LIVER AND KIDNEY FUNCTION TESTS IN EXPERIMENTAL RATS EXPOSED TO CAR PAINTS AND ADDITIVE

A RESEARCH PROJECT PRESENTED BY

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SUPERVISOR: DR. NEBEDUM, J.O.

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IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOCHEMISTRY, NNAMDI AZIKIWE UNIVERSITY, AWKA.

SUPERVISOR: DR. NEBEDUM J.O.

JUNE 2011

DEDICATION

This research project is dedicated to God Almighty who has always been with me at all times.

ACKNOWLEDGEMENT

I give thanks to God Almighty for His grace and mercies upon me during this research project. My wonderful and kind supervisor, Dr. Nebedum, J.O. who has been my guide and anchor from the beginning of this research work. I acknowledge in a special way, my Parents, Mr. and Mrs. Nwalutu, O. Joseph, I say thank you for your support and encouragement. I give thanks to the laboratory technologists- Mr. Anagonye Callistus and Miss Anajekwu Bene for their wonderful support during my bench work.

I want to use this medium to say a big thank you to the galvanizer of this defence, Dr Udedi, S., to my lecturers and staffs of Biochemistry department, Nnamdi Azikiwe university for their positive contributions and to Mr. Okeke, O. that saw to the typing of this thesis. To my brothers, sisters, friends and colleagues who in one way or another contributed positively to the success of this research project, I want to say a big thank you to you all.

TABLE OF COENTENTS

Assay for tissue damage

Liver function test

Alanine aminotransferase

Aspartate aminotransferase

Alkaline phosphatase

Bilirubin

The kidney

Nephrotoxicity

Acute renal injury

Kidney function test

Creatinine

i. Blood urea nitrogen

Chloride

Potassium

Bicarbonate

Chapter Three

Materials and methods

Grouping of experimental rats

Administration of car paint chemicals

Sacrifice of experimental and control rats

Collection of serum/sample

Determination of serum alanine aminotransferase

Determination of serum aspartate aminotransferase

ABSTRACT

The effects of fumes from car paints (hardener, primer, clear coat and their synergistic combination) and additive (thinner) used in car refurbishing industry were investigated on liver and kidney functions in exposed albino rats. Rats (n=64) were divided into 6 groups consisting of 12 rats each, 5 of which were exposed to individual chemicals (thinner, hardener, primer, clear coat, synergistic combination) for 28days and the sixth, being the control (unexposed) group. On the 3rd, 7th, 14th and 28th day, three rats from each group were killed and their sera analyzed for serum enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and bilirubin to asses liver function and sodium, potassium, urea, creatinine, bicarbonate and chloride ion concentration to assess kidney function. Results obtained indicated that serum enzymes and bilirubin increased significantly in all the test groups relative to the control group, but most significantly for AST, ALP and bilirubin at $P<0.05$. Parameters for kidney function increased in all the test groups when compared to the control but most significantly for creatinine, bicarbonate and potassium at $P<0.05$. Most of the parameters were increased by the 7th day but fell towards control values by the 28th day. In general, synergistic combination had the greatest effect in most cases while primer and thinner gave the least effects on both liver and kidney functions. It is therefore concluded that exposure to fumes of car paints and additive may cause both kidney and liver dysfunction.

LIST OF FIGURES

CHAPTER ONE

1.0 INTRODUCTION

Approximately 200,000 workers worldwide are employed in paint manufacture. The total number of painters is probably several millions in which large number of painters are employed in automotive industry (Janko et. al., 1992).

Thousands of chemical compounds are used in paint products as pigments, solvents, additives and binder. In addition to polyisocyanate, (which is a component of hardener) paint constituents include organic solvents (ethylacetate, toluene, mixed dibasic esters, petroleum naptha, butyl acetate, ethyl benzene, xylene and methyl ethyl ketone), metals (Lead and Chromium) and trace quantities of hexamethylene diisocyanate (HDI) monomers (Janko et. al., 1992). Hepatotoxic effect of some of these solvents was recognized early because they produce a chronic hazardous health effect. However, it usually takes decades to document such a problem, for example vinyl chloride was once thought to be safe and had been used for forty two years until it was found to cause liver angiosarcoma (Alelson, 1983).

During spray painting in autobody repair shops, workers are exposed to all of the paint components which are atomized. The

routes of exposure are usually through the airways or skin. Thus, many organ systems may be affected simultaneously (Lundberg and Marianne, 1984). Some solvents are metabolically activated and damage the liver in man. Many solvents are considered potentially hepatotoxic singly or in combination with other substances. This is through an increased formation of toxic solvent metabolites resulting from the induction of microsomal enzymes which damage liver (Lundberg and Marianne, 1984).

In addition to the deleterious effect on the liver, toxic solvent has been found to affect the renal system, skin, cardiovascular system, mucous membranes etc (Kaukainen, et. al., 2003).

Liver and kidney injury is associated with occupational exposure to a wide variety of chemicals (Franco, et. al., 2004). Liver enzymes are often determined in routine occupational health examination for the screening of latent liver disease and adverse liver effect caused by solvent exposure (Kaukianen, et. al. 2003).

Although many investigators (Kurppa and Husman, 1982; Lungberg and Hakensson, 1985 and Kurppa, et. al., 1983) have reported that spray painting operation and paint manufacture usually do not produce damage to the liver. However, the possible toxicities of individual paint components and the incessant and reckless exposure to paints by workers at local

car refurbishing factories necessitated a comprehensive investigation into health consequences of such exposures.

1.1 RATIONALE

The economic situation in the country which has led to the increase in importation of fairly used cars and the high rate of road accidents as a result of bad roads have brought a boost to the car refurbishing industry in Nigeria. Workers and painters often work in poorly ventilated environments without adequate protection. Thus, the possible toxicities of individual paint compounds and the incessant and reckless exposure to paints by workers at local car refurbishing factories necessitated a comprehensive investigation into health consequences of such exposures.

1.2 AIMS AND OBJECTIVE

The aim of this research is to investigate the effect of car paint chemicals and additive on the Liver and Kidney functions by applying routine diagnostic test for these organ system functions as biochemical probes to detect perturbation and early pathological manifestation associated with car refurbishing industry.

The objects of this study is therefore to determine which of the car paint or additive causes most deleterious effect on the liver.
To determine which of the car paint or additive causes most deleterious effect on the kidney.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PAINT

Paint is any liquid, liquefiable or mastic composition which after application to a substrate in a thin layer is converted to an opaque solid film (Heitbrink, et. al., 1995).

Paints are made up of

a) Pigments; granular solid incorporated into paint to contribute color, toughness, texture. They can be classified as either natural or synthetic types. Some pigments are toxic such as the lead pigments that are used in lead paint.
b) Binder or vehicle which are film forming component of paint
c) Solvent which serves the function of adjusting the curing properties and viscosity of the paint. It is volatile and does not become part of the paint film. It also functions as the carrier for the non - volatile components. Solvent borne (oil based) paint can have various combinations of solvents as the diluent, including aliphatic, aromatic, alcohols, ketones and white spirit. These include organic solvents

such as petroleum distillate, esthers, glycol ethers etc, sometimes volatile low molecular weight synthetic resins also serve as diluents.

d) Additives which are miscellaneous ingredients added to modify surface tension, improve flow properties, improve the finished appearance, increase wet edge, improve pigment stability and control foaming (Heitbrink. et. al., 1995).

2.2 CHEMICAL IN PAINT MATERIALS

The following are chemicals present as ingredients in the following types of paint coating;

Hardener: Hardeners are made up of ethyl acetate, toluene, aliphatic polyisocyanate and hexamethylene diisocyanate

Clearcoat; The ingredients in clearcoat are methyl ethyl ketone, toluene, mixed dibasic esters and petroleum naptha

Thinner; The solvents in thinner are butyl acetate, ethylbenzene, toluene/xylene and methyl ethyl ketone

Primers; Primer is made up of, barium sulfate, resins and fillers, toluene/xylene and isocyanates.

Ethyl acetate, toluene, mixed dibasic esters, petroleum naptha, butyl acetate, ethyl benzene, xylene, are all organic solvents which are common chemicals in paint coatings. (Tornling, et. al., 1990)

2.3 ADDITIVES

Paint can have a wide variety of miscellaneous additives, which are usually added in very small amounts and yet give a very significant effect on the product. Some examples include additives to modify surface tension, improve flow properties, improve the finished appearance, increase wet edge, improve pigment stability, impart antifreeze properties, control foaming, control skinning (Heitbrink, et. al., 1995). Other types of additives include catalysts, thickeners, stabilizers, emulsifiers, texturizers, adhesion promoters, ultraviolent stabilizers, flatteners (de-glossing agents) and biocides to fight bacterial growth (Heitbrink, et. al., 1995).

2.4 SOLVENTS

Millions of workers are exposed to solvents on daily basis. Solvents are used in a wide range of industries (construction, maritime, retail and general industries). They are used in the manufacture of paints, varnishes, lacquers, paint removers, plastics, adhesives, and printing inks (NIOSH, 1987).

Solvents include a variety of commonly used chemicals such as alcohol, mineral spirits, petroleum distillate, benzene, toluene, xylene, perchloroethylene, trichloroethylene, methyl ethyl ketone (MEK), gasoline and kerosene (NIOSH, 1992). The national institute for occupational safety and health (NIOSH)

USA estimates that there are almost 10 million workers potentially exposed to organic solvents in the workplace (NIOSH, 1992).

Most exposed workers come in contact with a combination of several solvents (e.g. paint formulation, paint thinner, varnishes etc). The principal health effects most typically associated with organic solvent exposure include nervous system damage, kidney and liver damage, adverse reproductive effect such as sperm changes and infertility, skin lesion and cancer (NIOSH, 1977).. These agents can also cause death from acute exposure resulting from depression of the brain's respiratory center and/or cardiac arrhythmias (Ford, et. al., 1995). Solvents associated with liver or kidney damage include toluene, carbon tetrachloride, 1,1,2,2-tetrachloroethane, chloroform (Lungberg, et. al., 1994), acetone, aliphatic hydrocarbon, aromatic hydrocarbon A and B, xylene, ethylacetate, formaldehyde, methyl isocyanate, 2-butoxy ethanol, ethylbenzene (Myer, et. al., 1993).

2.5 POTENTIAL TOXICITY DURING CAR PAINTING PROCESS

During filling of the spray gun the hand is the only region of the body exposed to the painting chemicals. For this reason the potential exposure of the hands is higher during the filling of

the spray gun than during the spraying of the vehicle or cleaning the spray gun than during the filling of the spray gun mainly due to the fact that the process is carried out inside a booth with a local exhaust system (Liu and Sparer, 2007). The potential dermal exposure for the body is greater than potential hand exposure, which is in contrast when filling the spray gum (Liu and Sparer, 2007)

The main body regions exposure during cleaning of the spray gun is the hands, though there might be splashes to other parts of the body. The head and the back of the body are not exposed to the paint (Liu and Sparer, 2007).

2.6 THE LIVER

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions including detoxification, protein synthesis and production of biochemical necessary for digestion (Suzuki, et. al. 2008). Liver plays a major role in metabolism and has a number of functions in the body including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification. It produces bile which aids in digestion via the emulsification of lipids. The Liver is highly specialized tissue regulates a wide variety of high volume biochemical reactions including the synthesis and breakdown of small and complex

molecules, many of which are necessary for normal vital function (Bramstedt, 2006). When the liver is damaged, the plasma membrane becomes permeable and enzymes and substance found in the liver leak out of it. The liver is the only internal human organ capable of natural regeneration of lost tissues: as little as 25% of the liver can regenerate into a whole liver, predominantly due to the hepatocytes re entering the cell cycle. (Chen and Chen, 1994).

2.7 HEPATOXICITY

The liver plays a central role in transforming and clearing chemicals and is susceptible to toxicity from these agents. Certain medicinal agents when taken in overdoses and sometime even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatoxicity (Ostapowicz, et. al., 2002). Chemicals often caused subclinical injury to liver which manifest as abnormal liver enzyme test. (Jaeschke, et. al., 2002) Chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (e.g. ALT, AST, ALP, Bilirubin) are often used to indicate liver damage. Liver injury is defined as a rise in either

a) ALT concentration more than three times its upper limit of normal (ULN)
b) ALP concentration more than twice ULN concentration
c) AST concentration more than twice ULN concentration
d) Total bilirubin concentration more than twice ULN when associated with increased ALT or ALP (Mumoli, et. al., 2006 and Benichon, 1990).

2.7.1 FACTORS CONTRIBUTING TO THE HEPATOTOXICITY BY SOLVENTS

Organic solvents utilized in various industrial processes may be associated with hepatotoxicity. Factors contributing to the hepatotoxicity of solvent include; species difference, liver blood flow, protein binding, point of binding intracellularly, genetic factors, different cellular enzymatic degradation, age, nutritional condition, interaction with alcohol, interaction with medications of use and abuse (Nachman and John, 2002).

2.8 MECHANISM OF LIVER DAMAGE

Due to the Liver's unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury by drugs and other substances. 75% of blood coming to the liver arrives directly from gastrointestinal organ and then spleen

via portal veins which brings drugs and xenobiotics to near undiluted form. (Patel, et. al., 1998).

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria- an intracellular organelle that produce energy. Its dysfunction releases excessive amount of oxidants which in turn injure hepatic cells. Activation of some enzymes in the cytochrome p-450 system such as CYpze1 also leads to oxidative stress (Jaeschke, et. al., 2002). Injuries to hepatocyte and bile duct cells leads to accumulation of bile acid inside liver, promoting further liver damage (Patel, et. al. 1998)

Apoptosis in liver disease is a ubiquitous form of cell death occurring in human liver. It has been defined morphologically by the presence of cytoplasmic shrinkage (pyknosis), chromatin condensation, nuclear fragementation (Karyorhexis), the presence of plasma membrane blebbing and the maintenance of an intact plasma membrane which retains its integrity as the cell fragments into apotptotic bodies (Kroemer, et. al., 2005).

Caspases are a group of intracellular enzymes that mediate the cellular demolition characterized phenotypically as apoptosis. Mitochondrial permeabilization is not only requisite but also sufficient for hepatocyte apoptosis: Therefore, regulators downstream of mitochondrial permeabilization cannot prevent cell death (Green and Kroemer, 2004). Unlike developmental

apoptosis which is carefully regulated in a spacio termporal pattern and does not involve secondary events, pathologic apoptosis is unregulated and can be massive and correlates with outcome i.e. liver transplantation or death (Feldstein, et. al. 2003). Hepatocyte apoptosis is evident in liver injury related to viral hepatitis, metabolic diseases, alcoholic steatohepatitis, chemical induced liver injury and autoimmune hepatitis (Kohli, et. al. 1999, Natori, et. al., 1999, Natori, et. al., 2001, Papakyriakon, et. al., 2002 and Feldstein, et. al., 2003).

Apoptosis of other cellular components such as sinusoidal endothelial cells and stallate cells also plays a role in liver injury. Fibrosis is the hallmark of ongoing liver injury. Hepatic stellate cells mediate hepatic fibrosis. In the normal liver, stellate cells maintain a quiescent phenotype, on activation they undergo a metamorphosis to become myofibroblasts secreting collagen which leads to liver fibrosis (Canbay et. al., 2004).

2.9 DISEASES OF THE LIVER

The liver supports every organ in the body and is vital for survival, because of its strategic location and multidimensional functions the liver is prone to many diseases, the most common include; infections such as hepatitis A, B, C, E, alcohol damage, fatty liver, cirrhosis, cancer, drying and chemical damage. (Cotran et. al., 2005)

Many diseases of the Liver are accompanied by jaundice caused by increased levels of bilirubin in the system. Diseases that interfere with liver function will lead to derangement of the above mentioned processes. However, the liver has a great capacity to regenerate and has a large reserve capacity. In most cases the liver only produces symptoms after extensive damage (Bramstedt, 2006).

2.10 ASSAY FOR TISSUE DAMAGE

Analysis of certain enzyme activities in blood serum gives valuable diagnostic information for a number of disease conditions.

AST/GOT (Aspartate transaminase/Glutamic oxaloacetic transaminase), ALT/GPT (Alanine transaminase/Glutammic oxaloacetic transaminase) are important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity or infection (David and Michael, 2007). The SGOT (serum glutamic oxaloacetic transaminase) tests are also important in occupational medicine, to determine whether people exposed to carbon tetrachloride, chloroform or other industrial solvents have suffered liver damage (David and Michael, 2007).

Liver degeneration caused by these solvents is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotransferases are most useful in monitoring people

exposed to these chemicals, because these enzyme activities are high in liver and can be detected in very small amount.

2.11 LIVER FUNCTION TESTS

Liver function tests (LFT or LFs), which include liver enzymes are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patients liver. Hepatic (liver) involvements in some diseases can be of crucial importance. Some tests are associated with functionality (e.g. albumin), some with cellular integrity (e.g. Transaminase) and some with conditions linked to the biliary tract (Alkaline phosphatase and Gamma-glutamyl transferase) (Nyblom, et. al. 2006).

Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to; detect the presence of liver disease, distinguish among different types of liver disorders, guage the extent of known liver damage and follow the response to treatment (Nyblom, et. al., 2006).

Standard liver panel include the following; Alanine transaminase, Aspartate transaminase, Alkaline phosphatase, Total bilirubin, Direct bilirubin and Gamma glutamyl transpeptidase. (Nyblom, et al., 2004).

In blood serum, measurement of the activity of specific enzyme in a sample of blood serum is usually for the purpose of identifying a disease. The enzymes normally are concentrated in cells and tissues where they perform their catalytic function. In disease, however, certain enzymes tend to leak into the circulation from the injured cells and tissues. More than 50 enzymes have been found in human serum, some of these enzymes are often used to identify liver damage or dysfunction. In routine clinical practice the most common ones used include:

Transaminase; They are found in most body tissues, but in particularly high concentrations in the liver and heart tissue and are substantially increased in serum in disorder involving the liver and heart.

Acid Phosphatase: An enzyme found in most body tissue but in unusually high concentration in the adult prostrate gland, it is released into circulation in metastatic cancer of the prostate.

Alkaline phosphatase: an enzyme found in most body tissues, notably in bone marrow and liver. Serum values is usually elevated in conditions as paget's disease (inflammation of the bone) and Osteomalacia (softening of the bone) as well as in hepatitis and obstructive jaundice.

Amylase: A starch-digesting enzyme that originate chiefly from the pancreas and salivary glands. Its serum activity is usually elevated in the early stages of acute inflammation of the

pancreas, in obstruction of the pancreatic duct and in mumps (Encyclopedia Britannica, 2009).

2.11.1 ALANINE AMINOTRANSFERASE (ALT)

Alanine transaminase (ALT) also called Glutammic pyruvic transaminase or Alanine aminotransferase (AAT) is a pyridoxal transaminase enzyme found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyse the transfer of an amino group from alanine to a ketoglutarate, the products of this reversible transaminase reaction being pyruvate and glutamate (Li-Min Lun, et. al., 2006)

Alanine aminotransferase is primarily synthesized in the liver (Segal, et. al., 1962; Gatehouse, et. al., 1967; Matsuzawa, et. al., 1977 and Walton and Cowey, 1982), though its activities exist in other tissues including heart, muscle, kidney and brain (Milton, et. al., 1967; Mckenna et. al., 1996)

Alanine aminotransferase is an index for the diagnosis of liver function (Sherman, 1991; Cahen, et. al., 1996, Dufour, et. al., 2000). Some authors such as Bardella, et. al. (1995) and Korones, et. al. (2001) believes that ALT is not exclusively an index for the diagnosis of liver function.

Alanine aminotranferase level is particularly high in the liver and its serum activity is significantly elevated during liver

damage caused by drug toxicity and infection while the total protein level remains constant (Patnaik and Kanungo, 1976; Sherman, 1991; Agarawal, et. al., 1996; Cahen, et. al. 1996).

ALT is thought to be more specific indicator than Aspartate aminotransferase- an enzyme found in the cytosol and mitochondria (Rej, 1971). Serum levels of ALT normally are low- 167 to 667 nkat/L (10-40 u/L) in most laboratories. However, normal values may vary greatly among laboratories.

Any type of liver cell injury can modestly increase ALT levels and values up to 5000nkat/L (300 V/L) are considered nonspecific. Marked elevations – that is, ALT levels greater than 8350nKat/L (500 u/L) occur most often in persons with diseases that affect primarily hepatocytes such as viral hepatitis, ischemic liver injury (shock liver) and toxin induced liver damage. Despite the association between greatly elevated ALT levels and hepatocellular diseases, the absolute height of the ALT elevation does not correlate with the extent of liver cell damage (Kallei, et. al.., 1964).

When elevated ALT levels are found in the blood, the possible underlying causes can be further narrowed down by measuring other enzymes for example; elevated ALT levels due to liver-cell damage can be distinguished from bilary duct problems by measuring alkaline phosphatase (Gopal and Rosen, 2000).

2.11.2 ASPARTATE TRANSAMINASE (AST)

Apartate transaminase also called serum glutamic oxaloacetatic transaminase (SGOT) or aspartate aminotransferase (AST) is an enzyme associated with liver parenchymal cells. It facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate and vice versa. The transfer is performed by pyridoxal-5-phosphate (Kondo, et. al., 1985).

Aspartate aminotransferase exist in human tissues as two distinct isoenzyme- one located in the cytoplasm (C-AST) and the other in mitochondria (M-AST). These different isoenzymes can be useful in evaluating damages to organs (striated muscle, myocardium, and Liver tissues) where they are found (Mauro, 1990). Aminotransferase shows the greatest magnitude of increase because these enzymes are released into circulation when hepatic cells become damaged or die. AST is present in high concentrations in the cytoplasm of the Liver, Kidney, myocardial and skeletal muscle and occur in other organs with lower activities (Smita, et. al., 2009).

The amount of AST is directly related to the number of cells affected by the disease or injury, but the level of elevation depends on the length of time the blood was tested after the injury. Serum AST level becomes elevated eight hours after cell injury, peak at 24-36 hours and return to normal in three to

seven days. If the cellular injury is chronic, AST level will remain elevated (Pagana, 1998).

The activities of AST in the liver, kidney, heart, and skeletal muscle are 7000-, 4500-, 8000-, and 5000- fold higher respectively, than the AST activity in nonpathologic serum. For ALT, these relative activities are 2800-, 1200-, 400-, and 300-, fold higher respectively. Thus, ALT has more specificity for the Liver than AST and damage to cardiac or skeletal muscle can cause greater increase in AST than ALT (Lott and Wolf, 1986).

2.11.3 ALKALINE PHOSPHATASE (ALP)

Alkaline phosphatase is a membrame bound metalloenzyme which catalyses the hydrolysis of phosphomonoesterase at an alkaline pH. (Dengshun and Andrew, 2002). It is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The optimal pH for the activity of E. coli ALP enzyme is 8.0 (Garen and Levinthal, 1960).

Most human tissues contain ALP. Kidney, liver, bone, intestine, recticuloendothelial tissue and placenta are particularly rich sources. ALP derived from different tissues share catalytic properties, but differ in their chemical and physical characteristics. (Wolf, 1978). At least four isoenzymes of ALP have been identified in humans: non-specific liver/bone/kidney,

intestinal, placental and germ cell ALPs. The ALP produced by osteoblasts (OBs) and neutrophilic granulocytes is of the nonspecific liver/bone/kidney type of ALP. (Hoof and Bore, 1994). Alkaline phosphatase has become a useful tool in molecular biology, it can be used in removing phosphate group on the 5-end. This prevents DNA from ligating, thereby keeping DNA molecular linear until the next step of the process for which they are being prepared. Removal of the phosphate groups allows radiolabelling (Garen and Levinthal, 1960).

All mamalian alkaline phosphatase isoenzyms except placental (PALP and SEAP) are inhibited by homoarginine and similarly all except the intestinal and placental ones are blocked by levamisole. Heating for approximately 2hours at 65^0c inactivates most isoenzymes except placental isoforms (PALP) (Garen and Levinthal, 1960).

ALP activity in normal adult serum is derived predominantly from hepatic and reticuloendothelial sources. Physiologic bone growth in children increases serum activity to 2 to 5 times the level observed in adults. Serum enrichment by intestinal isoenzyme may cause postprondial elevation particularly in individuals with blood group B or O who is also secretors of ABH blood group substance.

The location of hepatic ALP on the sinusoid membrane suggest involvement in transport function. Increased ALP synthesis is

observed during cholestasis with increased bile acid concentration caused by intrahepatic obstruction of the bilary tree (Wolf 1978). It appears that the enzyme is associated with lipid transport in intestinal epithelium and with calcification process in bone (Gupta, et. al. 2004).

The normal range of ALP is 20 to 140 IU/L (Rajasonya, and Li-Fern, 1999), but varies widely with methodology of preparation (Wolf, 1978).

The majority of sustained elevated ALP levels are associated with disorders of the liver or bone or both. A variety of primary and secondary hepatic conditions may be associated with elevated serum ALP levels. Since production is increased in response to cholestasis, serum ALP activity provides a sensitive indicator of obstructive and space occupying lesions of the liver. Hepatic cell lesion is manifested by hyperbilirubinemia and dominant serum elevation of parenchymal enzymes such as aminotransferases (Wolf, 1978).

2.11.4 BILIRUBIN (BIL)

Bilirubin (formerly refered as hematoidin) is the yellow breakdown product of normal heme catabolism. Bilirubin is excreted in bile and its levels are elevated in certain diseases. It is responsible for the yellow colour of bruises and the yellow discolouration in jaundice (Liu, et. al. 2008).

Bilirubin is synthesized by the activity of bilirubin reductase on biliverdin. Bilirubin when oxidized reverts to become biliverdin once again. Bilirubin has been demonstrated to have potent antioxidant activity. Bilirubin main physiological role is as a cellular antioxidant (Baranano, et. al., 2002).

Erythrocytes are disposed of in the spleen when they get old or damaged, releasing hemoglobin which is further degraded to heme and is further turned into unconjugated bilirubin in the macrophages of the spleen while the globin parts are turned into amino acids. Unconjugated bilirubin (which is not soluble in water) is bound to albumin and sent to the liver.

In the liver it is conjugated to glucoronic acid and much of it goes into the bile and out into the small intestitine. Much of the amount of bilirubin is excreted through the intestine, but tiny amount of bilirubin in the urine accounting for it light yellow colour. If the liver's function is impaired or when biliary damage is blocked, some of the conjugated bilirubin leaks out of the hepatocyte and appears in the urine turnig it dark amber.

Bilirubin is a potent antioxidant that may provide cytoprotection (Rigato, et. al., 2005 and Sedlak and Snyder, 2004).

Tissue concentrations of bilirubin, about 20-50nM are much too low to cope with the mM levels of reactive oxygen species that most cells encounter. Very high level of bilirubin are

neurotoxic and elevated serum levels of bilirubin are associated with diminished risk of coronary artery disease (Djousse, et. al., 2003). Bilirubin inhibits lipid oxidation and oxygen radical formation (Michael, 2000). As little as 10nM bilirubin protects against 10,000 higherfold concentrations of hydrogen peroxide H_2O_2 (Dore, 1999). The antioxidant actions of bilirubin are amplified by bilirubin reductase in a biliverdin-bilirubin cyle. When bilirubin acts as an antioxidant it is oxidized to biliverdin which is rapidly reduced by Biliverdin reductase (BVR) to bilirubin. Depletion of Biliverdin Reductase (BVR) by RNA interference markedly diminishes the cytoprotective effects of exogenous bilirubin and lead to increased cellular level of oxgen free radicals and cell death (Sedlak and Snyder, 2004).

2.12 THE KIDNEY

The kidney is an organ with several functions It serves regulatory roles in most animals including vertebrates and some invertebrates. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid base balance and regulation of blood pressure (Al-Kahtani et. al., 2004). They serve the body as a natural filter of the blood and remove wastes which are directed to the urinary bladder. In producing urine, the kidney excretes waste such as urea and ammonium. The kidney is also

responsible for the reabsorption of water, glucose and amino acids and production of some hormones including calcitriol, renin and erythropoietin (Weinberg, 1991).

The kidney participates in whole body homeostasis, regulating acid base balance electrolyte concentration, extracellular fluid volume and regulation of blood pressure (Haynes and webb, 1993).

Diseases of the kidney are diverse but individuals with kidney disease frequently display characteristic chemical features. Common chemical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury. Chronic kidney disease, urinary tract infection, nephrohthiasis and urinary tract obstruction (Corwin and Bonventre, 1984).

2.13 NEPHROTOXICITY

Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. The nephrotoxic effect of most drugs is more profound in patients who already suffer from renal impairment. Some drugs may affect renal function in more than one way. Nephrotoxicity is usually monitored through a decreased creatinine clearance which indicates poor renal function. Normal creatinine clearance levels are between 80 - 120 µmol/L. Serum creatinine

is another measure of renal function, which may be more useful clinically when dealing with patients with early kidney disease (Galley, 2000).

2.14 ACUTE RENAL INJURY

Acute kidney injury is the abrupt decline in renal function with a decrease in glomerular filtration rate (GFR) resulting in the retention of nitrogenous waste products. Acute renal failure has been classified as

a) Prerenal failure: denoting a disorder in the systemic circulation that causes renal hypoperfusion. Prerenal failure is often followed by
b) Intrinsic renal failure: where correction of the circulatory impairment does not restore the normal GFR and
c) Post renal failure (obstruction) (Lote, et. al., 1996)

2.15 KIDNEY FUNCTION TESTS

Kidney function tests are a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning. There are a number of urine tests that can be used to assess kidney function. A simple screening test—a routine urinalysis—is often the first test conducted if kidney problems are suspected. A small, randomly collected urine sample is examined physically for color, odor,

appearance, and concentration (specific gravity); chemically, for substances such as protein, glucose, and pH (acidity/alkalinity); and microscopically for the presence of cellular elements (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells), bacteria, crystals, and casts (structures formed by the deposit of protein, cells, and other substances in the kidneys' tubules) (Lote, et. al., 1996). If results indicate a possibility of disease or impaired kidney function, one or more of the following additional tests is usually performed to pinpoint the cause and the level of decline in kidney function.

Blood urea nitrogen test (BUN); Urea is a byproduct of protein metabolism. Formed in the liver, this waste product is then filtered from the blood and excreted in the urine by the kidneys. The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because BUN is also affected by protein intake and liver function, the test is usually done together with a blood creatinine, a more specific indicator of kidney function.

Creatinine test; This test measures blood levels of creatinine, a by-product of muscle energy metabolism. It is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on a person's muscle mass, which usually fluctuates very little. With normal kidney function, then, the

amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine level is a more sensitive indicator of impaired kidney function than the BUN.

Other blood tests can also be useful in evaluating kidney function. These include; sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus ions, protein, uric acid and glucose (Wallach, 2000).

2.15.1 CREATININE

Creatinine which is an indicator of kidney function is a breakdown product of creatine phosphate in muscle. It is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the body by the kidney, though a small amount is actively secreted by the kidney into the urine. There is little to no tubular reabsorption of creatinine, if the filtering by the kidney is defective, blood level rises (Delanghe, et. al., 1989).

Serum creatinine is decreased in individuals with small stature, cachexia, amputations or muscle disease. Advanced liver disease causes low serum creatinine because of decreased hepatic conversion of creatine to creatinine and increased renal tubular secretion of creatinine (Spencer, 1986).

Acute renal failure (ARF) is a sudden loss of renal function occurring over several hours to days. An increased serum creatinine concentration, accumulation of nitrogenous wastes and a decline in urinary output are the hallmarks of ARF (Mathew, 2005).

Causes of high creatinine level include intrinsic renal disease, urinary tract obstruction and reduced renal blood from congestive heart failure, shock or dehydration (Mathew, 2005).

The ratio of blood urea nitrogen (BUN) to creatinine can be used to determine the etiology of acute renal failure. Normally, the ratio is 10 to 1. The ratio usually exceeds 20 in prerenal failure due to decreased renal perfusion, heart disease, shock, dehydration, hemorrhage, etc. postrenal diseases such as urinary tract obstruction, bladder or urethra also increased the ratio between 10 and 20. It is normal (10 to 12) in intrinsic renal disease because BUN and creatinine rise proportionally (Lawson et. al. 2002)

2.15.2 BLOOD UREA NITROGEN (BUN)

The blood urea nitrogen test is a measure of the amount of nitrogen in the blood in the form of urea and a measurement of renal function. Urea is a by-product from metabolism of proteins by the liver and therefore removed from the blood by the kidneys (Johnson, et. al., 1972). The most common cause of

elevated BUN is poor kidney function (Johnson, et. al., 1972). A greatly elevated BUN (>60mg/dl) generally indicates a moderate to severe degree of renal failure. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock; it could also be due to acute or chronic disease of the kidneys (Johnson, et. al., 1972). An elevated BUN with normal creatinine may reflect a physiological response to a relative decrease of blood flow to the kidney without indicating any true injury to the kidney. Increased production of urea is seen in cases of moderate to heavy bleeding in the upper gastrointestinal tract.

A low BUN usually has little significance, its causes include liver problems, malnutrition etc. BUN will only be elevated outside normal when more than 60% of kidney cells are no longer functioning (Johnson, et. al.. 1972).

2.15.3 CHLORIDE ION (Cl^-)

Chloride is an inorganic anionic halogen distributed exclusively within the extracellular fluid compartment (ECF), it is the major anion associated with sodium in the ECF. (Blume, et. al., 1968). Chloride ions have important physiological roles-in the central nervous system, the inhibitory action of glycine and some of the action of GABA relies on the entry of Cl^- into specific neurons. Also, the chloride-bicarbonate exchanger biological tansport

protein relies on the chloride ion to increase the blood capacity of carbondioxide in the form of the bicarbonate ion. It works with other electrolytes such as potassium, sodium and carbon dioxide to help keep the proper balance of body fluid and maintain the body's acid base balance (Narins and Emmett, 1980).

Chloride levels can be used to help monitor high blood pressure, heart failure and kidney disease. High level of chloride (hyperchloremia) indicates dehydration, metabolic acidosis and other conditions. Decreased level of chloride (hypochloremia) can indicate kidney disorder, addison's disease, congestive heart failure etc. (Seldin and Rector, 1972).

2.15.4 POTASSIUM ION (K^+)

Potassium is the most abundant cation in the body. The vast majority of potassium is in the intracellular compartment with a small amount in the extracellular space. Normal serum potassium is 3.5 to 5.5 mEq/L; however, plasma potassium is 0.5 mEq/L lower.

Serum potassium concentration does not always reflect total body potassium; therefore, hypokalemia could coexist with both normal or low total body potassium. Hypokalemia with normal total body potassium is due to a shift of potassium into the cell. Hypokalemia without intracellular shift of potassium is seen

when there is marked potassium deficiency. Hypokalemia without intracellular shift of potassium is seen when there is marked potassium deficiency. Hypokalemia associated with low total body potassium is either due to poor dietary intake or increased potassium loss from the body. Poor intake is an uncommon cause. Nevertheless, patients with extremely poor dietary intake (most commonly alcoholics) and patients on severe weight-reduction programs are in danger of developing severe potassium deficiency. Hypokalemia due to an increase in potassium loss is most commonly secondary to gastrointestinal disorders (Adrouge and Madias, 1981).

Hyperkalemia with normal total body potassium is caused by the shift of potassium out of the cell and is commonly seen in acidemia, sudden increase in plasma osmolality, massive tissue breakdown and, in very rare circumstances, adrenergic blockade and hyperkalemic periodic paralysis. Beta blockade can result in significant hyperkalemia during and immediately after exercise. This is because the potassium initially released from the muscle cells is normally taken up by these cells through the stimulation of beta-2 receptors by catecholamines. A sudden rise in osmolality can result in a modest increase in serum potassium (Arruda, et. al., 1981).

2.15.5 BICARBONATE (HCO_3^-)

Bicarbonate is a waste product of mitochondria respiration. It serves a crucial biochemical role in the physiological pH buffering system. It is the conjugate base of carbonic acid (H_2C0_3) and the conjugate acid of $C0_3^{2-}$. Bicarbonate is a vital component of the buffering systems of the body (Worthley, 1977). 70%-75% of $C0_2$ in the body is converted into carbonic acid (H_2C0_3) which can quickly turn into bicarbonate ($HC0_3^-$). Bicarbonate in conjunction with water, hydrogen ions and carbon dioxide form a buffering system which is maintainined at the volatile equilibrium (Worthley, 1977). This buffering system provide prompt resistance to drastic pH changes in both the acidic and basic directions. This is especially important for protecting tissues of the central nervous system where pH changes too far outside of the normal range in either direction could prove disastrous (Worthley, 1977). Bicarbonate also acts to regulate pH in the small intestine (Pitts, 1952).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Sixty four (64) Albino Rats (weighing between 82-130g) bought from Zoology Department, University of Nigeria, Nsukka were used.

A container of Thinner, Hardener, Primer and Clear coat purchased from Ossy Paint coy at Nkpor were used.

3.2 REAGENT

Randox Kits (United Kingdom) purchased from Linsolec Divine Enterprise Onitsha were used for analysis.

3.3 METHODS

3.3.1 GROUPING OF EXPERIMENTAL RATS

Experimental rats were left for two weeks to acclimatize to the environment before the commencement of research.

After acclimatization the albino rats were grouped into six-group of 12 rats each apart from the control: thinner, hardener, primer, clear coat, synergistic combination and control.

The control group had a total of 4 albino rats while the other 5 groups had 12 albino rats each.

3.3.2 ADMINSISTRATION OF CAR PAINT CHEMICAL

Different spray gun was used for different groups. Car paint (hardener, primer, clear coat and synergistic combination of the above car paints) and additive (thinner) was administered as fume two times daily morning (9.00am) and afternoon (3.00pm). Perforated (perforation just enough to allow breathing) cartons were used to cover cages before car paint administration and 30 shots of car paint fume was administered. After administration, albino rats were left to inhale car paint fumes for 2 hours after which cartons were removed.

The negative control group received nothing, they were placed far away from the site of car paint and additive administration. The procedure for car paint administration was repeated daily for 28 days.

3.3.3 SACRIFICE OF EXPERIMENTAL AND CONTROL RATS

Three experimental rats from each group were sacrificed on the 3rd, 7th, 14th and 28th days of the research and whole blood collected was used for analysis after separation of serum from blood cells. The negative control group was sacrificed on the 14th and 28th days.

3.3.4 COLLECTION OF SERUM/SAMPLE

Experimental rats were anaesthetized with chloroform and 7ml of whole blood was drawned from the cardiac cavity. Collected whole blood was emptied into a plastic tube and allowed to clot for 30minutes. The plastic tube was placed in a centrifuge and spinned at 1000rpm for 10minutes to separate serum from blood. Serum was pipette away from blood into a clean dry plain bottle using pasteur pipette.

3.4.1 DETERMINATION OF SERUM ALANINE AMINOTRANSFERASE

PRINCIPLE

Oxoglutarate + alanine $\longrightarrow$ glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957).

METHOD

Two test tubes were labeled “Reagent blank” and “sample”. One hundred microlitre (0.1ml) of serum was pipette into the test tube labeled sample. While 0.1ml of distilled water was dispensed in the test lebelled smple. Solution R1 (phosphate buffer, L – alanine, α oxoglutarate) (0.05ml) were dispensed into the test tubes. The content in each test tube were mixed and incubated for 30min at 37^0c. To each test tube, 0.5ml of

solution R2 was added followed by the addition of 5ml sodium hydroxide (to stop the reaction).

The content in each test tube was mixed and the absorbance of sample was read against the reagent blank after 5 minutes at 546nm wavelength. ALT concentration was determined using the table below.

Absorbance	**U/L**
0.025	4
0.050	8
0.075	12
0.100	17
0.125	21
0.150	25
0.175	29
0.20	34
0.255	39
0.250	43
0.275	48
0.300	52
0.325	57
0.350	62
0.375	67
0.400	72
0.425	77
0.450	83
0.475	88
0.500	94

3.4.2 DETERMINATION OF SERUM ASPARTATE AMINOTRANSFERASE

PRINCIPLE:

α-oxoglutarate + L aspartate $\longrightarrow$ L-glutamate + oxaloacetate

AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957).

METHOD

Two test tubes labeled “reagent blank” and “sample” were used for the analysis. To the test tube labeled sample 0.1ml of serum was added, 0.5ml each of reagent 1 (buffer) were added to both test tubes (reagent blank and sample), while 0.1ml of distilled water was added to the test tube labeled reagent blank. The contents in both test tubes were mixed, and left to stand for 30 minutes. Reagents 2 (2, 4-dinitrophenylhydrazine) (0.5ml) was added to test tubes labeled reagent blank and sample. The content in the test tubes were shaked and allowed to stand for 20 minutes at 25^0c. To each test tube (reagent blank and sample) 5.0ml each of sodium hydroxide were added. The test tubes were shaked and absorbance of sample (A sample) was read against reagent blank after 5 minutes at 546nm wavelength. The procedure was repeated three times. AST concentration was determined using the table below.

Absorbance	U/L
0.020	7
0.030	10
0.040	13
0.050	16
0.060	19
0.070	23
0.080	27
0.090	31
0.100	36
0.110	41
0.120	47
0.130	52
0.140	59
0.150	67
0.160	76
0.170	89

3.4.3 DETERMINATION OF SERUM ALKALINE PHOSPHATE

PRINCIPLE

P-nitrophenylphosphate + H_20 $\xrightarrow{ALP}$ phosphate + p-nitrophenol (Rec. Gscc, 1972)

PREPARATION OF REAGENTS

One vial of substrate - R1b (p - nitrophenylphosphate) was reconstituted with 10ml of buffer - R1a (diethanolamine buffer and $MgCl_2$) and stored in a reagent bottle labeled reagent.

METHOD

Into three cuvettes labeled “macro”, semi micro and “micro” were dispenced. 0.01ml, 0.02ml and 0.05ml test serum respectively. The reagents (3.0ml, 1.0ml and 0.50ml) were pipetted into the cuvettes respectively. The contents were mixed by shaking, the absorbance were read at a wavelength of 405nm. In 1, 2 and 3 minutes and the values recorded. The procedure was repeated three times. ALP concentration was obtained using the formula below.

ALP (U/L) = 2760 X Absorbance at 405NM/MIN semi micro

3.4.4 DETERMINATION OF SERUM BILIRUBIN

PRINCIPLE

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue colored complex.

Total bilirubin was determined in the presence of caffeine which released albumin bound bilirubin by the reaction with diazotized sulphanilic acid (Jendiasik and Grof, 1939).

METHOD

Two cuvettes were labeled “sample blank” and “sample”. To both cuvettes, 0.20ml of reagent 1 (sulphanilic acid and hydrochloric acid) was pipetted. Reagent 2 (sodium nitrite) (0.05ml). Reagent 3 (caffeine and sodium benzoate) (1ml) was pipette into the two cuvettes.

To the cuvette labeled blank and sample, 0.2ml of serum was pipetted. The contents in the cuvette were mixed and allowed to stand for 10mins at 25^0C.

One milliliter of reagent 4 (tartrate and sodium hydroxide) was added to each of the cuvettes. The contents in the cuvettes were mixed and allowed to stand for 30 minutes at 25^0C. Absorbance of the cuvette labeled sample was read against the blank at a wavelength 578nm.

The concentration of Bilirubin was calculated using the formula below.

Total bilirubin (mg/dl) = 10.8 x A_{TB} (578nm)

3.4.5 DETERMINATION OF SERUM CREATININE

PRINCIPLE

Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration (Bartels and Bohmer, 1972).

PREPARATION OF REAGENT

Equal volumes of solution R1a (picric acid) and R1b (sodium hydroxide) were mixed in a reagent bottle and labeled working reagent.

METHOD

Three cuvettes were labeled “reagent blank,” “standard” and “test sample”. To the cuvette labeled reagent blank, 100μl distilled water was pipetted, to the cuvette labeled “standard” 100μl standard was pipetted and to the cuvette labeled sample 100μl of serum was pipetted. One millilitre of working reagent was pipetted into each of the three cuvettes.

The contents in the cuvettes were mixed and after 30 seconds absorbance A1 of standard and sample were read at a wavelength 492nm. At exactly 2 minutes later, absorbance A2 of standard and sample were read at the same wavelength.

The concentration of Creatinine was calculated using the formula below.

Manual calculation

A2-A1 Concentration of creatinine in serum

$$\text{Sample or A standard } (\mu/\text{mol}/\text{l}) = \frac{\text{Asample}}{\text{A standard}} \times \text{standard conc.}$$

3.4.6 DETERMINATION OF SERUM UREA

PRINCIPLE

Urea + H_20 $\xrightarrow{\text{Urease}}$ $2NH_3 + C0_2$

NH_3 + hypochloride + phenol $\longrightarrow$ Indophenol (blue compound)

Urea in serum was hydrolysed to ammonia in the presence of urease. The ammonia was then measured photometrical by Berthelot's reaction (Weatherburn, 1967).

PREPARATION OF REAGENTS

The reagents were reconstituted according to the manufacturer's instruction thus: The contents of vial R1a (sodium nitroprusside and urease) was transferred into reagent bottle R1b (EDTA) mixed gently and labeled R1 reagent (EDTA, sodium nitroprusside and urease). The contents of bottle R2 (phenol) was diluted with 660ml of distilled water and stored in a reagent bottle labeled R2.

The contents of bottle R3 (sodium hypochlorite) was diluted with 750ml of distilled water and stored in a reagent bottle labeled R3.

METHOD

Three test tubes were labeled "blank", "standard" and "sample. To the test tube labeled sample 10μl of serum was pipetted into, 10μl of standard was pipetted into the test tube labeled

standard and 10μl of distilled water was pipetted into the test tube labeled blank.

100μl of reagent 1(R1) was pipetted each into the test tubes. The contents in the test tubes were mixed and incubated at 37^0C for 10 minutes.

After 10minutes 2.50ml of reagent 2 was pipette into the test tubes and then 250ml of reagent 3 (R3) was also added into each of the tubes. The mixtures in the test tubes were mixed and incubated for 15minutes at 37^0C. Absorbance at wavelength of 546nm of the sample and standard were read against the blank. Urea concentration was calculated using the formula below.

The concentration of Urea was calculated using the formula below.

$$\text{UREA CONCENTRATION (mmol/l)} = \frac{A\ \text{sample}}{A\ \text{standard}} \times \text{standard conc}$$

3.4.7 DETERMINATION OF SERUM BICARBONATE

PRINCIPLE

$$\text{Phosphoenol pyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxalate} + H_2PO_4$$

$$\text{Oxalate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxaloacetate was reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD. This results in the decrease in absorbance at 340nm that is directly proportional to $C0_2$ concentration in the sample (Forrester, et. al., 1976).

REAGENT PREPARATION

One vial of $C0_2$ reagent (PEP, magnesium sulfate, porcine, PEPC, sodium oxamate, buffer, non reactive fillers and stabilizers with sodium azide) was reconstituted in equal volume of $C0_2$ free water and mixed by inversion 5-6 times.

METHOD

One milliliter of carbon dioxide reagent was pipetted into the test tubes labeled “blank”, “standard” and “patient” and allowed to stand for 3minutes. Five microlitres (5μl) of water, standard (aqueous sodium bicarbonate) and serum were pipetted into the cuvette labeled “blank”, “standard” and “patient” respectively. Contents of all test tubes were mixed gently by inversion and allowed to stand for 5 minutes, and then transferred into the cuvette. Absorbances of cuvettes were read at a wavelength of

340nm.The concentration of Bicarbonate was calculated using the formula below.

$C0_2$ content = Abs blank – Abs. sample x concentration

Of sample Abs blank – Abs standard of standard

3.4.8 DETERMINATION OF SERUM SODIUM ION (Na+) CONCENTRATION

PRINCIPLE

Sodium was precipitated as the triple salt. Sodium magnesium uranyl acetate with the excess uranium reacted with ferrocyanide producing a chromophore whose absorbance varies inversely with the concentration of sodium in the serum (Maruna, 1958).

METHOD

FILTRATE PREPARATION

Three plastic test tubes were labeled “blank”, “standard” and “patient”. One milliliter of filtrate reagent (uranyl acetate and magnesium acetate in ethyl alcohol) was pipetted into all the test tubes. 50μl of distilled water, standard (sodium chloride solution) and serum were pipetted into the test tubes respectively. All test tubes were shaked vigorously and continuously for exactly 3 minutes.

All test tubes were centrifuged at high speed (1500g) for 10minutes and test supernatant fluids were taken from all plastic test tubes.

COLOR DEVELOPMENT

Three test tubes were labeled filtrate blank, filtrate standard and filtrate patient.

One milliliter acid reagent (diluted acetic acid) was pipetted to all tubes and to each of the test tubes 50μl of supernatant was pipetted to the respective tubes and mixed. 50μl of colour reagent was added to all tubes and mixed. The spectrophotometer was adjusted to zero mark with distilled water at 550nm and the absorbance of all tube mixtures read and recorded. The concentration of Sodium ion was calculated using the formula below

$C0_2$ OF (MEq/L) = $\frac{\text{Abs blank} - \text{Abs. sample}}{\text{Abs blank} - \text{Abs standard}}$ x concentration of standard

Of sample

3.4.9 DETERMINATION OF SERUM CHLORIDE ION (Cl^-) CONCENTRATION

PRINCIPLE

$2Cl + Hg(SCN)_2 \longrightarrow HgCl_2 + 2SCN$

$SCN + Fe^{3+} \longrightarrow FeSCN^{2+}$

The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate and subsequent formation of a red ferric thiocyanate complex is measured calorimetically.

The intensity of the colour formed is proportional to the chloride ion concentration in the sample (Schoenfeld, 1964).

METHOD

Three cuvettes were labeled “blank”, standard” and “sample”. 1.0ml of reagent (thiocyanate-Hg) was pipetted into the cuvettes labeled blank, standard and sample. 10µl of standard and serum were pipetted into the cuvettes labeled “standard” and “sample” respectively. The contents in the cuvettes were mixed and incubated for 5 minutes at 37^0C. The colorimeter was set to the zero mark with distilled water at a wavelength of 480nm and the absorbance (A) of the samples and standard were read against the blank. The concentration of Chloride was calculated using the formula below

$$\text{Chloride (MEq/L) of sample} = \frac{(A)\ \text{sample}}{(A)\ \text{standard}} \times 125 \text{ concentration of standard}$$

3.4.10 DETERMINATION OF SERUM POTASSIUM ION (K^+) CONCENTRATION

PRINCIPLE

The amount of potassium was determined using sodium tetraphenylboron in a specifically prepared mixture to produce

a colloidal suspension. The turbidity of which was proportional to potassium concentration (Terri and Sesin, 1958).

METHOD

To the three test tubes were labeled “blank”, “standard” and “sample”. were pipetted 2.5ml each of potassium reagent (sodium tetraphenylboron, preservatives and thickening agent).

Twenty five microlitre of potassium standard (equivalent of 4mEq/L), distilled water and serum were pipetted into the test tubes labeled standard, blank and sample respectively.

The contents in each test tube were mixed and allowed to sit for 3 minutes at room temperature (25^{o}C). The absorbance at a wavelength of 500nm of the standard and sample were read and recorded after adjusting the spectrophotometer with reagent blank. Potassium ion concentration was calculated from absorbance using the formula below.

$$\text{Potassium ion (MEq/L)} = \frac{\text{Abs of unknown}}{\text{Abs of STD}} \times \text{concentration of standard}$$

Of sample

CHAPTER FOUR

SUMMARY OF RESULT

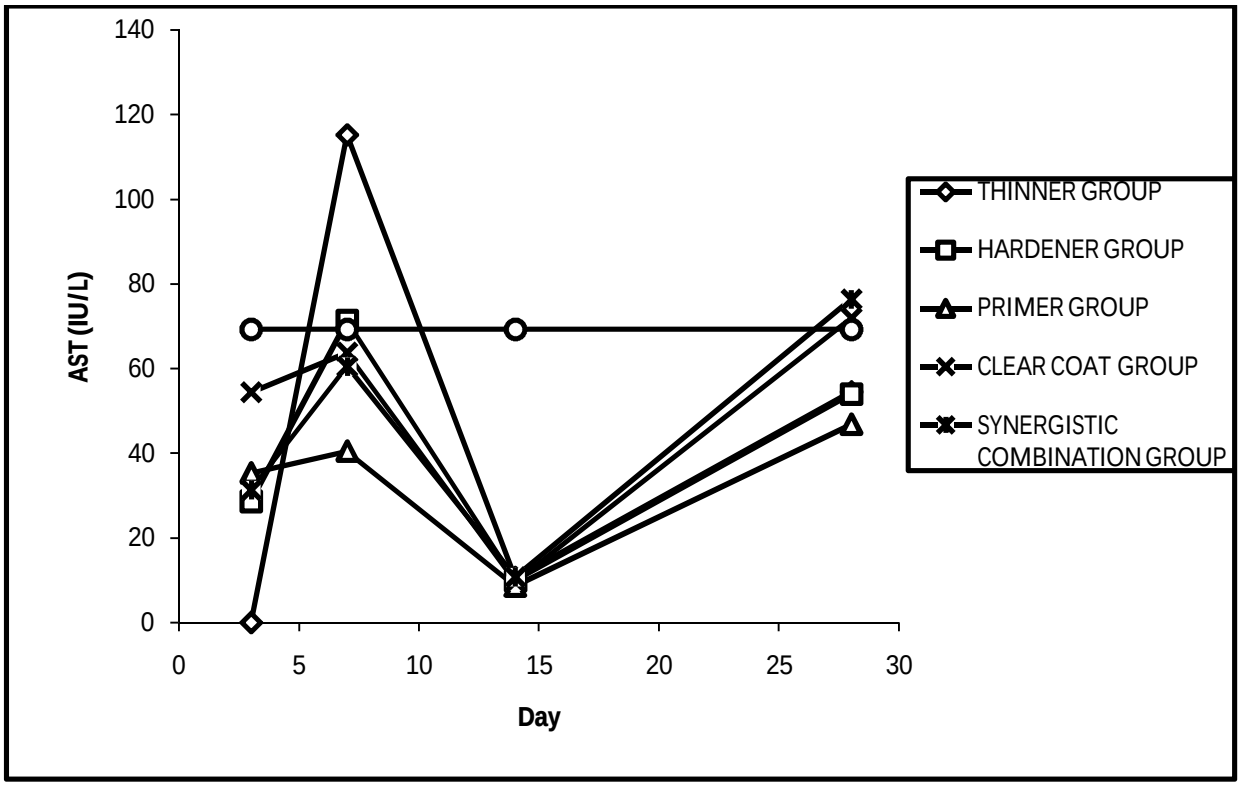

Fig 1: A plot of mean AST activity of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 1 shows a plot of mean activities of AST determined in all experimental rats sacrificed on day 3, 7, 14 and 28 and the negative control group.

AST activities in Thinner and hardener group were elevated (115.3±11.6 and 71.4±22.9 respectively) above the control group (69.3±10.3) on day 7. Thinner group showed the highest AST activity.

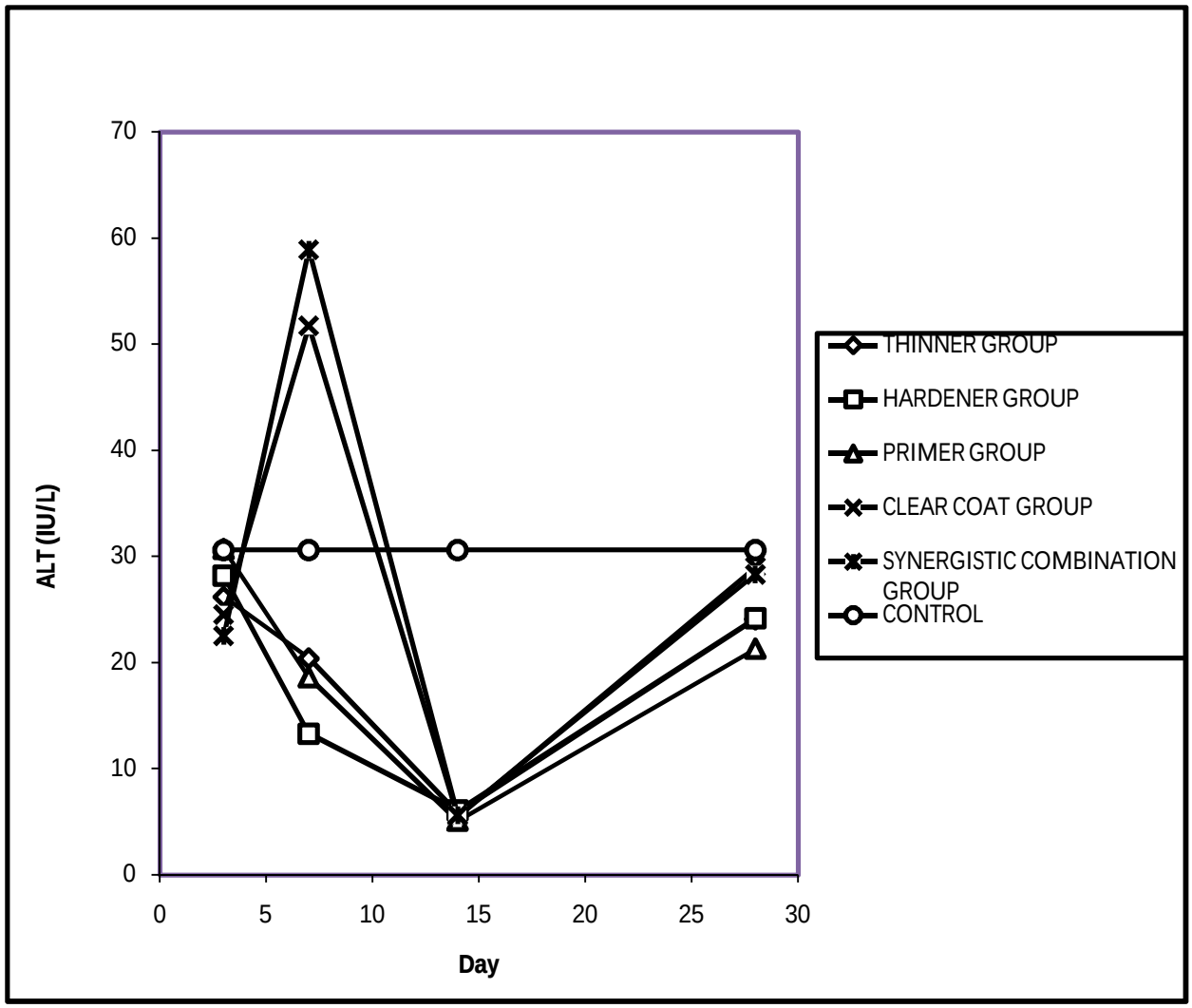

Fig 2: A plot of mean ALT activity of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 2 shows a plot of the mean activities of ALT in experimental rats sacrificed on day 3, 7, 14 and 28 and the negative control group. ALT activity of rats in synergistic combination and clear coat group sacrificed by 7th day was higher (58.9±27.1 and 51.7±22.1 respectively) than the control group (30.6±2.2). Synergistic combination group had the highest ALT activity.

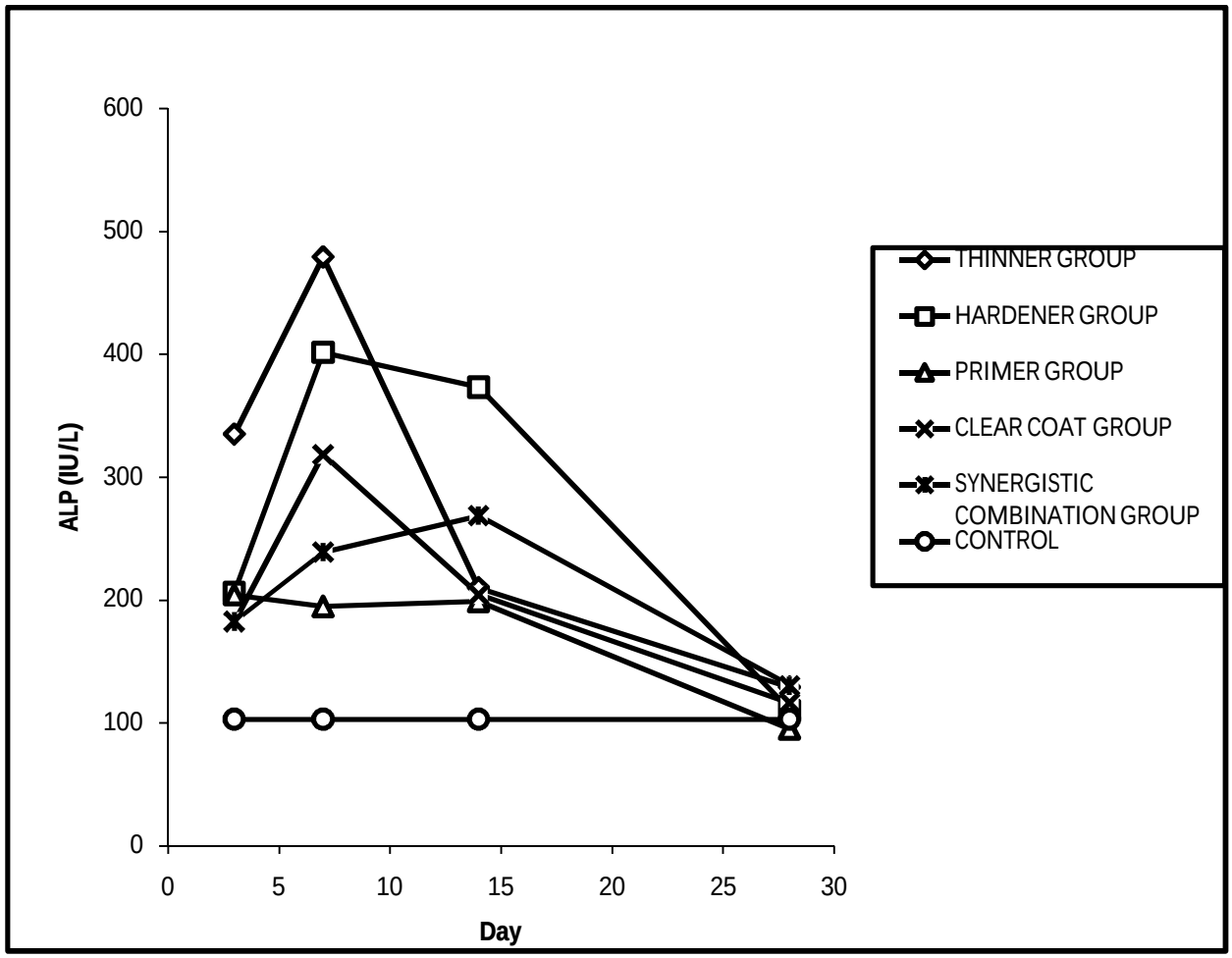

Fig 3: A plot of mean ALP activity of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 3 is a plot of the mean activity of ALP determined in all experimental groups sacrificed on day 3,7,14 and 28 and the negative control group. Serum ALP activity (479.1±166.8, 401.6±243.4, 318.4±36.4, and 268.8±72.4) in all groups were above that of the negative control group (103±21.5) and fell on the 28th day to the control value. Serum ALT activity in thinner group was highest.

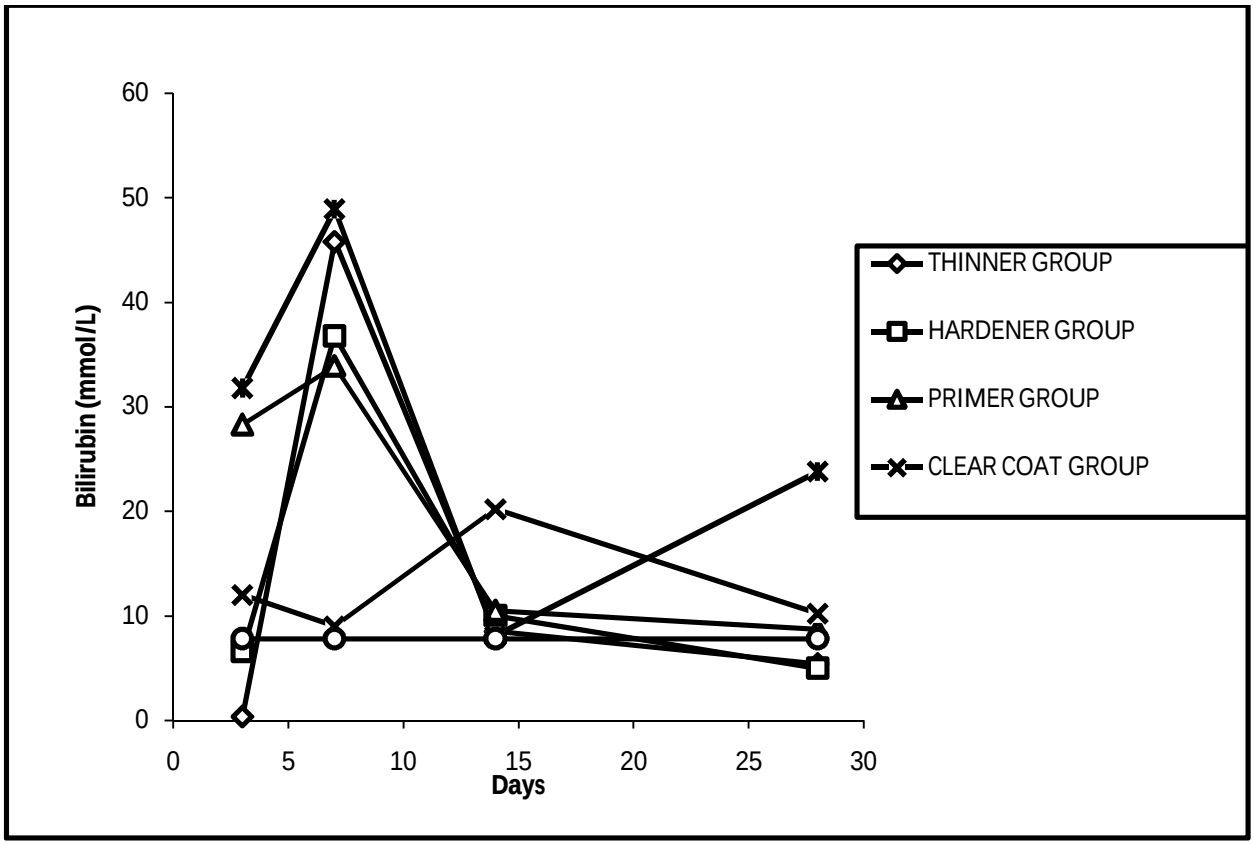

Fig 4: A plot of mean Bilirubin concentration of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 4 is a plot of the mean Total bilirubin concentration in experimental rats sacrificed on day 3, 7, 14 and 28 and negative control group. Bilirubin concentration (45.8±8, 36.8±4.1, 33.9±17.4, 9±1.2 and 48.9±5.6) in experimental rats in clear coat, thinner, hardener, primer and synergistic combination groups rose above the control group (7.8±1.2), especially by 7th and 14th day for clear coat and fell on the 28th day to the control value. Synergistic combination had the highest bilirubin concentration.

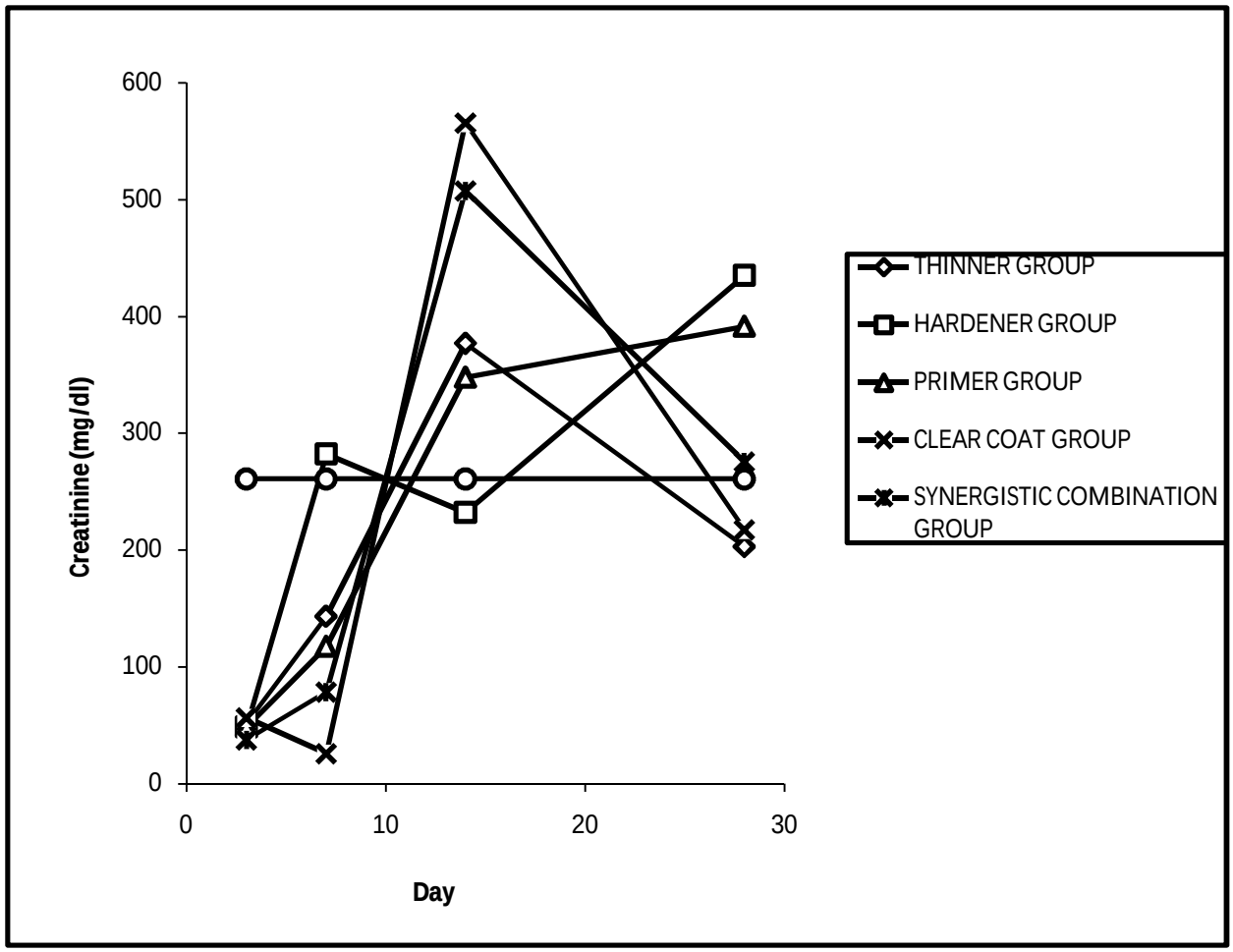

Fig 5: A plot of mean Creatinine values of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 5 is a plot of the mean creatinine (mg/dL) values in experimental rats sacrificed on day 3, 7, 14 and 28 and control group. Creatinine levels (377±135.3, 348, 565.5±29 and 507±96.7) mg/dl rose in all groups above the control group (261±21.8) except hardener on the 14th day and fell on the 28th day. Clear coat had the highest creatinine elevation.

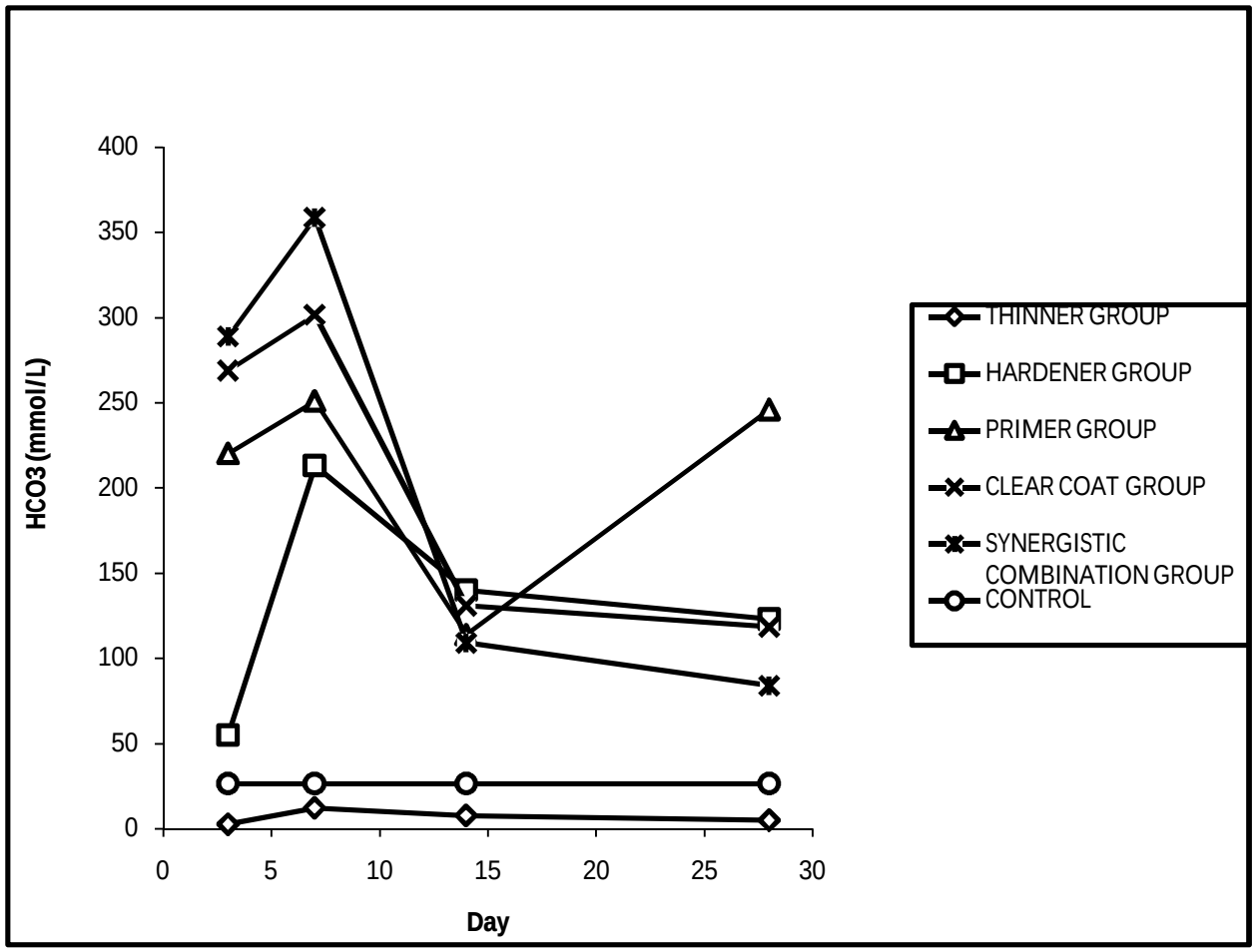

Fig 6: A plot of mean HCO_3 values of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 6 shows a plot of the mean $HC0_3$ values in rats sacrificed on day 3, 7, 14 and 28 and control group.

Bicarbonate values (213±28, 251±5.3, 301.5±23 and 358.6±33) in all groups were elevated on the 7th day above the control group (26.5±31.8), except in thinner. Synergistic combination had the highest bicarbonate elevation.

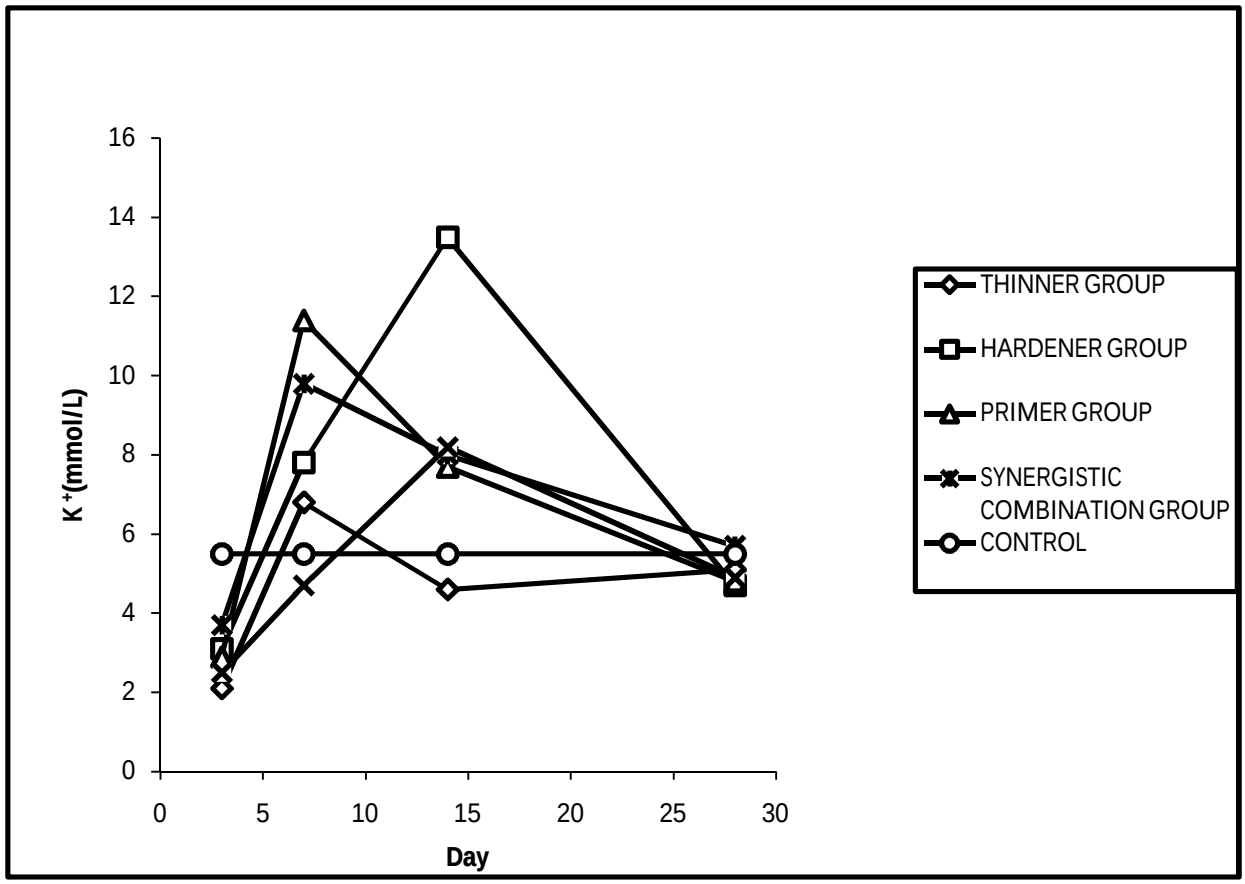

Fig 7: A plot of mean K ion concentration of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 7 shows a plot of the mean Potassium ion concentration in rats sacrificed on day 3,7,14 and 28 and the negative control group.

Potassium ion concentration (13.5±22 and 8.2±2.8) and (11.4±0.5, 9.8±0.9 and 6.8±1.9) were elevated in all groups (hardener and clear coat) and (primer, synergistic and thinner) respectively on the 14th and 7th day while thinner appeared with a least elevation, Hardener had the highest value on the 14 day.

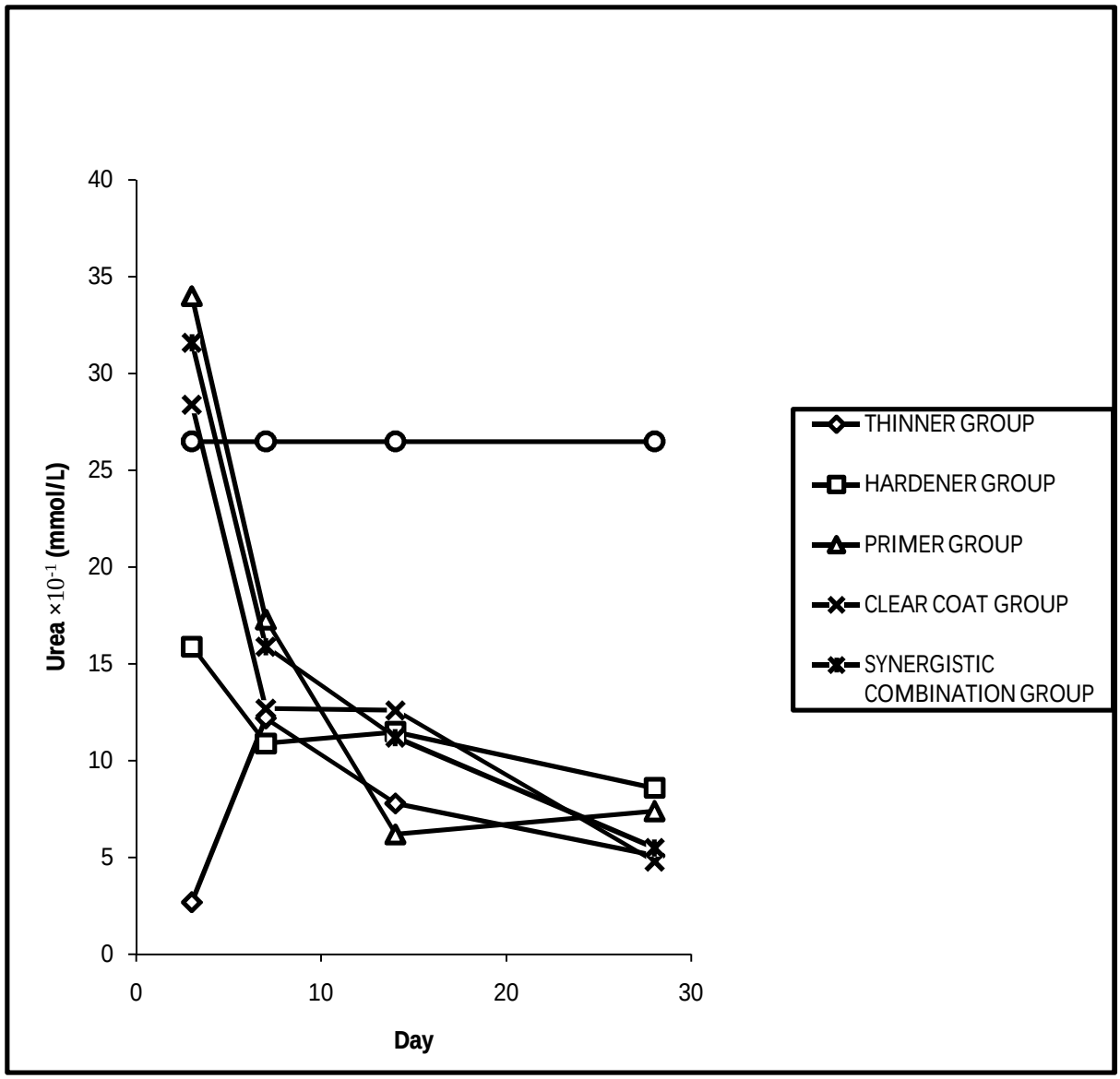

Fig 8: A plot of mean urea concentration of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 8 is a plot of the mean Urea concentration in albino rats sacrificed on day 3,7,14 and 28 and the control group.

Urea concentration in all groups fell below the negative control group.

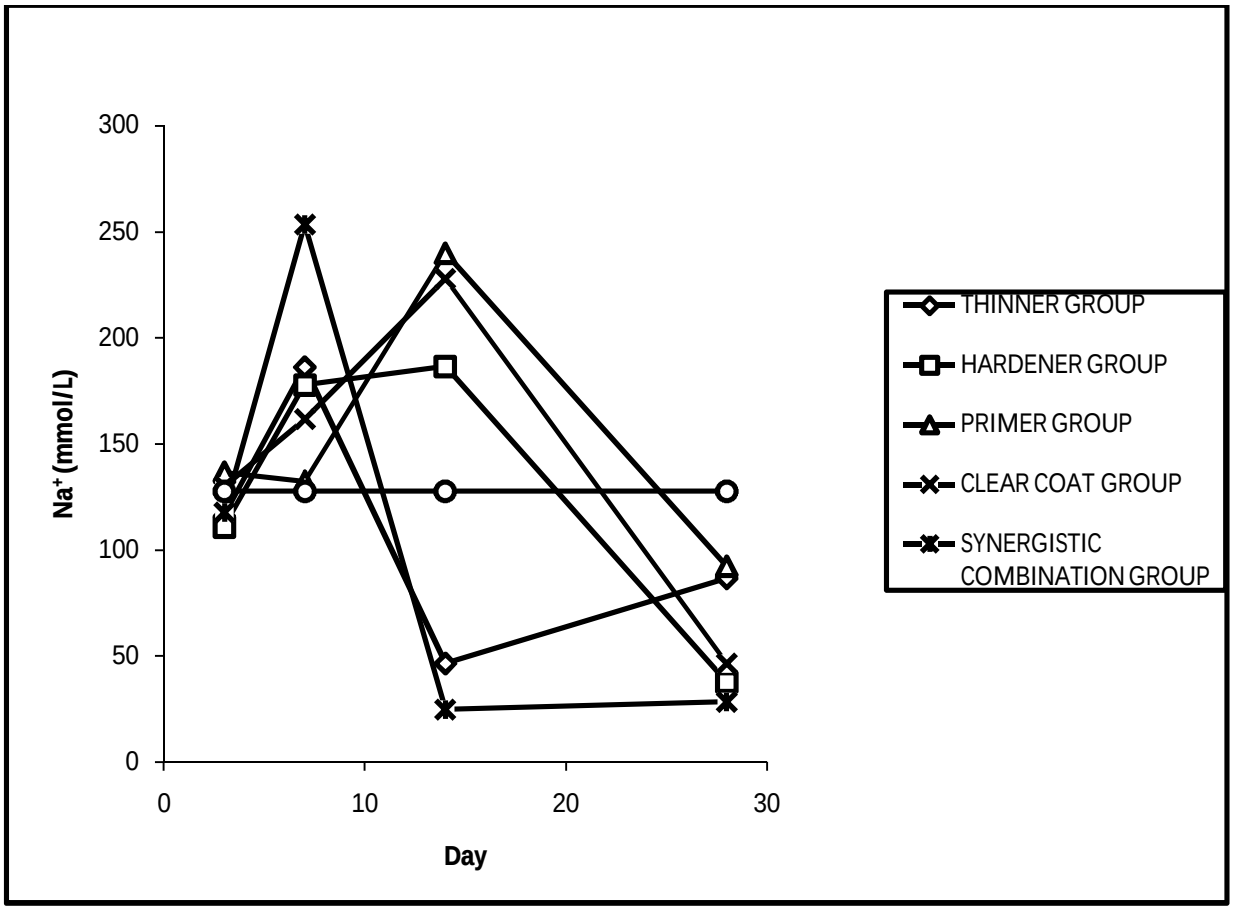

Fig 9: A plot of mean Na ion values of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig. 9 is a plot of the mean Sodium ion concentration in albino rats sacrificed on day 3, 7 14 and 28 and control group.

Sodium ion concentration was elevated in synergistic combination group (253.7±108.9) by 7th day, in Primer group (240±33.3), in clear coat group (228±12) and Hardener group (186.7±62.2) by 14th day.

Synergistic combination group had the highest elevation on the 7th day

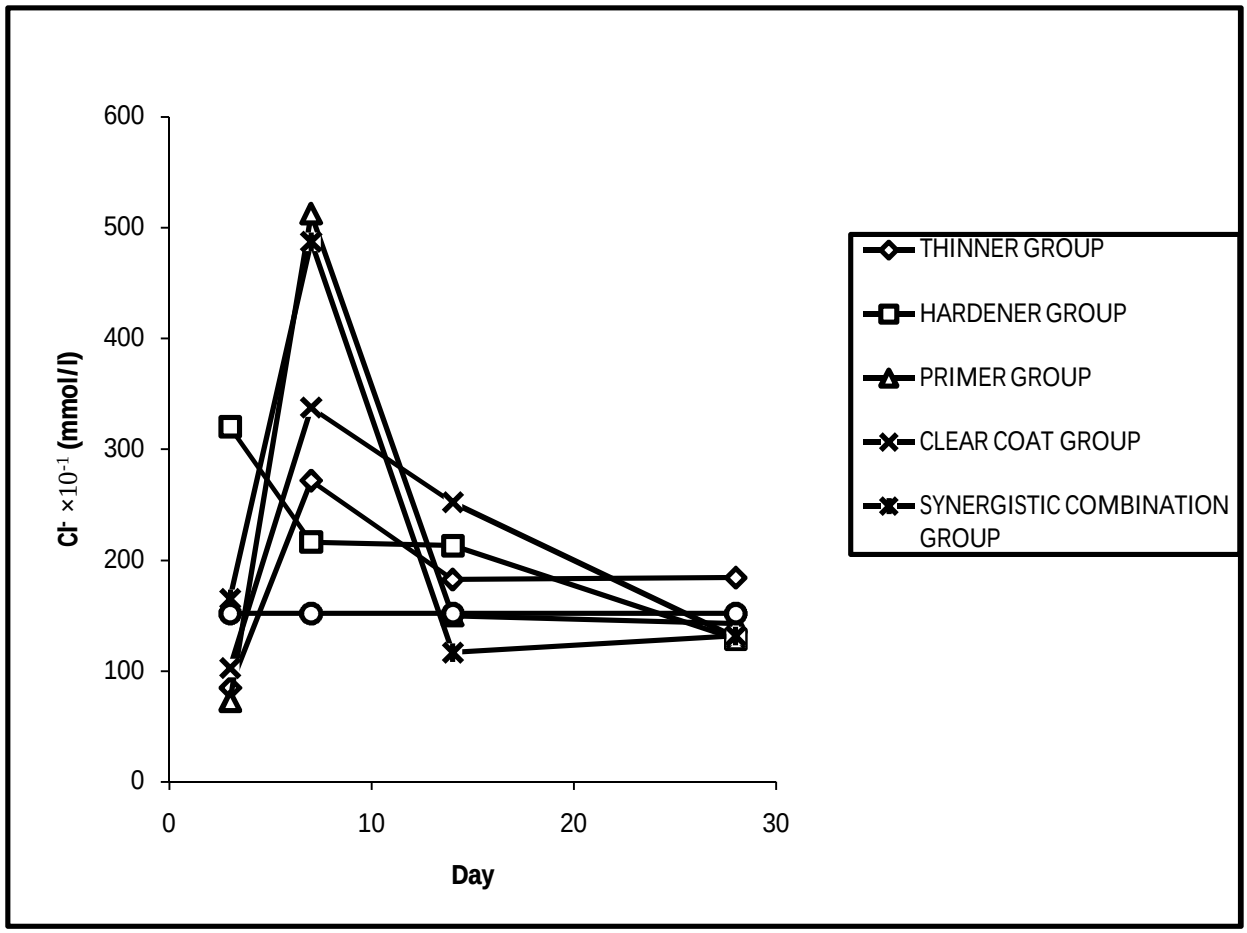

Fig 10: A plot of mean Cl^- concentration of Rats in different experimental groups sacrificed on day 3, 7, 14, and 28.

Fig 10 is a plot of the mean Chloride ion concentration in albino rats sacrificed on day 3,7,14 and 28 and negative control group. Chloride ion concentration (271.7±95.6, 216.3±27.5, 512.5±75, 337.5±8.3 and 487.5±58.3) in all groups were elevated above the control group (151.7±39.9) by 7^{th} day but subsequently reverted to the control values by 28^{th} day

CHAPTER FIVE

DISCUSSION

Liver and kidney injuries have long been associated with occupational exposure to a wide variety of chemicals (Franco, et. al., 2004).

The purpose of this research was to investigate the effects of car paints and additive on the liver and kidney functions by applying routine diagnostic test for these organ system functions, which act as biochemical probes to detect perturbation and early pathological manifestation associated with occupational hazards in car refurbishing industry.

Liver function test includes tests for serum AST, ALT, ALP and bilirubin (Smita, et. al., 2009). In this study serum enzymes generally show increased AST (115.3±11.6), ALT (58.9±27.1) and ALP (479.1±166.8) when compared with that in the control group (69.3±10.3, 30.6±2.2 and 103±21.5 respectively). Serum bilirubin also showed an elevation (48.9±5.6) when compared with that of the control group (7.8±1.2), but most significantly for AST, ALP and bilirubin at $p<0.05$. This result seemed to be in consonance with the report that transaminases have been showned to be significantly elevated ($p<0.05$) in serum activity when exposed to paint fume (Lundberg, et. al., 1994). Furthermore, transaminase elevation was reported in sera of

workers exposed to car paint in chestery (Nune, et al, 2005). The concentration of liver enzymes is low in the serum. However, if the liver is damaged hepatocyte cell membrane becomes more permeable and some of the enzymes normally located in the cytosol leak out into the blood stream resulting in their elevation (Giboney, 2005). Rasied level of transminase has serious implication for car paint workers. Such elevation is found in liver damage. The elevation of AST alongside ALT makes the liver a target of suspicion as this is the usual pattern in cases of hepatotoxicicty caused by toxic agents.

ALT and AST levels were elevated for more than 7 day and dropped by day 10, this agrees with Rosalki's (1974) work. Elevation of serum ALP has also been implicated in workers exposed to car paint (Nunes, et. al., 2005) and in factory workers exposed to paint fumes (Orisakwe, et. al., 2007). Although ALP is found in all tissues of the body at or in the cell membrane, it is highest in the liver and bone (Gupta, et. al., 2004). Rasied level of bilirubin have been reported to be associated with workers exposed to organic solvent (Rigato et. al., 2005). Impaired liver function, injury or blocked bile secretion can cause bilirubin to leak out into the blood stream resulting in its elevation. Lipid peroxidation is one of the characteristics of hepatoxicity (Jaeschke, et. al., 2002). A unique feature of the liver is its ability to regenerate, the repair

mechanisms being influenced by phospholipids coupled with rise in thymilidate synthetase and thymidine kinase level in the liver reaching a peak at 72hours indicating liver regeneration (Chen and Chen 1994).

The decrease by the 28^{th} day in the level of serum enzymes could be attributed to the synthesis of Cyt P_{450} or other intrinsic mechanisms that could be involved in the metabolism and clearance of toxic substances from circulation and hepatic cell recovery.

The test for kidney dysfunction shows significant increases in creatinine (565.5±29), bicarbonate (358.6±33), potassium (13.5±2.2) ($p<0.05$), sodium (253.7±108.9) and chloride (512.5±75) (mg/dL) from the control group (261±21.8, 26.5±31.8, 5.5±0.5, 127.8±19.8, and 151.7±39.9 respectively), but most significantly for creatinine, bicarbonate and potassium at $P<0.05$. However, raised levels of serum bicarbonate, Na^+ and creatinine had been reported in rats exposured to paint fumes (Orisakwe, et. al., 2007). Rasied concentration of creatinine and serum electrolytes have serious implication in kidney dysfunction (Seldin and Rector, 1972, Gross, et. al., 2005 and Mathew, 2005).

The result of this study demonstrated significant elevation ($P<0.05$) in kidney creatinine,bicarbonate, K^+ and Na^+

concentrations during the exposure of rats to fumes of thinner, hardener, primer, clear coat and synergistic combination of these chemicals, suggesting the renal impairment might have resulted from exposure to paint fume.

Three hypotheses could be used to explain the renal impairment induced by toxic solvents: firstly, it is possible that chronic renal tubular injuries could have lead to the release of sequestered tubular antigens, subsequently immune complex formation and glomerular deposition (Zum multi, et. al., 1981): secondly solvents in paint might have also caused generalized membrane perturbation either directly or through their reactive metabolites (Zimmermann and Norbach, 1980): thirdly, the toxic solvent might have had direct toxic effect in the glomerulus (Zimmer Mann and Norbach, 1980) Car paints (thinner, hardener, primer, clear coat) are composed of solvents (ethylacetate, toluene, aliphatic polyisocyanate, hexamethylene, diisocyanate, methyl ketone, mixed dibasic esters, petroleum naptha, buthyl acetate, ethylbenzene, xylene, barium sulfate, resins and filter and isocyanate) which are associated with liver and kidney dysfunction (Lungberg, et. al., 1994 and Myer, et. al., 1993). These solvents can cause liver and kidney damage by reacting molecularly with the mitochondria of intracellular organelles that produce energy, its dysfunction releases excessive amount of oxidants or by the activation of some

enzymes in the cytochrome P_{450} system which could injure hepatic cells (Jaechkhe, et. al., 2002). Injury to hepatocyte and nephritis had been reported to lead to accumulation of more enzymes and oxidants which could promote further liver and kidney damage (Patel, et. al., 1998).

Liver and kidney are vital organs in the body. While liver detoxifies toxins and excretes ALP, the kidney excretes electrolytes. Elevation of liver enzymes and bilirubin is an indication of liver dysfunction and elevation of serum potassium and chloride ions concentration an indication of kidney dysfunction.

The deleterious effect of car paint on liver was highest in synergistic combination>hardener>thinner>clearcoat>primer.

Deleterious effect of car paint on kidney was highest in synergistic combination>primer>clearcoat>hardener>thinner.

High deleterious effect of synergistic combination of car paint on liver and kidney is suggested to be to result of the combined/synergistic effect of car paints and additive.

CONCLUSION

The aim of this research is to investigate the effect of car paints and additive on the liver and kidney of experimental rats applying liver and kidney function test.

Exposure of car prints and additive to experimental rats caused both liver and kidney dysfunction.

Workers in car refurbishing industry could be at high risk of liver and kidney dysfunction. To prevent these organ system damage, workers should be educated on work related hygiene and hazards in the car refurbishing industry.

It can be concluded that car paints and additive causes both liver and kidney dysfunction in experimental rats.

Further study can be carried out on Lung and Skin histology to ascertain the effect of car paint fume on these organs during car painting.

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APPENDIX 1

AST REAGENTS COMPOSITION

Contents	initial concentration of solution
R1 buffer	
Phosphate buffer	100mmol/l pH 7.4
L-aspartate	100mmol/l
α-oxoglutarate	2mmol/l
R2. 2, 4-dinitrophenylhydrazine	2mmol

APPENDIX 2

ALT REAGENT COMPOSITION

Contents	initial concentration of solution
R1 buffer	
Phosphate buffer	100mmol/l pH 7.4
L-Alanine	100mmol/l
α-oxoglutarate	2.0mmol/l
R2. 2,4-dinitrophenylhydrazine	2mmol

APPENDIX 3

FORMULA USED IN OBTAINING ALP FROM ABSORBANCE

U/L = 2760 X a405NM/MIN semi micro

ALP REAGENT COMPOSITION

Contents	initial concentration of solution
R/a buffer	
Diethanolamine buffer	1mmol/l pH 9.8
$MgCl_2$	0.5mmol/l
R/b substrate	
p-nitrophenylphosphate	10mmol

APPENDIX 4

CALCULATION USED IN OBTAINING BILIRUBIN FROM ABSORBANCE

Total bilirubin (mg/dl) = 10.8 x A_{TB} (578nm)

BILIRUBIN REAGENT COMPOSITION

Contents		Initial concentration of solution
R1.	Sulphanilic acid	29mmol/l
	Hydrochloric acid	0.17N
R2.	Sodium Nitrite	38.5mmol/l
R3.	Caffeine	0.26mol/l
	Sodium benzoate	0.52mol/l
R4	Tartrate	0.93mol/l
	Sodium hydroxide	1.9N

APPENDIX 5

FORMULA FOR CALCULATING UREA LEVEL FROM ABSORBANCE

$$\text{UREA CONCENTRATION} = \frac{A \text{ sample}}{A \text{ standard}} \times \text{standard (mmol/l) conc}$$

UREA REAGENT COMPOSITION

Contents		Initial concentration of solution
R1.	EDTA	116mmol/l
	Sodium nitroprusside	6mmol/l
	Urease	1g/l
R2.	Phenol (diluted)	120mmol/l
R3.	Sodium hypochlorite (diluted)	27mmol/l
Sodium hydroxide		0.14N

APPENDIX 6

FORMULA USED IN CALCULATING CREATININE VALUE

Manual calculation

A2-A1 Concentration of creatinine in serum

Sample or A standard = $\frac{\text{A sample}}{\text{A standard}}$ x standard conc (μ/mol/l)

CREATINE REAGENT COMPOSITION

Contents		Initial concentration of solution
R/a.	Picvic acid	35mmol/l
R/b.	Sodium hydroxide	0.32mol/l

APPENDIX 7

CALCULATION OF BICARBONATE CONTENT

$C0_2$ content = Abs blank – Abs. sample x concentration

Of sample Abs blank – Abs standard of standard

BICARBONATE REAGENT COMPOSITION

When reconstituted $C0_2$ reagent contains PEP 1.8nM, magnesium sulfate 10mM, NADH 0.4mM, MDH (porcine) 1,250u/l, PEP (microbial) 200u/l, sodium oxamate 2.5mM. buffer, (pH 7.0), non reactive fillers and stabilizers with sodium Azide 0.1% as preservatives.

$C0_2$ standard contains 30mmol/l of sodium bicarbonate in a aqueous solution

APPENDIX 8

FORMULA FOR THE DETERMINATION OF SODIUM

$C0_2$ OF (MEq/L) = $\frac{\text{Abs blank – Abs. sample}}{\text{Abs blank – Abs standard}}$ x concentration

Of sample of standard

SODIUM REAGENT COMPOSITION

Filtrate reagent; Uranyl aceate 2.1nM and magenisum acetate 20mM in ethylalcohol

Acid reagent; a diluted acetic acid

SODIUM Colour reagent; potassium ferro cyanide, non reative stabilizers and fillers.

SODIUM STANDAR; sodium chloride solution; 150mEq/l of sodium

APPENDIX 9

FORMULA FOR CALCULATING POTASSIUM FROM ABSORBANCE

potassium (MEq/L) = Abs of unknown x concentration of standard

Of sample Abs of STD

COMPOSITION OF POTASSIUM REAGENTS

Potassium reagent; Sodium tetraphenylboron 2.1mM, preservative and thickening agents.

Potassium standard; equivalent to 4mEq/l

APPENDIX 10

FORMULA FOR DETERMINING CHLORIDE LEVEL

Chloride (MEq/L) = $\frac{(A)\ \text{sample}}{(A)\text{standard}} \times 125$ concentration of standard

Of sample

CHLORIDE REAGENT COMPOSITION

R Thiocyanate –Hg; mercuric thiocyanate

4mmol/l

Ferric nitrate

40mmol/l

Mercuric nitrate

2mmol/l

Nitric acid

45mmol/l

Chloride CAL; Chloride aqeous primary standard 125mmol/l

APPENDIX 11

PREPARATION OF 0.4MOL/L SODIUM HYDROXIDE

Na0H solution (0.4mol/l)

40g/mol

Molar = mole/litre of solution

$$\text{Molar} = \frac{\text{mass}}{\text{Molar mass/L of solution}}$$

0.4mol = mass/40g/mol ÷ 1L

0.4mol = mass/40g/mol x 1/1L

Mass = 16g/l

APPENDIX 12

FORMULA USED FOR STATISTICAL ANALYSIS

$$T_{cal} = \frac{X_1 - X_2}{Sp\sqrt{1/n_1 - 1/n_2}}$$

$$Sp^2 = \frac{(n_1 - 1)S^2 - (n_2 - 1)S^2}{n_1 + n_2 - 2}$$

T_{tab} at 5% significance i.e 0.05 significance

if $T_{tab} > T_{cal}$ ($P > 0.05$)

accepted i.e there is no significance variance

if $T_{tab} < T_{cal}$ ($P < 0.05$)

rejected i.e there is significance variance