EFFECTS OF BOUILLON CUBES AND FERMENTED SEEDS OF *PARKIA BIGLOBOSA* (G. DON) AT VARIED DIETARY INCLUSION LEVELS ON LIVER FUNCTION OF WISTAR RATS, *RATTUS NORVEGICUS*

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOLOGY

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AUGUST, 2015.

DECLARATION

I hereby declare that the work in this thesis titled ‘EFFECTS OF BOUILLON CUBES AND FERMENTED SEEDS OF *PARKIA BIGLOBOSA* (G. DON) AT VARIED DIETARY INCLUSION LEVELS ON LIVER FUNCTION OF WISTAR RATS, *RATTUS NORVEGICUS*’ was performed by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree at any institution. I take exclusive responsibility for all errors and oversights in this project.

Andrew Williams ALFA ___________________ ____________________

Signature Date

CERTIFICATION

This thesis titled 'EFFECTS OF BOUILLON CUBES AND FERMENTED SEEDS OF *PARKIA BIGLOBOSA* (G. DON) AT VARIED DIETARY INCLUSION LEVELS ON LIVER FUNCTION OF WISTAR RATS, *RATTUS NORVEGICUS*' meets the regulations governing the award of the degree of Master of Science (M.Sc.) of the Ahmadu Bello University, Zaria and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

To my dear parents and beloved wife: Williams, Mary and Esther.

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ABSTRACT

The ability of the liver to respond to environmental resistance is enhanced by suitable foods which are often seasoned with condiments which are either industrially prepared or locally by fermentation process. Condiments, particularly those prepared by the process of fermentation display probiotic properties while industrially prepared condiments tend to be potentially hazardous to health. A liver function test was carried out to determine total bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in *Rattus norvegicus* (wistar rats) after feeding on three brands of industrially manufactured condiments, bouillon cubes and traditionally fermented (condiment) seeds of *Parkia biglobosa* (FSPB). Four packets of 200 cubes each of three brands of bouillon cubes (BC): Maggi star, knorr and royco, were purchased at N260 each from the community market of the Ahmadu Bello University Zaria and seeds of *Parkia biglobosa* from Bagana Market in Kogi State. One hundred and nineteen young adult wistar rats of average weight of 102g were purchased from Institute of Veterinary Research, Vom, Plateau State, separated into 14 groups of 7 rats each and allowed to acclimatize for 7 days before the experimental feeding commenced with various percentage inclusions (10%, 20% and 30%) of the condiments in their feeds. Some quantity of table water, was used to mold the mixture into balls that were dried in the sun protected from dust and insects. Each group was fed with one of the percentages. One of the groups was fed with 100% FSPB and another, the control with 100% pelletised growers mash. The rats were liberally fed with the meals and table water for 28 days by allowing them free access to their meals and water so that each animal ate and drank what was enough for itself throughout the period of the experimental feeding and were all sacrified on the 29th day.

The blood and livers were harvested for enzyme assay using enzymatic assay kit obtained from Randox Laboratories Ltd, Co. Antrium, United Kingdom as well as light microscopy after staining the liver tissues with haematoxylin and eosin. Analysis of variance showed that levels of ALT and AST for those that fed on bouillon cube inclusion meals were significantly high ($P \leq 0.05$) with hepatocytes that showed degenerative changes and necrosis. Those fed with FSPB showed liver architecture, hepatocytes, sinusoids and hepatic central vein as normal with no significant effect ($P > 0.05$) on ALT and AST levels. Both bouillon cube and FSPB showed significant increase ($P \leq 0.05$) in the level of total bilirubin. BC inclusion meals at 10%, 20% and 30% appear to cause hepatocellular degeneration and necrosis thereby negatively affecting the ability of the liver of Wistar rats to detoxicate and biosynthesize. FSPB at those percentage inclusions as well as 100% appear not to show such changes with no negative effect on liver function of wistar rats. BC as a condiment therefore, could probably have negative health implications on the human liver.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Human activities, have been disturbing or destabilizing the ecological balance of the planet earth (Oladele, 2002) which makes the environment including natural forces and other living organisms to adversely change. This brings to bear man's ability to maximize the our biotic potentials especially when these changes constitute an environmental resistance.

A vital organ like the liver responds to this environmental resistance and it's ability to overcome the resistance is enhanced by suitable food and appropriate medication (ALF, 2015). Unlike medicine, ingested only when there is apparent abnormality in the physiological functioning of the body, food is ingested every day. This is because it provides the energy needed for physiological activities in addition to providing nourishment for the body (Chadwick, 2004).

Food is any substance consumed to provide nutritional support for the body. It is usually of plant or animal origin and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins or minerals. Food is ingested by an organism and assimilated by the organism's cells in an effort to provide, maintain life or stimulate growth (Encyclopedia Britannica Online, 2012). There are six major methods commonly employed in food processing and preservation. These methods are based on temperature, irradiation, moisture reduction, fermentation, chemical additives and controlled/modified atmosphere (Fashakin, 2008). Majority of the processed foods in homes and restaurants use the method of controlled temperature change. Among the method of controlled temperature change is cooking (ibid). Cooking is a process of heating in order to alter odour, flavour and texture and particularly to improve digestibility of the food components (ibid). Foods are often seasoned with condiments - bouillon cubes globally. In West Africa, condiments processed traditionally by fermentation of seeds of *Parkia* species or commercially emerging contemporary condiments in form of bouillon cubes are used.

Fermented seeds of *Parkia biglobosa* serve primarily as a condiment (Babalola, 2012). The brown seeds are ground into a pungent nutritious spice or condiment which is added to soups and stews throughout the savanna regions of sub-Saharan Africa (Campell–Platt, 1980). The use of these fermented seeds dates back many centuries and its use was already described in the 14th century (Sina and Traore, 2002). The production and sale of fermented seeds of *P. biglobosa* constitute an important economic activity for women across West Africa (Janeen, 2010).

They are consumed widely especially in developing and under-developed countries where animal protein is limited as a result of economic, social and cultural factors (Essenwah and Ikenebomeh, 2008). Hassan and Umar (2005) reported that seeds of *P. biglobosa* are good source of protein as well as essential amino acids including sulphur containing amino acids. Fermentation of these seeds enhances protein and lipid enrichment and reduces total carbohydrate present (Ogbonnaya *et al*., 2010). Souleyman (2009) reported that the fermented seed is believed to be good for the treatment of arterial hypertension.

Bouillon cubes are popular in Nigeria and beyond. Nestle company began the packaging of its bouillon product, the maggi cubes, in 1971 at its Ilupeju factory, Nigeria (Anonymous, 2011a). A bouillon cube constitutes among others sodium chloride, hydrogenated oil and monosodium glutamate (MSG) (RMRDC, 2003). According to Inuwa *et al*., 2011, MSG administered to adult wister rats in the proportion 200mg/kg, 300mg/kg and 400mg/kg body weight elicited an increase in body weight in an ascending order. The report also showed that MSG has hepatoxicity and nephrotoxicity tendencies especially when consumed at higher concentrations.

Optimal nutrition is a function, not just of what we ingest and digest, but of how well the liver bio-transforms food nutrients brought to the liver, into forms that can be transported by blood to all the cells of the body and that the cells can use to perform their metabolic functions. The cells can, therefore, still be greatly malnourished if the liver does not perform many functions effectively no matter how good the diet and digestion rate is. The liver performs some 500 different functions (Anonymous, 2011b). These functions include: processing food ingested by aiding digestion, extracting nutrients and breaking down harmful drugs and poisons including alcohol, stores and releases glycogen and glucose as a source of energy to the body, fights infections because of the presence of cells such as macrophages and releases important chemicals that activate immune responses when infection is detected, stores vitamins and minerals including iron; regulates fat metabolism and distribution in the blood stream, produces and maintains the balance of hormones as well as producing enzymes and other proteins responsible for most of the chemical reactions in the body such as blood clotting and tissue repair (ibid).

Liver function tests (LFT) are helpful screening tools and are effective modalities to detect hepatic dysfunction (Thapa and Anuj, 2007).

1.2 Statement of Research Problem

Some condiments, particularly those prepared by the process of fermentation display probiotic properties such as hypolipidemic, hepatoprotective and antibacterial properties (Manas *et al.*, 2014). Many commercially prepared condiments are typically mixtures of low quality genetically engineered ingredients, chemical preservatives, fillers and taste and texture enhancers that are potentially hazardous to health (Mercola, 2013; Cologuhoun and Bosch, 2014).

1.3 Justification

The nutritional, economic and health values of fermented seeds of *Parkia biglobosa* are evident (Hassan and Umar, 2005; Ogbonnaya *et al.*, 2010). However, Omeh *et al.* (2014) reported some hepatotoxic effect of the boiled seeds in the diet of wistar rats.

The popularity of contemporary emerging bouillon cubes and potential hazards associated call for more information on their functionality in vivo and more empirical scientific evidences on the health indices of food condiments could enhance man's biotic potential.

1.4 Aim of the Study

To evaluate the effect of diet with varied inclusion levels of bouillon cubes (maggi star, Royco, and knorr) and fermented seeds of *Parkia biglobosa* as condiments on total bilirubin, alanine aminotransferase and aspartate amino transferase serum levels as well as on the histology of hepatocytes of wistar rats.

1.5 Objectives of the Study

To determine the effect of fermented seeds of *Parkia biglobosa* and bouillon cubes (Maggi star, Knorr and Royco) on:

1. serum levels of total bilirubin, alanine aminotransferase and aspartate aminotrasnferase and
2. associated histological changes of hepatocytes.

1.6 Hypotheses

1. Fermented seeds of *P. biglobosa* and bouillon cubes (Maggi star, Knorr and Royco) have no significant effect on total bilirubin, alanine aminotransferase and aspartate aminotransferase serum levels in livers of wistar rats.
2. There is no significant histological change of hepatocytes of wistar rats caused by fermented seeds of *P. biglobosa* and bouillon cubes (Maggi star, Knorr and Royco).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bouillon Cube

Bouillon cubes are compressed stock that needs to be dissolved before use. Stock is the strained liquid left from slowly cooking vegetable, meat or fish in water with intense flavour added (Kathy, 2006). There are a range of different flavours available as there are different brands. The different brands include Maggi star bouillon, Royco bouillon and Knorr bouillon cubes. These are produced by two international companies with a world wide reputation: Nestle and Unilever companies.

Nestle (Nigeria) limited, a subsidiary of Nestle produces the Maggi star bouillon in Nigeria (Anonymous, 2011a). Maggi is an international brand of bouillon cubes, instant soups, stocks, ketchups, sauces, seasonings and instant noodles. Owned by Nestle since 1947; the original company was founded in Switzerland in 1872 by Julius Maggi (Anonymous, 2013). Nestle (Nigeria) limited was formed in 1961 (Anonymous, 2011a). Unilever was formed in 1929 by a merger between Lever Brothers, a pioneer of branded soap manufacturer founded by William Hesketh Lever and the Margarine Unie of the Netherlands (Geoffrey and Alison, 2004). In line with the global strategic direction of business, unilever Nigeria plc was formed in 2001. It produces Knorr bouillon and Royco bouillon cubes (UARFS, 2003). Bouillon cubes are generally used around the world as a seasoning agent and to add the base flavour to dishes such as sauces, soups and stews (Anonymous, 2015). The cubes contain three primary ingredients:

Monosodium glutamate (MSG), hydrogenated oil (trans fat) and sodium chloride (Bloom, 2013).

2.1.1 Monosodium glutamate

Monosodium glutamate (MSG), also recognized by its trade name as Agino Moto or Vedan (Bassey *et al.*, 2012) is glutamic acid produced outside the human (or animal) body to which a mineral ion, sodium, has been added (EDEN, 2015). Glutamic acid is an amino acid found in abundance in both plant and animal protein. In humans, it is a non-essential amino acid i.e. the body is capable of producing its own glutamic acid and not dependent upon getting glutamic acid from ingested food (ibid). In addition to its role as a building block of protein, glutamic acid serves as a neurotransmitter vital to the transmission of nerve impulses in many parts of the central nervous system.

Under certain circumstances, glutamic acid becomes a neurotoxin, causing neuro-degeneration and cell death which may be followed by neuroendocrine disorders (Gao *et al.*, 1994). When protein is digested, the glutamine acid in that protein is reduced for use in the body. However, in the absence of glutamic acid in the ingested protein, the body could synthesize all the glutamic acid needed for body function from other amino acids. The human brain is also capable of synthesizing glutamic acid according to its metabolic needs from endogenous materials (ibid). This is the L-form i.e. L-glutamic acid. Glutamic acid produced outside the human or animal body is characterized by impurities i.e. D-glutamic acid, pyroglutamic acid and L-glutamic acid produced by way of acid hydrolysis. This pathway is accompanied by carcinogenic mono and dichloro propanols.

Tchaou *et al*. (2013) showed that MSG consumption is hepatotoxic and may contribute to the emergence of prediabetes and the potential to increase metabolic syndrome in humans. MSG fed to the young has been shown to cause brain damage and subsequent learning, behaviour and endocrine disorders such as reproductive disorders and obesity (EDEN, 2015). Studies have shown that MSG can cross the blood brain barrier (BBB) in an unregulated manner during development and can pass through the five circuventricular organs (unique areas of the brain that lie outside the BBB) which are leaky at any stage of life (Skultetyova *et al*., 1998).

2.1.2 Hydrogenated oil

Hydrogenated oil is oil in which the essential fatty acid has been converted to a different form chemically, which has several effects (Richard, 2008). Hydrogenated oil is far more shelf stable and will not go rancid as quickly as untreated oil. It also has a high melting point and is often used in frying and pastries for this reason. Hydrogenated oil is made by forcing hydrogen gas into oil at high pressure changing the chemical structure with negative health effects (AHA, 2014). The molecules are closer to cellulose or plastics than oil (Richard, 2008). Oils of plant and animal origin can be and are hydrogenated. The blood becomes more viscous causing the heart to exert much pressure to pump blood throughout the body contributing to high blood pressure. It increases the “bad” low density lipoprotein cholesterol (LDL) and decreases “good” high density lipoprotein cholesterol (HDL).

High HDL-levels have cardioprotective effects whereas high LDL levels are an increased risk factor for heart disease (Eric, 2015). It slows the micro circulation of blood through the brain causing various emotional and physical ailments such as Alzheimer's and Parkinson's diseases and muddled thinking (Richard, 2008). Hydrogenated oil predisposes one to type II diabetes. The more enzyme dead food and ingredients one consumes, the more the body has to create and use up its own food enzymes during digestion. This places a large strain on the internal organs, especially the pancreas which has to create the necessary food enzymes for digestion. An overworked pancreas is one of the major causes of type II diabetes which is basically pancreatic failure (MFMER, 2015).

Cell walls are normally made of essential fatty acids (EFA's). These walls are the defense mechanism so crucial that they have sufficient EFA's. These essential fatty acids allow nutrients and waste matter to pass in and out of the cell yet keep foreign invaders and pathogens out (Richard, 2008). Once hydrogenated oil reach the cell, they assimilate them into the makeup of the cell wall without sufficient EFA's, the cells are now forced to incorporate them into its cellular matrix to make up the difference. This new cell wall now made up of hydrogenated oils is virtually useless and this compromises the very function of the cell wall (MFMER, 2015). The presence of hydrogenated oils in the cell prevents nutrients from entering the cell but at the same time allowing pathogens, microbes and viruses to pass through. It also prevents waste material getting out of the cell. Between the nutrient that are kept out of the cell and the waste matter that is now trapped in the cell, the cell often mutates or dies which can lead to cancer, tumors or other serious health issues (Richard, 2008).

2.1.3 Sodium chloride

A large number of studies have been conducted, all of which support the concept that sodium chloride (salt) intake is the major factor increasing blood pressure (BP) in the population. The diversity and strength of the evidence is much greater than other lifestyle factors, for example, overweight, low consumption of fruit and vegetables and lack of physical exercise (He and MacGregor, 2009). Numerous studies in rat, dog, chicken, rabbit, baboon and chimpanzee have all shown that salt intake plays an important role in regulating BP (Denton *et al*., 1995; Elliott *et al*., 2007). Furthermore, in all forms of experimental hypertension, whatever the animal model, a high salt intake is essential for BP to rise. A study in chimpanzees (98.8% genetic homology with man) demonstrates that a gradual increase in salt intake from 0.5g/day which is close to humans' evolutionary intake, to 10-15g/day which is similar to our current salt intake causes a progressive rise in BP (Denton *et al*., 1995).

There are several very rare genetic causes of high BP. All these result in a reduction in the kidney's ability to excrete salt, and thereby cause high BP (Lifton *et al*., 2001). The raised BP is considerably aggravated if salt is consumed. Rare genetic causes of low BP have also been described. These result in the kidney being unable to hold on to salt normally, thereby causing low BP. These forms of low BP are ameliorated by a high salt intake. Overall, these studies clearly indicate the importance of salt intake in regulating BP in humans.

A study in the pacific islands where one undeveloped community used sea water in their food and the other did not, showed that the community using seawater had high BP (Page *et al*., 1974). Another study in two rural communities in Nigeria, one of which had access to salt from a salt lake and the other did not, showed differences in salt intake and differences in BP and yet in all other aspects of lifestyle and diet, the two communities were similar (Uzodike, 1993). More recent epidemiological studies, for example, the international study of macro and micro nutrients and BP study (INTERMAP) (Zhou *et al*., 2003), and the Epic-Norfolk study (the Norfolk cohort of the European prospective investigation into cancer) (Khaw, *et al*., 2004), have lent further support for the important role of salt in determining the levels of BP in the population.

The damage that raised BP does, is mainly through its effect on cardiovascular disease (CVD), which is the major cause of death worldwide. It has been shown that raised BP, raised cholesterol and smoking account for over 80% of CVD (Emberson *et al*., 2003). However, raised BP is the single most important cause, responsible for 62% of stroke and 49% coronary heart-disease (World Health Report, 2002). Importantly, there is a continuous graded relationship between BP and CVD, beginning at 115/75 mm Hg (Lewington *et al*., 2002). Therefore, for most countries of the world, over 80% of all adult are at risk of CVD from their BP, the greatest number of CVD deaths attributable to BP occurs in the upper range of the usual BP (that is around 130/80mm gH). This could be due to many individuals having BP around this level in the population (MacMahon, 1996), that is, a level which would not currently be treated with drugs. Therefore, a population based approach through diet and lifestyle, for example, a reduction in salt intake, aimed at achieving a downward shift in the distribution of BP in the whole population, even by a small amount, will have a large impact on reducing the appalling burden of CVD.

Salt has other deleterious effects on health, independent of and sometimes additive to its effect on BP (He and MacGregor, 2009). When humans go from a low to a high salt intake, there is a retention of salt and thereby water, and this expands the extra-cellular volume. This triggers various compensatory mechanisms to allow an increase in urinary salt excretion but at the expense of continued retention of salt and water. This increase in extracellular fluid as long as higher salt intake is consumed exacerbates all forms of salt and water retention, for example heart failure (He *et al*., 2002) and is a major cause of oedema in women, aggravating both cyclical and idiopathic oedema (Cho and Atwood, 2002). Pery and Beevers (1992) performed an ecological analysis of the relationship between urinary sodium excretion (data from INTERSALT Study) and stroke mortality in western Europe. They found a significant positive correlation between 24-h urinary sodium excretion and stroke mortality. Several cross-sectional studies have shown a positive correlation between 24-h urinary sodium excretion and left ventricular mass in both hypertensive and normotensives, which is independent of BP (Kupari *et al*., 1994). A reduction in salt intake has been shown to decrease left ventricular mass in patients with essential hypertension (Liebson *et al*., 1995). Left ventricular hypertrophy is an important independent predictor of cardiovascular morbidity and mortality and is related to raised BP (Levy *et al*., 1990). An ecological analysis showed a significant direct association between salt intake and deaths from stomach cancer among 39 populations from 24 countries (Joossens *et al*., 1996). Foods that contain high concentration of salt are irritating to the delicate lining of the stomach. It is possible that this makes H-pylori infection, more likely or more severe and that the H-pylori infections then leads to stomach cancer. A modest reduction in salt intake may reduce H-pylori infection and therefore lead to stomach cancer prevention (He and MacGregor, 2009). Increasing salt intake increases urinary protein excretion and markedly increases the rate of deterioration of renal function in experimental forms of renal disease. Studies in humans have shown that salt intake relates, on population basis, to the amount of protein or albumin excretion (Verhave *et al*., 2004) which is an important risk factor for the development of kidney disease and CVD (Amlov *et al*., 2005). Salt intake is one of the major dietary determinants of urinary calcium excretion. Both epidemiological studies and randomized trials show that a reduction in salt intake causes a decrease in urinary calcium excretion (Cappuccio *et al.*, 2000). As calcium is the main component of most urinary stones, salt intake is therefore an important cause of renal stones (He and MacGregor, 2009). Other studies have shown that reducing salt intake causes a positive calcium balance and it is likely that reducing salt intake would slow down the loss of calcium from bone that occurs as one grows older (ibid). Although it is not thought that a high salt intake is a cause of asthma, epidemiological evidence suggests that the severity of asthma may relate to salt intake in different countries (ibid). A recent population-based study in children aged 6-7 years demonstrated that adding salt to food was strongly and independently associated with an increased risk of respiratory symptoms, that is, wheeze and asthma (Corbo *et al*., 2008). A high salt intake has been suggested as an indirect cause of obesity, through the effect it has on fluid intake. A carefully controlled metabolic study in adult humans showed that a reduction in salt intake caused a significant decrease in fluid consumption (He *et al*., 2001).

2.2 The Liver

2.2.1 Physiology of the liver

The liver is the largest internal organ of the body and the largest gland tissue (Hideo and Asuka, 2004). It is the organ in which nutrients absorbed in the digestive tract are processed and stored for use by other parts of the body. The liver cells have a multiplicity of functions (Anonymous, 2011b): metabolic e.g. glucose homeostasis, secretion of lipoproteins; synthetic e.g. albumin, coagulation prothrombin, fibrinogen, compliment and binding protein for iron, copper and vitamin A.; storage e.g. glycogen triglycerides, iron, copper; catabolic e.g. hormones, serum protein, detoxification of drugs, chemicals, products of bacterial metabolism and excretory e.g. bile (Zakim and Boyer, 2003). These functions play a central role in maintaining life (Hideo and Asuka, 2004). The liver receives blood through both the portal vein and hepatic artery. Most of its blood comes from the portal vein that conveys blood containing nutrients absorbed in the intestine. The hepatic artery, a branch of the celiac axis, is oxygenated in the liver (Hideo and Asuka, 2004).

The liver is composed of different populations of cells. These include the major parenchymal cells, the hepatocytes and a variety of non parenchymal resident cells including a population of macrophages termed kupffer cells (Naito *et al*., 2004); fat storing cells termed ito or stellate cells (Sato *et al*., 2003; Senoo, 2004), and endothelial cells.

The structural and functional unit of the liver is the acinus, in which are both the hepatic lobule and portal traid (also called Gilsson’s sheath or portal tracts). The hepatic lobule consists of hepatocytes that are the functional center of the liver and in which the hepatocytes–sinusoidal structures are formed. The sinusoids are capillary networks and are localized in the space between hepatic plates in which the hepatocytes are arranged (Motta, 1984 and Jane *et al*., 2009). In mammals and higher vertebrates, hepatic plates line the simple layered hepatocytes, so called one cell-thick plates, and pass through from the portal triad to the central vein located in the centre of the hepatic lobule (Jane *et al*., 2009). The portal triad is located in the portal spaces between the hepatic lobules, and contains branches of the portal vein, hepatic artery and bile ducts, lymph vessels and nerves. These vessels and ducts are surrounded by connective tissue (Motta, 1984).

The liver uses enzymes to help it get rid of the waste produced in the body by normal body processes and by the breakdown of drugs, alcohol and medications (Fausto 2000). When the liver is overly stressed by this waste or damaged by various infections, the liver enzyme tests done as part of the blood chemistry panel may show elevated values.

2.2.2 Liver function test

Liver enzyme tests, also called liver function tests (LFTs), are a group of blood tests that detect inflammation and damage to the liver. They can also check how well the liver is working (Melinda, 2014). Unlike most other organs, the liver has a remarkable ability to repair itself (Fausto *et al*., 2006).

The true liver function tests measure the liver's synthesis of proteins (albumin and platelets), its ability to properly breakdown substances and its ability to metabolise drugs (Taub, 2004).

Tests to measure the liver's ability to synthesis these proteins include: prothrombin test (PT) and international normalized ratio (INR). PT is a test of the time it takes for a blood sample to clot, under specific conditions in a laboratory; if low levels of clotting factors are present, the prothrombin time is longer (Ki-soo, 2013). INR is not really a test, but a standardized way for all laboratories to report PT, so their results can be compared accurately with each other. PT and INR rise in people with severe liver disease because the liver fails to make normal amounts of certain clotting factors. An elevated PT can have many other causes besides liver disease, however. PT is often checked together with PPT (partial thromboplastin time), which is not a liver function test. If PT and/or PPT are elevated, a problem with bleeding or clotting may be present (Melinda, 2014). Albumin, protein that circulates in blood, is also synthesized by the liver. Albumin levels are low in people with severe chronic liver disease, because the liver does not make normal amounts of albumin. However, albumin levels also may fall in a variety of medical conditions. A low albumin level is often temporary, so it is not a reliable way to diagnose liver disease (ibid).

Bilirubin is a waste product from the breakdown of red blood cells. The liver processes bilirubin so it can be excreted in stool. Bilirubin flows through the liver's bile ducts, dissolved in bile. Bilirubin blood levels may be elevated in people with impaired bile flow. This can occur in severe liver disease, gall bladder diseases, or other bile system conditions. Very high bilirubin levels cause jaundice. Bilirubin can be a useful liver function test in people with a known bile flow problem. An elevated bilirubin may also be present in people with a type of anaemia called hemolytic anemia (ACG, 2013).

The numerous metabolic functions of the liver (Anonymous, 2011b) are driven by proteins called enzymes which are contained in liver cells. When liver cells are damaged or destroyed, the enzymes in the cell leak out into the blood, where they can be measured by blood tests. Liver tests check the blood for two main liver enzymes: Aspartate aminotranferase (AST) and Alanine aminotransferase (ALT). AST enzyme is also found in muscles and many other tissues besides the liver while ALT is almost exclusively found in the liver. If ALT and AST are found together in elevated amounts in the blood, liver damage is mostly likely present (Green and Flamm, 2002). The liver also produces bile which helps digest fat. Bile flows through the liver in a system of small tubes (ducts), and is eventually stored in the gall bladder, under the liver. When bile flow is low or blocked, blood levels of certain liver enzymes rise: Alkaline phosphatase, 5′ nucleotidase and Gamma glutamyltranspeptidase (GGT) (EASL, 2009). Liver tests may check for any or all of these enzymes in the blood. If alkaline phosphatase and/or 5′ nucelotidase and GGT are elevated, a problem with bile flow is most likely present. Bile flow problems can be due to a problem in the liver, the gall bladder or the tubes connecting them (ACG, 2013). Since alkaline phosphatase is also found in bones, it can be elevated in certain medical conditions that affect bones, as well (Green and Flamm, 2002).

2.3 *Parkia biglobosa*

2.3.1 Taxonomy and distribution

The African locust bean tree, *Parkia biglobosa* (Sacande and Clethero, 2007), is a perennial leguminous tree which belongs to the family leguminosae and the subfamily mimosoideae (Peliga-Ba, 2009; Ihegwuagu *et al*., 2009). It grows in the savannah region of West Africa up to the southern edge of the Sahel zone 13^{o}N (Nnemeka *et al.,* 2009). In Nigeria, the tree is not normally cultivated but found in the wild in the savannah region (ibid).

2.3.2 Uses and effects

Parkia biglobosa (PB) is vital to life among the people of the savannah region of West Africa because of its multifunctional benefits. It plays an important role in recycling nutrients from the soil. It is a good source of timber and is useful in making pestles, mortars, bows and hoe handles (Nnemeka *et al.,* 2009).

Phytochemical screening reveals the presence of saponins, tannins, phenolics and cardiac glycoside in PB (Ajaiyeoba, 2002) and the invitro antioxidant property of the crude ethanol extract of the leaf and stem bark was reported by Millogo-kone *et al.* (2008). Concentrated hydroalcoholic extract of PB stem bark ameliorates positively biochemical alterations, prevents oxidative stress and histological and morphological changes induced by isoproterenol in male albino wistar rats (Adi *et al.,* 2013). Adi *et al.* (2013) therefore inferred that PB stem bark had cardioprotective effects and the therapeutic success of the plant in traditional medicine may be due to its antioxidant, antilipoperoxidative and antihyperlipidemic activities. Studies have shown that hexane extracts, methanol and aqueous extract of bark of PB have analgesic and anti inflammatory properties (Kouadio *et al*., 2000).

It has been reported that the husks and pods are good feed for livestock and the floury pulp can be made into a refreshing drink, rich in vitamin C and sugar (Nnemeka *et al.*, 2009). Bolariwa (2012) showed that extracts of PB seeds have termiticidal properties and could be effectively used for the control of termite infestations. The presence of some heavy metals as well as the interacting polar organic compounds in the raw seed extracts could be responsible for the termiticidal property (Bolariwa, 2012). Omeh *et al*. (2014) showed the effect of boiled PB seeds at different level of dietary incorporation on the liver and oxidative stress activity using Wistar albino rats to be significantly high ($P<0.05$) of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Total bilirubin (TB), Direct bilirubin (DB) and protein at 5%, 10% and 20% levels of incorporation. Result from oxidative stress showed that there is equally high level malodialdehyde (MDA) and catalase in the 5%, 10% and 20% ($P\leq0.05$) levels of incorporation. The work inferred that the high level of serum liver enzyme markers indicate hepatoxicity of PB boiled seeds. Amoa-Awu *et al*. (2005) reports that the raw PB seeds are naturally deficient and unpalatable but when fermented into condiment, the physical, chemical and nutritional characteristics of the seeds change. Fermented seeds of PB has cardioprotective property and its consumption, not only maintain health, but may prevent heart attack development in humans (Adi *et al*., 2013). Olakunle and Adebola (2012) defines fermented foods as those foods which have been subjected to the action of microorganisms, so that desirable biochemical changes cause significant modification to the food. Fermentation makes the food to be more nutritious, digestible and safer with better flavour. Fermented seeds of BP serve primarily as a condiment (Babalola, 2012). Regular consumption of fermented seeds of PB as condiment may represents a good dietary alternative for the control of hyperlipidaemia and associated conditions (Ayo-Lawal *et al*., 2014). The brown seeds are ground into a pungent nutritious condiment which is added to soups and stews throughout the savannah regions of sub-Saharan Africa (Campell-Platt, 1980). Hassan and Umar (2005) analyzed the seeds and pulp for protein and amino acids composition and reported the protein content (in % dry matter) of seeds with and without hull to be 28.20% and 32.40% respectively while that of pulp was 1.84%. The amino acid analysis indicates the seeds as a good source of most essential amino acids.

2.4 Wistar Rat

2.4.1 Nomenclature

The wistar rat is currently one of the most popular rats used for laboratory research. It is of the species *Rattus norvegicus* and an outbred albino rat (TWI, 2007). Takashi (2012) showed that the albino rats descended from hooded rats and all the albino rats descended from a single ancestor of an albino rat that emerged as a product of the breeding of hooded rats.

2.4.2 History

These stocks were developed in 1906 at the Wistar Institute which was founded in 1892 Philadelphia, Pennsylvania, United States of America. It is used in biological and medical research and is notably the first rat developed to serve as a model organism at a time when laboratories primarily use the common house mouse (*Mus musculus*) (Clause, 1998). More than half of all laboratory rat strains are descended from the original colony established by physiologist Henry Donaldson, scientific administrator, Milton J. Greenman and genetic researcher/embryologist Helen Dean King (TWI, 2007).

2.4.3 Description

The wistar rat is characterized by its wide head, long ears and having a tail length that is always less than its body length. The Sprague Dawley rats and long Evans rat were developed from Wistar rats. Wistar rats are more active than others like Sprague Dawley rats. The spontaneously hypertensive rat and the Lewis rat are other well-known stocks developed from wistar rats (TWI, 2007).

2.4.4 Uses

The rats found early use in laboratories researching in five areas. Small, INS suggested that the rate of learning could be measured by rats in a maze, a suggestion exploited by John B. Walson for his Ph.D dissertation in 1903 (John, 2011). The nutritive requirements of rats were used by Thomas Burr Osborne and Lafayette Mendel in 1909 in the United States to determine the details of protein nutrition. The reproductive function of rats were studied at the Institute of Experimental Biology at University of California (Paccola *et al.,* 2013). The genetics of rats was studied by William Ernest Castle at Bussey Institution of Harvard University until it closed in 1994 and rats have been the subject of cancer research, for instance at Crocker Institute for Cancer Research (TWI, 2007).

Using rats for scientific research has advantages (Remy, 2010). They are small, easily housed and maintained and adapt well to new surroundings, reproduce quickly and have a short lifespan of two to three years so that several generations can be observed in a relatively short period of time. They are relatively inexpensive, mild tempered and docile, making them easy for researchers to handle although some types can be more difficult to restrain than others. Most rats used in research are inbred so that, other than sex differences, they are almost identical genetically. According to the National Human Genome Research Institute in 2004, as a minimum requirement, rat/mice used in experiments must be of the same pure bred species. Rats have genetic, biological and behaviour characteristics that closely resemble those of humans, and many symptoms of human conditions can be replicated in rats. Their anatomy, physiology and genetics are well understood by researchers making it easier to tell causes of change in behaviour or characteristics (ibid).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The area of study was Bagana in Omala Local Government Area, the market town from which the seeds of Parkia biglobosa were bought. Omala L.G.A is in the Southern Guinea Savanna belt of Kogi State. It is located between latitude 8°0'0'N to 7°20'0'N and longitude 7°50'0'E to 7°20'0'E (www.onlinenigeria.com).

It has rich deciduous and occasional stunted trees. It is green in the rainy season with fresh leaves and tall grasses. The land is open during the dry season, showing charred trees and the remains of burnt grasses. The trees which grow in clusters are up to six metres tall, interspersed with grasses which grow up to about three metres. The trees include *Parkia* species (Locust Bean), Shea butter, oil bean and the Isoberlinia trees (ibid).

3.2 Experimental Procedures

3.2.1 Condiment collection

Seeds of *Parkia biglobosa* were purchased from Bagana market in Omala Local Government of Kogi State.

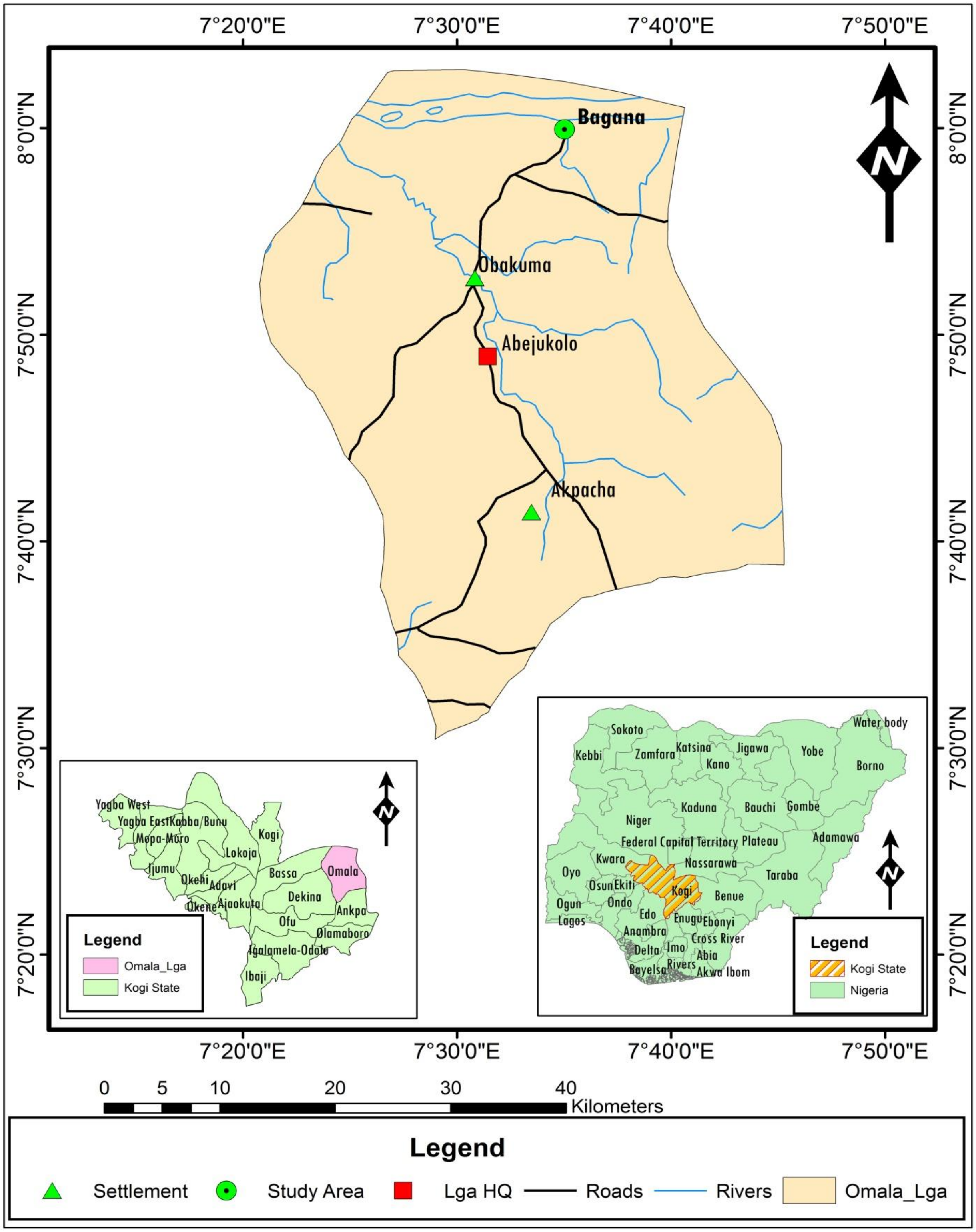


Figure: 3.1. Map of Study Area.
Source: Modified From Administrative Map of Kogi State.

3.2.2 Seed fermentation

Fermented seeds of *Parkia biglobosa* is called “dadawa” and “iru” by the Hausa and Yoruba speaking people respectively. It is called “Ugba” by the Igala speaking people of North Central Nigeria.

Traditional method of fermentation was used to ferment the seeds. The seeds were boiled in stream water, using a large metallic pot, with wood fire for 6 hours (this period may vary according to the intensity of the wood fire). Water was added at intervals to replace the lost quantity of water through vapourization. This kept the seeds always immersed in boiling water.

At the end of the 6 hours period, the seeds were pounded lightly using wooden mortar and pestle. This was to separate the seed coat from the cotyledons. The flaps of integument were removed by scrubbing between the palms of the hand and washed with water. The cotyledons were then boiled for 1 hour with fresh water after which they were poured into a basket made of oil palm fronds and covered with enough banana leaves to prevent rapid and excessive heat loss. The “incubated” seeds were then kept for about 72 hours at ambient temperature to obtain fermented seeds. The fermented seeds were then crushed using a millstone, into a brownish paste with characteristics pungent smell. The paste was molded into flakes and small triangular forms and kept in the sun, protected from dust and insects, to hasten drying. The sun drying was done for about 50 hours. They were then pulverised using wooden mortar and pestle. The powder was kept in an air-tight plastic container ready for use.

3.2.3 Bouillon cubes

Four packets each of three brands of bouillon cubes: Maggi star, Knorr and Royco cubes were bought at N260 each from the Ahmadu Bello University Community Market Samaru, Zaria.

3.2.4 Animal collection

One hundred and nineteen (119) young adults of wistar rats of average weight of 102 g were purchased from the Institute of Veterinary Research, Vom, Plateau State. They were transported in perforated plastic baskets with a commercial vehicle between the hours of 3:00 pm and 7:00 pm to the 200 level laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The rats were kept in captivity in metallic cages to acclimatize for 168 hours before experimental feeding commenced.

3.2.5 Feeding procedure

Four groups of 7 rats each were fed with 100% fermented seeds of *Parkia biglobosa* (FSPB), Royco, Maggi star and Knorr respectively. The 5th group was the control and was fed with 100% pelletised growers mash. There was 100% mortality within 168 hours in the groups fed with 100% Maggi star, Knorr and Royco respectively and 0% mortality within the same period in the groups fed with 100% FSPB and 100% pelletised growers mash.

The rest of the rats were separated into 12 giving a total of 14 groups of 7 rats each. The groups were labeled as control (C), 100% *Parkia biglobosa* (100% FSPB); 10% FSPB, 20% FSPB, 30% FSPB, 10% maggi star (10% S), 20% S, 30% S; 10% Royco (10% R), 20% R, 30% R, and 10% Knorr (10% K), 20% K, 30% K.

3.2.6 Feed preparation

The FSPB and bouillon cubes (BC) were included in the feeds at 10%, 20% and 30% using the formulae:

$x \times 10/90$

$x \times 20/80$

$x \times 30/70$

Where x = weight in grams (g) of the pure feed (100% pelletised growers mash)

$\left(\frac{x \times 10}{90}\right) g$ of FSPB or BC + x g of pure feed
= 10% inclusion

$\left(\frac{x \times 20}{80}\right) g$ of FSPB or BC + x g of pure feed
= 20% inclusion

$\left(\frac{x \times 30}{70}\right) g$ of FSPB or BC + x g of pure feed
= 30% inclusion

The appropriate weight of FSPB or BC in each case was thoroughly mixed with x g of the pure feed. Some quantity of table water was used to mold the mixture into balls that were dried in the sun protected from dust and insects.

3.2.7 Feed regimen

All the groups were fed for 672 hours. The rats, without restriction, had free access to their feeds and table water throughout the period. Group C was fed with 100% growers mash only. Group 100% FSPB was fed with 100% FSPB only. Group 10% FSPB was fed with 10% FSPB included in their growers mash Group 20% FSPB was fed with 20% FSPB included in their growers mash and group 30% FSPB was fed with 30% FSPB included in their growers mash. This procedure was replicated with the rest of the groups in the experiment.

3.3 Histology

3.3.1 Blood examination

The animals were sacrificed after 672 hours. Both the blood and livers were harvested.

Blood was collected into anticoagulant free bottles. The serum was separated from the blood cells by centrifugation for 5 minutes at 4,000 revolutions per minute (rpm) using centrifuge 5702 model and removing the serum into labeled specimen bottles using a pipette. The bottles were then kept in a refrigerator at 4°C until used. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Total bilirubin were assayed as described by Sherine and Safinaz (2008), using diagnostic kits for the assays of ALT, AST and total bilirubin obtained from Randox Laboratories Ltd, Co. Antrium, United Kingdom.

Alanine aminotransferase

$$\alpha\text{ – oxoglutarate + L-alanine} \xrightarrow{\text{GPT}} \text{L-glutamate + pyruvate}$$

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine.

Aspartate aminotransferase

$$\alpha\text{– oxoglutarate + L-aspartate} \xrightarrow{\text{GOT}} \text{L-glutamate + oxoaloacetate}$$

Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4 – dinitrophenyl hydrazine.

Total bilirubin

The principle is based on the formation of the purple compound Azo-bilirubin, when bilirubin reacts with the diazotized sulfanilic acid and introduced by Vander Bergh. Conjugated bilirubin reacts (direct-reaction) in an aqueous solution, whereas unconjugated bilirubin requires an accelerator or solubilizer such as alcohol (indirect reaction). The serum is diluted with water and methanol added in amount insufficient to precipitate the proteins, yet sufficient to permit all the bilirubin to react with diazo-reagent.

3.3.2 Liver examination

The livers were fixed in 10% buffered formalin for 24 hours. The formalin was removed by washing through running tap water for 4 hours dehydration. Dehydration was done by keeping the livers in series of graded concentration of alcohol (70%, 80%, 95% and 100%) for 24 hours each. The liver tissues were then transferred into concentrated (100%) xylene for 2 hours to remove the alcohol and embedded in paraffin wax. The paraffin wax displaced completely the xylene. The wax blocks were then fixed in a microtome, sliced into 7 µm thick sections, de-waxed and stained with hematoxylin and Eosin. They were then observed under light microscope for histological changes using eye piece of magnification x10 and objective x40. Photomicrographs of stained tissues were taken using Amscope digital camera for microscope (DCM500), 5M pixels, made in Japan.

3.4 Statistical Analysis

The results were expressed as the mean of seven replicates ± standard error and analysed using one way analysis of variance (ANOVA). $P \leq 0.05$ was regarded as significant. The Statistical Package for Social Sciences (SPSS) Computer Software version 21.0 was used for data analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 *Parkia biglobosa* Fed Group

ALT levels at varying percentage inclusion of *Parkia biglobosa* revealed highly significant difference (P≤0.05) with the 100% (66.50 IU/L) having the highest ALT level. Meanwhile, the lowest ALT level was observed in 20% (34.50 IU/L) which was not significantly different (P>0.05) from the control (41.63 IU/L) and 10% (39.67 IU/L) (Table 4.1).

The AST level in wistar rats was highly significant (P≤0.05) with the highest at 100% (128.88 IU/L) which was not significantly different from the control (119l.75 IU/L). Meanwhile, the lowest AST level was at 20% (91.38 IU/L) which was not significantly different (P>0.05) from the 10% (103.56 IU/L) and 30% (98.38 IU/L).

Total bilirubin level was highly significant (P≤0.05) with the highest (44.88IU/L) at 100% and the lowest (9.13 IU/L) which is the control was lower than all the percentages.

4.2 Maggi Star Fed Group

There was a highly significant difference (P≤0.05) of ALT levels at varying percentage inclusion with the highest (79.71 IU/L) at 30%. The lowest was 36.50 IU/L at 20% which was not significantly different from 10% (40.86% IU/L) and the control (41.63 IU/L) (Table 4.2).

AST level showed highly significant difference (P≤0.05) with the highest (163.29 IU/L) at 30% while the lowest (109.14 IU/L) was at 10% which was not significantly different

Table 4.1: Effect of Varying Percentage Inclusion of Fermented Seeds of *Parkia biglobosa* (FSPB) on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Concentration	ALT (IU/L)	AST (IU/L)	TB (IU/L)
FSPB (10:90)	39.67 ± 2.98^{c}	103.56 ± 3.10^{b}	10.22 ± 0.47^{cd}
FSPB (20:80)	34.50 ± 1.07^{c}	91.38 ± 3.75^{b}	10.63 ± 0.50^{bc}
FSPB (30:70)	50.75 ± 5.12^{b}	98.38 ± 6.71^{b}	11.88 ± 0.58^{b}
FSPB (100:00)	66.50 ± 2.69^{a}	128.88 ± 2.34^{a}	14.88 ± 0.30^{a}
Control	41.63 ± 2.50^{c}	119.75 ± 5.30^{a}	9.13 ± 0.35^{e}
Total	**46.44±2.21**	**108.27±2.88**	**11.32±0.36**

Means with the same superscript(s) along column were not significantly different ($P>0.05$).

Table 4.2: Effect of Varying Percentage Inclusion of Maggi Star on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Concentration	ALT (IU/L)	AST (IU/L)	TB (IU/L)
S (10:90)	40.86 ± 2.76^{b}	109.14 ± 3.49^{b}	10.00 ± 0.44^{bc}
S (20:80)	36.50 ± 9.03^{b}	115.88 ± 11.10^{b}	10.75 ± 0.56^{b}
S (30:70)	79.71 ± 2.69^{a}	163.29 ± 8.38^{a}	15.29 ± 0.42^{a}
Control	41.63 ± 2.50^{b}	119.75 ± 5.30^{b}	9.13 ± 0.35^{c}
Total	**48.97±4.05**	**126.40±5.34**	**11.20±0.48**

Means with the same superscript(s) along column were not significantly different (P>0.05).

from 20% (115.88 IU/L) and the control (119.75 IU/L). The highest level of AST was 163.29IU/L while the lowest was 109.14IU/L at 30% and 10% respectively, the highest value was significantly ($P \leq 0.05$) higher than the control.

There was a highly significant difference in the total bilirubin level. The highest level being 15.29 IU/L at 30% while the lowest was 9.13 IU/L, the control. Although 20% (10.75 IU/L) was significantly higher than the control, it was not significantly different from 10% (10.00 IU/L) while 10% was not significantly different from the control.

4.3 Knorr Fed Group

The varying percentage inclusion of knorr showed a highly significant effect ($P \leq 0.05$) on the level of ALT with the highest (55.14 IU/L) at 30% and the lowest (41.63 IU/L) as the control. This is significantly lower than the values at 20% (54.57 IU/L) and 10% (52.29) (Table 4.3).

The varying percentage inclusion of knorr showed no significant difference ($P > 0.05$) in AST level.

The varying inclusion of knorr showed a highly significant difference ($P \leq 0.05$) in the level of total bilirubin with the highest level at 30% (13.37 IU/L) which was not significantly different from 20%, the control which was not significantly different from 10% (10.71 IU/L). mean while 10% was not significantly different from 20% (12.00 IU/L).

Table 4.3: Effect of Varying Percentage Inclusion of Knorr on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Concentration	ALT (IU/L)	AST (IU/L)	TB (IU/L)
K (10:90)	52.29 ± 2.97^{a}	110.29 ± 6.17^{a}	10.71 ± 0.97^{bc}
K (20:80)	54.57 ± 2.80^{a}	111.29 ± 6.57^{a}	12.00 ± 0.82^{ab}
K (30:70)	55.14 ± 3.68^{a}	123.57 ± 5.80^{a}	13.57 ± 0.48^{a}
Control	41.63 ± 2.50^{b}	119.75 ± 5.30^{a}	9.13 ± 0.35^{c}
Total	**50.59±1.77**	**116.34±3.00**	**11.28±0.45**

Means with the same superscript(s) along column were not significantly different (P>0.05).

4.4 Royco Fed Group

Royco had a highly significant effect ($P \leq 0.05$) on the level of ALT. the highest value (89.14 IU/L) was at 30% and the lowest (36.57 IU/L) was at 10% which was not significantly different from the control (41.63 IU/L) but significantly lower than 20% (62.43 IU/L) (Table 4.4).

There was a highly significantly difference ($P \leq 0.05$) in the level of AST at varying inclusion of Royco with the highest at 30% (136.86 IU/L) and the lowest was at 10% (38.29 IU/L). The 10% inclusion was not significantly different from the control (119.75 IU/L) while the control was not significantly different from 20% (111.71 IU/L).

There was a highly significant difference ($P \leq 0.05$) in the level of total bilirubin with the highest at 30% inclusion (12.43 IU/L) which was not significant from 20% inclusion (11.71 IU/L). The lowest total bilirubin level was at 10% inclusion (9.00 IU/L) which was not significantly different from the control (9.13 IU/L).

4.5 Bouillon Brands' Group and *Parkia biglobosa* Groups

10% inclusion of bouillon cubes and FSPB showed a highly significant effect ($P \leq 0.05$) on the level of ALT with knorr producing the highest value (52.29 IU/L) which was significantly higher than maggi star (40.86 IU/L) and and FSPB (39.67 IU/L). The lowest (39.67 IU/L) was Royco (Table 4.5).

10% inclusion of bouillon cubes and FSPB had a highly significant effect ($P \leq 0.01$) on the level of AST. The highest (110.29 IU/L) was Knorr. The lowest (38.29 IU/L) was Royco which was not significantly different from (109.14 IU/L) maggi star and (103.56 IU/L)

Table 4.4: Effect of Varying Percentage Inclusion of Royco on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Concentration	ALT (IU/L)	AST (IU/L)	TB (IU/L)
R (10:90)	36.57 ± 2.98^{c}	38.29 ± 3.39^{c}	9.00 ± 0.53^{b}
R (20:80)	62.43 ± 3.50^{b}	111.71 ± 7.51^{b}	11.71 ± 1.02^{a}
R (30:70)	89.14 ± 2.16^{a}	136.86 ± 8.50^{a}	12.43 ± 1.13^{a}
Control	41.63 ± 2.50^{c}	119.75 ± 5.30^{ab}	9.13 ± 0.35^{b}
Total	**56.90±4.11**	**102.28±7.65**	**10.52±0.48**

Means with the same superscript(s) along column were not significantly different ($P>0.05$).

Table 4.5: Comparison of The Effect of Equal Percentage Inclusion of Maggi Star, Knorr, Royco and Fermented Seeds of *Parkia biglobosa* on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Concentration	Bouillon/FSPB	ALT (IU/L)	AST (IU/L)	TB (IU/L)
(10:90)	Maggi Star	40.86 ± 2.76^{b}	109.14 ± 3.49^{a}	10.00 ± 0.44^{a}
	Knorr	52.29 ± 2.97^{a}	110.29 ± 6.17^{a}	10.71 ± 0.97^{a}
	Royco	36.57 ± 2.98^{b}	38.29 ± 3.39^{c}	9.00 ± 0.53^{b}
	FSPB	39.67 ± 2.98^{b}	103.56 ± 3.10^{a}	10.22 ± 0.47^{a}
	Total	**42.16±1.76**	**91.20±5.76**	**10.00±0.31**
(20:80)	Maggi Star	36.50 ± 9.03^{b}	115.88 ± 11.10^{a}	10.75 ± 0.56^{a}
	Knorr	54.57 ± 2.80^{a}	111.29 ± 6.57^{a}	12.00 ± 0.82^{a}
	Royco	62.43 ± 3.50^{a}	111.71 ± 7.50^{a}	11.71 ± 1.02^{a}
	FSPB	34.50 ± 1.07^{b}	91.38 ± 3.75^{a}	10.63 ± 0.50^{a}
	Total	**46.23±3.33**	**107.30±4.11**	**11.23±0.36**
(30:70)	Maggi Star	79.71 ± 2.69^{a}	163.29 ± 8.38^{a}	15.29 ± 0.42^{a}
	Knorr	55.14 ± 3.68^{b}	123.57 ± 5.80^{b}	13.57 ± 0.48^{ab}
	Royco	89.14 ± 2.16^{a}	136.86 ± 8.50^{b}	12.43 ± 1.13^{b}
	FSPB	50.75 ± 5.12^{b}	98.37 ± 6.71^{c}	11.88 ± 0.58^{b}
	Total	**68.07±3.54**	**129.41±5.69**	**13.24±0.14**

Means with the same superscript(s) along column were not significantly different (P>0.05).

FSPB. The effect of bouillon 10% inclusion of bouillon cubes and FSPB on total bilirubin was not significant at P>0.05.

At 20% inclusion, the effect of bouillon cubes and FSPB on ALT was significant at P≤0.05. The highest (62.43 IU/L) was Royco. It was not significantly different from Knorr (54.57 IU/L). The lowest (34.50 IU/L) was FSPB which was not significantly different from Maggi star (36.50 IU/L).

The effect of 20% on AST level was not significant at P>0.05.

The effect of 20% on total bilirubin was not significant at P>0.05.

The effect of 30% on the level of ALT was highly significant at P≤0.05. The highest (89.14 IU/L) was Royco. The lowest value (50.75 IU/L) from FSPB was not significantly different from (55.14 IU/L) knorr and (62.43 IU/L) royco.

The effect on the level of AST was highly significant at P≤0.05 with maggi star as the highest (163.29 IU/L). The lowest (98.37 IU/L) was FSPB and was significantly different from knorr (123.57 IU/L) and Royco (136.86 IU/L).

The effect on the level of total bilirubin was highly significant at P≤0.05. The highest value from maggi star (15.29 IU/L) was not significantly different from knorr (13.57 IU/L). The lowest value from FSPB (11.88 IU/L) and was not significantly different from the value from Knorr (13.57 IU/L) and Royco (12.43 IU/L).

4.6 Condiments: Bouillon Cubes and FSPB

Effects of varying levels on ALT were highly significant at $P \leq 0.05$. The highest was royco (62.71 IU/L) which was not significantly different from maggi star (51.64 IU/L) and knorr (54.00 IU/L). The lowest was the control (41.63 IU/L) which was not significantly different from FSPB (42.77 IU/L), maggi star (51.64 IU/L) and knorr (54.00 IU/L) (Table 4.6).

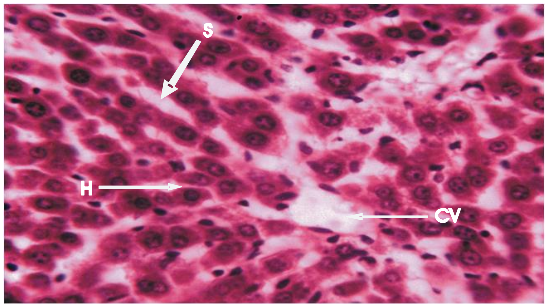
The effect on the level of AST was highly significant at $P \leq 0.05$ with the highest as maggi star (128.82 IU/L) which was not significantly different from the control (119.75 IU/L) and knorr (115.05 IU/L). The lowest value from royco (95.62 IU/L), was not significantly different from that of FSPB (98.92 IU/L) and knorr (115.05 IU/L).

The effect on the level of total bilirubin was significant at $P \leq 0.05$. The highest value which was from knorr (12.10 IU/L) was not significantly different from the values from maggi star (11.95 IU/L), royco (11.05 IU/L) and FSPB (11.00 IU/L). The lowest value was from the control (9.13 IU/L).

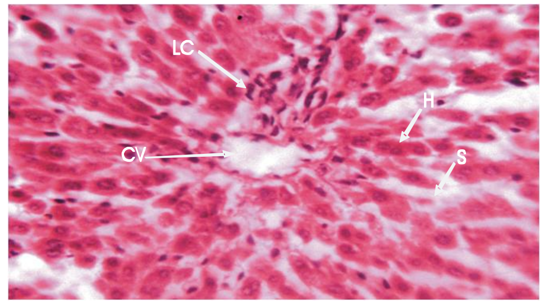
Table 4.6: Comparison of The Effects of Maggi Star, Knorr, Royco and Fermented Seeds of *Parkia biglobosa* on the ALT, AST and Total Bilirubin (TB) Levels in Wistar Rats

Bouillon	ALT (IU/L)	AST (IU/L)	TB (IU/L)
Maggi Star	51.64 ± 5.38^{ab}	128.82 ± 7.01^{a}	11.95 ± 0.57^{a}
Knorr	54.00 ± 1.76^{ab}	115.05 ± 3.65^{abc}	12.10 ± 0.50^{a}
Rayco	62.71 ± 5.06^{a}	95.62 ± 10.07^{c}	11.05 ± 0.61^{a}
Parkia	42.77 ± 2.55^{b}	98.92 ± 2.82^{bc}	11.00 ± 0.33^{a}
Control	41.63 ± 2.50^{b}	119.75 ± 5.30^{ab}	9.13 ± 0.35^{b}
Total	**51.35±1.93**	**110.08±3.15**	**11.31±0.24**

Means with the same superscript(s) along column were not significantly different ($P>0.05$).



(a)



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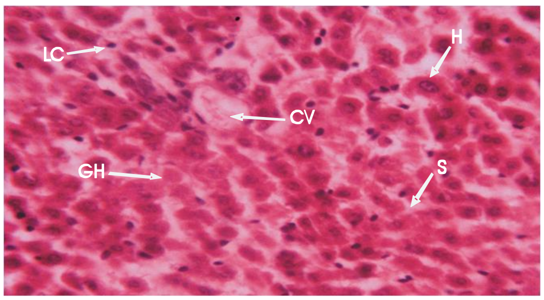
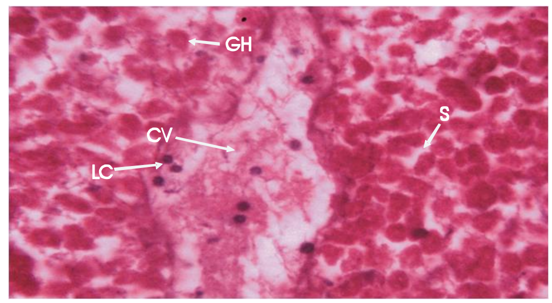
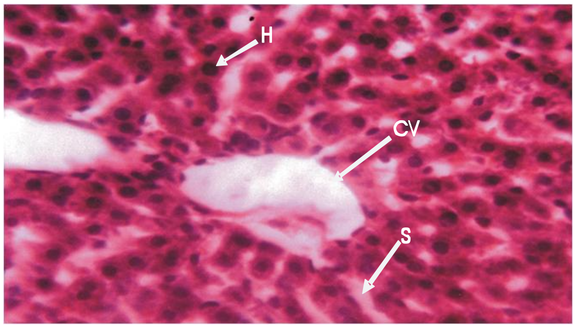


(c)

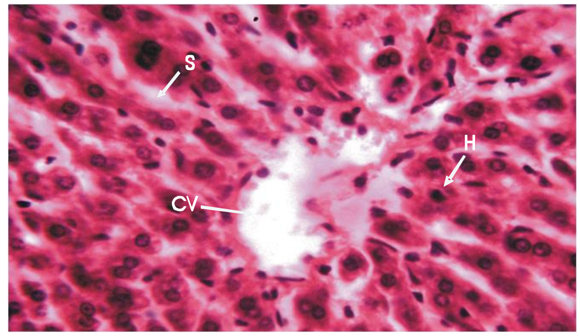


(d)

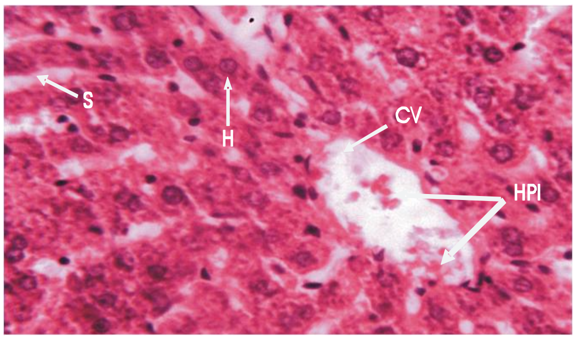
Plate I: Micrographs of histological changes of the liver tissues from the control and Knorr groups (a) liver section of Control group (C) with normal liver architecture. The hepatic central vein (CV), sinusoids (S) and most of the hepatocytes (H) appear normal (×400) H & E. (b) liver section of 10% K group. Most of the hepatocytes (H) and the terminal hepatic central vein (V) appear normal with few concentrated lymphocytic cells (LC) around the CV. The sinusoid appear normal (S) (×400) H & E. (c) liver section of 20% K group. There are few normal nucleated (H) and ghost hepatocytes (GH) and numerous lymphocytic cells (LC). The terminal hepatic central vein (V) appear normal with no clearly defined sinusoidal (S) architecture. There is cellular degeneration (×400) H & E. (d) liver section of 30% K group. The hepatocytes (GH) show ghost appearance without nucleus. There is distorted sinusoidal (S) architecture and the hepatic central vein (V) appear enlarged. Presence of few lymphocytic cells (LC) infiltration are also noted. There is necrotic evidence (×400) H & E.



(a)

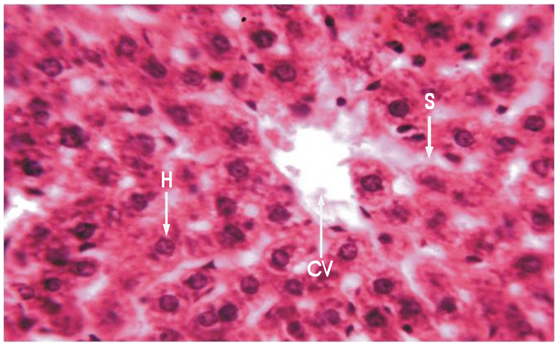


(b)

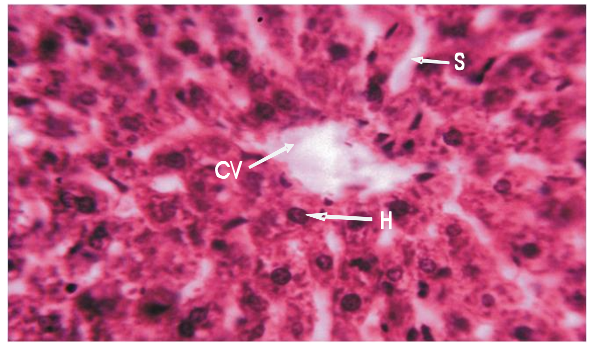


(c)

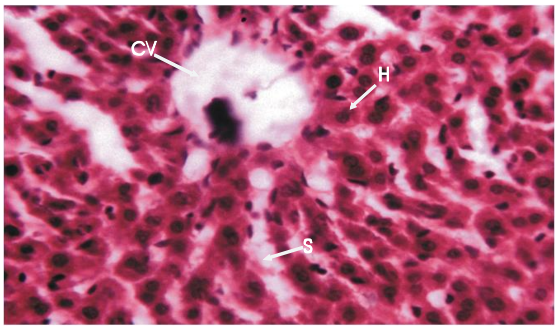
Plate II: Micrographs of histological changes of the liver tissues from Royco group (a) liver section of 10% R group. The liver architecture with most hepatocytes (H) appear normal. The sinusoids (S) and the hepatic central vein (CV) appear normal (×400) H & E. (b) liver section of 20% R group. The liver architecture showing the sinusoids (S) and most hepatocytes (H) as normal. The hepatic central vein (CV) appear dilated. (×400) H & E. (c) liver section of 30% R group. Most of the hepatocytes (H) appear viable. The terminal hepatic central vein (V) and the sinusoids appear dilated with lobular haemapoetic infiltration (HPI). Presence of mononuclear cells are also noted (×400) H & E.



(a)



(b)



(c)

Plate III: Micrograph of histological changes of the liver tissues from Maggi star group (a) liver section of 10% S group. The sinusoids (S), hepatic central vein (CV) and most hepatocytes (H) appear normal. There is a notable lack of inflammatory cell infiltration (×400) H & E. (b) liver section of 20% S group. The liver architecture appear normal with dilated sinusoids (S). The hepatic central vein (CV) and most hepatocytes (H) also appear normal (×400) H & E. (c) liver section of 30% S group. The hepatocytes (H) appear comparatively normal with dilated hepatic central vein (CV) and sinusoids (DS) (×400) H & E.

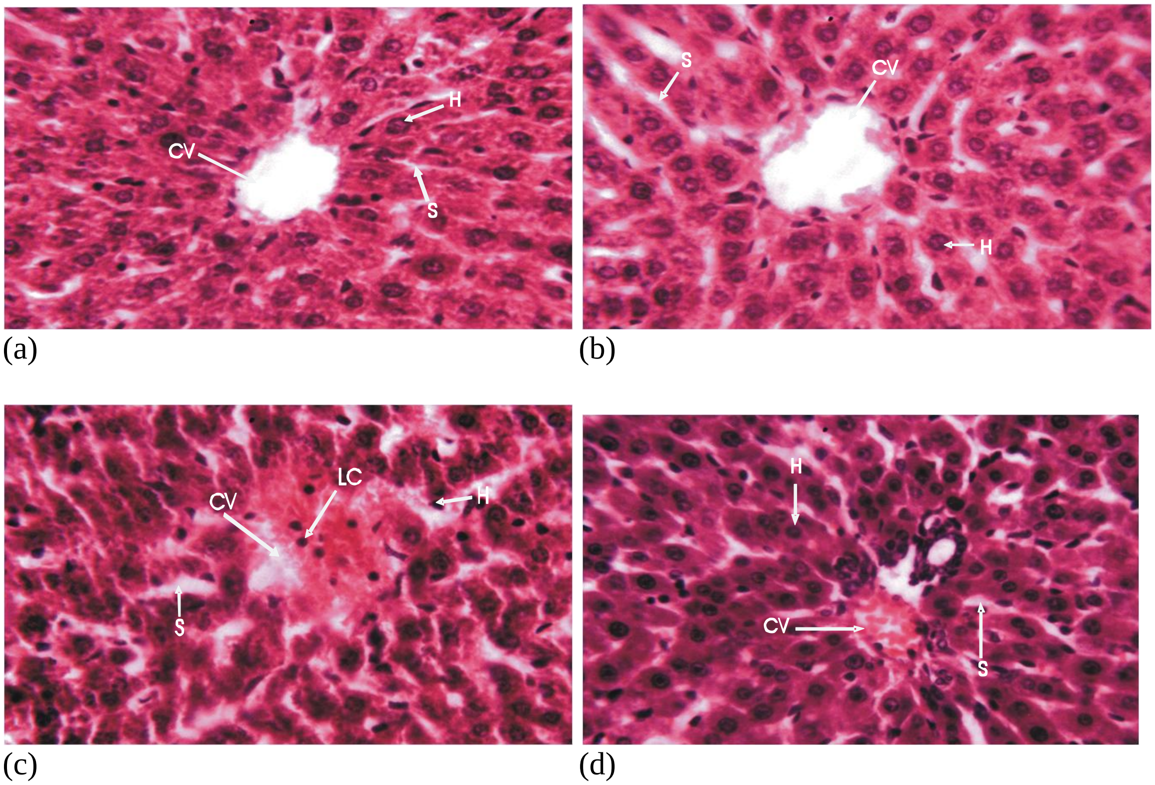


Plate IV: Micrographs of histological changes of the liver tissues from fermented seeds of *P. biglobosa* groups (a) liver section of 10% FSPB group. The liver architecture appear normal with slightly dilated hepatic central vein (CV). Most hepatocytes (H) and sinusoids (S) appear normal (×400) H & E. (b) liver section of 20% FSPB group. The liver general architecture and hepatocytes appear normal with slightly dilated hepatic central vein (CV) and sinusoids (S) (×400) H & E. (c) liver section of 30% FSPB group. Most of the hepatocytes (H) appear viable. The terminal hepatic central vein (CV) and the sinusoids appear dilated with notable inflammatory (lymphocytic) cells infiltration (LC) mostly within the hepatic lumen (×400) H & E. (d) liver section of 100% FSPB group. Most of the hepatocytes and sinusoids (S) appear normal with congested hepatic central vein (CV) (×400) H & E.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of Condiments on the Liver

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is more specific for hepatic injury because it is present primarily in the cytosol of the liver while AST has cytosolic and mitochondrial forms and is present in tissues of the liver, heart, skeletal muscle, kidney, brain, pancreas, lungs, white blood cells and red blood cells (Paul, 2005). Bilirubin is used by the liver to produce bile: an important factor in lipid metabolism.

ALT, AST and total bilirubin are traditional biomarkers used widely for detecting drug-induced liver injury (Yutaka *et al.,* 2004). The Food and Drug Administration of the United States proposed standard thresholds for human as HY's Law, however, those for animal have not been determined (ibid).

In this study therefore, increase or decrease in serum levels of these biomarkers is defined by comparing the values obtained from the test animals with the values obtained from the control. The liver enzyme assay showed marked and gradual increase in the serum levels of AST, ALT and total bilirubin (43.24, 51.17, 74.75; 85.91, 112.96, 141.24 and 9.90, 11.49, 13.76) IU/L respectively in the groups that were fed with varying percentages of bouillon cube (BC) inclusion meals. According to Karla (2012), necrosis causes higher-than-normal amount of ALT and AST. This result corroborates Ibrahim *et al*., (2010) where gradual increase in the serum levels of AST, ALT and Total bilirubin were observed. The work studied the possible ultra structural ameliorative effect of taurine in rats' liver treated with Monosodium glutamate confirms the hepatoprotective effect of taurine, especially when it is administrated as a protective as a protective substance than therapeutic. However, it does not totally agree with Tawfik and Al-badr (2012) where only ALT and AST levels were elevated while Total bilirubin significantly decreased. Monosidum glutamate was administrated to rats at doses of 0.6 and 1.6 mg/g body weight for 14 days. The result showed that monosodium glutamate at these doses per gramme of body weight could cause an adverse effect on hepatic and renal functions which might be due to oxidative stress induced by monosodium glutamate on the liver and renal tissues.

The groups fed with FSPB inclusion meal had ALT levels that did not significantly vary at lower percentages. However, there was an increase at higher inclusion levels. There was no marked variation in AST level while the total bilirubin showed a gradual increase in level. This result corroborates Ayo-Lawal *et al*., (2014) where the study showed that FSPB as condiment is a potential source of antioxidants of natural origin and posses significant DPPH (2,2-diphenyl-1-picrythydrazyl) radical and nitric oxide scavenging potentials. This suggests that regular intake of this condiment would be of immense health benefit since oxidative processes have been proposed to have a causative and/or contributory role in an increasing growing number of diseases such as certain types of cancers, neurodegenerative disorders, stroke and diabetes.

The result of the haematoxyline and eosin staining (H and E) reactions showed that with increasing percentage inclusion of BC in their meal, there was varying degrees of dilation of the hepatic central vein which contained lysed red blood cells, cell degeneration and necrosis. The degeneration and necrosis observed agree with the findings of Ibrahim *et al*. (2010) that reported monosodium glutamate (MSG), a major constituent of BC (RMRDC, 2003) has a destructive effect on hepatocytes of rats and Inuwa *et al*. (2011) who reported that MSG had hepatotoxicity and nephrotoxicity tendencies.

However, the groups fed with increasing percentage (10%, 20% and 30%) including 100% of FSPB appear to have normal liver architecture, hepatocytes and sinusoids with either slight dilation (10%, 20%, 30%) or congestion (100%) of hepatic central vein. This could be due to the quality of the FSPB as a result of the processing method used. This result corroborates that of Kpeme *et al*. (2012) on the oral administration of ethanol extract from fermented seeds of parkia biglobosa on male rats and Ayo-Lawal *et al*. (2014) on the investigation of the hypolipidemic potential of fermented seeds of *P. biglobosa* by supplementation in feeds of wistar rats.

5.1.1 Effects of FSPB

The presence of phytochemicals and the method of fermentation is key to the effect it has on hepatocytes. Methods of fermentation of seeds of *P. biglobosa* impart on its nutritional quality (Balogun and Oyeiola, 2012) and the plant is a source of phytochemicals (Ibrahim *et al*., 2010 and Modupe, 2014). Phytochemicals have the potential to stimulate the immune system, prevent toxic substances in the diet from becoming carcinogenic, reduce inflammation, prevent DNA damage, aid DNA repair, reduce oxidative damage to cells, trigger damaged cells to self destruction (apoptosis) before they can reproduce, help regulate intercellular signalling of hormones and gene expression and activate insulin receptors (Densie, 2013).

5.1.2 Effect of bouillon cubes

The distortion of the cyto-architecture of the liver of the rats fed with bouillon cube inclusion meals could be associated with functional changes that may be detrimental to the health of the wistar rats. The proliferating cells of the liver which produced red and white blood cells are normally found between the hepatic cells and the walls of the vessels (Eweka and Ominiabohs, 2007). Due to the distortion and dilation of the hepatocytes and their central vein with lobular haematopoetic infiltration the haemopoetic function of the liver may have been negatively affected as a result of the likely toxic effect of bouillon cubes.

5.2 Mechanism of Action

Cellular degeneration has been found to lead to cell death: apoptotic and necrotic cell death. Whereas apoptosis is a form of cell death that is generally triggered by normal, healthy processes in the body, necrosis is cell death that is triggered by external factors or disease such as trauma or infection (www.sciencedaily.com). Apoptosis causes cells to shrink, develop blebs (bubble-like spots) on the cell membrane, undergo degradation of genetic and protein materials in the nucleus and have their mitochondria broken down, thus releasing cytochrome. The fragments are each wrapped in their own membrane, with other chemicals (such as ATP and UTP) released freely. These chemicals lead macrophages to find and eliminate the dead cells and their fragments. This “eat me” message is triggered by a phospholipid normally inert in the cell membrane, and the macrophages in turn release cytokines that inhibit inflammatory responses.

In contrast, necrotic cells swell or may form vacuoles on their surfaces, with interior structures either distending or shrinking rapidly, destroying the cell's processes and chemical structures (Wilhelms *et al*., 1998). The unregulated release of cytochrome and the cell membrane's phosopholipid causes immediate reactions in surrounding tissue, leading to inflammation and oedema. Unlike apoptosis, necrotic cells are not targeted by macrophages for cleaning of their cellular debris (www.sciencedaily.com).

As the hepatocytes swell, damage to the cell membrane and interference with the cell's energy occur (www.sciencedaily.com). The disruption caused by bouillon cubes is key in this research. The research having eliminated other possible causes of cell swelling such as poisoning, water intoxication and hypoxia from asphyxia, showed bouillon cubes have acted as toxins to the hepatocytes. Their cellular integrity was therefore affected negatively, causing defects in cell volume homeostasis and membrane permeability.

The hepatocellular degeneration and necrosis noticed in this work could have been caused by the cytotoxic effect of bouillon cubes on the liver. Therefore, the function of the liver of wistar rats, particularly, detoxication and biosynthesis was affected. Bouillon cube as a condiment could probably have negative health implications on the human liver.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

FSPB did not have any significant difference ($P \leq 0.05$) in effect on the levels of ALT and AST except at 30% and 100% compared with the control. However, the integrity of the hepatocytes of the liver was not compromised. Bouillon cubes (Knorr, Royco and Maggi star) had a marked and progressive effect on the level for both ALT and AST and the liver architecture was compromised.

There was a significant difference in the effects of the bouillon cubes and fermented seeds of *Parkia* biglobosa on total bilirubin. Maggi star had the best performance among the bouillon cubes

6.2 Conclusions

The levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in wistar rats fed with varying inclusion levels of bouillon cubes (Knorr, Royco and Maggi star) were significantly high. Their livers showed histological changes in the architecture of the hepatocytes.

Consumption of feeds with varying inclusion levels of fermented seeds of *Parkia biglobosa* had no significant effect on ALT and AST levels in wistar rats. The consumption produced no histological changes in their livers. However, both bouillon cubes and fermented seeds of *Parkia biglobosa* showed a highly significant difference in effects on the level of total bilirubin in the rats

6.3 Recommendations

1. Local authorities should enlighten the people about the potentials of fermented seeds of *Parkia biglobosa* as a condiment to improve wellness of hepatocytes.
2. Food industries should be encouraged by the Federal and State Governments to commercially produce FSPB in the cube form as a condiment.
3. Rat farmers should be advised by relevant Authorities to feed their rats with FSPB inclusion meals.
4. Study of the performance of FSPB as feed inclusions in poultry and fisheries should be carried out.

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APPENDICES

Appendix I

Plate V: ***Parkia biglobosa* Plant with ripe fruits**

Appendix II

Plate VI: **Seeds of *Parkia biglobosa***

Appendix III

Plate VII: Mashed and flattened fermented seeds of *Parkia biglobosa.*