EFFECT OF ASPARTAME ON BIOCHEMICAL PARAMETERS OF MALE SPRAGUE DAWLEY RATS

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CERTIFICATION

We the undersigned certify that Deborah Esemuede Osegbe presented this Thesis to the Department of Medical Biochemistry, School of Basic Medical Science, University of Benin

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to God Almighty for keeping me alive throughout my academic sojourn and especially throughout the duration of this research.

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My profound gratitude goes to Almighty God for his grace, guidance all through my academic pursuit, and for the successful completion of my project work. My heartfelt thanks to my supervisor, and also my Head of Department, Prof. Akhere Omonkua, and Dr Blessing Francis for their immense guidance, support and contributions in this work.

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Table of Content

CHAPTER FIVE

LIST OF TABLES

ABSTRACT

Aspartame (ASP) is an artificial sweetener used in food products as an alternative to sugar. Concerns relating to the possible adverse health effects of its consumption have been raised due to aspartame's metabolic components which are formed during its breakdown. Some research studies have associated aspartame consumption with health disorders such as cancers, neurochemical changes, hepatotoxicity etc, since the liver helps in the metabolism and detoxification of harmful substances and drugs, it acts as a filter to clean the blood. Therefore, the purpose of this study was to investigate the effect of aspartame on liver function parameters in male Sprague Dawley rats. The rats were thirty-one (31) and were divided into five (5) groups: control, groups B-E. Group A (control) received 0.5ml of plain distilled water via gastric gavage. Group B, Group C, Group D, and Group E received (40, 80, 160, 320) mg/kg respectively for a duration of 75days. Results from this study showed a dose-dependent increase in serum Alkaline Transaminase (ALT) concentration between the control and the groups administered aspartame, but the liver ALT showed no significant difference. However, there was no significant difference between the means of the serum Aspartate Transaminase (AST) of the control group and groups administered aspartame. Also the result shows a dose dependent decrease in serum Alkaline Phosphatase (ALP), and a non significant difference in the means of liver ALP. Result from the present study showed significant difference in serum Gamma-glutamyl Transferase (GGT) only when the control group was compared with the group administered 40mg/kg. However, the serum protein, heart protein and liver protein results between all groups in this study, showed no significant difference, but however a significant decrease was observed in the kidney proteins of the rats administered aspartame, especially in the group that received 160mg/kg. The level of testis protein increased in the groups that received 80mg/kg and 160mg/kg when compared to control. However, the amount of serum globulin in the aspartame-administered groups was not different from that of the control group. Aspartame may act as a chemical stressor by altering organ function homeostasis and increasing protein oxidative damage. This might play a significant role in promoting apoptotic cell death leading to damage of the organs and subsequently death.

CHAPTER ONE

1.0 INTRODUCTION

Artificial sweeteners approximate the sweetness of natural sugar while having a low glycemic index and fewer calories.. These sweeteners are used in place of sucrose (table sugar) to sweeten meals and beverages since they are frequently 30 to 13000 times sweeter than sucrose (Nasim et al., 2021). Consumers and food manufacturers have long been interested in dietary sweeteners to replace sucrose in foods (Periyasamy, 2019). Due to their effects on glucose regulation, these products have recently attracted more attention. A lot of baked items, fizzy drinks, powdered drink combinations, jams, jellies, and dairy products contain artificial sweeteners (Findikli and Turkoglu, 2014).The five primary artificial sweeteners used in a range of food products are aspartame, saccharin, acesulfame potassium, neotame, and sucralose (Horio *et al.*, 2014). It was authorized by the Food and Drug Administration (FDA) for use in dry applications in 1981, carbonated soft drink applications in 1983, and general sweetener applications in 1996. (Butchko and Starge, 2001). It is currently known that, in terms of global consumption, aspartame accounts for 62% of the market value for artificial sweeteners. (Adaramoye *et al.*, 2016).

Aspartic acid, phenylalanine, and methanol are the two amino acids that make up aspartame. After consumption, it is converted to methanol and its component amino acids in the intestinal lumen by digestive esterases and peptidases. (Adaramoye *et al.*, 2016), resulting to an increase of its metabolites in the blood (Alwaleedi, 2016).

Due to the aspartame metabolic components that are produced during its breakdown, worries about potential negative health implications have been raised. These substances, which are taken into the blood, are what produce any negative consequences on health associated with consuming aspartame. (Alwaleedi, 2016).

Some research studies have associated aspartame with health disorders such as hepatotoxicity (Alkafafy *et al.,* 2015), cancers (Olney *et al.,* 1996), neurochemical changes in rats (Adaramoye *et al.*, 2016). Aspartame's metabolite methanol, which is converted to formaldehyde and then formate in the liver, is becoming more and more known to cause damage to liver cells. (Mourad, 2011). Elevated NADH levels and the production of superoxide anion, which may be related to lipid peroxidation, are associated with these processes.

1.1 JUSTIFICATION OF THE STUDY

This study's objective was to look into the impact of aspartame on liver function parameters.

The specific objectives were to determine the effects of aspartame on:

1. Alkaline phosphatase (ALP) levels in the liver and serum of Sprague Dawley rats
2. Alanine transaminase (ALT) levels in the liver and serum of Sprague Dawley rats
3. Aspartate transaminase (AST) levels in the liver and serum of Sprague Dawley rats
4. Gamma-glutamyl transferase (GGT) levels in the liver and serum of Sprague Dawley rats
5. Total protein concentrations in the liver and serum of Sprague Dawley rats, and
6. Albumin concentrations in the serum of Sprague Dawley rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. OVERVIEW OF ASPARTAME

It has a low calorific value and is about 180–200 times sweeter than sucrose. Aspartame is a dipeptide-based artificial sweetener. Around the world, aspartame is present in around 6000 products and has a significant economic impact under numerous brand names (Horio *et al.,* 2014). Because of its inexpensive cost, low calorie intake, appealing ads, and promise that it would help with weight loss, aspartame is immensely popular. The issues connected with sugar consumption are at the root of aspartame's popularity among consumers (Tandel, 2011). Regular sugar consumption is limited in diabetics who have difficulty managing their blood sugar levels. This is brought on by diabetics' low levels of insulin, a hormone that controls bloodstream sugar absorption. As a sugar substitute, aspartame helps limit sucrose consumption and only slightly releases energy. As a result of its slower rate of digestion compared to sucrose, blood sugar levels remain stable throughout time. (Zafar *et al*., 2017). Similar to diabetics, many people are compelled to replace high glycemic foods with artificial sweeteners. Sucrose encourages tooth decay because bacteria in the human oral cavity can utilise it as a food source effectively and produce byproducts that erode enamel. Aspartame is used in toothpaste manufacture because, unlike sucrose, it is not utilized by the microorganisms that cause dental plaque. (Sedghi et al., 2021).

Aspartame is increasingly being added to commonly consumed foods, and it is recommended for weight loss, those who are glucose intolerant, and those who have type 2 diabetes. (Choudhary, 2018).

2.2. BRIEF HISTORY OF ASPARTAME

Aspartame was unintentionally discovered in 1965 by James Schlatter, a chemist who was looking for new ways to heal gastrointestinal ulcers. The biologist tested prospective anti-ulcer medications using a tetrapeptide that is naturally synthesized in the stomach. Making the intermediate aspartyl phenylalanine methyl ester was one of the steps in the production of this tetrapeptide. A small quantity of the chemical unintentionally fell into the chemist's hand during one of the intermediate procedures used to create the gastrin inhibitor. The chemist licked his finger and enjoyed a delightful flavor despite all safety precautions. He decided the powder intermediate wasn't likely to be dangerous after tasting it and finding it to be incredibly sweet. (Mazur, 1984).

In a Science paper, Cloninger and Baldwin suggested using it as an artificial sweetener (Cloninger and Baldwin, 1970). Aspartame was first authorized by the Food and Drug Administration (FDA) in 1981 as a tabletop sweetener and then in 1996 as a universal sweetener for all foods and beverages. (Food and Drug Administration, 2006).

Aspartame has been used in over 6,000 items by hundreds of millions of individuals in countries all over the world since its approval (Butchko and Stargel, 2001). It comes in a range of flavors and is 200 times sweeter than sucrose. Several prepared foods (such as chewing gum, confections, gelatins, dessert mixes, puddings and fillings, frozen desserts, and yoghurt) as well as a number of medications include aspartame (e.g., vitamins and sugar-free cough drops) (Zafar et al., 2017).

2.3. PHYSICAL AND CHEMICAL PROPERTIES OF ASPARTAME

A white, crystalline, flavorless substance known as aspartame is extremely sweet. Aspartame is water soluble in alcohol, more soluble in acidic liquids than in neutral ones, and more soluble in acidic liquids at higher temperatures. (Zafar *et al*., 2017)

Aspartame is made up of two naturally occurring amino acids, L-aspartic acid and L-phenylalanine. The molar mass is 294.31 g mol1 and the chemical formula is C14H18N2O5. Its melting point is between 246 and 247 °C, and its density is 1.347g/cm3. (Solmaz et al., 2021). In both alkaline and acidic conditions, aspartame hydrolyzes. The maximum stability of aspartame in an aqueous solution is about pH 4.3, with a bell-shaped relationship between pH and aspartame stability. Temperature and pH fluctuations affect aspartame solubility. At room temperature, aspartame dissolves in water with a pH of 3 and a solubility of approximately 0.03 grams per milliliter.

Because aspartame is a dipeptide, it has some restrictions. It cannot be used for lengthy periods of time in baking, cooking, or liquid storage because it is thought to lose its integrity when heated. In solutions, aspartame breaks down to 3-carboxymethyl-6-benzyl-2,5-piperazine, or diketopiperazine (Zafar et al., 2017).

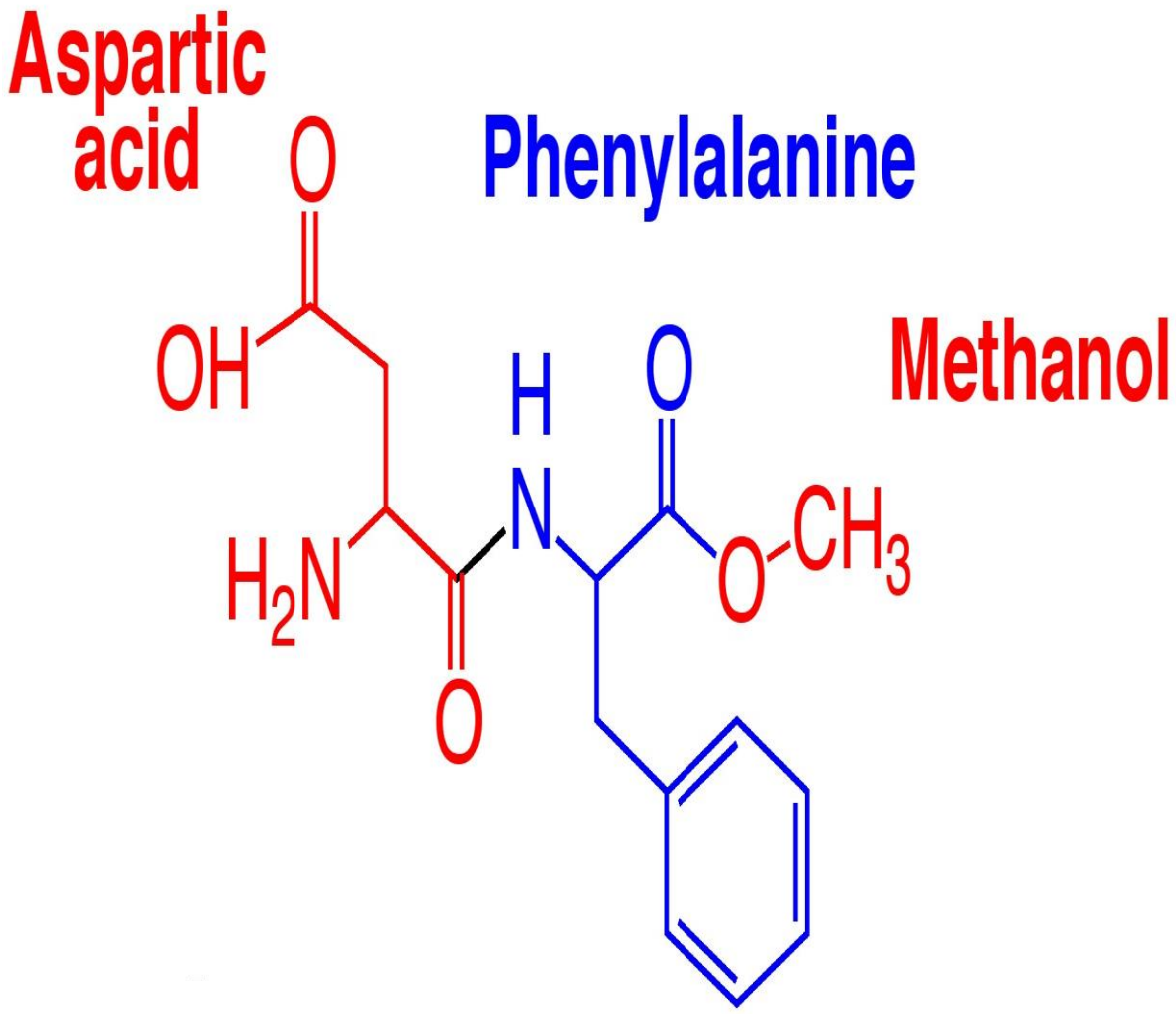


Fig 2.3: Chemical structure of Aspartame
Source: Khiraoui and Guedira , 2018

2.4 BIOCHEMISTRY OF ASPARTAME

2.4.1. SYNTHESIS OF ASPARTAME

Aspartame synthesis, on the other hand, is a little more complex. Direct incubation of L- aspartic acid and methyl ester of phenylalanine alanine with select bacteria also yields aspartame at the commercial level. Aspartame is synthesized using two key chemical processes known as the Z- and F- reactions. The major step in the Z-process is the dehydration of benzyloxy carbonyl L-aspartic acid with acetic anhydride. The anhydride is then mixed with the methyl ester of L-phenylalanine in toluene to produce benzyloxy carbonyl α-and β aspartames. Hydrogenolysis is used to remove the protective groups after crystallization, a mixture of α-and β aspartame isomers yields aspartame (Ager *et al.*, 2008). The F-process entails protecting the aspartic acid amino group with a formyl group, followed by natural dehydration to form anhydride. The formyl group is removed by acid hydrolysis after the anhydride is linked with L-phynylalanine or its methyl ester (Hill *et al.*, 2001).

2.4.2. METABOLISM OF ASPARTAME

Two amino acids make up aspartame (L-phenylalanine and L-aspartic acid) (Mazur et al., 2020). Through the action of esterase and peptidases, aspartame is hydrolyzed into its various constituents namely; methanol (10%), aspartic acid (40%) and phenylalanine (50%) (Choudhary and Lee, 2018). Following absorption through the intestinal mucosa, these metabolites are then transferred via the bloodstream to the liver, where they undergo additional metabolization. (Trocho et al, 1998; George et al., 2020). Phenylalanine, which is involved in the modulation of neurotransmitters, is largely transformed to tyrosine with trace amounts of phenylethylamine and

phenylpyruvate, whilst methanol is further broken down into formic acid, formaldehyde, and dikeketopiperazine. (Choudhary and Lee, 2018).

Aspartic acid is converted to alanine and oxaloacetate. It is an essential excitatory neurotransmitter and also a precursor to glutamate, asparagine, and glutamine (Cortney et al., 2017). Superoxide anions and hydrogen peroxide are also by products of aspartame metabolism. They are both implicated in protein denaturation and subsequent enzymatic alterations (Ashok et al., 2015). It also breaks down into formaldehyde, formic acid, and diketopiperazine, which are all metabolized in the body (Trocho *et al.,* 2008; George *et al.,* 2010). These metabolites are not extremely large, they nevertheless add to the toxicities (Heber, 2004), and because they might be toxic in high doses, long-term aspartame use may be a risk factor (Humphries *et al.,* 2008).

Indeed, aspartame metabolic products are thought to be more hazardous than the original molecule (Stegink, 2007; Ishak *et al.,* 2001).

2.5. OVERVIEW OF THE LIVER

The liver is the largest gland and the second-largest organ in the human body (after the skin) (weighing an average of 1500 g). The liver is roughly formed like a cone or a wedge, with the base to the right and the apex to the left. It is reddish-brown in color. It is extremely vascular, easy to friable, and has a soft consistency. (Kalra *et al.,* 2022It extends to the left upper abdomen and is located under the diaphragm in the right upper and mid-abdomen. The liver has four lobes: the caudate and quadrate lobes are smaller, and the right and left lobes are larger. The liver's left and right lobes are divided by the falciform ligament, which is named for its "sickle-shaped" location where it connects to the abdominal wall. The eight segments of the liver's lobes, which are comprised of thousands of lobules, can be further divided into (small lobes). The common

hepatic duct, which removes bile from the liver, is reached by a duct that runs from each of these lobules. (Kalra *et al.,* 2022). The liver's two primary surfaces are the visceral surface and the diaphragmatic surface. The visceral peritoneum covers everything except the gallbladder bed and the porta hepatis.

The right kidney, suprarenal gland, hepatic flexure of the colon, transverse colon, duodenum, gallbladder, and other anatomical organs are all closely connected to this surface. Except for the naked area, the visceral peritoneum covers the diaphragmatic surface, which rests against the inferior surface of the diaphragm. (Mahadevan, 2020).

Except for the bare spot where the liver contacts the diaphragm, the liver is entirely encapsulated in visceral peritoneum. There are five different ligament types with a direct connection to the liver.:

- ***Coronary ligament*** It connects this tissue to the liver and is created by peritoneal folds reflecting the inferior surface of the diaphragm; has two layers: (anterior and posterior.
- ***Left and right triangular ligament*** are the lateral extensions of the coronary ligaments and also connect the diaphragm to the left and right lobes of the liver, respectively.
- ***Falciform ligament*** is a peritoneal reflection that connects the liver to the upper anterior abdominal wall; has the round ligament of the liver on its free edge.
- ***Ligamentum teres hepatis***, also known as the round ligament of the liver, is a fibrous remnant of the umbilical vein, and extends from the internal aspect of the umbilicus up to the liver.
- ***Ligamentum venosum*** is also an embryonic remnant, in this case of the ductus venosus of the fetal circulation. In utero, the ductus venosus shunts blood from the umbilical vein to the inferior vena cava.(Abhilash et al., 2011; Finamor et al., 2017; Su et al., 2019)

Because it aids in blood purification through detoxification, the Being able to absorb more venous blood than arterial blood makes the liver special. The majority of the circulatory supply enters this organ through the portal vein, which carries blood from the gastrointestinal tract filled with metabolites absorbed in the intestines. The common hepatic artery, which emerges from the celiac trunk and supplies the liver with oxygenated blood, provides the remaining blood supply to this organ. The central veins combine to form the hepatic veins. Just prior to the diaphragm, they allow the liver to immediately drain blood into the inferior vena cava. The hepatic plexus, which travels with the hepatic artery and the portal vein, provides the liver's neural system. Additionally, the liver receives parasympathetic fibers from the anterior and posterior vagal trunks as well as sympathetic fibers from the celiac plexus. (Alexander, 2015; Su et al., 2019; Kalra et al., 2022).

2.5.1. FUNCTIONS OF THE LIVER

The liver is an essential organ of the body that performs over 500 vital functions. Here are some of its most important functions:

- **Albumin Production:** Circulatory fluids are prevented from leaking into surrounding tissue by the protein albumin. It also circulates hormones, enzymes, and vitamins throughout the body**.** Hepatocytes, which are the only cells that create endogenous albumin, manufacture it at a rate of 9 to 12 g per day. Transcytosis is used to transfer albumin from hepatocytes. Some hepatocytes in the liver have direct access to blood flow. Although the liver only produces albumin at a third of its maximum capacity, albumin synthesis accounts for around 50% of the liver's overall energy use. The body's requirements control

production. Changes in the extravascular liver space's osmolality and colloid osmotic pressure have an impact on the rate of production. Thyroxine, cortisol, and insulin all boost synthesis. Albumin production can be hampered by high potassium levels, exposure to toxins, or exposure of hepatocytes to excessive COP. Many significant physiologic functions of albumin exist. It aids COP, takes part in the metabolism of intermediate drugs, and is essential for recovery. Through albumin binding, toxic substances in the body can be detoxified and rendered inactive. Additionally, albumin has antioxidant qualities. (Martin, 2004; Wingfield, 2002; Caceci, 2005).

- **Bile Production:** Bile is a crucial fluid because it aids in the excretion of substances that the kidneys are unable to eliminate and facilitates the absorption and digestion of lipids by secreting bile salts and acids. Hepatocytes create bile, which is mostly made up of water, electrolytes, bile salts, bile acids, cholesterol, bile pigment, bilirubin, and phospholipids among other things. Hepatocytes secrete bile into the bile canaliculi, where it moves from smaller to bigger ducts before entering the duodenum or being deposited in the gallbladder for storage and concentration, depending on the duct and sphincter of Oddi pressures.. Following secretion of bile into the duodenum, it undergoes enterohepatic circulation, where it performs its job in the bowel, and bile components that are not excreted are recycled by conversion into bile acids by gut bacteria for reuse by absorption in the ileum and transport back to the liver (O'Brien et al., 2015; Klevay and Hegsted, 2008).

- **Bilirubin Metabolism:** Bile is a fluid that is critical to the digestion and absorption of fats in the small intestine. The breakdown of heme involves the liver significantly. Hemolysis takes place throughout the body, including in the liver, spleen, and bone marrow. Unconjugated bilirubin is created from biliverdin, which is made from heme. Unconjugated bilirubin that is coupled to albumin is delivered to the liver from the bloodstream. The unconjugated bilirubin then undergoes conjugation via the uridine diphosphate glucuronyltransferase (UGT) system, a phase II process, to become hydrophilic. The newly conjugated bilirubin then is secreted via bile canaliculi into the bile or small amounts dissolve in the blood where it then gets filtered for excretion by the kidneys. Most conjugated bilirubin enters the bile and is excreted with bile in feces as it is not absorbable by the intestinal wall. Some bilirubin is converted to urobilinogen or unconjugated bilirubin by gut bacteria for reabsorption to undergo enterohepatic circulation (O'Brien et al., 2015; Stec et al., 2016).
- **Filters Blood:** The liver filters out poisons, waste products, and other hazardous things as all blood leaving the stomach and intestines flows through it**.** The liver filters or detoxifies the blood. Almost all the blood in your body passes through the liver. As blood passes through the liver, it breaks down substances, such as prescription or over-the-counter drugs, street drugs, alcohol, and caffeine. Our bodies naturally produce some harmful (toxic) chemicals or poisons, and

those are also broken down by the liver. In this way the liver acts as a filter to clean your blood (Martins and Carvalho, 2007).

- **Regulates Amino Acids:** The liver makes sure amino acid levels in the bloodstream remain healthy. The liver also plays an important role in the metabolism of proteins: liver cells change amino acids in foods so that they can be used to produce energy, or make carbohydrates or fats. A toxic substance called ammonia is a by-product of this process. The liver cells convert ammonia to a much less toxic substance called urea, which is released into the blood. Urea is then transported to the kidneys and passes out of the body in urine (Menche, 2012; Schmidt et al., 2011).
- **Regulates Blood Clotting:** Blood clotting coagulants are created using vitamin K, which can only be absorbed with the help of bile, a fluid the liver produces. With the help of vitamin K, the liver produces proteins that are important in blood clotting. It is also one of the organs that break down old or damaged blood cells (Pschyrembel and Klinisches, 2014).
- **Stores Vitamins and Minerals:** The liver stores significant amounts of (Lipid-soluble) vitamins A, D, E, K, and B12, as well as iron and copper. The hepatocytes take up many types of vitamins and minerals from the blood and store them. These include vitamins A, B_{12}, D, E, K and minerals such as iron and copper. Vitamin A is stored within stellate cells in the liver as retinyl ester. The active form, retinol, is converted to this by lecithin retinol acyltransferase. This provides an easily retrievable source of Vitamin A and regulates its availability for other pathways. Vitamin A may be stored or removed from storage several times a day, regulating the amount in circulation and preventing damage that may occur as a result of excess. This process is known as retinol

recycling. Vitamin D can either be produced in the body (cholecalciferol) or found in food (ergocalciferol). It must be metabolised in the liver before becoming the active form. Vitamin E is a family containing various chemicals, including anti-oxidants. It can be stored in either the liver or adipose tissue. Around 2-5mg of Vitamin B12, cobalamin is stored in the body, with around 50% of this being in the liver. (Dioguardi *et al*., 2016; Iguchi *et al*., 2016; Su *et al*., 2019; Adams *et al*., 2005)

- **Processes Glucose:** Glucose is a vital energy source for cells and levels in the blood stream must remain constant. The liver helps maintain blood glucose levels in response to the pancreatic hormones insulin and glucagon. After a meal, glucose enters the liver and levels of blood glucose rise. This excess glucose is dealt with by glycogenesis in which the liver converts glucose into glycogen for storage. The glucose that is not stored is used to produce energy by a process called glycolysis. This occurs in every cell in the body. In between meals or during starvation, blood glucose levels fall. The hepatocytes detect this change, and restore glucose levels by either glycogenolysis which converts glycogen back to glucose, or gluconeogenesis in which non-sugars such as amino-acids are converted to glucose (Schmidt *et al*., 2011; Iguchi et al., 2016; Adams *et al*., 2005).

2.6. LIVER FUNCTION TESTS

A variety of tests are used to measure the blood levels of various proteins and liver enzymes, including a liver function test. Proteins called liver enzymes quicken the body's chemical processes. In addition to creating bile and chemicals that aid in blood coagulation, these chemical processes also break down food and poisons and combat illness. Common liver enzymes include:

Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma-glutamyl transferase (GGT), Albumin etc. (Pandey & Cascella, 2022).

2.6.1. ALANINE TRANSAMINASE (ALT)

Alanine aminotransferase (ALT) is a transaminase enzyme that was formerly known as serum glutamate pyruvate transaminase (SGPT). In the alanine cycle, pyruvate and glutamate are created when an amino group is transferred from alanine to alpha-ketoglutarate by an enzyme called alanine aminotransferase. (Hughes and Jefferson, 2008). Serum and organ tissues, especially the liver, contain considerable amounts of the ALT enzyme, as do the kidney, skeletal muscle, and myocardium. In the pancreatic, spleen, and lung, ALT levels are lower. When there is considerable cellular necrosis, the serum level of alanine aminotransferase increases and is utilized as a marker of liver function. In cases of hepatitis, congestive heart failure, liver or bile duct injury, or myopathy, ALT levels may be high. Rodent plasma ALT may be impacted by diet, constraint, and medication administration. (Peltz-Sinvani *et al*., 2016).

2.6.2. ASPARTATE TRANSAMINASE (AST)

Aspartate aminotransferase (AST) is a transaminase enzyme that catalyzes the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate (Zhang et al., 2017). The AST enzyme is found in cerebrospinal fluid, exudates, and transudates in proportion to the amount of cellular damage. The AST enzyme, formerly known as serum glutamate oxalate transaminase (SGOT), is present in all tissues except bone and is mostly located in the liver and skeletal muscle. After bleeding, trauma, necrosis, infection, or neoplasia of the liver or muscle, the concentration of AST is raised.

2.6.3. ALKALINE PHOSPHATASE (ALP)

Alkaline phosphatase refers to a set of isoenzymes that are broadly dispersed throughout the body. (e.g., liver, bile ducts, intestine, bone, kidney, placenta, and leukocytes) that catalyze the release of orthophosphate from ester substrates at an alkaline pH. The isoenzymes of greatest clinical importance in adults are in the liver and bone because these organs are the major sources of serum ALP (Yokoyama, 2007). The placenta, small intestine, and kidneys are the sources of other isoenzymes. In the liver, ALP is found on the canalicular membrane of hepatocytes; its precise function is undefined. Although the locations of ALP's deterioration are unknown, its elimination from serum is unaffected by the liver's functional ability or the biliary tract's patency. ALP has a serum half-life of roughly 7 days. Through enhanced production of the enzyme and leakage into the serum, a process mediated by bile acids, hepatobiliary illness raises blood ALP levels. (Hughes and Jefferson, 2008). The testing technique, age, and sex all have a significant impact on the (Revzin *et al*., 2017)**.**

2.6.4. GAMMA-GLUTAMYLTRANSFERASE (GGT)

A transferase enzyme called gamma-glutamyltransferase catalyzes the transfer of gamma-glutamyl functional groups from molecules like glutathione to an acceptor, which could be an amino acid, a peptide, or water (forming glutamate). GGT is present in the cell membranes of many tissues, including the kidneys, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles (Kolli & Devaraj, 2018). It is involved in the transfer of amino acids across the cellular membrane Loomba *et al*., 2012) and leukotriene metabolism (Yokoyama, 2007). It is also involved in glutathione metabolism by transferring the glutamyl moiety to a variety of

acceptor molecules including water, certain L-amino acids, and peptides, leaving the cysteine product to preserve intracellular homeostasis of oxidative stress (Koehler *et al*., 2014).

2.6.5. ALBUMIN

The most prevalent serum protein and greatest quantitatively produced synthetic byproduct of the liver is serum albumin. Additionally, it is the main transporter of free fatty acids in blood. Serum albumin can also indiscriminately bind steroids, thyroid hormones, hemin, and other strange compounds in addition to fatty acids. (Green and Flamm, 2002). Serum albumin is a water-soluble, anionic globular protein of molecular weight ~65,000. The protein's structure is dominated by several long α-helices that make the protein rigid. Serum albumin houses 11 distinct hydrophobic binding domains and so is capable of simultaneously carrying multiple fatty acids (Unalp-Arida, 2016).

2.7 ASPARTAME AND THE LIVER

Despite being declared safe by the FDA, aspartame appeared to have some unfavorable side effects in a number of case studies. Although the majority of research has been on aspartame's neurotoxic consequences (Bergstrom et *al.,* 2007; Ashok *et al.,* 2014); Abhilash *et al.,* (2011) found that when rats were given 1000 mg/kg BW for 180 days, aspartame caused liver damage. Increased amounts of liver function enzymes, which were substantiated by histological findings, confirmed the liver damage in their investigation. In a study that looked at its negative effects on experimental animals, it was discovered that aspartame consumption of 250, 500, and 1000 mg/kg/day significantly elevated the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Pehlivan *et al.,* 2020).

According to some research, aspartame can cause dose-dependent hepatotoxicity (Alkafafy *et al.*, 2015). Rats were given 50, 100, and 200 mg/kg aspartame intraperitoneally in a study to see how it affected alterations in acetylcholinesterase (AChE) activity in liver, lung, kidney, and brain tissues. At 12 hours after injection, AChE activities were considerably increased in the liver and kidney at all dosages. The lungs and brain, on the other hand, did not show the same high levels. However, 24 hours after the injection of 200 mg/kg, liver and kidney activities were comparable to the control (Polat *et al.*, 2017).

An indication of liver damage is also increased ALP activity (Fernandez & Kidney, 2007). According to some theories, aspartame may be a substantial source of formate in humans who ingest large amounts of it, which could have detrimental physiological effects. (Borkum, 2016).

Most importantly, those who have phenylketonuria, a genetic disorder in which sufferers are unable to convert phenylalanine to tyrosine, must avoid aspartame. Due to aspartame's harmful effects on those with phenylketonuria, the FDA mandates that all products containing aspartame bear a label indicating the presence of phenylalanine. (Fitch and Keim, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. CHEMICALS AND REAGENT

- Food grade Aspartame (HSWT®)
- Sodium hydroxide (NAOH)
- Sodium chloride (NACL)
- Albumin Kit (Randox)®
- Alanine transaminase Kit(Randox) ®
- Aspartate transaminase Kit(Randox ®)
- Gamma-glutamyl transferase Kit (Randox) ®
- Alkaline phosphatase Kit (TECO®)
- Distilled water- This was used in all biochemical assays.

3.1.2. EQUIPMENTS

The major equipments used for the assay are

- Sensitive electronic balance (TYPE: LAC214C, 704010)
- UV- spectrophotometer-A&E lab UK (Model: AE-560-20)
- Hisense refrigerator (Model: REF302DR), Haier thermocool chest freezer (Model: HTF-319H)

- Thermostat oven (Model: DHG-9053A)
- Centrifuge (Model: D-37520 Osteorode
- Kendro laboratory products, Germany)
- HH-S6 Water Baths (Searchtech instruments British Standard)
- Mortar and pestle
- Micro-pipette
- Electronic compact balance (S. Mettler).

3.2. METHODS

3.2.1. ANIMAL AND EXPERIMENTAL PROTOCOL

Thirty one (31) prepubertal male Sprague Dawley rats were purchased from Faculty of Life Sciences, University of Benin, Edo State, Nigeria. They were between 3 to 4 weeks and weighed between 71g to 137g. The animals were housed in a well-ventilated area at TRIGAS Research laboratories at the Department of Medical Biochemistry, University of Benin, with 12h light and 12h dark cycles. They were fed twice a day (standard pelleted) and given clean water *ad libitum*. The animals were acclimatized for two weeks before the study started. The animals were treated according to the Laboratory Animal Care and Use Guidelines (NAS, 2011). At the end of acclimatization, the experimental animals were then grouped into cages of five (5) labeling A-E in such way that the mean across each group was 102 ± 2. Group A (control) had 6 rats, while Groups B-D had 6 rats each, and Group E had 7 rats.

3.2.2. PREPARATION AND ADMINISTRATION OF ASPARTAME

The Aspartame was reconstituted appropriately in distilled water and orally administered to the experimental animals via gastric *gavage*. Group A received 0.5ml of plain distilled water throughout the course of the experiment. The weights of the experimental animals were determined weekly using electronic weighing balance, and a fresh stock solution of aspartame was prepared each week using the newly calculated weight for each group, making sure that each animal received a dose less than 1ml. The stock was then stored in the refrigerator. Administration went on for 75 days.

3.2.3. BLOOD COLLECTION

All 31 animals were fasted overnight at the end of administration. Animals were sacrificed via cervical dislocation technique. Blood samples (2mls) were collected into plain bottles, while various organs (liver, kidney, heart, and testes) were harvested, rinsed in normal saline, blotted, weighed, kept in plain bottles, and stored in ice pack for biochemical analysis. Blood collected into plain bottles were put into ice to enable clotting; they were then centrifuged at 1000 rpm for 5 minutes using a centrifuge. The serum was carefully decanted for further biochemical analysis.

3.2.4. TISSUE HOMOGENIZATION

Following the sacrifice, portions of all tissues (liver, kidney, heart, and testes) harvested were rinsed in cold normal saline and further homogenized in ice cold normal saline 4:1 w/v. The homogenate was centrifuged at 1,000 rpm for 15 minutes; the separated supernatant was stored in a freezer until analysis.

3.3. BIOCHEMICAL ANALYSIS

3.3.1. SERUM AND TISSUE TOTAL PROTEIN ASSAY USING BIURET METHOD

Reagents/ Procedure:

Standard bovine serum albumin (BSA) - 20mg/ml, 1N NaOH, Biuret reagent – 3 g of $CuSO_4$ and 9 g of Na-K tartrate was dissolved in 500 ml of 0.2 N NaOH. 5 g of KI was then added and the solution made up to the 1litre mark using 0.2 N NaOH.

Assay Protocol:

Test tube	1	2	3	4	5	6
Sample						
Standard BSA (ml)	-	0.2	0.4	0.6	0.8	1.0
Sample (ml)	-	-	-	-	-	-
0.2						
DistilledH_2O (ml)	1.0	0.8	0.6	0.4	0.2	0.8
1N NaOH (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Biuret (ml)	3.0	3.0	3.0	3.0	3.0	3.0
Absorbance (540)	0.000	0.058	0.236	0.324	0.470	0.524
-						
Amount of Protein (mg)	0.0	4.0	8.0	12.0	16.0	20.0

The amount of protein in each sample was extrapolated from a protein standard curve.

3.3.2. SERUM AND TISSUE ALANINE TRANSAMINASE (ALT) ASSAY USING RANDOX KI T® CAT. NO. AL 146

Reagent Composition:

R1.	Buffer	1 x 100 ml 200 tests
R2.	2, 4-Dinitrophenylhydrazine	1 x 100 ml
R3.	Sodium Hydroxide	1 x 100 ml
CAL.	Pyruvate Standard	1 x 10 ml

Principle:

I-oxoglutarate + L-alanine ⟶ L-glutamate + pyruvate

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

Contents		Initial Concentration Solutions
R1.	Buffer Phosphate buffer	100 mmol/l, pH7.4
	L-alanine	200 mmol/l
	I-oxoglutarate	2.0 mmol/l
R2.	2,4-dinitrophenylhydrazine	2.0 mmol/l
R3.	Sodium Hydroxide	4.0 mol/l

Procedure:

Wavelength:	Hg 546 nm (530 - 550 nm)
Cuvette:	1cm light path
Incubation Temperature:	37°C
Measurement	against Reagent Blank

Pipette into test tubes:	Reagent Blank	Sample
Sample	---	0.1 ml
Buffer (R1)	0.5 ml	0.5 ml
Distilled Water	0.1 ml	--

Mix, incubate for exactly 30 min. at 37°C

2, 4-DNP (R2)	0.5 ml	0.5 ml

Mix, allow to stand for exactly 20 min. at 20 to 25°C

Sodium Hydroxide (R3)	5.0 ml	5.0 ml

Mix, read the absorbance of sample (Asample) against the reagent blank after 5 mins

Calculation:

Obtain the activity of ALT in the serum from the table:

Absorbance	U/l	Absorbance	U/l
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.17	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

3.3.3. SERUM AND TISSUE APARTATE TRANSAMINASE (AST) ASSAY USING RANDOX KIT ®Cat. No. AS 101

Reagent Composition:

Contents	Initial Concentration of Solutions
R1. Buffer	
Phosphate buffer	100 mmol/l, pH 7.4
L-aspartate	100 mmol/l
I-oxoglutarate	2 mmol/l
R2. 2, 4-dinitrophenylhydrazine	2 mmol/l

Principle:

I-oxoglutarate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + Oxaloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Procedure:

Wavelength:	Hg 546 nm

Cuvette: 1cm light path

Incubation Temperature: 37°

Measurement against Reagent Blank

Pipette into test tubes:

	ReagentBlank	Sample
Sample	---	0.1ml
Reagent 1	0.5ml	0.5ml
Distilled Water	0.1ml	---

Mix, incubate for exactly 30 min. at 37°C

Reagent 2	0.5 ml	0.5 ml

Mix, allow to stand for exactly 20 min. at 20 to 25°C

Sodium Hydroxide	5.0 ml	5.0 ml

Mix, read the absorbance of sample (Asample) against the reagent blank after 5 minutes.

Calculation:

Obtain the activity of AST in the serum from the table:

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

3.3.4. SERUM AND TISSUE GAMMA-GLUTAMYL TRANSFERASE (GGT) ASSAY USING RANDOX KIT ® Cat. No. GT 2750

R1a.	Buffer/Glycylglycine	1 x 70 ml
R1b.	Substrate	20 x 3 ml

Reagent Composition:

Contents	Concentration in the Test
R1a. Buffer/Glycylglycine	
Tris buffer	100 mmol/l, pH 8.25
Glycylglycine	100 mmol/l
R1b. Substrate	
L-γ-glutamyl-3-carboxy-4-nitroanilide	2.9 mmol/l

Principle:

Colorimetric Method

The substrate L-B-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by B-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405nm.

L-B-glutamyl-3-carboxy-4-nitroanilide + glycylglycine

$$\xrightarrow{\gamma\text{-GT}}$$

L-B-glutamylglycylglycine + 5-amino-2-nitrobenzoate

Test Method:

Wavelength:	Hg 405 nm (400 - 420 nm)
Cuvette:	1 cm light path
Temperature:	25°C, 30°C, 37°C
Measurement:	against air

Pipette into cuvette:

Sample	0.10 ml
Reagent (25°C, 30°C, 37°C)	1.00 ml

Mix, read initial absorbance and start timer simultaneously. Read again after 1 ,2 and 3 min.

Calculation:

To calculate the GGT activity use the following formula. U/L = 1158 x A 405 nm/min

3.3.5. SERUM AND TISSUE ALBUMIN CONCENTRATION (ALB) ASSAY USING RANDOX KIT (R) Cat. No.

AB 362

Principle:

The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample

Reagent Composition:

Contents Initial	Concentration of Solutions
R1. BCG concentrate	
Succinate buffer	75 mmol/l; pH 4.2
Bromocresol green	0.15 mmol/l
Brij 35	

Procedure:

Wavelength:	Hg578 nm or Hg623 nm
Spectrophotometer:	630 nm (600-650 nm)
Cuvette:	1 cm light path
Incubation Temperature:	20 - 25°C

Measurement: against reagent blank

Pipette into test tubes:

	Reagent	Standard	Sample
Distilled H2O	0.01 ml	----	----
Standard (CAL)	----	0.01 ml	----
Serum or Plasma	----	----	0.01 ml
BCG reagent (R	3.00 ml	3.00 ml	3.00 ml

Mix and incubate for 5 minutes at +20 to +25°C. Measure the absorbance of the sample (Asample) and of the standard (Astandard) against the reagent blank.

Calculation:

The albumin concentration in the sample may be calculated from the following formula:

Albumin Concentration (g/l or g/dl

$$\frac{\text{Asample}}{\text{Astandard}} \times \text{Concentration of standard}$$

3.3.6. SERUM AND TISSUE ALKALINE PHOSPHATASE (ALP) ASSAY USING TECO KIT(R)

Principle:

The alkaline phosphatase acts upon the AMP-buffered sodium thymolphtalein. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure:

1. For each sample, dispense 0.5mL of Alkaline Phosphatase substrate into labeled test tubes and equilibrate to 37 ^{0}C for three (3) minutes.
2. At timed intervals, add 0.05Ml (50µl) of each standard, control, and sample to its respective test tube. Mix gently. Use deionized water as sample for Reagent Blank.
3. Incubate for exactly ten(10) minutes at 37^{0}C
4. Following the same sequence as in step 2 add 2.5mL Alkaline Phosphatase colour Developer at timed intervals. Mix well.
5. Set the wavelength of the spectrophotometer at 590nm. Zero with Reagent Black. (Wavelength range : 580-630).
6. Read and record absorbance of samples.

Calculation:

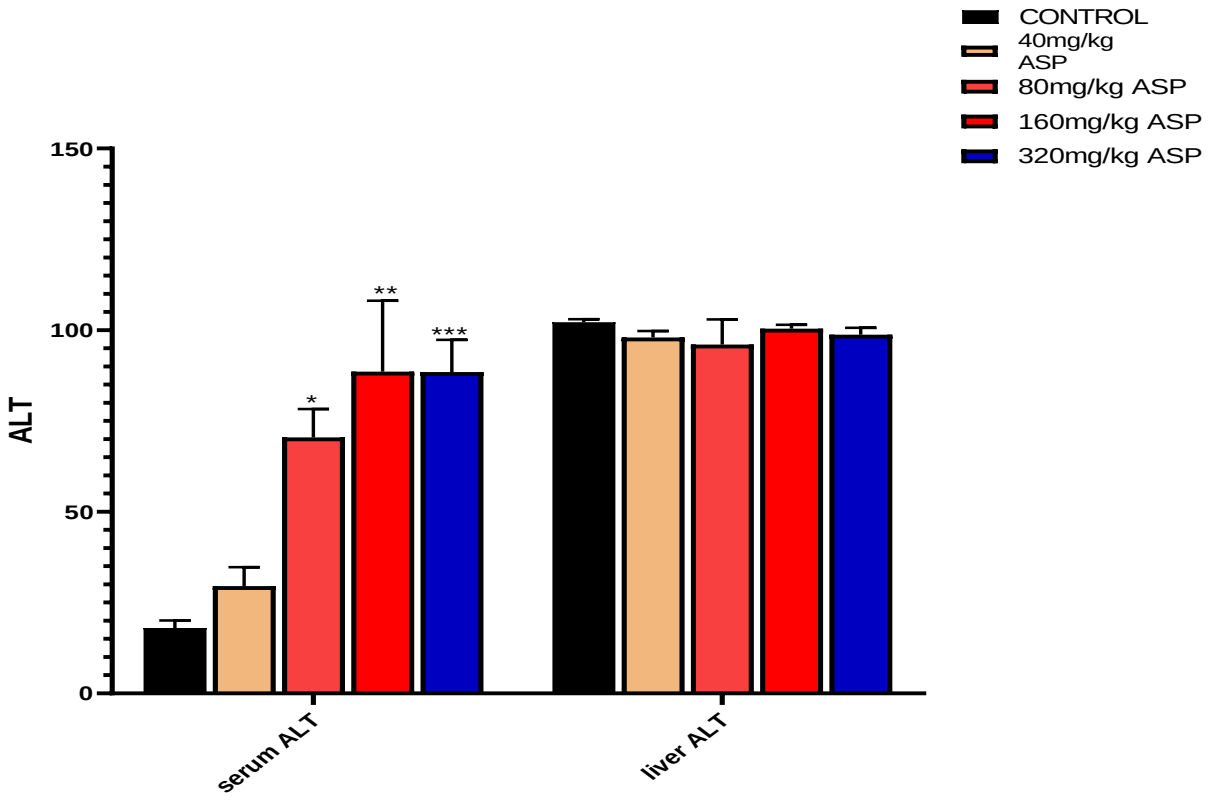
$$\frac{\text{Abs of Unknown}}{\text{Abs of Standard}} \times \text{Value of Std (IU/L)} = \text{Unk. (IUL)}$$

3.4. STATISTICAL ANALYSIS

The data were expressed as means of 4 to 7 determinations ± S.E.M. The differences between groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done by Tukey. A value of $P < 0.05$ was accepted as significant. Graph pad, version 8.0.2 was used for the analysis.

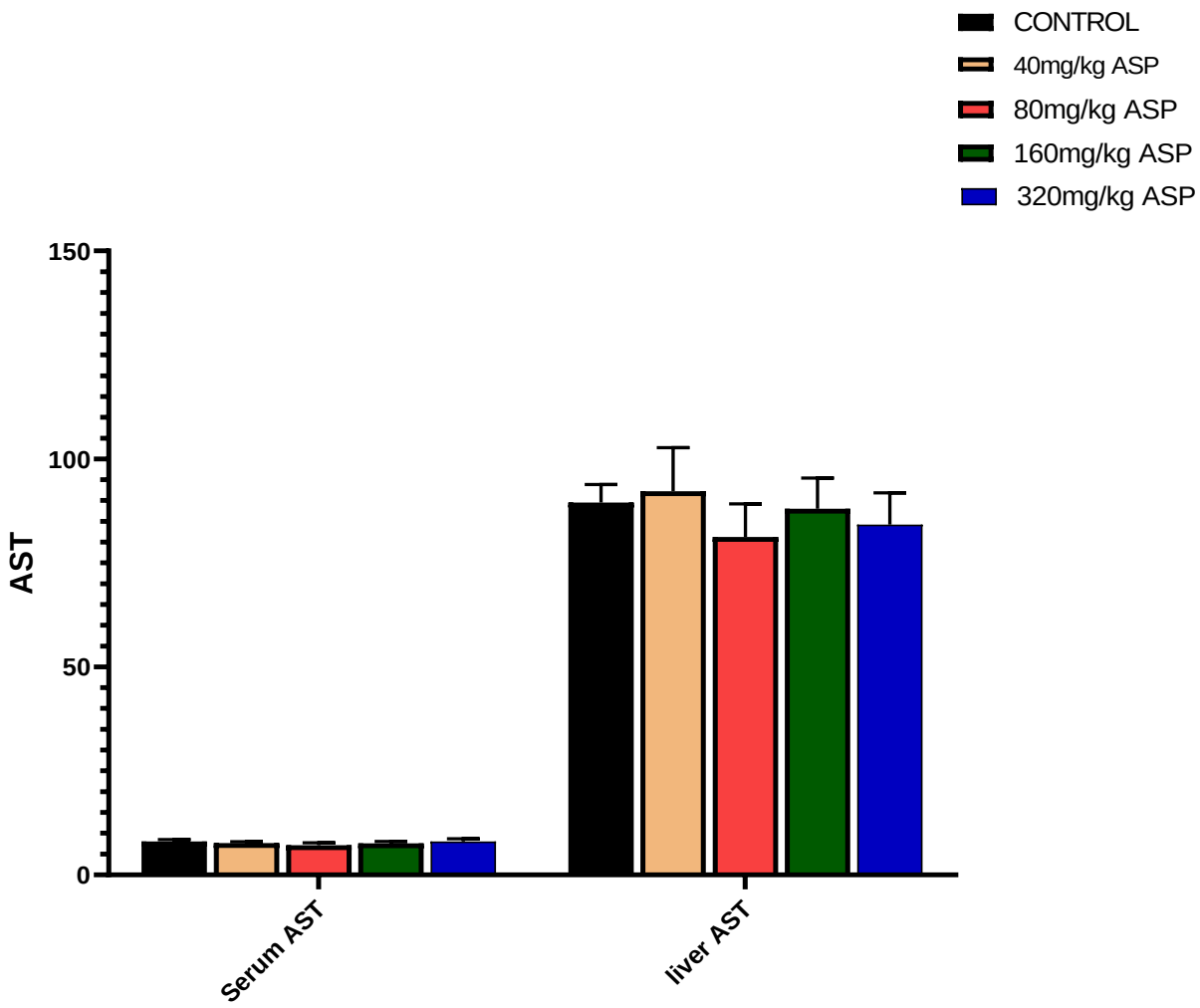
CHAPTER FOUR

4.0 RESULTS



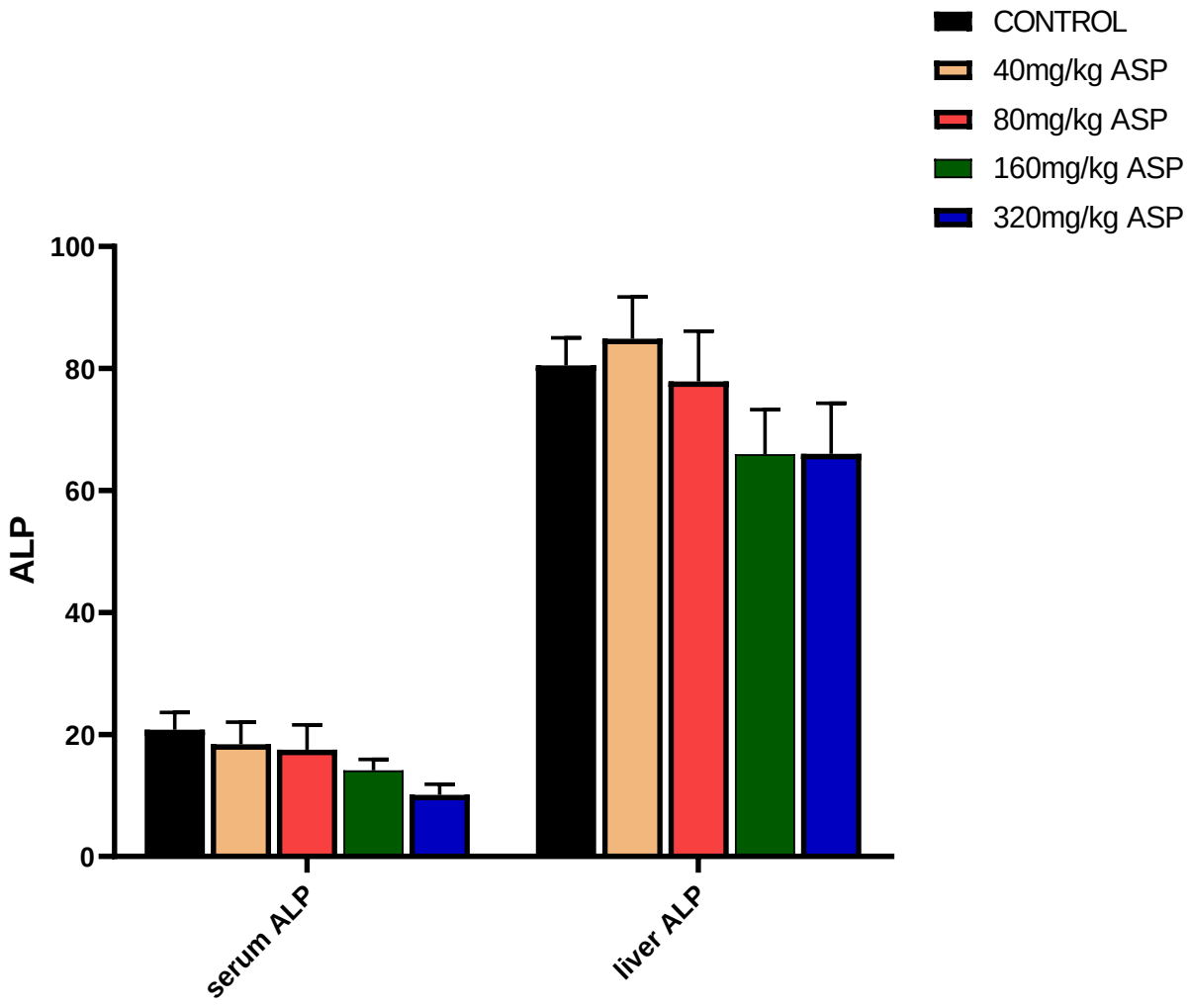
Effect of Aspartame on Alanine Transaminase (ALT) activity in male Sprague Dawley rats. Data are represented as Mean ± SEM.

There was no significant difference in serum ALT when the control was compared to the group administered 40mg Asp. But there was significant difference ($P < 0.05$) between the control and groups administered 80mg/kg, 160mg/kg, and 320mg/kg Aspartame. In addition, there were significant differences ($P<0.05$) between the various groups administered aspartame. However, there was no significant difference in the liver ALT when the control was compared to other groups.



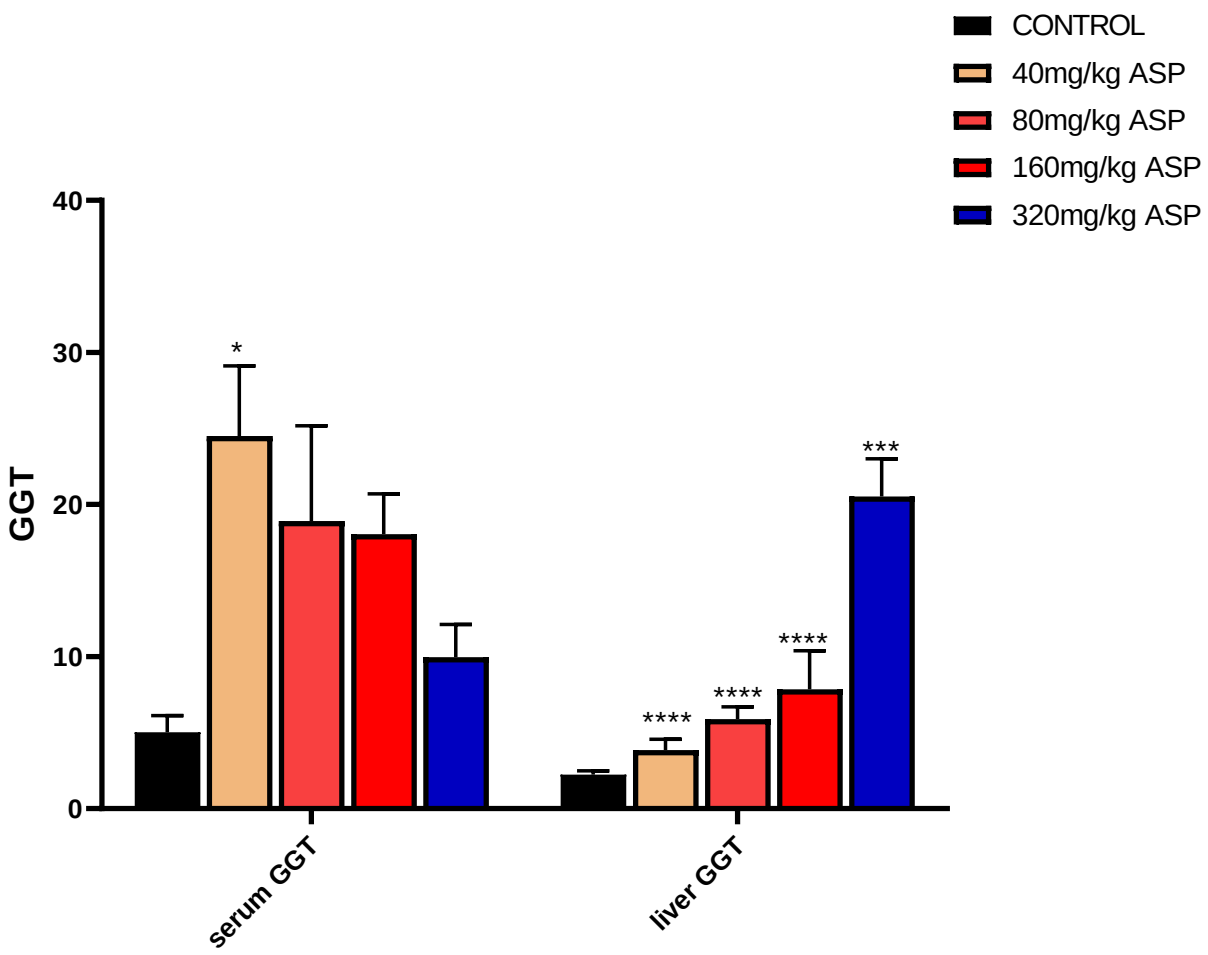
Effect of Aspartame on Aspartate Transaminase(AST) Activities in the serum and Liver of male Sprague Dawley rats. Data are represented as Mean ± SEM.

Both the serum and liver AST showed no significant difference ($P>0.05$) when control was compared to other groups administered Aspartame , and also when comparison was made within the groups.



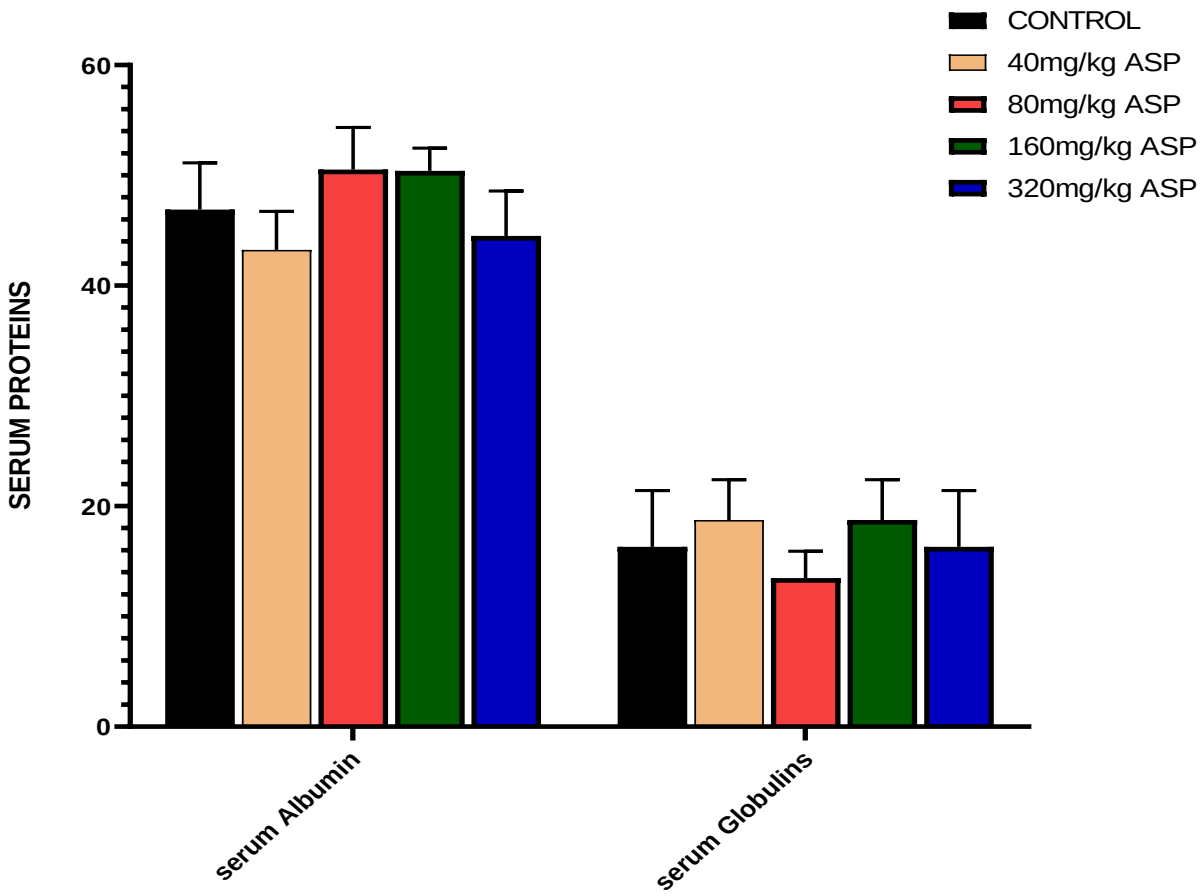
Effect of Aspartame on Serum and Liver Alkaline Phosphatase(ALP) Levels of male Sprague Dawley rats. Data are represented as Mean ±SEM.

A dose dependent slight decrease in the serum ALP was observed among the aspartame fed groups. However, the liver ALP showed no significant difference ($P > 0.05$) between the control and aspartame fed groups.



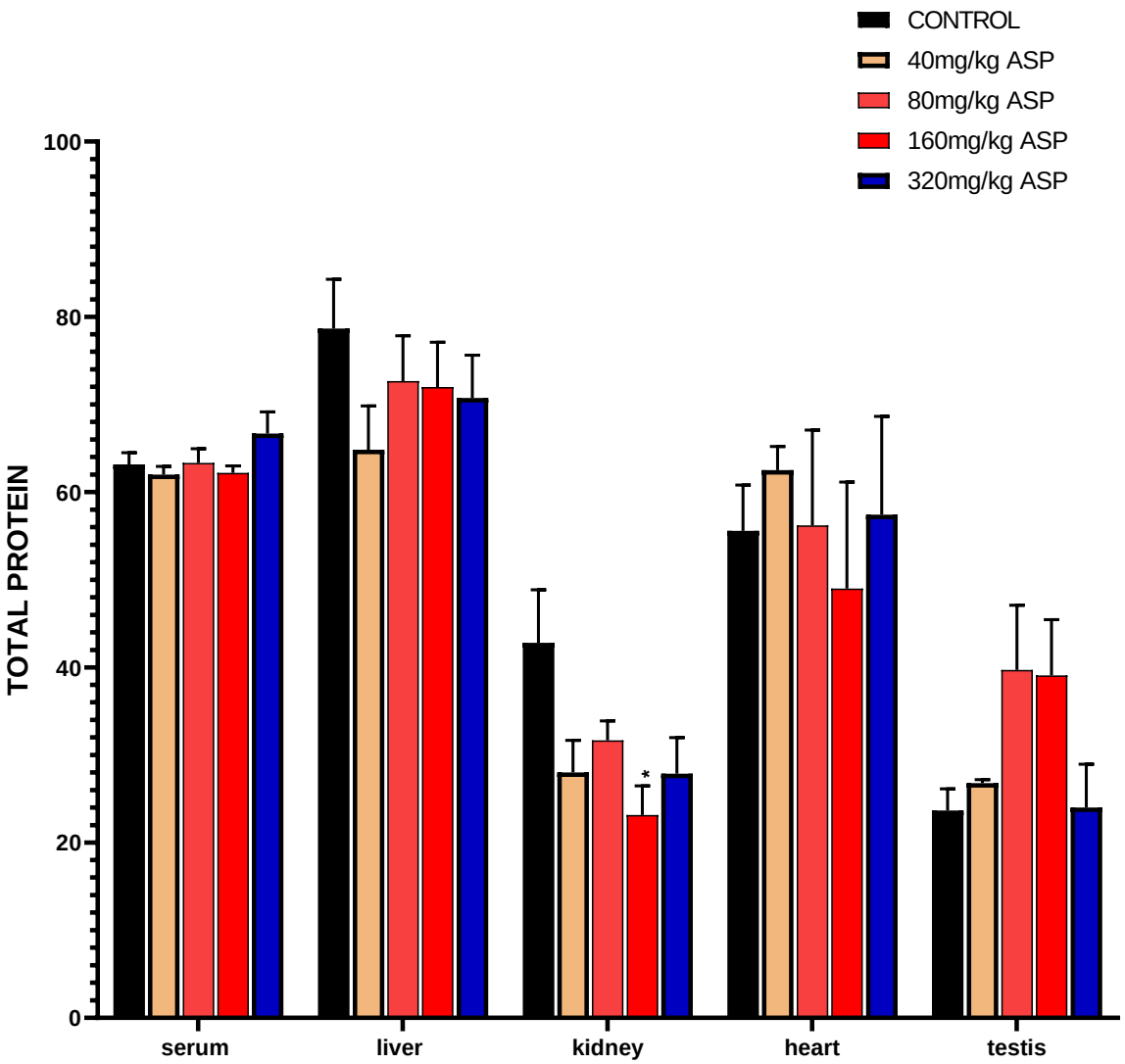
Effect of Aspartame on Serum and Liver Gamma-Glutamyl Transferase (GGT)) Levels of male Sprague Dawley rats. Data are represented as Mean ± SEM.

In the serum, there was a significant ($P<0.05$) increase when the control was compared with the aspartame-fed groups. However, it was observed that the GGT level decreases as the concentration of aspartame increases. Comparison between the Control and aspartame fed groups showed a dose dependent increase in liver GGT. There was also significant difference ($P < 0.05$) when comparison was made within groups.



Effect of Aspartame on Serum Albumin and Globulin Levels of male Sprague Dawley rats. Data represented as Mean ±SEM.

Effect of aspartame on serum albumin showed no significant difference ($P > 0.05$) when control was compared to the aspartame fed groups. Also, the serum globulin was also not significantly different ($P > 0.05$) when control was compared to other groups.



Effect of Aspartame on Total Protein Levels of male Sprague Dawley rats. Data represented as Mean ±SEM.

There was no significant difference ($P > 0.05$) between the serum proteins of the control and the aspartame fed groups for all the tissues However, the kidney proteins decreased in the aspartame fed groups when compared to the control.

CHAPTER FIVE

5.0 DISCUSSION

Alanine aminotransferase (ALT) - an enzyme found inside liver cells helps the liver to break down proteins for easy absorption.

A significant increase in ALT enzyme has been reported to indicate a degree of damage done to the liver, as inflamed or damaged liver releases ALT into the bloodstream causing plasma ALT levels to rise (Peltz-Sinvani, 2016; Trocho *et al.,* 2008; Ashok *et al.,* 2015). Result from this study showed a dose-dependent increase in serum ALT concentration between the control and the groups administered aspartame, thereby suggesting some degree of damage to the liver.

Aspartate Transaminase (AST) is another liver enzyme but it is not specific to the liver. A high AST level is a sign of liver damage, though it can also mean damage to other organs that produce it like the heart or kidneys (Ozer et al., 2008). This study shows that there was no significant difference between the means of the serum AST of the control group and those of the experimental groups, same applies to the liver AST levels. This however does not agree with the study by Finamor *et al*., (2021), who reported that aspartame caused liver injury by increasing ALT and AST levels in the serum of aspartame-treated mice as compared to control mice.

Although high levels of ALP may indicate liver disease or certain bone disorders and mildly elevated levels can be caused by many different factors other than a diseased condition (Sharma, Pal and Prasad, 2014), this study showed a non-significant slight decrease in the serum ALP as the concentration of aspartame increases. It is worthy of note that ALP test alone cannot be used to estimate a liver injury. This however was in contradiction with study by AbdElwahab *et al.,*

(2017) that reported a marked increase in ALP level in the liver, thus suggesting liver dysfunction caused by administration of aspartame. This contradiction could be as a result in the difference of the length of administration in both experiments.

Gamma- glutamyl Transferase (GGT) activity in the serum is a marker of hepatobiliary injury, as high levels of GGT in the blood has been linked to liver disease or damage to the bile ducts (Ramaiah, 2007). Result from this present study showed elevated levels of serum and liver GGT in the aspartame fed rats compared to the control group, indicating a liver injury. This agrees with the findings by Abhilash *et al*., (2011) that observed similar increase in the level of serum and liver GGT in rats treated with aspartame.

The serum protein, heart protein and liver protein results between all groups in this study, showed no significant difference. However, a significant decrease was observed in the kidney proteins of the aspartame treated rats, especially in the group that received 160mg/kg. This decrease could be due to the presence of hydrogen peroxide (a byproduct of aspartame's metabolism) in the kidney (Halliwell *et al*., 2002) Hydrogen peroxide has been implicated in protein denaturation and subsequent enzymatic alterations (Ashok *et al*., 2015).

Assessing serum albumin levels is considered a test of liver function owing to the fact that hepatic albumin synthesis tends to decrease in end-stage liver disease (Giannini *et al.,* 2005). In this study, the levels of serum albumin and globulin in the aspartame-administered groups were not significantlydifferent from that of the control group. This is in agreement with Abdel *et al*., (2012), who experimented on 10, 50 and 100mg/kg of aspartame and observed no significant difference between aspartame treated rats when compared with control.

CONCLUSION

Aspartame may act as a chemical stressor by altering organ function homeostasis and increasing protein oxidative damage. This study has shown that the consumption of aspartame could result in liver injury and so, the intake of aspartame should be cautioned.

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INDEX

Table 1: Serum Biochemical Parameters

	CONTORL	40mg ASP	80mg ASP	160mg ASP	320mg ASP
SERUM ALT	18±2.098	29.53±5.195	70.5±7.805	88.56±19.54	88.46±19.54
SERUM AST	8.067±0.4432	7.667±0.349	7.133±0.5789	7.6±0.4561	8.067±0.6401
SERUM ALP	20.77±2.858	18.41±3.601	17.51±4.078	14.13±1.803	10.1±1.766
SERUM GGT	5.032±1.094	24.51±4.596	18.91±6.27	18.05±2.653	9.953±2.162
SERUM PROTEIN	63.17±1.327	62±0.9309	63.33±1.606	62.2±0.8	66.67±2.472
SERUM ALBUMIN	46.9±4.245	43.25±3.491	50.53±3.811	50.42±2.057	44.52±4.065
SERUM GLOBULINS	16.3±5.115	18.75±3.652	13.47±2.431	11.78±1.826	22.16±5.241

ASP = Aspartame

Table 2: Tissue Biochemical Parameters

L IVER ALT	102.2±0.8632	97.97±1.775	96.03±6.92	100.4±1.044	98.74±1.917
LIVER AST	89.5±4.334	92.25±10.48	81.25±7.91	88±7.427	84.2±7.671
LIVER ALP	80.53±4.522	84.87±4.522	77.87±8.258	65.96±7.315	66.03±8.253
LIVER GGT	2.24±0.257	3.86±0.7016	5.897±0.8085	7.85±2.527	20.54±2.469
LIVER PROTEIN	78.67±5.608	64.83±5.009	72.67±5.181	72±5.092	70.75±4.888
KIDNEY PROTEIN	42.8±6.037	28±3.658	31.67±2.216	23.17±3.321	27.86±4.137
HEART PROTEIN	55.58±5.226	62.5±2.693	56.2±10.88	49±12.14	57.43±11.21
TESTIS PROTEIN	23.67±2.472	26.8±0.3742	39.7±7.385	39.08±6.346	24±4.946
L IVER ALT	102.2±0.8632	97.97±1.775	96.03±6.92	100.4±1.044	98.74±1.917
LIVER AST	89.5±4.334	92.25±10.48	81.25±7.91	88±7.427	84.2±7.671