EFFECT OF AQUEOUS EXTRACT OF DATE FRUIT (*Phoenix dactylifera*) IN CARBON TETRA CHLORIDE - INDUCED HEPATOTOXIC RATS

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DECLARATION

I hereby declare that this work is the product of my own research efforts; undertaken under the supervision of Professor M.K. Atiku and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent preparation of this dissertation by MISBAHU SAMBO (SPS/11/ MBC/00020) were carried out under my supervision.

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APPROVAL

This is to certify that this thesis by Misbahu Sambo has been examined and approved for the award of the degree of Master of Sciences (Biochemistry) of Bayero University, Kano for its contribution to knowledge and literary presentation.

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In the name of Allah I begin. May His infinite mercy, peace and blessings continue to be upon His messenger Muhammad (SAW), his households, companions and those that follow their steps up to the last hour. Oh Allah! I owe You all thanks and appreciations. It is with Your might all this is possible. Thank you LORD.

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Thank you all. Oh Allah! Help me throughout my life here and hereafter. In Your most Gracious and Merciful name I conclude.

DEDICATION

I dedicate this thesis to my mentor, guardian and Uncle; **Dr. Mustapha Isa Qasim**.

TABLE OF CONTENT

ABSTRACT

The aqueous fruit extract of *Phoenix dactylifera* was investigated for its phytochemical composition and hepatocurative effect of its 249mg/kg and 580mg/kg concentrations on carbon tetra chloride (CCl_4) - induced liver damage in rats. A comparison was also made between the hepatocurative effect of standard drug (Livolin) with that of the various concentrations of the extract. Furthermore, the effect of controlled diet (a diet which contains minimal amount of protein and lipids) in treating liver disease was also studied. Phytochemical screening indicates the presence of flavonoids, saponins, tannins and cardiac glycosides, while alkaloids were absent. Administration of 249mg/kg and 580mg/kg of the extract seperately did not cause any harm to the liver of normal rats. The 249mg/kg and 580mg/kg of the extract independently significantly ($P < 0.05$) reduce the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total and direct bilirubin, as well as, significantly ($P < 0.05$) increase the level of total protein and albumin in treated groups compared to positive control groups. Comparatively, the hepatocurative effect of Livolin has no significant ($P > 0.05$) difference with that of 580mg/kg of the extract, but is significantly ($P < 0.05$) higher than that of 249mg/kg of the extract. The hepatocurative effect of the drug combined separately with 249mg/kg and 580mg/kg of the extract is not significantly ($P > 0.05$) different with that of the drug alone. In this research, controlled diet seems not to have any effect in treating liver disease as comparative studies between the controlled-diet group and the uncontrolled-diet group shows no significant ($P > 0.05$) difference interms of hepatocurative effect. These results suggest that the aqueous fruit extract of *Phoenix dactylifera* has hepatocurative effect against CCl_4 - induced liver damage in rats.

CHAPTER ONE

INTRODUCTION

The liver is the largest gland of the body and the second largest organ of the body, weighing about 1800g in men and 1400g in women. It is triangular in shape and is divided into four lobes. A thin layer of connective tissue surround each lobe, extending into the lobe itself and dividing the liver mass into small functional units called lobules (Kerry and Janice, 2013). It is situated in the upper right portion of the abdominal cavity (Ham, 1974). This location of the liver is very essential in carrying its functions because it receives nutrient-rich blood directly from the gastro intestinal (GI) tract and then store those nutrients that need to be stored, degrade those that are supposed to be degraded, leave those that need not to be changed unaltered and transform those that need to be transformed into substances that are used elsewhere in the body for metabolic use. The liver support almost every organ in the body and is therefore necessary for survival (Cotran *et al.,* 2005).

Because of its multi dimensional functions and strategic location, the liver is prone to many diseases. The most common include infections such as hepatitis A, B, C, D and E, alcohol damage, fatty liver, cirrhosis, and cancer. Most of these diseases are accompanied by remarkable change in biochemical parameters (Nelson and Cox, 2005).

Liver disease has a worldwide distribution. Very often, affected individual are asymptomatic for a long period of time, making it very difficult to generate accurate incidence and prevalence data in the general population (Kerry and Janice, 2013). However in Nigeria, a studies carried out from January 1, to December 31, 2010 revealed that liver disease accounted for 7.9% of medical admission, with primary liver cancer and liver cirrhosis accounting for 44.3% and 20.4%

respectively. On the basis of the final diagnosis, the following categories of liver disease were identified: Acute hepatitis, chronic hepatitis, liver cirrhosis, primary liver cancer, secondary liver cancer, liver abscess and unclassified groups (Nwokediuko *et al.,* 2013).

Among the different types of liver disease, viral hepatitis remains a leading cause of morbidity and mortality affecting millions of individuals worldwide. According to WHO, two billion people have been infected with the hepatitis B virus (HBV), and more than 350 million have chronic HBV infection. In addition, it has been estimated that up to 3% of the world population have been infected with hepatitis C (HCV) of which 170 million people are chronically infected (WHO, 2008).

It was also found that hepatocellular carcinoma (HCC) is a major cause of death by cancer worldwide, accounting for over half million death per year. In men, HCC is the fifth most frequently diagnosed cancer worldwide, and is also the second leading cause of cancer-related death in the world (WHO, 2008).

The statistics on the incidence and prevalence of liver disease is always increasing, hence, quite alarming. The liver being the central focus for metabolic activities and other multifarous functions in the human body and whose damage can negatively affect almost every organ in the body, need to be protected from being damaged or need to be properly cured when infected. This urgent need led to the development of many synthetic drugs for the protection of liver or for curing the liver when infected. The most common among these drugs are Livolin, Lamivudine, interferon alpha-2b, Telbivudine, Interferon alpha-2a and Entecavir. Some of these drugs are antiviral (e.g Lamivudine and interferon) while others are nutrient supplements (e.g Livolin).

The type of drug used depend on the cause of the disease, and sometimes doctors often combine different antiviral drugs to combat the virus (Consumer information by audience, 2013).

Despite these efforts in developing drugs for liver disease, still, the prevalence and incidence of the disease is not significantly decreasing. This is due to the fact that almost all the drugs have significant side effects, and sometimes may even complicate rather than alleviate the infection. Furthermore, many of these treatments are expensive (Nuhu, 2001).

These and many other reasons led to the search for alternative drugs (i.e drugs from plants).The use of plants for medicine is as old as man himself. Despite the doubt in the efficacy and safety of the traditional herbal medicine, no one can argue their existence and practice in our societies (Nuhu, 2001). However, this doubt can be discarded if systematic research methodology is employed in evaluating the scientific basis for the traditional herbal drugs (medicine). The curiosity for exploring plants as alternative drugs has been on the rise, and the protective and curative effects of various parts of many plants on various diseases were tried. Liver disease is among the diseases that receive such trials. Many plants that are claimed to possess hepatoprotective and curative effects were tried and the results were positive. Among such plants is date palm fruit (Alqarawi *et al.,* 2004).

Phoenix dactylifera tree belong to the palm family (*Arecaceae*). It grows vertically to form unbranched trunk driven by the activity of a single terminal shoot apex. Date fruits are single seeded fruits of cylindrical, rounded or ovoid shape with fleshy sweet mesocarp covered with a thin epicarp, somewhat yellowish to reddish brown in colour (Jain *et al.,* 2011). Date palms are grown in more than forty countries. Some of the major palm producing countries are Iraq, Saudi Arabia, Egypt, Tunisia, Morocco and Algeria. Also, it is grown in Nigeria for consumption and

local trade. However, the fruit is found in abundant quantity and cheap price because it is imported with ease from Niger Republic which serve as a commercial depot of date fruits from Morocco, Tunisia and Algeria. Date fruit has a lot of socio economic importance, nutritional values and medicinal values

(El Hadrami and Alkhayri, 2011). Researches reported that it contains substances capable of activating enzymatic and non enzymatic antioxidant system (El Hadrami *et al.,* 2011). It also has anti tumural and anti ulcer properties. It also has antioxidant and neuroprotective effects against bilateral common carotid artery occlusion in rats. It also has anti inflammatory activity (Mohammmad and Al okbi, 2004), anti diarrheal activity (Agbon *et al.,* 2013) and hepatocurative effect (Alqarawi *et al.,* 2004).The medicinal value of this noble fruit are numerous, the above are just a few.

Considering the increase rise in liver disease cases, and considering the abundance, availability and cheap price of this fruit coupled with its numerous medicinal values and the high probability of its application as hepatocurative agent, it becomes an obligation on researchers to explore it and carry out a research on its hepatocurative effects.

1.1 STATEMENT OF RESEARCH PROBLEM

Hepatic damage is a global metabolic and epidemic disease affecting essential biochemical activities in almost every age group. Conventional drugs used in the treatment of liver disorders are often inadequate. Also, the spectrum of liver abnormalities caused by allopathic drugs is found to be broad. In view of severely undesirable effect of synthetic agents, it is necessary to search for alternative drugs, which are of doubtful efficacy and safety. Therefore, there is a

growing focus to follow systematic research methodology, and to evaluate scientific basis for the traditional herbal medicine that are claimed to posses hepatocurative activities.

Therefore, the current study focuses on the use of date fruit (*Phoenix dactylifera*) in treating liver disease.

1.2 JUSTIFICATION

The liver is the central focus for metabolic and other multifarous functions in human body. Because of its multi dimensional functions and strategic location, it is prone to diseases caused by microbes and chemicals. Also, certain drugs when taken in over dose and even when administered at therapeutic dose may cause injury to the liver (Venkatehwar *et al.,* 2013). Other chemical agents that are used in the laboratories, industries and herbal remedies may also cause hepatotoxicity. So also, synthetic liver drugs may cause adverse effect. Morever, the rate at which the number of patients with liver disease is increasing is quite alarming.These necessitate for the search of alternative drugs (drugs from plants) that are claimed to possess hepatocurative effect. *Phoenix dactylifera* is among such plants. *Phoenix dactylifera* is readily available in abundant quantity and cheap price in the northern part of Nigeria. However, its application especially as medicine is untraceable or very negligible. But reports from North African and Asian countries highlighted its application in the treatment of many diseases including liver disease. Hence, there is the need to try these claims on our readily available date fruits and to ascertain its hepatocurative effects.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this research project is:

To investigate the effect of aqueous extract of *Phoenix dactylifera* in carbon tetrachloride hepatotoxic rats.

The objectives of this research are:

i. To carry out the phytochemical screening of the aqueous extract of a variety of date fruit.
ii. To determine the levels of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, total and direct bilirubin as markers of liver disease.
iii. To investigate the effect of controlled diet in treating liver disease using aqueous extract of date fruit.
iv. To investigate the hepatocurative effect of a combination of aqueous extract of date fruit and Livolin.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE LIVER

The liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44 – 1.66 kg (Cotran *et al.,* 2005), and is a soft, triangular organ. It is both the largest internal organ and the largest gland in the human body. The liver is found in all vertebrates and is typically the largest visceral organ. Its forms varies considerable in different species and is largely determined by the shape and arrangement of the surrounding organs. Nonetheless, in most species, it is divided into right and left lobes; exception to this general rule include snakes, where the shape of the body necessitate a simple cigar-like form. The internal structure of the liver is broadly similar in all vertebrates (Romer and Pareons, 1997).

The liver is the only human internal organ capable of natural regeneration; as little as 25% of the liver can regenerate into a whole liver (Dieter, 2011).This is however not true regeneration but rather compensatory growth (Cotran *et al.,* 2005).The lobes that are removed do not regrow and the growth of the liver is a restoration of function, not original form. This contrast with the true regeneration where both original function and form are restored (Council man, 1993).

The liver is large and meaty. It cannot be felt because it is protected by the rib cage. It does not pulsate, it does not move much, only passively, and you do not ordinarily see it secreting anything. Without it the body is kaput. Therefore, it is necessary for survival.

2.1.1 Cells of the Liver

Two major types of cells populate the liver lobes: Parenchymal and non parenchymal cells.

Parenchymal cells are also referred to as hepatocytes.They perform the majority of numerous liver functions. 80% of the liver volume are occupied by parenchymal cells (Arthur and John, 1995).

Non parenchymal cells constitute 40% of the total number of liver cells but only 6.5% of its volume. They are localized in the sinusoidal compartment of the tissue. The non parenchymal cells include: (i) Sinusoidal endothelial cells(SEC) (ii) Kupffer cells (KC) and (iii) Hepatic stellate cells (HSC), formally known as fat storing cells (Kmiec, 2001).

Also, intrahepatic lymphocyte (IHL) including pit cells i.e liver-specific natural killer cells are often present in the sinusoidal lumen (Kmiec, 2001).

Liver sinusoidal endothelial cells constitute the lining or wall of hepatic sinusoid (common capillary bed that are formed by terminal branches of blood vessels).They perform important filtration function due to the presence of small fenestrations that allow free diffusion of many substances, but not of particles of the size of chylomicrons, between the blood and hepatocyte surface. SEC show huge endocytic capacity of many ligands including glycoprotein components of extracellular matrix (ECM; such as hyaluronate, fibronectin, or chondroitin sulphate proteoglycan), immune complexes, transferin and ceruloplasmin. SEC may function as antigen-presenting cells (APC) in the context of both MHC-I and MHC-II restriction with resulting development of antigen-specific T-cells tolerance. They are also active in the secretion of cytokines, eicosanoids, nitric oxide and some ECM components (Naganna, 1989).

Kupffer cells are the phagocytic cells found in the liver. As the most common phagocyte in the human body, their main function is to engulf particulate matter (e.g bacteria) that enters the liver through the portal blood. Kupffer cells are intra sinusoidally located tissue macrophages with a pronounced endocytic and phagocytic capacity. They are in constant contact with gut-derived particulate materials, and soluble bacterial products so that a sub threshold level of their activation in the normal liver may be anticipated. Hepatic macrophages secrete potent mediators of the inflammatory response (reactive oxygen species, eicosanoids, nitric oxide, CO, TNF-alpha and other cytokines) and thus control the early phase of liver inflammation, playing an important part in the innate immune defence. High exposure of kupffer cells to bacterial products especially endotoxins (lipopolysaccharide LPS), can lead to the intensive production of inflammatory mediators, and ultimately to liver injury. Besides typical macrophage activities, kupffer cells play an important role in the clearance of senescent and damaged erythrocytes (Guyton and Hall, 2001).

Hepatic stellate cells are present in perisinusoidal space. They are characterized by abundance of intra cytoplasmic fat droplets and the presence of well-branched cytoplasmic process, which embrace endothelial cells and provide focally a double lining for sinusoid. In the normal liver, HSC store vitamin A, control turnover of extracellular matrix, and regulate the contractility of sinusoid. Acute damage to hepatocyte activate transformation of quiescent stellate into myofibroblast-like cells that play a key role in the development of inflammatory fibrotic response (Kmiec, 2001).

Pit cells represents a liver associated population of granular lymphocyte i.e natural killer (NK) cells. They spontaneously kill a variety of tumor cells in MHC-unrestricted ways and this anti tumor activity may be enhanced by the secretion of interferon-gamma.

Beside pit cells, the adult liver contains other subpopulation of lymphocytes such as gamma delta T cells and both "conventional" and "unconventional" alpha beta T cells, the kupffer containing liver-specific NK T cells (Juan *et al.,*2007).

Parenchymal and non parenchymal cells secrete tens of mediators that exert multiple paracrine and autocrine actions (Kmiec, 2001).

2.1.2 Anatomy of the Liver

The liver is roughly triangular organ that extends across the entire abdominal cavity just inferior to the diaphragm. Most of the liver's mass is located on the right side if the body where it descends inferiorly towards the right kidney. The liver is made up of very soft, pinkish-brown tissue capsule. This capsule is further covered and reinforced by the peritoneum of the abdominal cavity, which protects the liver and holds it in place within the abdomen (Ham, 1974).

The peritoneum connects the liver in four loacations; The coronary ligament, the left and right triangular liagaments, and the falciform ligaments. These connections are not true ligaments in the anatomical sense; rather, they are condensed regions of peritoneal membrane that support the liver (Kelly and Derith, 1989).

The liver consist of four distinct lobes; the right, left, caudate and quadrate lobes.

- The right and left lobes are the largest lobes and are separated by falciform ligament. The right lobe is about five to six times longer than the tapered left lobe.
- The small caudate lobe extends from the posterior side of the right lobe and wraps around the inferior vena cava.

- The small quadrate lobe is inferior to the caudate lobe and extents from the posterior side of the right lobe and wraps around the gall bladder (Shneider and Sherman, 2008).

So also in the liver are found the bile ducts. These are tubes that carry bile through the liver and gall bladder. They form a branched structure known as the biliary tree. Bile produced by liver cells drain into microscopic canals known as bile canaliculi. The countless bile canaliculi join together into many larger bile ducts found throughout the liver. These bile ducts next join to form the larger left and right hepatic ducts, which carry bile from the left and right lobes of the liver. These two hepatic ducts join to form the common hepatic duct that drains all bile away from the liver. The common hepatic duct finally joins with the cystic duct from the gall bladder to form the common bile duct, carrying bile to the duodenum of the small intestine. Most of the bile produced by the liver is pushed back up the cystic duct by peristalsis to arrive in the gall bladder for storage, until it is needed for digestion (William, 1991).

2.1.3 Blood Flow In and Out of the Liver

The circulation of blood in and out of the liver is of major importance to liver functions. Approximately 75% of the supply comes from the hepatic portal veins. This is because blood travelling to the spleen, stomach, pancrease, gall bladder and intestines passes through the capillaries in these organs and is collected into the hepatic portal vein. The blood is rich in nutrients. The hepatic portal vein then delivers this blood to the tissues of the liver where the contents of the blood are divided up into smaller vessels and processed before been passed to the rest of the body (Shneider and Sherman, 2008).

The remainder of the blood supply enters by way of hepatic artery and rich in oxygen. Terminal branches of the blood vessels (veins and arteries) join to form a common capillary bed,

which constitute sinusoids of the liver. Thus, a mixture of venous and arterial blood bathes the liver cells (hepatocytes). The sinusoid empty into venule that occupy the centre of each liver lobule and are called the central veins. The central veins join to form the hepatic vein, which constitute the venous drainage from the liver and empties into the inferior venacava, close to the diaphragm (Longo, 2012).

2.1.4 Liver as Food

The liver of mammals, fowl, and fish are commonly eaten as food by humans. Lamb, calf, chicken and goose livers are widely available from butchers.

Animals liver are rich in iron and vitamin A. Cod liver oil is commonly used as a dietary supplement (Longo, 2012).

2.2 FUNCTIONS OF LIVER

The various functions of the liver are carried out by the liver cells or hepatocytes. The liver is thought to be responsible for up to 500 separate functions usually in combination with other systems and organs. The following are some of the functions of liver:

2.2.1 Carbohydrate Metabolism

Carbohydrates are the most abundant biomolecules on earth. Certain carbohydrates (sugars and starch) are dietary staple in most parts of the world, and the oxidation of carbohydrate is the central energy yielding pathway in most non photosynthetic cells. The end product of carbohydrate metabolism is glucose. Glucose occupies a central position in the metabolism of plants, animals and many microoraganisms. Some tissues depend on glucose for their metabolic

energy (Heirinch *et al.,* 1999). The liver is involved in the following aspects of carbohydrate metabolism:

2.2.1.1 Gluconeogenesis

The supply of glucose from the blood is the sole or major fuel source. However, the supply of glucose from this store is not always sufficient; between meals and during longer fasts, or after vigorous exercise, glycogen is depleted. For these times, organisms need a method of synthesizing glucose from non carbohydrate precursors. This is accompolished by a pathway called gluconeogenesis, which converts pyruvate and related three and four-carbon compounds to glucose. In mammals, gluconeogenesis takes place mainly in the liver.

After vigorous exercise, lactate produced by anaerobic glycolysis in skeletal muscle returns to the liver and is converted to glucose, which moves back to muscle and is converted to glycogen – a circuit called the cori cycle (Hers and Hue,1983).

2.2.1.2 Glycogenesis and Glycogenolysis

Excess glucose in the blood is taken to the liver and converted to glycogen via a process called glycogenesis. In vertebrates, glycogen is found primarily in the liver and skeletal muscle. The glycogen in the muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted in less than an hour during vigorous activity. Liver glycogen serve as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast). So, in conditions in which the readily available glucose is exhausted, the glycogen in the liver is broken down (degraded) to glucose via a pathway called glycogenolysis, and the glucose can be used by tissues in demand of it. However, liver glycogen can be depleted in 12 – 24 hours (Nelson and Cox, 2005).

2.2.2 Lipid Metabolism

Lipids are the principal form of stored energy in most organisms and major constituent of cellular membranes. Specialised lipids serve as pigments, cofactors, detergents, transporters, hormones, extracellular and intra cellular messengers and anchors of membrane protein. The liver is involved in the following aspects of lipid metabolism:

2.2.2.1 Cholesterol synthesis

Cholesterol is a component of cellular membrane and precursor of steroid hormones and bile acids. It is an important essential molecule in human but is not required in diet because all cells can synthesize it from simple precursors. Much of the cholesterol synthesis in vertebrates takes place in the liver. A small fraction of the cholesterol made in the liver is incorporated into the membranes of hepatocytes but most of it is exported as biliary cholesterol, bile acid or cholesteryl esters (Gurr and Harwood, 1991).

2.2.2.2 Fatty acid synthesis and B-oxidation

When a cell or organism has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipid such as triacylglycerols (Nelson and Cox,2005).So also, when in need, fatty acids are oxidized to yield acetyl CoA and NADH via B-oxidation. Under most circumstances, fatty acids are the primary oxidative fuel in the liver. (Gurr and Harwood,1991).

2.2.2.3 Lipoprotein synthesis

When diet contains more fatty acids than are needed immediately as fuel, they are converted in the liver and packaged with specific apolipoprotein into very low density lipoprotein (VLDL).

Excess carbohydrate in the diet can also be converted to triacylglycerols in the liver and exported as VLDLs. High density lipoprotein (HDL) also originates from the liver and small intestine (Gurr and Harwood, 1991).

2.2.2.4 Ketogenesis (synthesis of ketone bodies)

Excess acetyl coA released by oxidation of fatty acids and not required by the liver is converted to ketone bodies; these circulate in the blood to other tissues to be used as fuel for citric acid cycle. Ketone bodies can also be regarded as a transport form of acetyl groups. They can supply a significant fraction of energy in some extrahepatic tissues (Nelson and Cox, 2005).

2.2.3 Bilirubin Metabolism and Bile Production

At the end of the life span of erythrocyte and on daily basis, they are destroyed in the reticuloendothelial system. The protein part of the haemoglobin molecule is catabolised while the porphyrin ring is opened to form bilirubin.

The iron in the molecule is released bound to transferin. It either enters the iron store or is used for further haemoglobin synthesis. Over 80% of bilirubin is derived from haemoglobin (Nduka,1999). The free bilirubin released from the haem moiety circulates in the plasma in water soluble form as bilrubin–albumin complex. This bilirubin which is unconjugated is taken to the liver and then conjugated by the enzyme glucuronyl bilirubin transferase (Nelson and Cox, 2005). The conjugated bilirubin which is water soluble is secreted in the biliary canaliculi and reaches the gut via the biliary system.

Bile is produced by the liver of most vertebrates. It aids in the digestion of lipids in the small intestine. In humans, bile is produced continuesly by the liver, stored and concentrated in

the gall bladder. When an organism eats, it is discharged into the duodenum (Guyton and Hall, 2011).

2.2.4 Detoxification and Drug Metabolism

The liver also detoxifies foreign organic compounds such as drugs, food additives, preservatives and other possibly harmful agents with no food value. Detoxification often involves the cytochrome P-450-dependent hydroxylation of relatively insoluble organic compounds, making them sufficiently soluble for further break down and excretion (Simon *et al.,* 2010).

2.2.5 Protein Metabolism

The liver constantly renews its own proteins, which have a relatively high turnover rate, and is also the site of biosynthesis of most plasma proteins. The liver is involved in the following aspects of protein metabolism:

2.2.5.1 Deamination and transamination of amino acids

Amino acids not needed as biosynthetic precursors are transaminated or deaminated followed by conversion of non nitrogenous parts of those molecules to pyruvate and citric acid cycle intermediates with various fates. Several of the enzymes used in these pathways (e.g alanine and aspartate aminotransferase) are commonly used as markers of liver disease. This function of deamination and transamination and conversion is carried out in the liver (Kieth and Robert, 2001).

2.2.5.2 Removal of Ammonia from the body by synthesis of Urea

This is one of the most important functions of the liver. Urea is synthesized either from the oxidation of amino acids or from ammonia. Ammonia is very toxic and need to be removed. Free ammonia is produced from so many processes such as nucleotide degradation. Excess ammonia in tissues is added to glutamate to form glutamine, a process catalysed by glutamine synthetase. After transport in the blood stream, the glutamine enters the liver and NH_4 is liberated in mitochondria by the enzyme glutaminase. So also, during amino acid catabolism, in most cases, the amino group is transferred to alpha - ketogluterate to form glutamate. The glutamate is transported to liver mitochondria where glutamate dehydrogenase liberates the amino group as ammonium ion (NH_4^+). Now both the NH_4^+ from ammonia and from the amino acids catabolism are combined with CO_2 (as HCO_3^-) to form carbamyl phosphate, thus initiating the urea cycle. Finally, urea is produced and thus ammonia is removed from the body in form of urea by the liver (Ansley *et al.,*1978).

2.2.5.3 Synthesis of Albumin

Albumin and most other proteins (excepting immunoglobin) including blood coagulation factors are synthesized in the parenchymal cells of the liver. Albumin is the main protein of human blood plasma. It binds water, cations (such as Ca^{2+}, Na^+, and K^+), fatty acids, hormones, bilirubin, thyroxine, and pharmaceuticals. Its main function is to regulate the colloidal osmotic pressure of blood (Farrugia, 2010).

2.2.6 Storage of Vitamins and Immunological Function

The liver is a store of various compounds that are essential for body metabolism such as glycogen, vitamin A, B_{12} and iron (Nduka, 1999). The kupffer cells of the liver which are

specialized macrophages are involved in the immunological activities performed by the reticulo endothelial system. Thus, the liver contributes to the body immune system (Nduka, 1999).

All these functions of the liver are necessary for survival. However, when the liver is infected (diseased), it will not be able to carry out its functions. Then what causes liver disease?

2.3 CAUSES OF LIVER DISEASE

Liver disease may occur suddenly or develop slowly over the course of many decades. There are many causes of liver disease. Among them are: microorganisms, chemicals, toxins, food, alcohol, heredity, drugs and infections of other organs.

2.3.1 Microorganisms and Parasites

Micro organisms that cause liver disease include virus, bacteria and other parasites.

2.3.1.1 Virus

Five viruses have been identified to cause inflammation of the liver (viral hepatitis).They are: hepatitis A, B, C, D and E (Kerry and Janice, 2013). In viral hepatitis, the presence of the virus in liver cells causes the immune system to attack the liver, resulting in inflammation and impaired function (Nakamoto and Kaneko, 2003).

2.3.1.2 Bacteria and other parasites

Staphylococcus aureus has been identified as one of the bacteria that cause liver disease (pyogenic or bacterial abscess).The infection is classified based on the route of entry of the organism. Infections may arise from the biliary tract, portal vein and hepatic artery or by direct extension.

Other parasites that may cause liver disease are (i) *Entamoeba histolytica* which cause amoebic liver abscesses. (ii) *Fasciola hepatica*,*Clonorchis sinensis* and *Ascaris lumbricoids* which all cause liver fluke disease.

2.3.2 Chemicals / Drugs/ Toxins and Alcohol

Chemicals such as CCl_4 are found to cause liver disease by production of radicals which destroy the cell membrane of the liver (Recknegel and Glende,1973).

Drugs such as paracetamol, rifampicin or mono amine oxidase inhibitors also cause liver disease by several mechanisms when taken in over dose (Kumar *et al.,* 2004).

Toxins when taken beyond the detoxification ability of the liver may also result to liver disease through a variety of mechanism including cell damage and disrupting cell metabolism (Akunna *et al.,* 2012).

So also alcohol, excess of 80g in men and 40g in women is associated with development of alcoholic hepatitis. Also, excess alcohol may destroy or alter liver cells resulting in fat deposit (fatty liver) and even permanent scarring – cirrhosis (Cotran *et al.,* 2005).

Unscreened herbal remedies and certain dietary supplements may also cause liver disease.

2.3.3 Metabolic syndrome/ Other diseases/ Heridity

Metabolic syndromes, obesity, diabetes and hyperlipidaemia may cause non alcoholic fatty liver disease (NAFLD).Severe NAFLD may lead to inflammation and cirrhosis (Masuoka and Chalasani, 2013).

Some diseases such as leptospirosis, relapsing fever, Brucellosis and typhoid can lead to liver disease. Liver disease may also be due to genetic abnormalities such as hemochromatosis, Wilson disease, tyrosinemia and glycogen storage disease (Sambo, 2008).

Also, auto immune disorder in which the immune system may begin to attack the liver or bile ducts causing inflammation and scarring can lead to a progressive form of liver disease. Examples are primary biliary cirrhosis and autoimmune hepatitis (Jaun *et al.,* 2007).

2.4 TYPES OF LIVER DISEASE

Liver disease are of so many types, among which are: hepatitis, cirrhosis,Wilson disease, hemochromatosis, cancer of the liver and fatty liver.

2.4.1 Hepatitis

This means inflammation of the liver. It is a medical condition that is characterized by the presence of inflammatory cells in tissue of the organ (Mnddrey, 2001).The condition can be self limiting (healing on its own) or can progress to fibrosis. Fibrosis is where scar tissue is formed on the inflamed liver. It can take a variable time to develop. Although fibrosis is present, the liver keeps on functioning quite well. Treating the cause of the inflammation may prevent the formation of further liver damage and may stop or reverse some or all of the scarring. Untreated hepatitis can lead to cirrhosis.

Hepatitis may occur with limited or no symptoms, but often leads to Jaundice, poor appetite and malaise. Hepatitis is acute when it last less than six months and chronic when it persist longer.

Worldwide, hepatitis viruses are the most common cause of the condition, but hepatitis can be caused by other infections, toxic substances (notably alcohol, certain medications, some industrial organic solvents and plants) and autoimmune diseases (WHO, 2013). So also, injury to liver cells due to insufficient blood or oxygen results in ischemic hepatitis or liver shock (Medline Plus, 2012).

2.4.2 Cirrhosis

This is a disease in the liver that is characterized by scarring which produces marked nodularity of the liver. It results from permanent damage or scarring of the liver. It is the end stage of many different forms of liver disease and is known to cause a number of other health problems, including variceal bleeding, ascites and hepatic encephalopathy.

Cirrhosis may result from almost any type of injury to the liver that does not heal but instead lead to progressive inflammation and scarring. Obstruction of the bile duct, when the duct cannot drain bile from the liver, may result to secondary biliary cirrhosis (Mc Graw Hill, 1997).

2.4.3 Wilson Disease

This is also called hepatolenticular degeneration. It is a rare inherited disorder of copper metabolism in which the liver cannot adequately metabolise and remove copper from the body. In wilson disease, cirrhosis of the liver is associated with the degeneration of the brain (Mc Graw Hill, 1997).

2.4.4 Hemochromatosis

This is also a hereditary disease of the liver marked by excessive deposition of iron due to faulty iron metabolism. In this condition, the body absorbs more Iron than normal. It causes cirrhosis and is frequently associated with primary cancer of the liver (MC Graw, 1997).

2.4.5 Cancer of the Liver

Although primary liver cancer is relatively uncommon, many other forms of cancer often metastasize in the liver. Because the liver filters a high volume of blood which may be carrying cancer cells, it is susceptible to developing a form of secondary cancer. If cancer originates in the liver, it is often caused by hepatitis B or C or it can develop in cases of advanced liver disease when cirrhosis is present (Kmiec, 2001).

2.4.6 Fatty Liver

This is the result of excess fat in liver cells. Fatty tissue slowly build up in the liver when a person diet exceeds the amount of fat his body can handle. A person has fatty liver when fat makes up atleast 5% of the liver (Mnddrey, 2001). Simple fatty liver can be a completely benign condition and usually does not lead to liver damage. However, once there is a buildup of simple fat, the liver becomes vulnerable to further injury which may result in the inflammation and scaring of the liver (Duan *et al.,* 2014). Obesity, starvation and protein malnutrition, diabetes, alcohol and CCl_4 are among the causes of fatty liver (Levine *et al.,* 2000).

Classic symptoms of liver disease include: Nausea, Vomiting, right upper quadrat abdominal pain, and Jaundice. Fatigue, weakness and weight loss may also occur. However, since there are

a variety of liver disease, the symptoms tend to be specific for that illness until late-stage liver disease and liver failure occur (Kerry and Janice, 2013).

Most of liver diseases are accompanied by remarkable changes in biochemical parameters of liver disease.

2.5 CONSEQUENCE OF LIVER DISEASE

The consequence of liver disease are numerous and varied. Their ultimate effects are often incapacitating or life threatening, and their presence is ominous. These consequences are not general, but specific to particular liver disease conditions. However, more than one consequence can be a result of a particular liver disease condition.

Among the consequence of liver disease are:

(1) Jaundice: this is a yellow discoloration of skin due to elevated bilirubin concentration in the blood stream. It could be prehepatic, hepatic or post hepatic.

(2) Portal hypertension, ascites and varices resulting from circulatory changes within the diseased liver and producing severe GI hemorrhage and marked sodium and fluid.

(3) Nutritional deficiency, which results from the inability of the damaged liver cells to metabolize certain vitamins (responsible for impaired functioning of the central and peripheral nervous system and for abnormal bleeding tendencies) and to synthesize proteins.

(4) Hepatic encephalopathy or coma, reflecting accumulation of ammonia in the serum due to impaired protein metabolism by the diseased liver. This occur in decompensated liver disease condition (Mnddrey, 2001).

2.6 MARKERS OF LIVER DISEASE AND THEIR CLINICAL SIGNIFICANCE

When the liver is diseased, the liver itself and other organs become affected. Many functions of the liver are affected and a lot of biochemical parameters which are used in measuring how well the liver is, are altered. These biochemical parameters are what we called markers of liver disease. And they are:

2.6.1 Albumin

Albumin is synthesized in the parenchymal cells in the liver. When large number of parenchymal cells are destroyed, synthesis of albumin is consequently impaired (meaning there will be albumin lower than the normal level). Low albumin level however may be due to other factors such as increased metabolism. As such, albumin alone cannot be used as yard stick in assessing liver disease. Nevertheless, in conjunction with other more specific test, it assists in the confirmation of hepatocellular dysfunction (Nduka, 1999).

2.6.2 Aspartate aminotransferase (AST)

Also known as glutamate - oxaloacetate transaminase (GOT). It catalyse the reaction:

$$Aspatate + alpha - ketoglutarate \xrightarrow{AST} oxaloacetate + glutamate$$

It is found in skeletal, muscle, heart and liver (Nyblom *et al.,* 2004).

AST concentration is lower in tissues that contain both AST and ALT except in the liver where they exist in virtually equal amounts (Nduka, 1999).

AST rises up to 100 times the upper limit of normal in severe tissue injury such as acute hepatitis and liver necrosis. This rise may occur before any clinical sign or symptom such as Jaundice manifest (Nduka, 1999).

2.6.3 Alanine aminotransferase (ALT)

Also known as glutamate pyruvate transaminase (GPT). It catalyse the reaction:

$$Alanine + alpha - ketoglutarate \xrightarrow{ALT} Pyruvate + glutamate$$

Its greatest activity is found in the liver. Very high values of ALT are seen in acute hepatitis, either toxic or viral in origin. Moderate increase in ALT level is seen in hepatic diseases such as infective hepatitis, alcoholic hepatitis or hepatocellular carcinoma. Very high activity of serum ALT than normal may be noticed in extra hepatic obstruction or cholestasis. ALT is also produced by epithelial cells of biliary canliculi, and obstruction of bile with consequent irritation of epithelial cells lead to secretion of ALT into serum (Nduka, 1999).

2.6.4 Alkaline phospphatase (ALP)

This is an enzyme that hydrolyse phosphate esters (e.g ATP). Normal circulating levels are contributed by intestine, bone, liver and placenta. ALP level is affected during liver disease. An increase in ALP level of above 5 fold its upper reference range is suggestive of cholestasis. In acute liver cell damage, the rise is usually no more than about twice its upper range (Nduka, 1999). So, the highest ALP rise in liver disease occurs in obstructions in the bilirubin metabolic processes (intrahepatic and extrahepatic obstruction) (Medline, 2013).

2.6.5 Bilrirubin

Bilirubin is also another important marker of liver disease. The measurement of bilirubin could be that of indirect (unconjugated), direct (conjugated) or total (direct + indirect) bilirubin. Each type of bilirubin mentioned give us a kind of clue about the disease to be suspected (Nduka, 1999).

Increase in direct bilirubin indicate bile obstruction, cirrhosis, hepatitis, intrahepatic cholestasis etc. (Keith and Robert, 2001).

Increase in direct bilirubin may indicate Crigler najjar syndrome, erythroblastosis, hemolytic disease of new born, sickle cell anemia, Gilberts syndrome etc. (Keith and Robert, 2001).

Additional conditions in which the test of bilirubin may be performed are biliary stricture, cholangiocarcinoma, haemolytic anaemia due to glucose-6-phosphate DHG deficiency, hepatic encephalopathy etc. (Sambo, 2008).

2.6.6 Urea and others

Urea is formed almost solely in the liver. Hepatic necrosis (which results in absence of metabolism of amino acids) and liver cirrhosis are among the clinical condition that can cause low urea level in blood. In essence, liver damage result to low urea level than normal (Ansley *et al.,* 1978).

Other markers of liver disease include 5^1- nucleotide, Gamma-Glutamyl transpepetidase (GGT), Total serum bile acids, Lactate DHG, Ornithine carbamyl transferase (OCT), Sorbitol DHG (SDH), Creatine phosphokinase (CPK), Choline esterase (ChE) etc. (Jaun *et al.,* 2007).

2.7 TREATMENT OF LIVER DISEASE

If the liver is damaged, and the biochemical parameters indicate that, then it has to be treated before it resume its normal function. The treatment can be achieved by the use of synthetic drugs, herbal drugs and controlled diet.

2.7.1 Synthetic Drugs

There are no commonly accepted, effective conventional drugs therapy regimes to prevent or reverse liver damage (Simon *et al.,* 2010). Treatment mainly consist of identifying the underlying cause (s), determining possible steps to slow or stop progression of degeneration and manage symptoms. More so, the number of drugs actually used successfully in human is very small. Some of the synthetic drugs used in the treatment of liver disease include:

(i) Ribavirin : this is an antiviral drug, it is an oral drug used for treatment of chronic hepatitis C. This drug can cause anemia due to haemolysis. Most importantly, it can cause damage to developing fetus.

(ii) Lamivudine : is an oral drug, also an antiviral used for the treatment of chronic hepatitis B. Unfortunately, in a large fraction of treated patients, the virus learns to mutate or change to avoid drug's effect (Consumer Information by Audience, 2013).

(iii) Interferon injections are used for treatment of both chronic hepatitis B and C, in different doses. Interferon causes fevers, chills and flu like symptoms especially with the first few doses. Drops in white blood cells counts can be seen and can require dose adjustment. New interferon drugs are pegylated, meaning they

contain polyethylene glycol combine with interferon (Consumer Information by Audience, 2013).

(iv) PEG-intron is a drug that is approved for once-weekly therapy for Hepatitis C. Another drug PEGASYS was approved as therapy for treating hepatitis C.

(v) Livolin : this drug is not antiviral but contain an important phospholipid called phosphatidyl choline. This lipid is one of the most important substance for liver protection and health, and is a primary constituent of cell membranes. It acts by several mechanisms: exerting potent antioxidant effects, inhibiting the tendency of stellate cells to progress to cirrhosis, decreasing apoptoic death of liver cells there by prolonging the life of liver cells, stabilizing the cell membrane etc. However, at higher dosages, it may cause reduced apatite, nausea, abdominal bloating, gastrointestinal pain and diarrhea (Consumer Information by Audience, 2013).

There are many other liver drugs such as Entecavir, telbivudine, tenofovir and simeprevir (Consumer Information by Audience, 2013).

All these synthetic drugs are associated with one or more side effects.

2.7.2 Controlled Diet

A liver patient need to adjust, regulate and monitor the kind of diet he consume. This is in order to avoid over working the liver, or taking anything that will increase the intensity of the disease. As such, a liver patient need to take some precautions regarding diet intake. Some of these are specific to certain liver diseases, others relate to how advanced the liver disease is (Kerry and Janice, 2013). Diet control is mostly observed in cirrhosis and advance liver disease,

most especially in decompensated cirrhosis - where the liver is not capable of performing all of its normal functions resulting in a number of complications including fluid retention and mental confusion – encephalopathy (Ansley *et al.,* 1978).The following are some diet control measures that a liver patient may observe:

(i) Protein-rich diet food need to be limited, most especially in decompensated cirrhosis. Much protein when taken by liver patient cannot be properly processed, as a result, ammonia may accumulate leading to hepatic encephalopathy (coma). However, protein should not be limited too much, because it can cause deficiency of certain amino acids.

(ii) Salt intake has to be limited in cirrhosis because as liver disease progresses, fluid can be stored around the stomach area-this is called ascites, so also swelling of the feet and legs (oedema) may occur. This occur due to the fact that salt cause the retention of fluid in the body.

(iii) Carbohydrate intake should be increased in proportion to protein.

(iv) A moderate amount of fat should be taken. Much fat cannot be digested in liver disease patient, most especially if the disease concern bile production or flow.

(v) Extra amount of certain vitamins and minerals need to be taken because a damaged liver has problems storing many vitamins and minerals (British Liver Trust, 2011).

All the above listed precautions and many others not mentioned can help in managing liver disease.

2.7.3 Herbal Drugs

An estimated 4000 million inhabitants of the world, that is about 80% of world's population, are thought to rely chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs. In recent years, there is a growing interest in herbal medicine (Nuhu, 2001).

In the absence of reliable synthetic drugs for the treatment of liver disease in modern medicine, there are a number of medicinal plants recommended for the treatment of liver disorders (Etuk *et al.,* 2009).

Among the plants that are believed to have hepatoprotective / curative effects are *Khaya senegalensis, Calotropis procera*, *Aegle mameloes*, Aloevera, *Eclipta alba*, *Anogeissus leiocarpus* and *Phoenix dactylifera* (Simon *et al.,* 2010). Almost all herbal drugs are of doubtful efficacy and safety. Therefore, there is a growing focus to follow systematic research methodology, and to evaluate scientific basis for the traditional herbal medicine that are claimed to possess hepatocurative activity.

The current study focuses on the use of *Phoenix dactylifera* in treating liver disease.

2.8 *PHOENIX DACTYLIFERA* (DATE FRUIT)

Phoenix dactylifera (Date fruit, *Dabino* in Hausa) is a tree that belongs to the palm family *(Arecaceae*). It grows vertically to form un-branched trunk driven by the activity of a single terminal shoot apex. Date fruits are single seeded fruits of cylindrical, rounded or ovoid shape with fleshy sweet mesocarp covered with a thin epicarp, somewhat yellowish to reddish brown in

colour (Jain *et al.,* 2011). The Arab world is the major producer and exporter of dates in the world. It is grown in more than forty countries in the world.

In Nigeria, it is grown locally in Jigawa, Kano, Sokoto, Zamfara, and Katsina States, though not in an amount that can be enough for local consumption talkless of commercial activities. There are more than 320 varieties in Saudi Arabia (Nasser, 2011). However, a survey conducted during this research in one of the major markets of Date fruit at Babaldu junction, in Birnin Kudu Local Government of Jigawa State, Nigeria, seven varieties of Date fruits where identified by one of the major whole seller of date fruits. Among the seven varieties, four are cultivated locally. The three foreign varieties are Digila (Dan Agadas), Dan Mali and Dan Targal. While the locally produced date fruits are (i) Siki (wet and dry) which has three sub varieties; Yellow, Tafarnuwa and Zabiya (ii) Dan Kila (iii) Zabiya busasshe and (iv) Dan gida.

The importation of foreign date is the major and only factor that makes the fruit available in abundant quantity and cheap price. Among all the seven varieties at Babaldu Junction date fruit market, Digila (Dan Agadas) is the most commonest and most available in Nigeria. It is imported with ease from Niger Republic which serve as a commercial depot of the Date fruits which is believed to be cultivated and imported originally from Algeria. Date fruits are consumed for the pleasant flavor, odor, and their biting texture in addition to their use for flavoring foods, beverages and medicine (Asibey-berko and Tayie,1999).

Sadiq *et al.* (2013) revealed in their experiment that both flesh and seeds of date fruit contain essential and non essential amino acids. Minerals found in both flesh and seeds are Na, K, Ca, Mg, Fe, Zn and P. Phytochemical analysis of *Phoenix dactylifera* revealed the presence of tannins, saponins, cardiac glycosides and steroids in both flesh and seeds of date fruit. Their

work also revealed that the fruit flesh has moisture content 3.50 ± 0.6/100g, crude protein 17.15 ± 0.15g/100g, crude lipid 0.52± 0.01g/100g, ash content 1.50 ± 0.07g/100g, carbohydrate 75.15±0.15g/100g, calorific value 337.7±9.70kj/1000g and crude fibre is below detection limit. The result also reveals that phoenix dactylifera contains antioxidants such as falavonoids (Sadiq *et al.,* 2013).

Antioxidants are defined as molecules which have the property of inhibiting or slowing oxidation reactions and which act to maintain a reducing environment. Both plants and animals have both enzymatic (e.g catalase, glutathione peroxidase, super oxide dismutase etc.) and non enzymatic (i.e small molecules) antioxidants systems. Antioxidants have the primary ability to quench free radical chain reactions, often initiated by reactive oxygen and nitrogen species (RONS) such as peroxide or superoxide (Gazzani *et al.,* 1998).

In the context of health, a plant-based antioxidant is any plant-derived compound that either directly or indirectly contribute to invivo redox balance in humans. It is well understood that antioxidants such as vitamin C, vitamin E and carotenoids are among the major dietary antioxidants we have. There are several other micro nutrients such as selenium, copper and zinc that are so essential for humans to maintain an effective antioxidant system. Selenium is an essential co-factor for glutathione peroxidase, while copper and zinc are essential cofactors for superoxide dismutase (Lii *et al.,* 1998).

The body is subject to a wide range of physiological oxidants. They often have a dual role which can be either beneficial or harmful, depending on where they are produced and their concentration. Some of these oxidants are free radicals, with one or more unpaired electron,

while others are not radicals but are reactive oxygen or nitrogen species that readily form free radicals (Droge, 2002). RONS is the term normally used to describe these oxidants collectively.

Date fruit has been found to be a good source of energy and mixture of antioxidants including vitamin C (ascorbic acid), carotenoids, flavonoids and polyphenols (Faqir *et al.,* 2012).The result of the studies conducted by Faqir *et al.* (2012), indicated that the inclusion of date fruit and its extract in the food will increase the content of antioxidant, and thus probably prevent oxidative deterioration of food.The same research concluded that date fruit have good antioxidant potential and can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products.

The antioxidant activity of date fruit is among the reasons why its hepatocurative activity in hepatotoxic rats caused by CCl_4 was tried. This is due to the fact that CCl_4 causes liver damage via the formation of radicals in chain reaction which eventually destroys the lipid membrane of liver cells. A research conducted by Alqarawy *et al.* (2004) shows that date fruits has the ability to restore the normal functional status of the poisoned liver, and also protect it against subsequent CCl_4 hepatotoxicity.

The mechanism by which date flesh and pits induces its hepatoprotective activity is not certain. However, it is possible that B - sitosterol, a constituent of *Phoenix dactylifera*, is partly responsible for the protective activity against CCl_4 hepatotoxicity (Alqarawy *et al.*, 2004). CCl_4, the inactive metabolite, is transformed to a free radical through the microsomal cytochrome P-450-dependent enzyme, resulting in the activation of CCl_4 toxicity via chain reaction. An additional and important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit aromatase activity of cytochrome P-450, thereby favoring liver

regeneration. On that basis, it is suggested that flavonoids in *Phoenix dactylifera* could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase (Kowalska *et al.,*1990).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Experimental Animals

Male and female albino rats weighing between 200g to 250g were purchased from Biological Sciences Department of Bayero University, Kano. The animals were housed in a well ventilated animal house in the Biological Sciences Department of Bayero University, Kano. The rats were allowed to acclimatize for one week prior to the experiment and had access to food and clean water ad libitum.

3.1.2 Plant Material

Date fruit- *Phoenix dactylifera* (*Dabino* in Hausa); Dan Agadas variety was bought in the month of September, 2014 from Babaldu junction date fruit market in Birnin Kudu Local Government Area of Jigawa State, Nigeria.

3.1.2.1 Preparation of plant extract

The extract was prepared as explained by Agbon *et al.,* 2013 with little modifications. The date fruit obtained was air dried after which the flesh was separated manually from the pits. The flesh was further dried under shed at room temperature. The dried flesh was pulverized into powder and 650g was soaked in a container of 2 litres of cold distilled water for 24 hours. The solution was filtered and the filtrate collected in a container.

A measuring cylinder was used to collect 10ml of the filtrate which was transferred into a well labelled petri dish. The petri dish was placed in a drying cabinet and the filtrate evaporated at a temperature of 55 - 60^0C. The extract was obtained and weighted. The amount per 10ml of the filterate obtained after the evaporation was 2.48g, and was used as a reference point for the extract preparation and administration.

3.1.3 Preparation of Carbon Tetrachloride

Carbon tetrachloride was prepared as CCl_4 solution in olive oil using the formular :

$$Volume\ to\ Administer = \frac{weight\ of\ experiment\ rats\ (kg) \times dose\ of\ CCl_4\ (mg/kg)}{Concentration\ of\ CCl_4\ (mg/ml)}$$

3.1.4 Preparation of Standard Drug

The standard drug used was Livolin. Each tablet contains;

Phosphatidyl choline	300mg
Vitamin B1	10mg
Vitamin B2	6mg
Vtamin B12	10mcg
Vitamin B6	10mg
Nicotinamide	30mg
Vitamin E	10mg

The drug was prepared as Livolin solution in water using the formular;

$$Volume\ to\ Administer = \frac{weight\ of\ experiment\ rats\ (kg) \times dose\ of\ drug\ (mg/kg)}{Concentration\ of\ drug\ (mg/ml)}$$

3.1.5 Diet Preparation

3.1.5.1 Preparation of controlled diet

Dried maize grain (3000g) was bought from Kabuga gate market, Kano. It was grounded into powder with grinding machine. Spinach was also bought from Kabuga gate market and was dried under shade at room temperature. The dried spinach was grounded to powdered form. Maize powder (50g) was mixed with 5g of the powdered spinach, and little water was added to make bolus. The bolus was given as controlled diet to rats in group X to XIV (rats treated with controlled diet).

3.1.5.2 Preparation of uncontrolled diet

The normal chick grower mash feed (Vital feeds company) was bought at Kabuga gate market.

The grower mash was given as food to rats in group I to IX (rats treated with uncontrolled diet).

3.1.6 Animal Groupings and Treatment

A total number of seventy (70) albino rats were used. The animals were grouped into two sets.The first was the uncontrolled diet set, and the second set was the controlled diet set. Each group in the two sets consists of five animals. The animal grouping in the two sets was as follows:

(A) UNCONTROLLED DIET SET :

The animals in this set were grouped and treated as follows:

Group I: Negative control (Food + water only).

Group II: Positive control (100mg/kg CCl_4 once) administered subcutaneously as reported by Alhassan *et al* (2009).

Group III: Food + 249mg/kg body weight of extract for thirty days.

Group IV: Food + 580mg/kg body weight of extract for thirty days.

Group V: 100mg/kg of CCl_4 once only + 249mg/kg body weight of extract for thirty days.

Group XI: 100mg/kg of CCl_4 once only + 580mg/kg body weight of extract for thirty days.

Group VII: 100mg/kg of CCl_4 once only + 5.2mg/kg body weight of standard drug (Livolin) for thirty days as reported by Olukiran *et al* (2014).

Group VIII: 100mg/kg of CCl_4 once only + 249mg/kg body weight of extract + 5.2mg/kg body weight of standard drug for thirty days.

Group IX: 100mg/kg of CCl_4 once only + 580mg/kg body weight of extract + 5.2mg/kg body weight of standard drug for thirty days.

(B)CONTROLLED DIET SET:

The animals in this set (Group X to XIV) were fed with controlled diet (which contain minimal amount of protein and lipids).This feed (which contain minimal amount of protein and

lipids) was given to them (group X to XIV) after inducing liver damage. This set (Group X to XIV) were treated as follows:

Group X: 100mg/kg of CCl_4 once only + controlled diet + 249mg/kg body weight for thirty days.

Group XI: 100mg/kg of CCl_4 once only + controlled diet + 580mg/kg body weight of extract for thirty days.

Group XII: 100mg/kg of CCl_4 once only + controlled diet + 5.2mg/kg body weight of standard drug (Livolin) for thirty days.

Group XIII: 100mg/kg of CCl_4 once only + controlled diet + 249mg/kg body weight of extract + 5.2mg/kg of standard drug for thirty days.

Group XIV: 100mg/kg of CCl_4 once only + controlled diet + 580 mg/kg body weight of extract + 5.2mg/kg of standard drug for thirty days.

CCl_4 administration was done subcutaneously. And any other treatment in animals treated with CCl_4 was done 48hrs after CCl_4 administration.

3.1.7 Collection of Blood Samples

At the end of the experimental period (30 days), the animals were sacrificed by cervical decapitation. The blood of each animal was collected in a separate centrifuging tube. The blood samples were centrifuged and the sera were pipetted into separate well labelled containers. These sera were used in the estimation of the various biochemical parameters.

3.1.8 Instruments, Equipment and Reagents

3.1.8.1 Instruments

The instruments used include:

Test tubes, plain serum bottle, centrifuging tubes, micropippete, syringes(1ml), razor blades, roll of masking tape, conical flask, 10ml measuring cylinder (250ml), test tube hangers, packet of filter paper, petri dish etc.

3.1.8.2 Equipment

The equipment used include :

Equipement	Model
Spectrophotometer	spectrum lab 752s, New life medical instrument, England
Digital weighing balance	kilo tech Elite 210-4
Electronic thermostat water tank	model-HH-W21.Cr42II
Centrifuge	model 800D
Drying oven	Gallenkamp vacuum oven, made in England.

3.1.8.3 Reagents

The reagents used include:

Ferric chloride solution, Dragendroff's reagent, Elacial acetic acid, Ethyl ether, N-butanol, Sodium chloride solution, Starch solution, Baljet reagent, Methyl red, etc.

3.2 METHODS

3.2.1 Methods for the Determination of Biochemical Parameters

All the biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, total bilirubin, direct bilirubin and total protein) were determined using Randox test kits specific for each of the parameters.The procedure were carried out according to manufacturers protocol.

3.2.2 Methods for Phytochemical Analysis

3.2.2.1 Qualitative Test

(a) Determination of Tannins, Ferric chloride test (Trease and Evans,1989)

Distilled water (10ml) was added to 0.5g of the sample. The mixture was filtered.

Few drops of 1% ferric chloride solution was added to 2ml of the filterate. The color change (faint green) was observed.

(b) Determination of Saponins, Froth test (Sofowora,1993)

Distilled water (5ml) was added to 1g of the sample, the mixture was boiled and then filtered. distilled water (3ml) was added to the filtrate and then shaken vigorously for five minutes. Frothing was observed on warming.

(c) Determination of Flavonoids, Ferric chloride test (Aiyelaagbe and Paul, 2009)

Distill water (5ml) was added to 0.5g of the sample. The mixture was boiled then filtered. Few drops of 10% ferric chloride solution was added to 2ml of the filterate. The colour change (violet color) was observed.

(d) Determination of Alkaloids, Dragendroff's test (Brain and Turner,1975)

Aqueous HCl (5ml) was added to 5g of the sample. The mixture was stirred on water bath (55 – 60^{o}C) and filtered. Few drops of dragendroff's reagent was added to 1ml of the filterate. The color change (Dark brown) was observed.

(e) Determination of Cardiac glycoside, Keller killanis test (Aiyelaagbe and Paul,2009)

Extract (100mg) was dissolved in glacial acetic acid (1ml) containing 1 drop of ferric chloride solution. This was then underlayer with concentrated H_2SO_4 (1ml).

A brown ring at the interface was observed.

3.2.2.2 Quantitative Test (Harborne,1992)

(a) Quantitative Determination of Tannins (Harborne, 1992)

This involves two procedures (a and b).

(i)Sample (5ml) was put in a conical flask.0.1N iodine (1ml) was then added. 4% NaOH (10ml) was then added. The mixture was then kept in the dark for 15 min. 4% H_2SO_4 (10ml) was added. The mixture was then titrated with 0.1N sodium thiosulphate, until a milky or clear solution is obtained. Note: three drops of starch was added as indicator before the titration.

The titre value = Tannins + Pseudotannins

Same procedure was done for a blank sample (in which only water was used in place of the original sample).

(ii) Sample (5ml) was put in a conical flask.1% gelatin (15ml) was added. The volume was made up to 100ml with distill water. The mixture was filtered. 0.1N iodine (25ml) was added to 20ml of the filtrate. 4% NaOH (10ml) was added. The mixture was kept in dark for 15 minutes. Distilled water (10ml) was then added.4% H_2SO_4 (10ml) was added. The mixture was then titrated with 0.1N sodium thiosulphate solution, until a clear solution was obtained. Three drops of starch was used as indicator.

The titre value = Pseudotannins only.

Same procedure was done for blank sample.

$$\%\ Pseudotannin + Tannin = \frac{[\ (Blank\ a - \ Expt\ A\ ) \times 0.029\ \times 100}{5\ (\ volume\ of\ sample\ taken\ )}$$

$$\%\ Pseudotannin = \frac{[\ (Blank\ b - Expt\ B)\ \times 0.029\ \times 100}{5\ (\ volume\ of\ sample\ taken)}$$

% true Tannins = [A] – [B]

(b) Quantitative Determination of Saponins (Harborne, 1992)

Extract (50ml) was placed in 500ml flask.50% alcohol (300ml) was added and boiled under reflux for 30minutes. The mixture was then filtered while hot through a coarse filter paper. Charcoal (2g) was then added to the filtrate, it was boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate saponin. The precipitated saponin was collected by decantation and dissolved in small amount of boiling 95% alcohol and filtered while hot. The filterate was cooled at room temperature to separate the saponin in a relatively pure form. The clear supernatant fluid was decanted and the saponins suspended in about 20ml of alcohol and filtered. The filter paper was transferred to a dessicator containing anhydrous calcium chloride and leave to dry.

The amount of the saponin is the total weight of the dessicator and its content – (weight of dessicator + weight of filter paper + weight of anhydrous calcium chloride).

(c) Quantitative Determination of Flavonoids (Harborne, 1992)

80% of Aqueous methanol (100ml) was added to 10g of the sample extract. The mixture was filtered using whatman No 1 filter paper. The filtrate was transferred into crucible and evaporated to dryness over water bath (at 55 – 60^{o}C).It was dried to constant weight. Flavonoid was calculated as percentage of starting material.

(d) Quantitative Determination of Alkaloids (Harborne, 1992)

10% acetic acid (200ml) in ethanol was added to the sample (5g) in 250ml beaker. The mixture was covered and allowed to stand for four hours. It was then filtered. The extract was reduced in water bath to ¼ volume. Concentrated ammonium hydroxide was added drop wise until

precipitation was complete. The solution was allowed to settle and the precipitate collected. The precipitate was washed with dilute ammonium hydroxide. It was then filtered and the residue dried to constant weight. The weight obtained is the amount of alkaloid.

(e) Quantitative Determination of Cardiac glycoside (Harborne, 1992)

Extract (8ml) was transferred into 100ml volumetric flask and water (60ml) and 12.5% lead acetate (8ml) were added. All was mixed and filtered. Filterate (50ml) was transferred into another 100ml flask and 4.7% Na_2HPO_4 (8ml) were added to precipitate excess Pb^{++} ions. This was mixed and completed to volume with water.

The mixture was filtered twice through same filter paper to remove excess lead phosphate. Purified filtrate (10ml) was transferred into clean flask and treated with Baljet reagent (10ml). These were allowed to stand for 1 hour for complete color development. An orange-red color was observed. The colour intensity was measured calometrically at 495nm. The % of total glycoside was calculated using the formular ;

$$\%\ of\ total\ Glycoside = \frac{A \times 100}{17}$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical Analysis of Date fruit

Table 1 shows the result of qualitative analysis of phytochemicals present in the aqueous extract of Date fruit.The result shows the presence of tannins, saponins, cardiac glycosides and flavonoids in the aqueous extract of the Date fruit while alkaloids are absent.

Table 1: Phytochemical qualitative analysis of aqueous extract of Date fruit

PHYTOCHEMICAL	INFERENCE
Tannins	Positive
Saponins	Positive
Cardiac glycoside	Positive
Flavonoids	Positive
Alkaloids	Negative

The result for quantitative analysis of phytochemicals present in the aqueous extract of date fruit is presented in table 2. The result shows the amount of phytochemical per 100g of the extract to be 0.44g/100g for tannins, 0.73g/100g for saponins, 2.09g/100g for flavonoids and 1.51g/100g for cardiac glycosides.

Table 2: Quantitative estimation of phytochemicals present in aqueous extract of Date fruit.

PHYTOCHEMICAL	QUANTITY (g / 100g of extract)
Tannin	0.44g
Saponins	0.73g
Flavonoids	2.09g
Cardiac glycoside	1.51g

4.1.2 Biochemical Analysis

Table 3 shows comparison between negative control group (group I) and the positive control group (group II). The result shows a significant ($P < 0.05$) increase in the level of serum AST, ALT, ALP, total bilirubin and direct bilirubin in group II compared to group I. This indicates that hepatotoxicity is induced in group II. The result also shows significant decrease ($P < 0.05$) in the level of total protein and albumin in group II compared to group I. This further reaffirm the inducement of hepatotoxicity in group II.

Table 3: Comparison of biochemical parameters between negative control group (group I) and positive control group (group II)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	ALBUMIN (g/L)
GROUP I	36.36^{a} ± 1.17	44.75^{b} ± 1.19	78.55^{c} ± 1.20	74.00^{d} ± 0.99	0.95^{e} ± 0.04	0.50^{f} ± 0.07	35.68^{g} ± 2.41
GROUP II	105.23^{a} ± 2.74	93.22^{b} ± 1.31	114.53^{c} ± 3.75	40.20^{d} ± 1.58	1.97^{e} ± 0.05	1.26^{f} ± 0.18	20.73^{g} ± 0.96

Values are expressed as mean ± SD

Values in the same column bearing the same superscript are significantly different at $P< 0.05$

n (number of rats)= 5 rats for each group

Table 4 shows the results of biochemical parameters (AST, ALT, ALP, total protein, total bilirubin, direct bilirubin and albumin) in the negative control group (group I), positive control group (group II), group that were administered 249mg/kg of the extract (group III) and the group that were administered 580mg/kg of the extract (group IV). The result shows no significant ($P > 0.05$) difference in the level of biochemical parameters between group III and I except in the level of total protein. There is also no significant ($P > 0.05$) difference in the level of biochemical parameters between group IV and I except in the level of ALP. All these indicate that both 249mg/kg and 580mg/kg of the extract may have no harmful effect on the liver.

Table 4: Comparison of biochemical parameters between negative control group (group I), positive control group (group II), group administered 249mg/kg of extract (group III) and 580mg/kg of extract (group IV)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	ALBUMIN (g/L)
GROUP I	36.36^{a} ±1.17	44.75^{d} ±1.19	78.55^{g} ± 1.20	74.00^{l} ±0.99	0.95^{r} ±0.04	0.50^{u} ±0.07	35.68^{x} ±2.41
GROUP II	105.23^{abc} ± 2.74	93.22^{def} ±1.31	114.53^{gh} ±3.75	40.20^{lo} ±1.58	1.97^{rst} ±0.05	1.26^{uvw} ±0.18	20.73^{xyz} ± 0.96
GROUP III	35.28^{b} ± 1.71	47.54^{e} ± 1.32	80.04^{hk} ± 2.06	65.09^{ql} ±1.21	0.98^{s} ± 0.09	0.49^{v} ±0.03	32.73^{y} ±0.96
GROUP IV	37.73^{c} ±1.75	45.58^{f} ± 1.15	72.65^{kg} ± 1.16	70.17^{oq} ±1.80	0.97^{t} ±0.10	0.49^{w} ±0.15	34.13^{z} ±0.74

Values are expressed as mean ± SD

Values in the same column bearing the same superscript are significantly different at $P < 0.05$

n(number of rats)= 5 for each group

The result of biochemical parameters of uncontrolled diet set (group I, II, V, VI, VII, VIII and IX) are shown in table 5.The result shows significant ($P < 0.05$) decrease in the level of AST, ALP, ALT, Direct bilirubin and Total bilirubin, as well as, significant ($P < 0.05$) increase in the level of Albumin and Total protein in hepatotoxic group that were treated with 249mg/kg of the extract (group V) and the positive control group (group II).This suggest that the 249mg/kg of the extract may have hepatocurative effect. Comparison between group V and the negative control group (group I) shows that the levels of AST, ALT, ALP, Direct bilirubin and Total bilirubin are significantly ($P < 0.05$) higher in group V, and the level of Albumin and Total protein and Albumin are significantly lower in group V. This indicate that even though the 249mg/kg of the extract may possess hepatocurative effect, but it does not result to complete healing of the liver. The result as presented in table 5 also shows that the level of AST, ALP, ALT, Total bilirubin and Direct bilirubin are significantly ($P < 0.05$) lower in hepatotoxic groups treated with 580mg/kg of extract (group VI) compared to the positive control group (group II),It also shows that the level of Albumin and Total protein are significantly ($P < 0.05$) higher in group VI compared to group II. This also suggest that the 580mg/kg of the extract may have hepatocurative effect. Comparison between group VI and group I shows that the level of AST, ALP, and ALT is significantly ($P < 0.05$) higher in group VI, and the level of Albumin and Total protein is significantly ($P < 0.05$) lower in group VI than in group I. This also might mean that the 580mg/kg of the extract does not lead to total healing of the liver. Comparison between hepatotoxic group treated with 249mg/kg of extract (group V) and hepatotoxic group treated with 580mg/kg of the extract (group VI) shows that the level of AST, ALT, ALP, direct and total bilirubin are significantly ($P < 0.05$) lower in group VI compared to group V. It also shows that the level of albumin and total protein are significantly ($P < 0.05$) higher in group VI than in

group V. These all may suggest that the 580mg/kg of the extract has more hepatocurative effect than the 249mg/kg of the extract.

Also in table 5, the result of comparison between hepatotoxic groups that were treated with 5.2mg/kg of standard drug (group VII) and the positive control group (group II) shows that the levels of AST, ALT, ALP, Direct bilirubin and Total bilirubin are significantly ($P < 0.05$) lower in group VII compared to group II. It also shows that the level of Albumin and Total protein are significantly ($P < 0.05$) higher in group VII than in group II.This also suggest that 5.2mg/kg of the standard drug may have hepatocurative effect. However, comparison between group VII and group I shows no significant ($P > 0.05$) difference in the level of biochemical parameters except in the level of ALT where it is significantly ($P < 0.05$) higher in group VII than in group I, and in the level of Total protein and Albumin where they are significantly ($P < 0.05$) lower in group VII than in group I. Comparison between hepatotoxic group treated with 249mg/kg of extract (group V) and hepatotoxic group treated with 5.2mg/kg of Livolin (group VII) shows that the level of AST, ALT, ALP, Total bilirubin and Direct Bilirubin are significantly ($P < 0.05$) in group V than in group VII.And the level of Albumin and Total protein are significantly ($P < 0.05$) lower in group V than in group VII.This indicates that Livolin might have more hepatocurative effect than 249mg/kg of the extract. However, when group VI and group VII were compared, there is significant ($P < 0.05$) increase only in the level of AST and ALP. This indicates that the 580mg/kg of the extract has almost the same hepatocurative effect as Livolin. The result shows significant ($P < 0.05$) decrease in the level of AST, ALP, ALT, Direct bilirubin, and Total bilirubin, and significant ($P < 0.05$) increase in the level of Albumin and Total protein in hepatotoxic group treated with 249mg/kg of extract + 5.2mg/kg of standard drug (group VIII) compared to the positive control group (group II).This indicate that treatment with 5.2mg/kg of

standard drug + 249mg/kg of the extract may result to hepatocurative effect. Furthermore, the result shows significant ($P < 0.05$) decrease in the level of AST, ALP, ALT, Direct bilirubin and Total bilirubin, as well as, significant ($P < 0.05$) increase in the level of Albumin and Total Protein in hepatotoxic groups treated with 580mg/kg of extract + 5.2mg/kg of standard drug (group IX) compared to the positive control group (group II).This further indicates that treatment of hepatotoxic rats with 5.2mg/kg of standard drug + 580mg/kg of the extract may result to hepatocurative effect.

Table 5: Effect of oral administration of aqueous extract of *P. dactylifera* on biochemical parameters in rats treated with uncontrolled diet (Group I, II, III, IV, VII, VIII and IX).

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PRO (g/L)	TOTAL BIL (mg/dl)	DIRECT BIL (mg/dl)	ALBUMIN (g/L)
GROUP I	36.36^{a} ± 1.17	44.75^{abcd} ± 1.19	78.55^{a} ± 1.20	74.00^{abc} ± 0.99	0.95^{a} ± 0.04	0.50^{ab} ± 0.07	35.68^{abcd} ± 2.41
GROUP II	105.23^{abcd} ± 2.74	93.22^{aefg} ± 1.31	114.53^{abcd} ± 3.75	40.20^{aefg} ± 1.58	1.97^{abcde} ± 0.05	1.26^{bcdef} ± 0.18	20.73^{aefg} ± 0.96
GROUP V	90.26^{aefgl} ± 2.24	87.81^{ahik} ± 1.69	98.39^{aefg} ± 1.31	50.06^{ahi} ± 1.32	1.47^{afghi} ± 0.18	1.07^{aghjk} ± 0.13	25.14^{ahij} ± 1.19
GROUP VI	45.59^{ahij} ± 2.44	49.65^{a} ± 1.37	90.07^{ahik} ± 1.88	60.80^{a} ± 1.71	1.02^{bf} ± 0.17	0.52^{cg} ± 0.07	31.73^{a} ± 0.96
GROUP VII	39.31^{beh} ± 0.10	48.36^{beh} ± 1.50	79.06^{beh} ± 0.80	65.03^{beh} ± 3.73	0.99^{cg} ± 0.18	0.50^{dh} ± 0.05	31.42^{beh} ± 2.82
GROUP VIII	38.38^{cfi} ± 2.08	49.28^{cfi} ± 1.17	80.10^{cfi} ± 2.65	65.03^{cfi} ± 3.73	0.95^{dh} ± 0.04	0.48^{ej} ± 0.05	30.21^{cfi} ± 0.69
GROUP IX	37.51^{dgj} ± 0.97	48.32^{dgk} ± 1.84	77.28^{dgk} ± 1.38	68.92^{agj} ± 3.08	1.02^{ei} ± 0.26	0.51^{fk} ± 0.07	29.05^{dgj} ± 0.84

Values are expressed as mean ± SD n=5

Values in the same column bearing the same superscript are significantly different at $P < 0.05$

Table 6 shows the result of biochemical parameters in the controlled diet set (group X, XI, XII, XIII and XIV). These sets were treated with controlled diet after liver damage caused by CCl_4.

Table 6: Effect of oral administration of aqueous extract of *P. dactylifera* on biochemical parameters in rats treated with controlled diet (Group 1X, XI, XII, XIII and XIV)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	ALBUMIN (g/L)
GROUP X	92.24^{abc} ±2.14	88.72^{abc} ± 1.69	97.61^{acd} ± 1.54	50.80^{abcd} ±1.93	1.47^{abcd} ± 0.09	1.13^{abcd} ±0.16	24.03^{abcd} ± 0.59
GROUP XI	45.32^{ae} ± 2.32	50.27^{a} ± 1.32	91.08^{aefg} ± 0.65	62.14^{ae} ±2.19	1.02^{a} ± 0.07	0.49^{a} ± 0.08	31.35^{a} ±1.53
GROUP XII	41.15^{b} ± 1.57	50.86^{bd} ± 2.52	81.44^{ae} ±1.58	66.40^{b} ±2.44	0.97^{b} ± 0.11	0.51^{b} ±0.12	29.80^{b} ± 1.15
GROUP XIII	39.38^{ce} ± 1.52	50.79^{ce} ±1.19	81.49^{cf} ± 1.80	67.37^{ce} ± 2.44	0.97^{c} ± 0.08	0.50^{c} ± 0.02	29.26^{c} ±0.80
GROUP XIV	40.22^{a} ± 2.50	49.82^{ade} ±1.11	79.90^{dg} ± 2.08	65.71^{d} ±1.37	1.02^{d} ±0.11	0.52^{d} ± 0.05	30.20^{d} ± 1.88

Values are expressed as mean ± SD

Values in the same column bearing the same superscript are significantly different at $P < 0.05$

n(number rats) = 5 for each group

Table 7 shows the result of the assessment of the effect of controlled diet in treating liver disease. The result shows no significant ($P > 0.05$) difference in the level of biochemical parameters between hepatotoxic group treated with 249mg/kg of extract only (group V) and hepatotoxic group treated with 249mg/kg of extract + controlled diet (group X). The result also shows no significant ($P > 0.05$) difference in the level of biochemical parameters between hepatotoxic group treated with 580mg/kg of extract only (group XI) and hepatotoxic groups treated with 580mg/kg of extract + controlled diet(group XI). Also, there is no significant ($P > 0.05$) difference in the level of biochemical parameters between hepatotoxic groups treated with 5.2mg/kg of standard drug only (group VII) and hepatotoxic group treated with 5.2mg/kg of standard drug + controlled diet (Group XII). Furthermore, the result shows no significant ($P > 0.05$) difference in the level of biochemical parameters between hepatotoxic group treated with 249mg/kg of extract + 5.2mg/kg of standard drug (group VIII) and hepatotoxic group treated with 249mg/kg + 5.2mg/kg of standard drug + controlled diet (group XIII). The result also shows no significant ($P > 0.05$) difference in the level of biochemical paramaters between hepatotoxic group treated with 580mg/kg of extract + 5.2mg/kg of standard drug (group IX) and hepatotoxic group treated with 580mg/kg of extract + 5.2mg/kg of standard drug + controlled diet (group XIV). These all suggest that the control diet used in this research may have no any effect in treating liver disease.

Table 7: Comparison of biochemical parameters between uncontrolled diet Set (V, VI, VII, VIII and IX) and controlled diet Set (X, XI, XII, XIII and XIV).

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	ALBUMIN (g/L)
GROUP V	90.26 ±2.24	87.81 ± 1.69	98.39 ±1.31	50.06 ±1.32	1.47 ±0.18	1.07 ± 0.13	25.14 ± 1.19
GROUP X	92.24 ±2.14	88.72 ± 1.69	97.61 ± 1.54	50.80 ± 1.93	1.47 ± 0.09	1.13 ± 0.16	24.03 ± 0.59
GROUP VI	45.59 ± 2.44	49.65 ± 1.37	90.07 ± 1.88	60.80 ±1.71	1.02 ± 0.17	0.52 ±0.07	31.73 ± 0.96
GROUP XI	45.32 ± 2.32	50.27 ± 1.32	91.08 ± 0.65	62.14 ± 2.19	1.02 ± 0.07	0.49 ± 0.08	31.35 ±1.53
GROUP VII	39.31 ±0.10	48.36 ± 1.50	79.06 ±0.80	65.03 ±3.73	0.99 ±0.18	0.50 ±0.05	31.42 ±2.82
GROUP XII	41.15 ± 1.57	50.86 ± 2.52	81.44 ± 1.58	66.40 ± 2.44	0.97 ± 0.11	0.51 ± 0.12	29.80 ± 1.15
GROUP VIII	38.38 ± 2.08	49.28 ± 1.17	80.10 ± 2.65	65.03 ±3.73	0.95 ±0.04	0.48 ± 0.05	30.21 ± 0.69
GROUP XIII	39.38 ± 1.52	50.79 ±1.19	81.49 ± 1.80	67.37 ± 2.44	0.97 ± 0.08	0.50 ±0.02	29.26 ± 0.80
GROUP IX	37.51 ± 0.97	48.32 ±1.84	77.28 ±1.38	68.92 ±3.08	1.02 ± 0.26	0.51 ± 0.07	29.05 ± 0.84
GROUP XIV	40.22 ± 2.50	49.82 ± 1.11	79.90 ± 2.08	65.71 ± 1.37	1.02 ±0.11	0.52 ± 0.05	30.20 ±1.88

Values are expressed as mean ± SD n=5

Values in the same column bearing the same superscript are significantly different at $P < 0.05$

4.2 DISCUSSION

In the present research, phytochemical analysis of aqueous extract of date fruit reveals the presence of flavonoids, tannins, saponins and cardiac glycosides. This finding is similar to that of Sadiq *et al.,* (2013). Contrary to the findings of Agbon *et al.,* (2013), the presence of alkaloids was not noticed.

The result of this research shows that administration of 100mg/kg of CCl_4 subcutanously once to normal (healthy) rats induce hepatotoxicity after 48 hours. Literature search shows that certain concentrations of CCl_4 can induce liver damage but natural healing started after three days of liver damage inducement (Shehu, 2008). Eventhough, Alhassan *et al.* (2009) concluded in their experiment that natural healing of the liver occurs 72hrs after administration of low doses of CCl_4, but they also confirmed that high doses (90 -120mg/kg) can induce massive liver damage and may persist for longer period compared to lower dosage, hence giving rise to an ideal hepatotoxicity rat model. In a research conducted by Ilanchezhian and Roshy (2008), the biochemical parameters level and most especially the histopathological studies in the natural recovery group, support that natural healing occurs but the degree of the healing is not matcheable with the group in which drug was administered. As such, in this project,100mg/kg as reported by Alhassan *et al.* (2012) was administered to healthy rats (group II, V, VI, VII, VIII, IX, X, XI, XII, XIII and XIV), and the result shows that 100mg/kg of CCl_4 caused liver damage. This result is similar to that reported by Alhassan *et al* (2009) and Alhassan *et al.* (2012).

The hepatotoxic effects of CCl_4 occurs when CCl_4 is metabolized by the cytochrome-P-450 systems in the liver into a highly reactive metabolite – trichloro methyl radical, $\cdot CCl_3$, which binds covalently to macromolecules and induce peroxidative degradation of membrane lipids of

endoplasmic recticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, thus initiating lipid peroxidation (Rechnagel *et al.,* 1973). Lipid peroxidative degradation is one of the principal causes of hepatotoxicity of CCl_4 (Muriel and Escobar, 2003). This is evidenced in this research by the elevation in the serum level of AST, ALP and ALT in group II. These are all non plasma specific enzymes and mostly found in kidney, heart, liver etc. These enzymes were reported to be higher in hepatotoxic rats than in normal rats when there is liver necrosis (Keith and Robert, 2001). The concurrent elevation of serum AST together with ALP and ALT more likely indicates hepatotoxicity induced by CCl_4 (Nduka, 1999). The results showed an increase in total and direct bilirubin level in the positive control group (group II) compared to the negative control group (group I), this indicates the presence of hyperbilirubunaemia. These observations are all indicators of hepatic cell damage and is in accordance with the report of Venukumar and Latha (2002).This study also shows a significant decrease in serum albumin and total protein concentrations in hepatotoxic rats.This is also an indication that liver damage is induced by CCl_4, because the liver is the primary site for the synthesis of plasma proteins; as such, hepatic injury will cause a decrease in serum albumin and total protein concentrations (Etuk *et al.,*2009).

The administration of different concentrations (249mg/kg and 580mg/kg) of aqueous extract of date fruit to healthy rats resulted in no significant ($P > 0.05$) increase in AST, ALT, ALP, total and direct bilirubin. In addition, it also shows no siginificant ($P > 0.05$) decrease in total protein and albumin concentrations. All these indicate that aqueous extract of date fruit may have no harmful effect on liver. This is in accordance to the findings of Agbon *et al.,* (2013) who reported that the oral acute toxicity (LD_{50}) of the aqueous extract of date fruit is greater than 5000mg/kg.

Date fruit have been used extensively for traditional cure of liver illness in Arab peninsular (Alshoaibi *et al.,* 2012). In this research, the administration of 249mg/kg of aqueous extract of date fruit to hepatotoxic rats resulted in significant ($P < 0.05$) decrease in the level of AST, ALT, ALP and total bilirubin. It also resulted in significant ($P < 0.05$) increase in the level of total protein and albumin concentrations. Moreso, the administration of 580mg/kg of aqueous extract of date fruit to hepatotoxic rats resulted in significant ($P < 0.05$) decrease in the level of AST, ALT, ALP and total bilirubin. It (580mg/kg) also resulted in significant ($P < 0.05$) increase in the level of total protein and albumin concentrations. All these results suggest that administration of either 249mg/kg or 580mg/kg of aqueous extract of date fruit has hepatocurative effect. This is in accordance to the findings of Alqarawi *et al.* (2004) who reported that the extract of date fruit has hepatocurative effects.

The reason why the aqueous extract of date fruit induces hepatocurative effect could be due to the presence of antioxidants such as flavonoids and vitamin C (Faqir *et al.,* 2012). Flavonoids are phenolic substances that have the ability to reduce free radical formation and scavenge free radicals. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess radical scavenging ability. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity (Pietta, 2000). It was also found that phenolic and polyphenolic compounds are very efficient scavengers of free radicals (Halliwell, 1994) because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogen. Morever, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion, which catalyse lipid peroxidation (Gazzani *et al.,* 1998). The hepatocurative effect of the aqueous extract of date fruit could also be due to the ability of flavonoids to inhibit the activity of cytocrome P-450 aromatase, this is because one of the

important factor in the hepatocurative / protective activity of any drug is its ability of its constituents to inhibit aromatase activity of cytochrome P-450, thereby favoring liver regeneration (Kowalska *et al.,* 1990).

The result of this research also shows that the administration of 5.2mg/ kg/ day of Standard drug (Livolin) to hepatotoxic rats result in significant ($P < 0.05$) decrease in the level of AST, ALT, ALP, total and direct bilirubin. It also resulted in significant ($P < 0.05$) increase in the level of total protein and albumin in the hepatotoxic rats. These all suggest that the administration of 5.2mg/ kg/ day of the standard drug (Livolin) may have hepatocurative effect. This is in accordance to the findings of Olukiran *et al.,* (2014). Although, the level of some of the biochemical parameters (ALT, total protein and albumin) in the hepatotoxic rats treated with the standard drug are significantly ($P < 0.05$) different from that of negative control group (healthy rats), but still it (level of biochemical parameters) is the closest to that of the healthy rats, than that in rats treated with 249mg/kg and 580mg/kg of extract. The standard drug (Livolin) is not antiviral, but it contain an important phospholipid called phosphatidyl choline. This lipid is one of the most important substances for liver protection and health, and is a primary constituent of cell membranes. It acts by several mechanisms: exerting potent antioxidant effects, inhibiting the tendency of stellate cells to progress to cirrhosis, decreasing apoptoic death of liver cells (thereby prolonging the life of liver cells) and stabilizing the cell membrane.

In addition, this research shows that administration of 249mg/kg and 580mg/kg concentrations of the aqueous extract of date fruit combined separately with 5.2mg/kg of the standard drug to the hepatotoxic group result to hepatocurative effect. This is with no surprise, as each of the extract (249mg/kg and 580mg/kg) separately alone exert hepatocurative effect.

As earlier stated, an assessment of the effect of controlled diet on liver disease was carried out. It is expected that controlled diet may help the liver to resume normal function and prevent other consequences that may result from the liver disease. It is clear that too much protein, salt and lipids is not good for liver patients, most especially if the liver disease condition is very severe to the extent that the liver cannot process them (protein, salt and lipids). Failure of the liver to process these food may lead to greater complications (British Liver Trust, 2011). However, the result of this research showed that there is no significant ($P > 0.05$) difference in the level of biochemical parameters between hepatotoxic rats treated and fed with uncontrolled diet and those treated and fed with controlled diet. This indicates that controlled diet used in this research has no effect in treating liver disease or probably the liver disease is not too chronic and severe, and it is not a decompensated type, hence the treatment may not require the restriction and control of certain diets (British Liver Trust, 2011).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

The present research reveals the presence of flavonoids, saponin, tannin and cardiac glycosides in the aqueous extract of *Phoenix dactylifera*, while alkaloids are absent. It also reveals that 249mg/kg and 580mg/kg concentrations of the aqueous extract of *Phoenix dactylifera* has no any negative effect on normal liver of rats. It also shows that both concentrations of the extract have hepatocurative effects, although, the 580mg/kg concentration have more hepatocurative effect than 249mg/kg concentration of the extract. The hepatocurative effect of the extract could be due to the presence of phytochemicals such as flavonoids as evidenced in the results of the phytochemical analysis conducted in the research. Flavonoids has antioxidant activity, and that could have contributed in curing liver disease by either quenching free radical chain reaction or through other mechanisms. It was also found that the controlled diet used in this research has no effect in treating liver disease.

5.2 CONCLUSION

The present research reveals that *Phoenix dactylifera* has hepatocurative effect. As such, it is hoped that it will contribute immensely in the treatment of liver disease.

5.3 RECOMMENDATIONS

1. It is recommended that the possibility of inclusion of date fruit in the diet of liver patients should be looked into.

2. The amount of date fruit that will exert the same or greater hepatocurative effect than the standard drug (Livolin) should be determined.

3. It is also recommended that healthy individuals should be eating date fruit once in a while, so as to benefit from its content.

4. It is also recommended that the control diet to be used for subsequent research on liver disease should be deproteinised or defattened.

5. It is recommended that the other varieties of date fruit should be used to compare and fish out the most effective.

REFERENCE

Agbon,A.N., Kwanashie,H.O. and Hamman,W.O (2013) "Evaluation of antidiarrheal effect of the aqueous fruit extract of *Phoenix dactylifera L* . on the histology of the small intestine of wister rats". *British Journal of pharmacology and toxicology*. **4(3)**: 121-127.

Aiyelaagbe,O.O. and Paul,M.O. (2009) "Phytochemical Screening for Active compounds in *Mangifera indica* from Ibadan,Oyo State". *Plant Science Research.*Volume **2**,Issue 1.

Akunna,G.G., Saalu,C., Ogunmodede,O.S., Ogunlade,B. and Bello,A. (2012) "Aqueous extract of date fruit (*Phoenix dactylifera*) protect Testis against Atrazine-induced toxicity in rats". *World Journal of Life Science and Medical Research* 2012 ; **2(2)**:100

Alhassan,A.J., Sule,M.S., Hassan.J.A., Baba,B.A. and Aliyu,M.D. (2009) "Ideal hepatotoxicity model in rats using carbon tetrachloride (CCl_4)".*Bayero Journal of pure and applied sciences*, **2(2)** :185-187.

Alhassan,A.J., Sule,M.S., Atiku,M.K., Wudil,M.A., Dangambo,M.A., Mashi,J.A., Zaitun,M. and Uba,G.K. (2012) "Effect of *Caliptropis procera* aqueous root extract gainst CCl_4 induced liver toxicity in rabbits". *Bayero journal of pure and applied sciences*, **5(1)**: 38-43.

Al-qarawi,A.A., Mousa,H.M., Ali,B.H., Abdel-Rahman.H. and Almagy,S. (2004) "Protective effect of extracts from dates (*Phoenix dactylifera L.*) on carbon tetrachloride-induced hepatotoxicity in rats". *International Journal of App/Rev Veterinary Medicine* .Vol. **2**, No. **3**.

Alshoaibi,Z., Amamary,M.A., Al-Habori,M.A., Al Zubair,A.S. and Abdelwahab,S.I (2012) "Invivo antioxidant and hepatocurative effect of palm date fruit (*Phoenix dactylifera*)". *International Journal of pharmacology*, **8** : 185-191.

Ansley,J.D., Isaacs,J.W., Rikkers,L.F., Kutner.M.H., Nordlinger,B.M. and Rudman,D. (1978). "Quantitative test for Nitrogen metabolism in cirrhosis; Relation to other manisfestations of liver disease".*Gastroentrology*, **75** :570 -579.

Arthur,C.G. and John,E.H. (1995). " *Textbook of medical physiology*". John E Hall.

Asibey-berko,E. and Tayie,F.A.K. (1999). "Proximate analysis of some under utilized Ghanaian vegetables" *Ghana Journal of science* **39**.,91-9 future in: *International Journal of food sciences and nutrition*.Vol **54(4)** :247-259.

Brain,K.R. and Turner,T.D. (1975) "Scientific evaluation of phytochemicals" First Edition.Bristol.

British Liver Trust (2011) " The use of controlled diet in treating liver disease". Available at www.british liver trust.org.uk

Consumer information by Audience (2013). U.S Food and drug Administration,10903 New Hampshire Avenue, Silver Spring, M D 20993.

Cotran,R.S., Kumar,V., Fausto,N., Nelson,F., Robbins,S.L., Abbas,A.K. (2005). "*Robbins and Cotran pathologic basis of disease*" (7th ed.) St. Louis, M O: Elsevier Saunders. P878.

Council man,W.T.(1993). "*Diseases and its causes*". New York Henry Holt and company,London Williams and Norgate.The University Press,Cambridge,U.S.A.

Dietter, H. ed (2011). Liver Regeneration". Berlin: De Gruyter. Pp1.

Droge,W. (2002). "Free radicals in the physiology control of cell function".*Physiological reviews* **82**, 47-95.

Duan X-Y., Zhang,L., Fan J-G., Qia, o.L. (2014). "NAFLD leads to liver cancer:do we have sufficient evidence?". *Cance Lett*.2014; **345(2)**:230-4. Doi: 10:1016/.

El Hadrami,A. and Alkhayri,J.M. (2012) "Socio economic and traditional importance of date fruit". *Emir.J.Food Agric* 2012. **24(5)**:371-385.

El Hadrami,A., Daayf,F. and El Hadrami,I (2011). "Secondary metabolites of date palm" In:Jain,S.M.,Alkhayri,J.M. and Johnson,D.V.

Etuk,E.U., Agaie,B.M., Ladan,M.J. and Garba,I. (2009). "The modulatory effect of *Cochspermum tinctoriuma* rich aqueus root extract on liver damage induced by carbon tetrachloride in rats". *African Journal of pharmacy and pharmacology*.Vol **3 (4)**. Pp 151-157.

Faqir,M.A., Sardar,I.B., Ahmad,H.E., Muhammad,I.K. and Muhammad,S.A. (2012). "Phytochemical characteristics of date palm (*Phoenix dactylifera*) fruit extracts". *Pakistan Journal of Food Science*. **22(3)**:117-127.

Farrugia,A. (2010) "Albumin usage in clinical medicine:tradition or therapeutic?". *Transfus Med Rev* **24(1)**:53-63.

Gazzani,G., Papetti,A. and Massolini,G. (1998) "Anti-and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment". *Journal of Agric. And Food chemistry.* **46**:4118 - 4122

Gurr,M.I and Harwood,J.L. (1991) "*Lipid Biochemistry:An introduction*". 4th edition,Chapman and Hall. London.

Guyton,A.C. and Hall,J.E. (2001). "*Text book of medical physiology*". Harcourt international edition.10th edition. W.B Saunders.Philadelphia.

Halliwell,B. (1994). "Free radicals and antioxidants:A personal view" *Nutr. Res.* **52**:253 - 265.

Ham,A.A. (1974) "*A text book of Histology*" 7th edition. JB Lippincot company,Philadelphia and Toronto.Pp 686.

Harborne,J.B (1992) "A guide to modern technique of plant analysis". London: Chapman and Hill.

Heirinch,R., Melendex-Hevia,E., Montero,F., Nurio,J.C., Stephani,A. and Waddell,T.D. (1999). "The structural design of glycolysis: an evolutionary approach". *Biochem.soc.Trans* **25**, 294-298

Hers,H.G. and Hue,L. (1983). "Gluconeogenesis and related aspects of glycolysis". *Annu Rev.Biochem* **52**, 617-653.

Ilanchezhian,R. and Roshy,J.C. (2006). "Hepatoprotective and hepatocurative activity of the traditional medicine keteki (Pandarous odoratissimus Rox b.)- An experimental evaluation." Dept of Dravyaguna, Institute for postgraduate Teaching and Research in Ayurveda, Gujurat Ayurved University, India.

Jain,S.M., Alkhayri,J.M. and Johnson,D.V. (Eds)(2011) "Date palm Biotechnology". Springer, Netherlands.

Jaun,R., Jean-pierre,B., Andres,B., Juerg R. and Mario,R.W. (2007) "*The text book of hepatology: from basic science to clinical practice*".

Keith,G.T. and Robert,R.E.J. (2001). "*Liver function In: Tietz fundamentals of clinical chemistry*". 5th edition. W.B Saunders company. Pp 747-70.

Kelly,W.N. and Derry,V.T. (1989)(eds). " *Text book of internal medicine*". JB Lippincott company. U.S.A. Pp 471-472.

Kerry,H.C. and Janice,L.H. (2013). "*Brunner Text book of medical-surgical nursing*". Lippincott Williams and Wilkins.

Kmiec,Z. (2001). " Cooperation of liver cells in health and disease". *Adv Anat Embryol cell Biol* **161**: III-XIII.

Kowalska,M.T., Brandt,M.E. and Puettt,D. (1990) "Inhibition of cytochrome P-450 aromatase activity by plant extracts". *Plant Med* 1990;56:675-77.

Kumar,G.S., Vartha,G.P., Sundarajan,P.M. and Rejesekara,P.M. (2004). "Hepatoprotective activity of *Trianthema portula castrum L.* against paracetamol and thioacetamide intoxication in albino rats". *Journals of ethno pharmacology*, **92(1)**:37-40.

Levine,M., Rumsey,S.C., Wang.Y., Park,J.B., Daruwala,R. (2000). "Vitamin C In: Stipanuk,M.H (Ed) Biochemical and Physiological aspects of human nutrition". WB Saunders, Philadelphia. Pp 541-567.

Lii,C.K., Ko,Y.J., Chiang,m.T., Sung,W.C. and Hen,H.W. (1998). " Effect of dietary vitamin E on antioxidant enzyme activities in Sprague-Dawley rats". *Nutr Cancer* 1998; **32**:95-100.

Longo,D.L. ed (2012). " Chapter 305. Chronic hepatitis". (Onlline).Harrison's principles of internal medicine (18th ed). New York: Mc Graw -Hill.

Masuoka,H.C., Chalasani,N. (2013). "Non Alcoholic fatty liver disease: an emerging threat to obese and diabetic individuals". *Annals of the New York Academy of sciences*. **1281(1)**:106-122.

Mc Graw Hill (1997). "*Encyclopedia of science and technology*". Volume 10, 8th edition. Ice Leo. Pp 166-170.

Medline plus (2012). "Hepatic ischemia". National library of medicine.

Mnddrey,W.C. (2001). "Update in hepatology". *Ann Int Med* 2001; **134**:216-23.

Mohammed,D,A. and Al okbi,S.Y. (2004) " Invivo evaluation of antioxidant and anti inflammatory activity of different extracts of date fruit in adjuvant arthritis". *Polish Journal of food and Nutrition Sciences*.**Vol.13154, No 4**.Pp 397-402.

Muriel,P. and Escober,Y. (2003). " Kupffer cells are responsible for liver cirrhosis induced by carbon tetra chloride". *Journal of Applied Toxicology;* **23** : 103 – 108. Pub Med.

Naganna,B. (1989). " Plasma Proteins ". In Talwar,G.P., Srivastava,L.M., Maidgil,K.D. (eds) "*Textbook of Biochemistry and human Biology*". 2nd edition.Prentice9-61.Hall of Indiana, New Delhi. Pp 59-61.

Nasser,S.A (2011). " First Arab Palm conference on the development of date palm and dates sector in the Arab world". *Emirates Journal of food and Agriculture*.Vol **24 (5)**.

Nduka,N. (1999) "*Clinical Biochemistry for students of pathology*" Longman Nigeria Plc.

Nelson,D.L. and Cox,M.M. (2005). "*Lehninger Principle of Biochemistry*". Fourth edition. W H Freeman and company, New York.

Nuhu,H. (2001). "*Pharmacognosy in A.B.U*" Graphic media production. Zaria.

Nwokediuko,S.C., Osuala,P.C., Uduma,U.V., Alaneme,A.K., Onwuka,C.C. and Mesigo,C. (2013) "Pattern of liver disease admission in Nigerian tertiary hospital". *Nijer J Clin Pract* 2013; **16**:339-42.

Nyblom,H., Berggren,U., Balldin,J., Olsson,R. (2004). "High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking". *Alcohol* **39(4)**: 336-339.

Olukiran,O.S., Akomolafe,R.O., Bamitale,K.D., Ajayi,A.O., Okonji,R.E. and Bejide,R.A. (2014). "Protective and Curative effects of Livolin forte on CCl_4-induced liver damage in wister rats" *J Exp Integr Med*.2014; **4(1)**:57-65.

Pietta,P,G. (2000) "Flavonoids as antioxidants". *J Nat Prod.* **63(7)**:1035-42.

Recknegel,R.O and Glende,E.A. (1973). "CCl_4 hepatotoxicity: an example of lethal cleavage".*CRC critical review toxicology*, **2**:263-297.

Romer,A.S. and Pareons,T.S. (1997) "The vertebrate body".Philadelphia, P A:Holt-Saunders International. Pp 354-5.

Sadiq,I.S., Izuagie,T., Shuaibu,M., Dogoyaro,A.I., Garba,A. and Abubakar,S. (2013). "The Nutritional evaluation and medicinal value of date palm (*Phoenix dactylifera*)".*International Journal of modern chemistry*. Vol **4(3)**: 147-154.

Sambo,M. (2008) "Effect of organic solvents extracts of the bark of *Anogeissus leiocarpus* on serum bilirubin level of CCl_4 –induced liver damage in rats". A BSc thesis submitted to the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto.

Schneider,B.L., Serman,P.M.(2008). Pediatric gastro intestinal disease". Connecticut: PMPH-USA.

Shehu,M. (2008) "Phytochemical analysis and potency of aqueous leaves extract of *Azadiracha indica* and *Carica papaya* against lipid peroxidation and liver damage in rats". Unpublished manuscript, MSc research thesis, Department of Biochemistry, Post graduate school, BUK.

Simon,R.P., Patel,H.V and Kiran,K. (2010). "Hepatoprotective activity of some plant extracts against paracetamol induced hepatotoxicity in rats". *Journal of herbal medicine and toxicology*. Vol **4(2)**. Pp 101-106.

Sofowora,A. (1993) "Medicinal plants and medicine in Africa". John Whilley Spectrum.

Tim,T. (1999) at www.innerbody.com/ir.

Treaase,G.E. and Evans,W.C. (1989) "Pharmacognosy" 13th Edition. ELBS/Bailliere Tindall, London,UK.

Venkatechwar,R., Atlarama,R.T. and Naidu,A. (2013). "Evaluation of hepatoprotective activity of ethanolic extract of aerial parts of *Smilax perfoliata* and *Flemingia wightiana*". *Research Journal of pharmaceutical,Biological and Chemical sciences*. **Volume 4, issue 2**,Pp 1178.

Venukumar,M.R and Latha,M.S (2002). "Hepatoprotective effect of the methanolic extract of Curculigo orchioides in CCl4-treat male rats". *Internationa Journal of Pharmacol.* **34**:269-75.

WHO (2008) "Hepatitis B fact sheet,Revised 2008.Available at http://www.who.int/media central fact sheet/fs 204/en/index.html.

William, F.G. (1991). "*Review of medical physiology*" 15th edition.

APPENDIX I

Calculations for Quantitative Analysis of date fruit (*Phoenix dactylifera*)

(a) Tannins

The following formular was used to calculate % pseudotannin + tannin :

$$\% \text{ pseudotannin + tannin } = \frac{[\text{(Blank a - Expt A) x 0.029 x 100}]}{5 \text{ (vol of sample taken)}}$$

And the following formular was used to calculate % pseudotannin:

$$\% \text{ pseudotannin} = \frac{[\text{(Blank b - Expt B) x 0.029 x 100}]}{5 \text{ (vol of sample taken)}}$$

Hence,to get true tannins,the following formular was used

% true tannins = [A] – [B]

(b) Saponins

Total weight of saponin = 0.09g in 50ml of extract, which is equivalent to 0.73g/100g of extract.

(c) Flavonoids

Total weight of flavonoids = 0.26 in 50mls of extract,which is equivalent to 2.09g/100g of extract.

(e) Cardiac glycoside

The following formular was used in calculating the % of cardiac glycoside:

$$\% \text{ of total glycoside} = \frac{A \times 100}{17}$$

APPENDIX II

Result for Qualitative Analysis

PHYTOCHEMICAL	**OBSERVATION**	**INFERENCE**
(1)Tannins	Faint green colour	Positive
(2)Saponins	Frothing persist on warming	Positive
(3)Cardiac Glycoside	Brown ring	Positive
(4)Flavonoids	Violet color	Positive
(5)Alkaloids	Dark brown colour	Negative

APPENDIX III

Statistical Software and Anova for Biochemical Parameters

The statistical software used was Minitab 17.

(1)Anova for Total Protein

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 5462.2 420.169 94.04 0.000

Error 56 250.2 4.468

Total 69 5712.4

Means

Group n Mean St Dev

G I 5 74.000 0.986

G II 5 40.200 1.583

G III 5 65.088 1.209

G IV 5 70.172 1.800

G V 5 50.058 1.322

G VI 5 60.800 1.708

G VII 5 65.03 3.72

G VIII 5 65.058 2.020

G IX 5 68.92 3.08

G X 5 50.800 1.934

G XI 5 62.144 2.188

G XII 5 66.40 2.44

G XIII 5 67.37 2.44

G XIV 5 65.714 1.371

(2)Anova for Total Bilirubin

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 5.9015 0.45396 27.34 0.000

Error 56 0.9297 0.01660

Total 69 6.8312

Group n Mean StDev

G I 5 0.9500 0.0400

G II 5 1.9740 0.0522

G III 5 0.9760 0.0956

G IV 5 0.9660 0.1019

G V 5 1.4740 0.1791

G VI 5 1.0180 0.1721

G VII 5 0.9900 0.1825

G VIII 5 0.9480 0.0396

G IX 5 1.022 0.259

G X 5 1.4720 0.0993

G XI 5 1.0240 0.0650

G XII 5 0.9700 0.1138

G XIII 5 0.9740 0.0786

G XIV 5 1.0220 0.1092

(3)Anova for Direct Bilirubin

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 5.0565 0.38896 38.50 0.000

Error 56 0.5658 0.01010

Total 69 5.6222

Group n Mean StDev

G I 5 0.5020 0.0709

G II 5 1.2560 0.1804

G III 5 0.4940 0.0297

G IV 5 0.4960 0.1474

G V 5 1.0740 0.1258

G VI 5 0.5240 0.0723

G VII 5 0.5040 0.0541

G VIII 5 0.4820 0.0507

G IX 5 0.5060 0.0733

G X 5 1.1280 0.1579

G XI 5 0.4980 0.0789

G XII 5 0.5140 0.1246

G XIII 5 0.5020 0.0228

G XIV 5 0.5200 0.0464

(4)Anova for AST

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 38512.8 2962.52 779.93 0.000

Error 56 212.7 3.80

Total 69 38725.5

Group n Mean StDev

G I 5 36.360 1.167

G II 5 105.24 2.75

G III 5 35.280 1.708

G IV 5 37.728 1.753

G V 5 90.26 2.24

G VI 5 45.59 2.44

G VII 5 39.312 0.999

G VIII 5 38.376 2.077

G IX 5 37.512 0.966

G X 5 92.244 2.139

G XI 5 45.32 2.34

G XII 5 41.150 1.571

G XIII 5 39.384 1.519

G XIV 5 40.22 2.50

(5)Anova for ALT

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 20344.4 1564.96 688.49 0.000

Error 56 127.3 2.27

Total 69 20471.7

Group n Mean StDev

G I 5 44.754 1.190

G II 5 93.218 1.309

G III 5 47.542 1.324

G IV 5 45.642 1.262

G V 5 87.814 1.694

G VI 5 49.654 1.374

G VII 5 48.358 1.502

G VIII 5 49.276 1.172

G IX 5 48.320 1.839

G X 5 88.716 1.689

G XI 5 50.268 1.322

G XII 5 50.86 2.52

G XIII 5 50.788 1.188

G XIV 5 49.818 1.108

(6)Anova for ALP

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 8256.4 635.110 182.46 0.000

Error 56 194.9 3.481

Total 69 8451.4

Group n Mean StDev

G I 5 78.550 1.204

G II 5 114.53 3.75

G III 5 80.036 2.057

G IV 5 72.650 1.157

G V 5 98.398 1.305

G VI 5 90.066 1.876

G VII 5 79.064 0.801

G VIII 5 80.10 2.65

G IX 5 77.282 1.380

G X 5 97.610 1.541

G X 5 91.080 0.646

G XII 5 81.440 1.579

G XIII 5 81.498 1.802

G XIV 5 79.904 2.082

(7) Anova for Albumin

Source DF Adj SS Adj MS F-Value P-Value

Factor 13 1044.8 80.368 38.34 0.000

Error 56 117.4 2.096

Total 69 1162.2

Group n Mean StDev

G I 5 35.68 2.41

G II 5 20.726 1.550

G III 5 32.726 0.956

G IV 5 34.126 0.741

G V 5 25.138 1.193

G VI 5 31.726 0.959

G VII 5 31.42 2.82

G VIII 5 30.214 0.693

G IX 5 29.052 0.836

G X 5 24.026 0.588

G XI 5 31.352 1.532

G XII 5 29.800 1.148

G XIII 5 29.264 0.801

G XIV 5 30.204 1.875