring. Therefore, haem synthesis is reduced. Because haem is important for the function of the cytochrome system and cellular respiration, lead poisoning has tremendous impact on the entire organism. Lead also inhibits the Na^+/K^+-ATP pump and attaches to the RBC membranes, leading to their lysis (Gurer *et al*., 1998).

2.7.3 Neurologic effects of lead poisoning

Lead affects the central nervous system by different mechanisms, most of which are unexplored. In the brain, lead is known to alter the function of cellular calcium and inactivate the blood-brain barrier (Tsao *et al*., 2000). These alterations result in leakage of proteinaceous fluid and brain oedema, which affects all, parts of the CNS, predominantly the cerebellum and the occipital lobes (Vaziri, 1998). Lead-induced cerebral oedema is manifested initially by headaches, clumsiness, vertigo, and ataxia, followed by seizures, coma, mortality, or recovery with permanent neurologic loss (West *et al*., 1994). Lead also impairs the function of several protein kinases and neurotransmitters (Gurer *et al*., 1998). In the peripheral nervous system, lead poisoning causes segmental demyelination of motor neurons and destruction of Schwann cells, resulting in motor neuron dysfunction (Philip and Gerson, 1994).

2.7.4 Gastrointestinal effects of lead poisoning

Lead causes contractions of the smooth muscle lining of intestinal walls, leading to severe, excruciating, colicky abdominal pains (lead colic), anorexia and diarrhoea (Pirkle *et al*., 1998).

2.7.5 Renal effects of lead poisoning

Lead nephropathy develops because of the inhibitory effects of lead on cellular respiration (West *et al*., 1994). Lead causes a generalized dysfunction of proximal, tubular, energy-dependent functions, manifesting as a Fanconi-like syndrome, with aminoaciduria, glycosuria, and phosphaturia (Meister and Anderson, 1983). While this effect generally is limited and reversible by chelation, chronic industrial exposure to lead has been associated with an irreversible interstitial nephropathy (Gurer *et al*., 1998). This chronic nephropathy may result in hyperuricaemia with gout, called saturnine gout (Levin and Goldberg, 2000).

2.7.6 Other effects of lead poisoning

Lead has negative effects on the reproductive system, causing low sperm count and abnormal sperm morphology in men and infertility, menstrual irregularity, spontaneous abortion, and stillbirths in women (Meggs *et al*., 1994).

In children, lead impairs the release of human growth hormone and insulin growth factor and interferes with skeletal calcium and cyclic adenosine monophosphate (cAMP) functions, resulting in abnormalities of bone growth (Suzuki and Yoshida, 1979). Chronic exposure to lead also may result in reduced thyroid function (Dawson *et al*., 1999). Rarely, acute lead poisoning results in hepatitis, pancreatitis, or cardiac dysfunction (Weng-Yew *et al*., 2009).

2.8 Mechanisms of lead toxicity

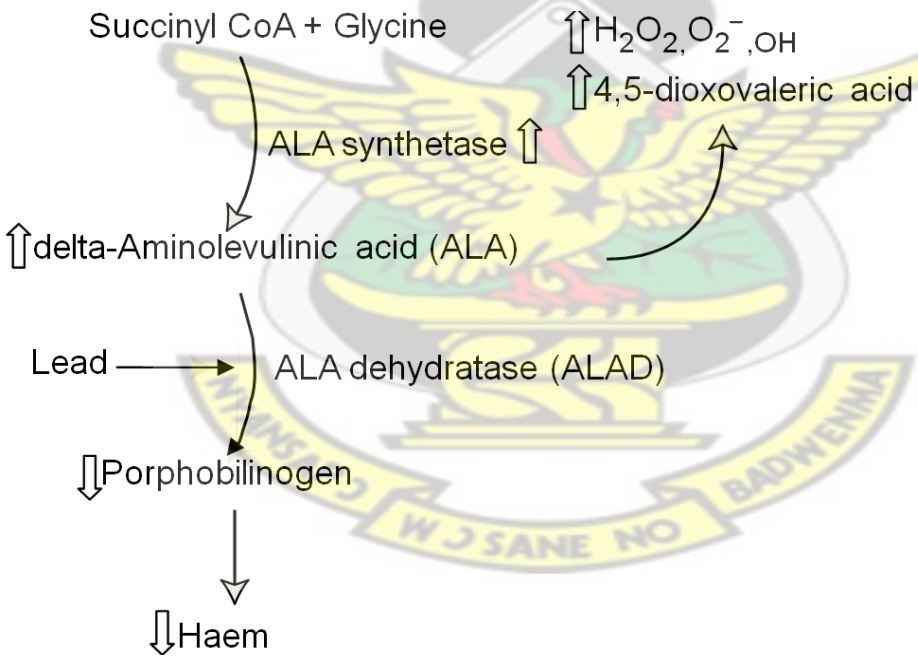
Lead toxicity leads to free radical damage via two separate, although related, pathways:

(1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide, and

(2) the direct depletion of antioxidant reserves (Ercal *et al*.,2001). In any biological system where ROS production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human system's ability to deal with increased oxidant stress occur via independent pathways. One of the effects of lead exposure is on glutathione metabolism. Glutathione is a cysteine-based, molecule produced in the interior compartment of the lymphocyte. More than 90 percent of non-tissue sulphur in the human body is found in the tripeptide glutathione (Meister and Anderson, 1983). In addition to acting as an important antioxidant for quenching free radicals, glutathione is a substrate responsible for the metabolism of specific drugs and toxins through glutathione conjugation in the liver (Meister and Anderson, 1983).The sulphydryl group of glutathione also directly binds to toxic metals that have a high affinity for sulphydryl groups. Mercury, arsenic, and lead effectively inactivate the glutathione molecule so it is unavailable as an antioxidant or as a substrate in liver metabolism. Concentrations of glutathione in the blood have been shown to be significantly lower than control levels both in animal studies of lead exposure and in lead-exposed children and adults (Vaziri *et al*., 1998).

Lead also binds to enzymes that have functional sulfhydryl groups, rendering them nonfunctional and further contributing to impairment in oxidative balance. Levels of two specific sulphydryl-containing enzymes that are inhibited by lead are delta-aminolevulinic acid dehydratase (ALAD) and glutathione reductase (GR).

These enzymes have been demonstrated to be depressed in both animal and human lead-exposure studies (Bechara, 1996). In a study of paediatric lead exposure in Lucknow, India, children with blood lead levels of 11.39 μg/dL had significantly depressed levels of ALAD, compared to children with levels of 7.11 μg/dL or lower. Depressed levels of ALAD in these children correlated with depressed levels of glutathione. In a study of lead-exposed battery plant workers, significant correlations were seen between ALAD activity, blood lead levels, and erythrocyte malondialdehyde (MDA) level (Flora *et al*., 2010). Malondialdehyde is a clinical marker of oxidative stress, specifically lipid peroxidation, which occurs in lead exposure. ALAD is a crucial enzyme in lead toxicity because the inhibition of ALAD lowers haem production and increases levels of the substrate delta-aminolevulinic acid (ALA) (Figure 2.8).

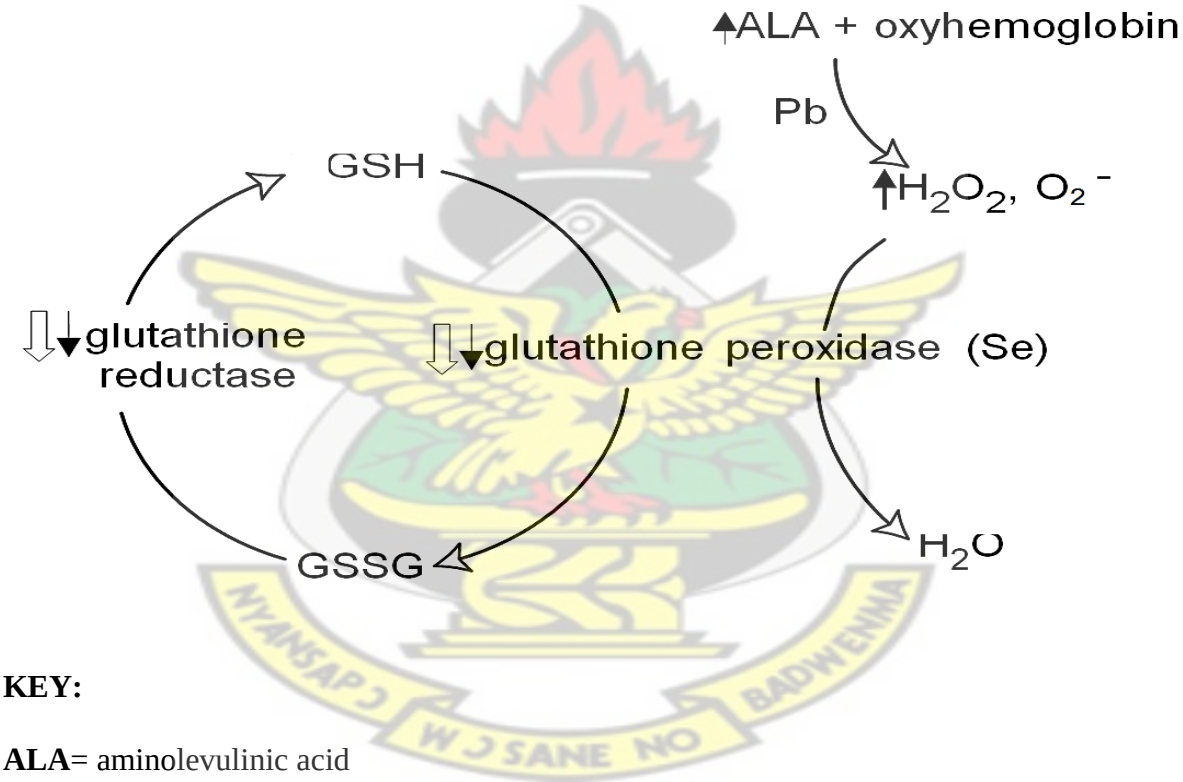


(KEY: ⇧ or ⇩ indicates increase and decrease respectively in enzymes or substrate as a result of lead exposure.

Figure 2.8 Inhibition of ALAD resulting in elevated ALA.

Source: Fischer *et al*. (1998)

Elevated levels of ALA, found in both blood and urine of subjects with lead exposure, are known to stimulate ROS production (Sugawara *et al*., 1991). Glutathione reductase, the enzyme responsible for recycling of glutathione from the oxidized form (glutathione disulphide; GSSG) to the reduced form (reduced glutathione; GSH) is also deactivated by lead. Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase were all found to correlate with depressed glutathione levels in occupationally-exposed workers (Suzuki and Yoshida, 1979). Figure 2.9 demonstrates the effect of lead on glutathione metabolism.



KEY:

ALA= aminolevulinic acid
↑↓ = reduction or elevation in enzymes or substrate due to upregulation or decreased availability
⇩ = reduction due to direct binding to Pb
GSH = reduced gluthathione
GSSG = oxidized gluthathione

Figure 2.9 Effect of lead on glutathione metabolism
Source: Shalan *et al*. (2005).

2.8.1 The generation of reactive oxygen species (ROS) by lead

Erythrocytes have a high affinity for lead, binding about 99 percent of the lead in the bloodstream (Lee *et al*., 2006). Lead has a destabilizing effect on cellular membranes, and in red blood cells (RBCs) the effect decreases cell membrane fluidity and increases the rate of erythrocyte haemolysis. Haemolysis appears to be the end result of ROS-generated lipid peroxidation in the RBC membrane. Lead can also bind directly to phosphatidylcholine in the RBC membrane, leading to a decrease in phospholipid levels (Simon and Hudes, 1999). Lipid peroxidation of cellular membranes has also been identified in tissues from various regions of the brain of lead-exposed rats. Hypochromic or normochromic anaemia is a hallmark of lead exposure; it results from ROS generation and subsequent erythrocyte haemolysis (Hsu *et al*., 1997). Lead is considered, along with silver, mercury, and copper, to be a strong haemolytic agent, able to cause erythrocyte destruction through the formation of lipid peroxides in cell membranes. In addition to membrane peroxidation, lead exposure causes haemoglobin oxidation, which can also cause RBC haemolysis. The mechanism responsible for this reaction is lead-induced inhibition of ALAD (Flora *et al*., 2010). ALAD is the enzyme most sensitive to lead's toxic effects – depressed haem formation. As a result, elevated levels of the substrate ALA are found in both the blood and urine of lead-exposed subjects. These elevated levels of ALA generate hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$), and also interact with oxyhaemoglobin, resulting in the generation of hydroxyl radicals ($^{\cdot}OH$), the most reactive of the free radicals (Figure 2.8). As ALA is further oxidized, it becomes 4, 5-dioxovaleric acid (Shalan *et al*., 2005). The generation of this potentially genotoxic compound is a possible mechanism for the metal-dependent DNA carcinogenicity of lead (Lee *et al*., 2004). By the same mechanism, ALA may be responsible for the increased frequency of liver cancer in acute intermittent porphyria, another condition where elevated levels of ALA occur (Tsao *et al*.,

2000). Lead has also been shown to either elevate or suppress blood levels of the antioxidant enzymes; superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Elevations of these enzymes have been seen at lower levels of exposure, while suppression can occur at higher exposure levels over longer periods of time. In one study of 137 lead-exposed workers, those with high blood lead levels (over 40 μg/dl) had significant reductions in blood GPx that correlated with elevated erythrocyte MDA levels. Those with lower exposures (25-40 μg/dl) had elevated levels of GPx, due probably to compensatory reaction for increased lipid peroxidation (Simon and Hudes, 1999).

2.8.1.1 The role of oxidative stress in the pathology of lead toxicity

Lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning (Hsu and Guo, 2002). Oxidative stress has also been implicated in specific organs with lead-associated injury, including liver, kidneys, and brain tissue. Reactive oxygen species generated as a result of lead exposure have been identified in lung, endothelial tissue, testes, sperm, liver, and brain (Hsu and Guo, 2002). Workers with occupational lead-exposure have a significantly higher frequency of infertility, stillbirths, miscarriages and spontaneous abortion, reduced sperm counts and motility, increased rates of teratospermia, and decreased libido (Levin and Goldberg, 2000). Studies of ROS in lead-exposed animals have shown alterations in SOD activity in testicular tissue and increased sperm ROS production that have been associated with decreased sperm counts, decreased motility, and decreased sperm-oocyte penetration (Hsu *et al*., 1997).

2.9 Treatment of lead poisoning

In all cases of suspected lead intoxication in adults, the first step in management should be removal of the individual from the exposure (Lawton and Donaldson, 1991). Whether discontinuation of exposure is sufficient treatment or chelation therapy should be

administered depends on the blood lead concentration, the severity of clinical symptoms, the biochemical and haematologic abnormalities, and the nature of the exposure. It is not recommended that specific blood lead concentrations be used to determine when treatment with a chelating agent is indicated. As a general rule, however, such a level is usually well above 80 µg/dl (3.85 µmol/L), which is also the level frequently associated with more severe symptoms (Fischbein, 1992). The primary indication for treatment of adults is brief, high-level exposure causing acute manifestations (Ribarov and Benov, 1981).

The use of chelation is not generally indicated in cases of long-term occupational exposure. The Occupational Safety and Health Administration (OSHA) lead standard specifically prohibits 'prophylactic' chelation for the prevention of elevated blood lead levels (Saryan and Zenz, 1994). In prophylactic chelation, chelating or similarly acting drugs are used routinely to prevent elevated blood levels in workers who are occupationally exposed to lead or to lower blood lead levels to predesignated concentrations thought to be safe. In patients with on-going lead exposure, chelation therapy is not considered appropriate medical treatment (Royce and Rosenberg, 1994). Chronic administration of oral agents to workers who continue to be exposed to unacceptably high levels in their workplace is a technique that has been used by employers in the past and is considered inappropriate (Landrigan, 1994).

The occupational safety and health administration standard allows the use of "therapeutic" or "diagnostic" chelation only if administered under the supervision of a licensed physician in a clinical setting. This should be done in conjunction with thorough and appropriate medical monitoring (Kaufman *et al*., 1994). When treatment with a chelating agent is indicated, calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) is often the drug of choice. Dimercaprol (British anti-lewisite, or BAL in oil) is a dithiol compound (2,

3-dimercapto-1-propanol) and penicillamine (Cuprimine), a dimethyl cysteine, popular agents in the past, are used less frequently (Fischbein, 1992).

The role of the new oral agent succimer (Chemet) in the treatment of adults has not been determined; it is approved by the U.S. Food and Drug Administration for treatment of children only (Porru and Alessio, 1996). Although isolated reports document effectiveness of succimer in treating adults, no clinical trials with this agent for treatment of adult lead toxicity have been reported (Meggs *et al*., 1994). The efficacy of chelating agents in treating patients with subtle neurologic and renal abnormalities has not yet been fully studied (Porru and Alessio, 1996) and is therefore not indicated.

2.9.1 Mechanism of action of chelating agents

The medical treatment of lead poisoning is carried out by means of chelating agents. These drugs are heavy metals antagonists which form a complex (a coordination compound, with a heterocyclic ring), preventing or reversing the binding of metallic cations to body ligands, i.e. reactive biological groups (Klaassen, 1990). Ideally, a chelating agent should have a number of characteristics:

- high affinity for the toxic metal, greater than that possessed by endogenous ligands
- low affinity for essential metals such as calcium, zinc, iron, copper
- ability to compete with the active biological ligands and to prevent or reverse the binding between them and metals
- ability to bind metals in a stable form
- capacity to form non-toxic complexes with the toxic metals
- distribution in the body compartments should be similar to that of the metal to be chelated

- ability to reach the sites of metal storage
- resistance to biotransformation
- high hydrosolubility, which allows an efficient elimination through the kidney. In addition, the drug should be well tolerated, conveniently administered and cheap (Alessio and Dell'Orto, 2003).

2.9.2 Side effects of pharmaceutical chelating treatments

It is essential to remember that the chelating agents themselves may have significant adverse effects, which represent a risk, apart from the lead toxicity. Treatment with these agents usually represents a failure in preventing lead overexposure of the patient and should initiate investigation of other workers at risk. Physicians contemplating chelation therapy for treatment of lead poisoning are advised to consult with colleagues experienced with use of up-to-date treatment protocols, in view of the lack of clear and consistent guidelines addressing the issue of when to chelate in such cases (Royce and Rosenberg, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of samples

The palm fruits used in this work were obtained from a palm tree plantation located close to Goaso. The *Tenera* variety was selected for this study.

3.2 Extraction of palm oil from oil palm fruits

Ten kilograms of freshly harvested ripe oil palm fruits were collected and boiled in a large container for about four hours. The mass of pulp was produced by pounding the boiled fruits using wooden pestle and mortar. The pulp was immersed in water and stirred. The fibres and the seeds were filtered out using a basket as a sieve. The filtrate was poured into an aluminium pot. It was boiled for five hours. Heating was discontinued and the mixture allowed to cool. Two hundred millilitres of water was sprinkled on it. The palm oil which set on top of the boiled filtrate was scooped into a pot. The oil collected was heated gently for ten minutes to remove traces of water.

3.3 Fractionation of crude palm oil

3.3.1 Saponification to produce unsaponifiable extract

A 10 ml sample of crude palm oil was placed in a 250 ml conical flask, 15 ml of 20% sodium hydroxide added. The flask was corked and connected to a vacuum pump and the mixture was stirred using a magnetic stirrer, as shown in figure 3.1.

Figure 3.1 Saponification of crude palm oil

The mixture was boiled on a hot plate until the solution thickened. The flask was removed from the hot plate and disconnected from the vacuum pump. Stirring was continued as the solution cooled slowly. The cooled solution was transferred into a separating funnel and 200 ml of hexane was added. It was shaken and mounted on a retort stand to settle. The tap was opened to elute the bottom layer (soap). The upper layer was washed several times with distilled water until the waste water tested neutral to phenolphthalein. This upper layer was labelled as the unsaponifiable extract of palm oil. This was then stored in a cold dry place. This method was performed as described by Meloan, (2009) with some modifications.

Figure 3.2 Extraction of unsaponifiable matter using hexane

3.3.2 Adsorption chromatography of crude palm oil

This was based on the method described by Ahmad *et al*. (2008). A chromatographic glass tube, 3.0 cm in diameter, was mounted on a retort stand. A small glass wool was plugged at the bottom of this tube. An adsorbent, silica gel (70 to 230 mesh), was used to fill the column up to a height of 5.0 cm. The sides of the column were tapped four times to remove air spaces. A glass rod was used to press down the adsorbent and flattened the surface. The eluting solvent used was 100 ml n-hexane. Ten milllilitres of crude palm oil was dissolved in 30 ml of n-hexane and introduced into the column. The eluent was collected into a flask until the adsorbent in the column became colourless. The collected fraction was stored in a cold, dry place.

3.3.3 Production of bleached extract

Bleaching was carried out based on a method described by Patterson (1992). A mass ratio of 1 silica gel to 11 CPO was used. An amount of 110 grams of CPO was poured into a

beaker and heated on a hot plate. A magnetic stirrer was put into the beaker containing CPO and continuously stirred until it reached 100 °C. Thereafter, 6 drops of phosphoric acid was added to the CPO, followed by the addition of 10 grams silica gel. The adsorbent was poured slowly to ensure it mixed well with CPO and bubbles formation was prevented. Then, the mixture was heated for an hour until it reached 150 °C. After bleaching was done, the mixture was allowed to cool. The spent adsorbent was separated from the bleached palm oil (BPO) by filtration using filter paper. Filtration was carried out in the oven at 80 °C, for a period of 22 hours.

3.4 Evaporation of solvents in collected extracts

Rotary evaporator was used to evaporate solvents in extracted samples. Figure 3.3 shows the set-up used.

Figure 3.3 Vacuum evaporation of sample

3.5 Quantitative analysis of carotenes and tocols

3.5.1 Carotene

The carotene content of crude palm oil was determined according to the method described by Coursey (2000). A known amount of CPO (3g) was dissolved in 20 ml hexane in a 50 ml volumetric flask. Hexane was used as the blank solution. A Spectrophotometer, V-1100, J.P Selectra (Barcelona, Spain) was used to measure the absorbance at 446 nm. Using the absorbances obtained the concentration of carotenes in the sample was calculated, using the formula below;

[Carotene] = [383 × Absorbance (446 nm) × Volume (ml)]/[100 × Sample weight (g)]

Where*; [Carotene] = concentration of carotenes in ppm*

Volume = volume of hexane mixed with palm oil

383 = diffusion coefficient

The same procedure was repeated with other palm oil extracts.

3.5.2 Vitamin E (Tocols)

Crude palm oil (0.5 ml) was weighed and dissolved in hexane in a 1.0 ml vial. The prepared CPO sample was then injected into a High Performance Liquid Chromatography (HPLC) system, SMI3000SE, Hitachi (Waldbronn, Germany) with a fluorescence detector (excitation at 295 nm and emission at 325 nm) and an analytical silica column (25cm × 4.6mm ID, stainless steel, 5µm) was used to analyse vitamin E, according to the method described by Ng and Tan (1999). The mobile phase used was hexane: tetrahydrofuran: isopropanol (100:6:4 v/v) at a flow rate of 1.0 ml/min. The HPLC system was calibrated

using the standard vitamin E, obtained from Merck (Darmstadt, Germany) and the concentration of vitamin E in CPO was determined. The procedure was repeated with other palm oil extracts. Using the results obtained the concentrations of vitamin E in the samples were calculated, using the formula below;

[Vitamin E] = A sample × [STD] (ppm) × V hex (ml)/A STD × Wt sample (g)

Where;

[Vitamin E] = concentration of vitamin E in ppm

[STD] = concentration of standard

A sample = area of sample

A STD = area of standard

V hex = volume of hexane

Wt sample = weight of sample

3.6 Determination of free fatty acid (FFA) of palm oil extracts

Free fatty acids content in the palm oil samples was determined by titration method, according to the Palm Oil Research Institute of Malaysia (PORIM) test method (1990). The samples for the analysis were melted at 60 ^{o}C to 70 ^{o}C and 10 g was weighed into 50 ml Erlemeyer flask. Twenty ml of ethanol and three drops of phenolphthalein were added and placed on the hot plate. The temperature was maintained at 40 ^{o}C. The flask was swirled gently while titrating with 0.1M NaOH to the first permanent pink colour. The volume of NaOH used was recorded. The percentage FFA in the sample was calculated, using the formula below,

$$\text{FFA \% as palmitic acid} = \frac{25.6 \times M(NaOH) \times V}{W}$$

Where;

M =Molarity of NaOH solution

V = volume of NaOH solution used in ml

W = weight of sample

3.7 Investigation of lead toxicity and use of palm oil as antidote

Design and dosing of experimental animals were based on the method of Ashour (2002) with some modifications. Adult male albino rats, weighing 100-120 g were used in this study. They were purchased from the Breeding Unit of the Faculty of Pharmacy, KNUST in Kumasi. The rats were made to acclimatise for one week in ventilated plastic cages with wire mesh covers. The cages contained saw dust which is good for absorbing urine. Each cage contained six rats under normal laboratory conditions of temperature and humidity with an alternating 12-hour light/dark cycle. Diet and water were supplied *ad libitum* throughout the experimental period. The diet was prepared using dried maize and fish (5:1 kilograms) milled together. A sublethal dose of 2 g/L lead acetate was used. The dosage of lead acetate and palm oil were based on other studies (Flora *et al*., 2010; Wang and Quinn, 1999). The entire experimental treatment lasted for 40 days. The first group (A) represented the control group and was orally administered deionized water daily for 40 days. The second group (B) were orally administered lead acetate (2 g/L) daily for 40 days. In the third group (C) of animals, lead acetate treatment lasted for 35 days and there was no treatment for 5 days. Rats in the fourth group (D) were given lead acetate and crude palm oil at the same time for the entire 40 days. This group was used to show the prophylactic properties of palm oil against lead poisoning. The fifth group (E) was divided into four sub-groups (E1, E2, E3 and E4). Each sub-group contained six rats. Sub-group E1 was administered lead acetate for 35 days and was orally injected with 0.5 ml/kg body weight/day crude palm oil for 5 days. Sub-group E2 was also administered with lead

acetate for 35 days and subsequently given 0.5 ml/ kg body weight/day of silica gel extract for 5 days. For sub-group E3, after 35 days of lead acetate treatment, rats were given 0.5 ml/kg body weight/day of bleached palm oil for the remaining 5 days. Sub-group E4 were given lead acetate for 35 days and treatment was continued with 0.5 ml/kg body weight/day of unsaponifiable extract for 5 days. The various groupings are summarised in the table below.

Table 3.1 Summary of grouping and treatments given to rats

Groups	Treatment 1	Duration (Days)	Treatment 2	Duration (Days)	Total duration (Days)
A	Deionised water	40	_	_	40
B	Lead acetate	40	_	_	40
C	Lead acetate	35	_	_	40
D	Lead acetate	40	Crude palm oil	40	40
E1	Lead acetate	35	Crude palm oil	5	40
E2	Lead acetate	35	Silica gel extract	5	40
E3	Lead acetate	35	Bleached palm oil	5	40
E4	Lead acetate	35	Unsaponifiable matter	5	40

NB: For rats in group D, the lead acetate and CPO were administered together giving a total duration of 40 days.

3.7.1 Blood sampling and processing

On the 40th day, animals were anesthetized and blood was taken through cardiac puncture, into two different blood sample tubes. The first tube contained EDTA and was used for complete blood count (CBC) analysis. Blood sample in the other tube was without EDTA, was left for a short time to allow clotting. Serum samples were later obtained by centrifugation at 3000 rpm for 20 minutes and then kept in the refrigerator for analysis.

3.8 Measurement of various haematological and biochemical parameters of experimental animals

Haematological parameters (red blood cells, haemoglobin and haematocrit) were determined using Sysmex analyzer, KS21N, (Lincolnshire, USA) and biochemical parameters (serum total protein, serum alanine transaminase (ALT), serum aspartate aminotransferase (AST), creatinine, urea and uric acid) were obtained from Selectra analyzer (Selectra junior), (Leopoldsburg, Belgium).

3.9 Statistical analysis

Results were expressed as mean values ± SE (n = 6). Means of samples were compared by one-way analysis of variance (ANOVA). Significant differences between means were determined by Bonferroni's multiple comparison test ($P<0.05$). Percentage changes were also calculated and graphs were also drawn. The software used was GraphPad prism version 4.0 (San Diego California, USA).

CHAPTER FOUR

4.0 RESULTS

4.1 Carotenes, tocols and free fatty acids contents of CPO and palm oil extracts

Table 4.1 Concentration of carotenes and tocols in crude palm oil and palm oil extracts.

Phytonutrients	Concentration (ppm)			
	Crude palm oil	Silica gel extract of palm oil	Bleached palm oil	Unsaponifiable matter of palm oil
Carotenes	530	16310	209	19570
Tocols	1040	19870	640	39290

Table 4.1 shows the difference in concentrations of carotenes and tocols in crude palm oil, silica gel extract, bleached and unsaponifiables of crude palm oil in parts per million. The unsaponifiable palm oil recorded the highest concentration of carotenes (19570 ppm) and tocols (39290 ppm). This was closely followed by silica gel extract which recorded 16310 ppm of carotenes and 19870 ppm of tocols. The bleached palm oil had the least amount of both carotenes and tocols.

Table 4.2 Free fatty acid (FFA) content of crude palm oil and palm oil extracts

Crude palm oil and fractions	Free fatty acid content (%) v/v
Crude palm oil	4.6
Silica gel extract of palm oil	3.3
Bleached palm oil	13.8
Unsaponifiable matter of palm oil	2.7

Table 4.2 shows the level of free fatty acids in crude palm oil and palm oil extracts. The bleached palm oil contained the highest amount of free fatty acids (13.8 % v/v). The crude

palm oil was second highest and about three times lower than bleached oil. Silica gel extract contained 3.3 % v/v of FFA, about four times lower than that in bleached oil. The unsaponifiable matter had the least level of FFA.

4.2 Haematological measurements

Table 4.3 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on red blood cells count (count × 10^6 cell/µl) of albino rats

Treatments	Deionised water (control)	Lead acetate	Lead acetate discontinuation	Lead acetate + palm oil	Crude palm oil	Silica gel extract	Bleached palm oil	Unsaponifiable matter
Mean ± SE	8.09 ± 0.2^a	6.58 ± 1.0^b	6.92 ± 1.0^c	7.57 ± 1.4^d	7.93 ± 0.2^a	7.87 ± 0.9^a	6.87 ± 0.5^c	7.96 ± 0.7^a
% Decrease		18.7	14.5	6.4	1.8	2.8	15.1	1.6
p-value		< 0.01	< 0.01	< 0.05	> 0.05	> 0.05	< 0.05	> 0.05

Values are means ± SE (n=6). Values not sharing a common superscript are significantly different (p< 0.05).

From Table 4.3, oral administration of lead acetate at a dose of 2 g/L for a period of 40 days caused a highly significant decrease (P<0.01) in RBC count. When lead acetate was administered for 35 days, RBC count still exhibited a highly significant decrease. The percentage decrease was 14.5. In animals orally gavaged with palm oil (0.5 ml/kg body weight) simultaneously with lead acetate, a significant decrease was observed as compared with the control group, a percentage reduction of 6.4. The oral gavaging of crude palm oil and palm oil fraction to animals given 2 g/L lead acetate for 35 days increased RBC count (particularly the unsaponifiable extract of palm oil).

Table 4.4 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on haemoglobin levels (g/dl) of albino rats.

Treatments	Deionised water (control)	Lead acetate	Lead acetate discontinuation	Lead acetate + palm oil	Crude palm oil	Silica gel extract	Bleached palm oil	Unsaponifiable matter
Mean ± SE	14.7 ± 0.7^{a}	11.6 ± 0.8^{b}	11.5 ± 0.6^{b}	12.2 ± 0.9^{a}	12.9 ± 0.9^{a}	13.2 ± 0.5^{a}	12.0 ± 0.6^{b}	13.0 ± 0.8^{a}
% Decrease		21.0	22.0	17.3	12.5	10.2	18.1	11.6
p-value		< 0.01	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05

Values are means ± SE (n=6). Values not sharing a common superscript are significantly different ($p < 0.05$).

Table 4.4 shows haemoglobin levels in control and test groups of animals. The groups administered lead acetate (2 g/L) for 35 days showed a significant decrease in haemoglobin level, compared to the control. The difference was also significant for the group given lead acetate for a longer period of 40 days. The groups given palm oil or palm oil fractions had increases in their haemoglobin levels, except the group that was given bleached palm oil. Among these groups, the group given silica gel extract was most effective in maintaining haemoglobin level, as the percentage reduction was only 10.2. The group given bleached palm oil fraction produced the least effect, with a percentage reduction of 18.1 which is close to the group given lead acetate without any further treatment. The group that was given lead acetate alongside palm oil showed increase in haemoglobin content when compared with the group that was given lead acetate only.

Table 4.5 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on blood haematocrit value (%) of albino rats

Treatments	Deionised water (control)	Lead acetate	Lead acetate discontinuation	Lead acetate + palm oil	Crude palm oil	Silica gel extract	Bleached palm oil	Unsaponifiable matter
Mean ± SE	47.7 ± 1.2^{a}	36.6 ± 1.8^{b}	37.2 ± 1.8^{b}	41.2 ± 1.1^{a}	44.1 ± 1.2^{a}	42.6 ± 2.6^{a}	39.6 ± 3.0^{c}	42.7 ± 1.5^{a}
% Decrease		23.4	21.9	13.8	7.5	10.7	17.0	10.6
p-value		< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05

Values are means ± SE (n=6). Values not sharing a common superscript are significantly different ($p < 0.05$).

The mean values of blood haematocrit values of both control and test animals are shown in Table 4.5. There was significant reduction in haematocrit (Hct) values when animals were given lead acetate. The percentage reduction was 23.4. Also, for the group in which lead acetate was discontinued, there was still a significant difference in Hct values when compared with the control group. Administration of palm oil and palm oil extracts to animals treated with lead acetate generally increased Hct values of rats. The treatment with crude palm oil maintained the Hct values close to the control level.

Table 4.6 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on blood platelet count (count × 10^6 cell/µl) of albino rats.

Treatments	Deionised water (control)	Lead acetate	Lead acetate discontinuation	Lead acetate + palm oil	Crude palm oil	Silica gel extract	Bleached palm oil	Unsaponifiable matter
Mean ± SE	1165.3 ± 98.1[a]	766.0 ± 40.3[b]	790.3 ± 25.3[c]	1005.0 ±15.1[d]	1081.0 ± 102.1[a]	1079.0 ± 75.2[a]	796.7 ± 40.4[c]	1036.0 ± 25.9[a]
% Decrease		34.2	32.2	13.7	7.3	7.4	31.6	11.1
p-value		< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Values are means ± SE (n=6). Values not sharing a common superscript are significantly different ($p < 0.05$).

According to Table 4.6, the mean values of platelet count of lead-treated group showed significant decrease when compared to the control group ($p<0.05$). The group administered palm oil alongside lead acetate had their mean values also showing significant decrease, compared to the control group. However, a look at the percentage differences indicate that the group with palm oil administered simultaneously with lead acetate had a lower decrease of 13.7%. The groups given crude palm oil and palm oil extracts recorded platelet counts close to the normal levels.

4.3 Renal function parameters

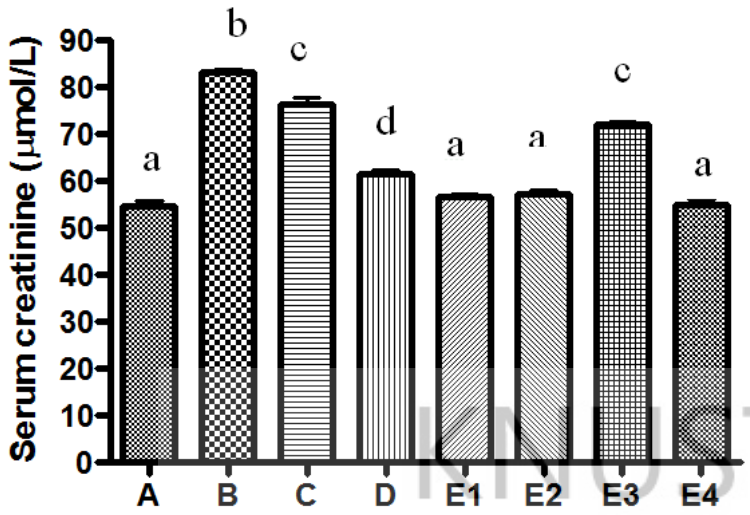


Figure 4.1 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on serum creatinine of albino rats. A (control), B (lead acetate, 40 days), C (lead acetate, 35 days), D (lead acetate and palm oil), E1 (treated with crude palm oil), E2 (treated with silica gel extract), E3 (treated with bleached palm oil) and E4 (treated with unsaponifiable matter). Values are means ± SE (n=6). Bars not sharing common letters are significantly different ($p < 0.05$).

Lead acetate at a dose of 2 g/L (B) caused significant increase in creatinine (Figure 4.1). The percentage increase was 52.5, compared to the control. When bleached palm oil was administered the percentage increase was 42.0 and this was significantly different from the control. In the presence of crude palm oil, silica gel extract and unsaponifiable matter, normal serum creatinine levels were seen.

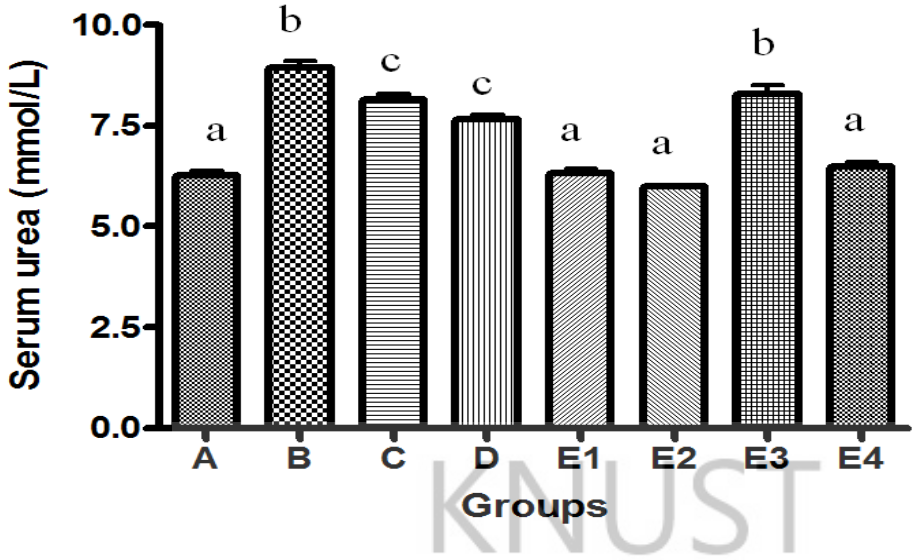


Figure 4.2 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on serum urea of albino rats. A (control), B (lead acetate, 40 days), C (lead acetate, 35 days), D (lead acetate and palm oil), E1 (treated with crude palm oil), E2 (treated with silica gel extract), E3 (treated with bleached palm oil) and E4 (treated with unsaponifiable matter). Values are means ± SE (n=6). Bars not sharing common letters are significantly different ($p < 0.05$).

Administration of lead acetate at a dose of 2 g/L provoked a significant increase in serum urea, according to Figure 4.2. The effect was similar for the group where lead acetate was orally administered for 35 days. Palm oil and its extracts (E1, E2 and E4) decreased serum urea levels of rats.

4.4 Liver function parameters

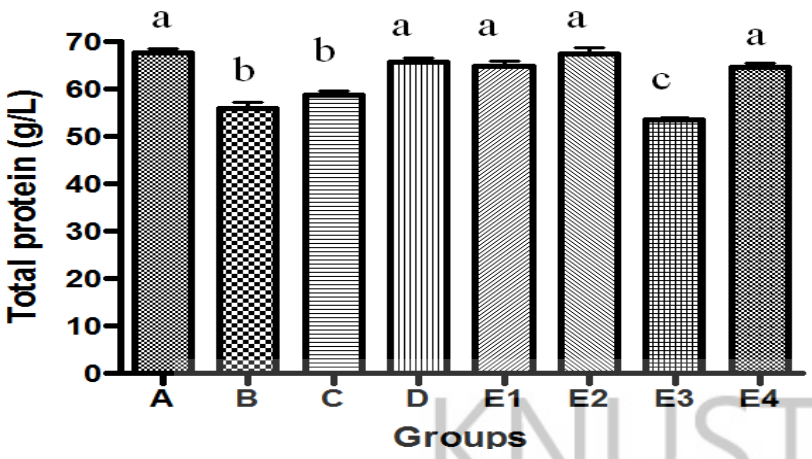


Figure 4.3 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on total protein of albino rats. A (control), B (lead acetate, 40 days), C (lead acetate, 35 days), D (lead acetate and palm oil), E1 (treated with crude palm oil), E2 (treated with silica gel extract), E3 (treated with bleached palm oil) and E4 (treated with unsaponifiable matter. Values are means ± SE (n=6). Bars not sharing common letters are significantly different ($p < 0.05$).

Figure 4.3 shows that 40 days of 2 g/L lead acetate treatment of rats resulted in a significant decrease in total protein. Treatment to alleviate the effect of lead using palm oil and palm oil extracts produced varying results. Crude palm oil, silica gel extract and unsaponifiable matter (E1, E2 and E4) were effective in increasing the total protein levels of rats administered lead acetate. Treatment with bleached palm oil could not effect any change in total protein level of rats.

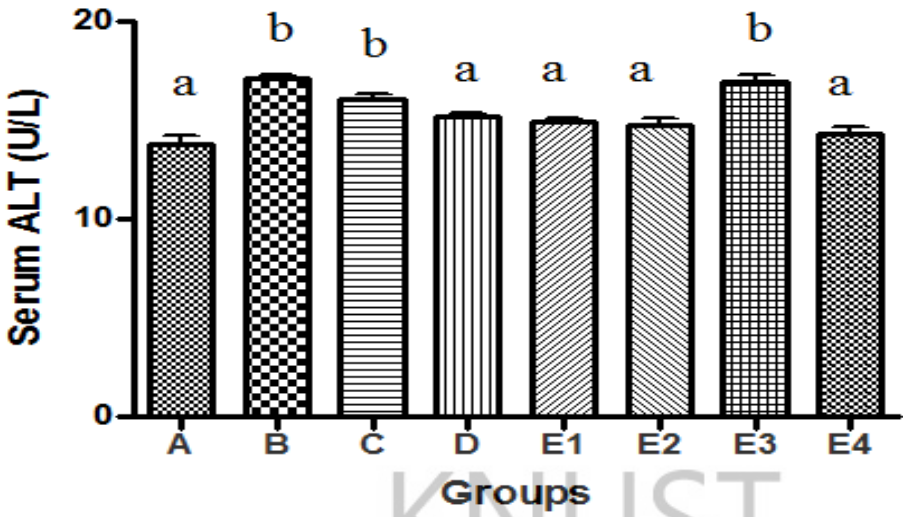


Figure 4.4 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on serum ALT of albino rats. A (control), B (lead acetate, 40 days), C (lead acetate, 35 days), D (lead acetate and palm oil), E1 (treated with crude palm oil), E2 (treated with silica gel extract), E3 (treated with bleached palm oil) and E4 (treated with unsaponifiable matter). Values are means ± SE (n=6). Bars not sharing common letters are significantly different ($p < 0.05$).

Figure 4.4 shows that lead acetate (2 g/L) treatment for 40 days increased serum ALT levels of rats. The ALT levels decreased when whole palm oil and its extracts were administered to rats already treated with lead acetate for a period of 35 days. The group treated with unsaponifiable extract (E4) had the least percentage increase of 3.3.

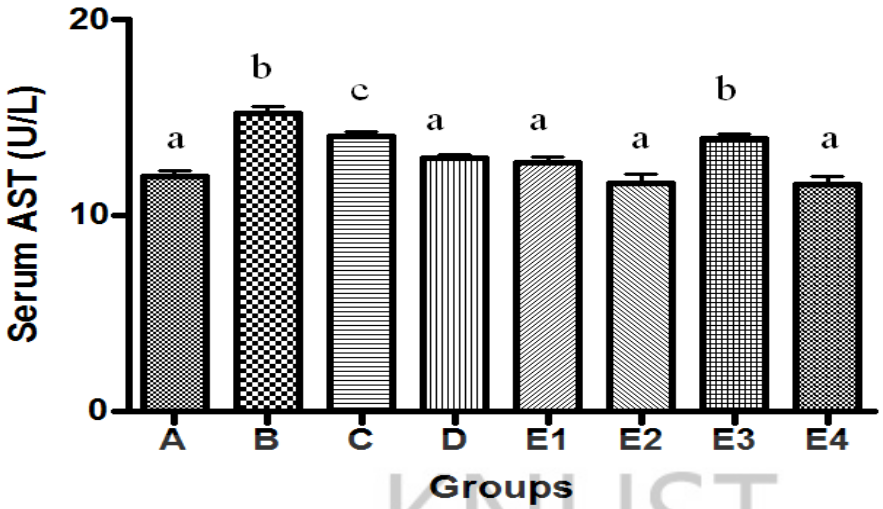


Figure 4.5 Effects of oral administration of lead acetate (2g/L), CPO and palm oil extracts on serum AST of albino rats. A (control), B (lead acetate, 40 days), C (lead acetate, 35 days), D (lead acetate and palm oil), E1 (treated with crude palm oil), E2 (treated with silica gel extract), E3 (treated with bleached palm oil) and E4 (treated with unsaponifiable matter). Values are means ± SE (n=6). Bars not sharing common letters are significantly different ($p < 0.05$).

The administration of palm oil and its extracts generally led to a decrease in serum aspartate aminotransferase (AST) levels (Figure 4.5). These groups had their AST levels significantly lower than the control group. The mean AST value of rats treated with bleached palm oil (E3) was close to that of the untreated groups.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Palm oil extraction and fractionation

The amounts of phytonutrients in palm oil were studied by Choo *et al*. (1997) and they found out that crude palm oil posseses 1% minor components which include carotenoids, tocopherols and tocotrienols and sterols. The results from the present study (Table 4.1) have shown that saponification and adsorption chromatography are good techniques for concentrating tocols and carotene contents of palm oil. It has been possible to use these processes to produce palm oil extracts with tocols and carotene levels ten times higher, compared to that in crude palm oil. Heat destruction on the contrary, probably due to its effect on stability of tocols and carotenes, led to significant reductions in the levels of the phytonutrienents in palm oil.

The free fatty acid analysis (Table 4.2) of these extracts also revealed that thermal bleaching of palm oil led to increased liberation of free fatty acids (FFA) from triglycerides, resulting in high amount of FFA. The bleached palm oil contained 13.8 % v/v of FFA which is 300 % higher than the amount in the crude palm oil. Fractionation using saponification and adsorption chromatography produced extracts with reduced levels of free fatty acids. Results from Tables 4.1 and 4.2 indicate that thermal bleaching reduces carotene and tocol levels of palm oil but increases free fatty acid content. Saponification and adsorption chromatography produced opposite effect of high carotene and tocol levels, with minimal free fatty acids level.

5.2 Effects of palm oil extracts on lead poisoning

A study by Flora *et al*. (2010) compared the effects of different doses of lead acetate on blood lead levels (BLL) of male albino rats weighing 100-120g. Their results showed that BLL was time-and-dose dependant. When 2 g/L of lead acetate was orally administered for 40 days, there was a highly significant increase in BLL. In the second part of the present study, the effects of oral administration of lead acetate (2 g/L) on haematological parameters and organs such as kidney and liver were determined. The results show that oral administration of 2 g/L lead acetate to male albino rats for a period of 35 and 40 days is effective in causing haematological, renal and liver function impairments. This is demonstrated by comparing the mean values of groups A (control) and B (2 g/L lead acetate for 40 days) (Tables 4.3-4.6). There were significant differences among these groups in all the parameters measured. Again, there were significant differences, comparing groups A and C (2 g/L lead acetate for 35 days) on haematology, kidney and liver function parameters (Figures 4.1-4.5).

The lead-poisoned rats showed significant decreases in their RBC count (Table 4.3). Patrick (2006) earlier reported in a study that erythrocytes have high affinity for lead, binding 99% of the lead in the bloodstream. Lead destabilizes the membranes and the result is decrease in RBC fluidity and increased erythrocytes haemolysis (Lawton and Donaldson, 1991).

The current data also show that haemoglobin content (Table 4.4), haemotocrit value (Table 4.5) and platelet count (Table 4.6) were significantly decreased, parallel to the decrease in RBC count (Table 4.3) in experimental rats with lead acetate intoxication at dose of 2 g/L. Lead may inhibit the body's ability to produce haemoglobin by interfering with several enzymatic steps in the haem biosynthetic pathway. Specifically, lead causes the inhibition

of aminolevulinic acid dehydratase (ALAD) (Masci *et al.*, 1998), which is very sensitive to lead's toxic effect (Farrant and Wigfield, 1998). The decrease in haematocrit and platelets counts was in agreement with that reported by Redig *et al*. (1991). The results indicate the ability of lead poisoning to induce anaemia, both by interfering with haem biosynthesis and by diminishing RBC survival.

Treatment of animals with crude palm oil and palm oil extracts presented in Tables 4.3, 4.4, 4.5 and 4.6 resulted in some improvement in the RBC count, haemoglobin concentration, haematocrit and platelet count, respectively. The prophylactic effect of palm oil on lead-poisoned rats, characterized by the improvement of haematological parameters has been shown by this study. The unsaponifiable matter of palm oil, as well as silica gel extract had high concentration of palm phytonutrients including carotenes, tocopherols and tocotrienols. These were effective in alleviating the toxic effects of lead on the blood. These palm oil extracts reversed the decrease in the haematological parameters to near normal levels. Crude palm oil was also effective in correcting the heavy metal toxicity. The efficiency was however, less compared to the silica gel extracts and the unsaponifiable fraction. It had been pointed out that tocols and carotenes are antioxidants (Parker *et al*., 2001; Brian, 1991). The greater efficacy of the silica gel extract and the unsaponifiable fraction over crude palm oil might be due to the fact that the antioxidant capacity via presence of carotenes and tocols, in these extracts were higher than they were in the crude palm oil. Bleached palm oil, on the other hand, did not have any significant effect ($p<0.05$) on the haematological parameters under consideration. In fact, biochemical and haematological parameters of rats treated with bleached palm oil was comparable to those of rats given lead acetate without any form of treatment. The measured amounts of carotenes and tocols in the bleached sample were just a small fraction, compared to

quantities observed in the crude palm oil. This might account for the bleached fraction's inability to reverse the effects of lead poisoning.

There was a significant increase ($p<0.05$) in serum urea upon administration of 2 g/L of lead acetate, when compared to the control (Figure 4.2). The observed elevation in serum urea concentration in response to lead acetate administration is in agreement with previous studies (Schraishuhn *et al.*, 1992). Urea is the principal end product of protein catabolism. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis could be a probable cause for the elevated levels of urea. Additionally, the elevated serum urea levels could be as a result of degradation of RBC.

The results presented in Figure 4.1 shows that lead acetate intake at a dose of 2 g/L caused significant increase in creatinine, compared to control. Increase in creatinine concentrations is in agreement with previous reports (Ghorbe *et al.*, 2001). Such increase may indicate impairment in kidney function. Generally, about 50 % of kidney function is lost before an appreciable rise in serum creatinine concentration is detected (Kaptan and Szabo, 1983). Treatment of the albino rats with CPO and palm oil extracts alleviated the toxic effects of high levels of lead. Silica gel extract and unsaponifiable matter were more effective than the other treatments (Figure 4.2).

This study also showed an elevation of aminotransferases in the serum when lead acetate (2 g/L) was administered. Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels in the serum indicates damage to cells rich in these enzymes (Champe and Harvey, 1995). The activities of aminotransferases in the serum are hence useful indicators of liver damage for the diagnosis and study of acute hepatic disease. The elevation of serum aminotransferases (Figures 4.4 and 4.5) is an indication of damage to the structural integrity of the liver (He and Aoyama, 2003). The observed elevation of

alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of animals treated with lead acetate suggest liver damage. The findings of the present study show that CPO and palm oil extracts were able to reduce serum levels of ALT and AST close to control levels. The healing effects of the unsaponifiable matter and the silica gel extract were much more pronounced, when compared to the other forms of treatment. Crude palm oil was also effective and its prophylactic ability was also established.

The total serum protein test measures albumin and globulin, the major blood protein. Low total proteins suggest liver biosynthetic function impairment (Nair *et al.*, 2007). From Figure 4.3, serum total protein decreased under lead toxicity. This study has further shown that CPO and some fractions were effective in improving serum total protein levels. Bleached palm oil could not produce any effect because bleaching reduces the phytonutrients of palm oil to a minimal level.

Oxidative stress generated through reactive oxygen species (ROS) and the depletion of antioxidant reserves had been proposed as a mechanism of lead toxicity (Ercal *et al.*, 2001). Palm oil has been shown to elevate blood levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (Wang and Quinn, 1999). In that study, 0.5 ml/kg body weight of rats per day, of palm oil administered for four days enhanced the activities of these enzymes. In line with this finding, the current study administered the same dose (0.5 ml/kg body weight/day) for a period of five days to treat lead-poisoned rats. It can therefore be suggested that antioxidant action of crude palm oil and palm oil extracts might play a role in the treatment of lead poisoning.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The physicochemical composition of palm oil extracts were evaluated in this study. Palm oil extracts derived from saponification and chromatographic isolation had high content of tocols (tocopherols and tocotrienols) and carotenes. The extracts derived from saponification had 39290 ppm of tocols and 19570 ppm of carotenes. The chromatographic isolated extract also contained 39290 ppm of tocols and 16310 ppm of carotenes. On the contrary, these extracts had low levels of free fatty acids. A fraction obtained through thermal bleaching, while found to have low levels of tocols (640 ppm) and carotenes (209 ppm) had high amount of free fatty acids (13.8 % v/v). Oral administration of lead acetate (2 g/L) for 35 and 40 days to rats was found to have toxic effects which manifested in haematological disorders, impairment of kidney and liver functions in rats. When 0.5 ml/kg body weight/day of crude palm oil and palm oil extracts were administered to the rats, the effects of lead toxicity were reversed. Palm phytonutrient concentrate, derived from saponification and chromatographic separations with silica gel column used for treatment were more efficient than the crude palm oil. Bleached palm oil was not effective in the treatment of lead toxicity.

6.2 Recommendations

1. Further studies must be conducted to ascertain whether palm oil extracts are dose dependant in the treatment of lead poisoning.
2. The amount of lead that actually accumulates in the body after a known amount had been administered should be determined.

3. How lead poisoning affects reproductive health of rats and how palm oil extracts respond to any possible effects, be investigated.
4. Studies should be conducted to find out the role of palm oil consumption in influencing blood lipid concentrations. Many people avoid its consumption because they consider palm oil among the risk factors in the development of cardiovascular diseases.

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APPENDICES

APPENDIX A: DATA FOR RATS IN GROUP A

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	7.9	13.7	47.7	1036	59.4	6.4	65.8	14.2	10.9
2	8.3	14.4	46.6	1124	55.9	5.9	67.0	11.9	12.9
3	8.4	15.2	47.9	1330	52.1	6.3	70.8	14.8	12.7
4	7.9	15.5	48.9	1161	52.4	6.2	65.8	14.3	11.5
5	8.1	14.4	45.6	1132	54.1	6.7	67.8	13.6	12.4
6	7.9	15.1	48.3	1203	53.3	6.2	69.2	14.1	11.3

APPENDIX B: DATA FOR RATS IN GROUP B

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	6.7	11.9	35.9	796	85.4	9.0	57.6	16.4	14.2
2	7.9	12.1	37.2	688	80.2	9.1	59.1	17.5	16.3
3	5.5	12.4	35.6	772	83.7	8.2	50.1	16.6	14.1
4	6.6	10.2	38.2	782	85.1	8.7	50.7	17.2	15.4
5	5.4	11.2	33.1	764	82.2	9.2	54.2	17.5	14.9
6	7.6	12.0	37.6	795	81.9	9.4	58.2	17.4	16.1

APPENDIX C: DATA FOR RATS IN GROUP C

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	6.7	11.4	36.7	800	79.5	8.4	55.6	15.9	14.5
2	7.0	10.6	38.6	790	70.4	8.3	59.2	16.9	14.7
3	7.4	11.5	36.4	752	80.1	7.6	60.1	16.2	13.0
4	6.3	11.3	39.6	824	76.5	8.3	58.2	15.6	13.7
5	8.3	12.0	35.4	772	74.9	7.8	57.6	15.2	14.2
6	5.4	12.2	34.9	823	77.2	8.5	67.1	16.5	13.9

APPENDIX E: DATA FOR RATS IN GROUP D

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	6.7	11.1	40.9	1022	59.4	7.5	63.6	14.4	12.9
2	8.4	12.2	41.4	998	60.3	7.3	68.6	15.3	13.0
3	7.8	13.4	42.1	1014	62.5	8.0	65.3	15.6	12.6
4	5.6	11.6	39.3	1019	64.2	7.9	64.4	15.5	13.6
5	9.6	11.2	42.1	992	62.1	7.5	67.1	14.9	12.4
6	7.2	13.2	40.2	986	60.7	7.8	65.6	15.6	13.1

APPENDIX F: DATA FOR RATS IN GROUP E1

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	7.9	14.1	45.4	1005	55.0	7.4	63.4	15.4	11.7
2	7.8	13.3	42.1	1210	57.4	6.3	67.3	14.6	13.7
3	8.0	12.8	44.1	1037	57.9	6.1	60.4	14.3	12.7
4	8.1	12.5	44.9	1212	57.4	6.7	64.2	15.1	12.3
5	7.7	12.6	44.9	1009	54.9	6.5	65.4	14.8	12.6
6	8.2	11.4	45.0	1007	56.7	6.9	68.3	15.3	13.2

APPENDIX G: DATA FOR RATS IN GROUP E2

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	7.9	13.2	44.0	1004	60.1	6.0	65.8	15.6	12.1
2	7.7	14.0	38.5	1101	57.2	6.0	69.1	14.4	10.1
3	6.4	13.2	45.3	1034	55.7	6.1	70.5	13.3	12.2
4	9.2	12.6	39.7	1106	58.4	5.8	66.2	15.0	13.2
5	7.6	13.5	42.3	1024	54.5	6.1	61.9	15.6	10.6
6	7.8	12.7	40.7	1207	56.7	5.9	70.8	14.7	11.6

APPENDIX H: DATA FOR RATS IN GROUP E3

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	6.2	11.4	42.1	790	70.9	7.5	52.6	17.0	14.7
2	7.6	12.1	37.5	860	72.2	8.9	53.5	16.0	13.9
3	6.6	12.4	43.8	770	71.8	8.4	53.1	16.4	13.7
4	6.4	10.8	41.1	785	72.5	8.2	52.6	17.5	14.2
5	6.8	11.2	42.3	747	74.3	8.0	53.8	18.1	13.6
6	6.6	11.9	40.9	825	70.1	8.8	55.2	16.7	13.6

APPENDIX I: DATA FOR RATS IN GROUP E4

RAT	RBC	HGB	HCT	PLT	CTE	UREA	T. PTN	ALT	AST
1	7.7	12.5	43.1	1018	60.4	6.5	63.5	15.7	10.2
2	8.9	13.2	44.0	1004	55.1	6.4	66.9	13.7	12.1
3	7.9	13.3	44.2	1044	53.9	7.0	62.5	13.4	12.4
4	6.9	12.1	40.7	1057	52.6	6.3	67.9	14.1	10.6
5	8.6	14.4	41.1	1027	54.3	6.4	61.4	15.1	11.7
6	7.5	13.3	43.5	1074	52.4	6.3	65.2	13.7	12.5