International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Therapeutic Assessment of Aqueous Leaf Extract of Sterculia Tragacanta (Bitter Tree) on Biochemical Parameters and Histology of the Liver of Sprague Dawley Rats

Ikpan Oyama Ogbe[1], Eyo Emem Efeffiom[2], Usang Akedor Ibor[3], Ogar Rose[4], Sunday Julius Iyoma[5]

Abstract: ***Medicinal plants when consumed have been known to cause various degrees of toxicity to the liver. Sterculia tragacantha has been used for therapeutic remedies for the treatment of disease such as diabetics, diarrhoea, dysentery, arthritis, witlow and jaundice. The aim of this study was on the assessment of aqueous leaf extract of Sterculia tragacantha (bitter tree) on biochemical parameters and histology of the liver of Sprague dawley rats. Twenty nine Sprague dawley rats weighing 100-136g were randomly divided into four groups of five animals per group and administered orally for fourteen days. Group I: Normal controls were given food and distilled water, Group II: Low dose were given 44.72mg/kg/day, medium dose were given 89.44mg/kg/day and high dose given 134.16mg/kg/day. The animals were sacrificed by chloroform inhalation method, blood samples were obtained by cardiac puncture into plain specimen containers for analysis of liver function. The liver tissue were removed and fixed in 10% neutral buffered formalin, grossed and histologically processed, microtomed and stained with Hematoxylin & Eosin and Periodic Acid Schiff (PAS) staining technique respectively. Results from this study showed that the LD50 was 447.21kg/body weight, no significant differences between the test and control result of the serum levels of the liver function parameters (total bilirubin, aspartate transaminase, alanine transaminase and alkaline phosphatase) although, all are statistically insignificant. Histologically, with Hematoxylin and Eosin stain, medium and high dose of S. tragacantha extract might cause mild inflammatory cell to the portal triad area. While on periodic acid Schiff stain, S. tragacantha extract showed no morphological changes on the glycogen of the liver. Hence, from this findings administration of S. tragacantha was found to be safe at low dose and do not cause significant changes in the liver function test parameters on the liver histology and glycogen deposits. It is recommended that a low dose of S. tragacantha is safe for twenty nine animals for study use.***

Keywords: Aqueous Leaf Extract, Sterculia tragacanta, Bitter Tree, Liver, Sprague Dawley Rats

1. Introduction

1.1 Background of the Study

Medicinal plant have gained populace since ancient times (Asadbeigi, Mohammadi, Rafieian-Kopaei, Saki, Bahmani 2014). One of such medicinal plant is *Strerculia tragacantha*. *Sterculia tragacantha* is of the family, *sterculiaceae*, which is widespread in the savannah woodlands often characterized by stony hills. *Sterculia*, belongs to the largest *Malvaceae* genera, comprises about 300 species. It is widespread in Nigeria and called by different tribes with different names. For instance, it is called “Kukuuki” in Hausa, “Kokongiga”in Nupe “Boboli” in Fuljida, Ufia” in Igala and “Idot-eto”in Efik and Ibibio (Igolie, Ogaji, Igoli, 2005). This plant has a wide application as a traditional remedy to various ailments in Nigeria. For instance, theYorubas use its burnt sterm bark in the preparation of black soap (Rita, Chinaka and Sunday 2014). The Igedes also employ the sterm bark decoction to treat diarrhoea (Igolie *et al.*, 2003). Several tribes in north central Nigeria use the macerated bark from the plant for the treatment of dysentery (Igolie, Igwe and Igoli 2003).). *Sterculia tragacantha* leaves for years have been used by traditional healers in Eastern Nigeria in the treatment of arthritis, edema, gout, withlow and cold (Rita *et al.*, 2014). The sweet and cold herb of *S. species* has been used to relieve pain, smooth the muscle and blood vessels constriction, treat inflammation of mucous and promote intestine movement, as it clears lung and intestines and stimulates lung dispersing function by enhancing the functions of lung and large intestine (Barwick, 2004) *Strerculia tragacantha leaves*, barks and seeds are used by local African healers to alleviate diarrhoea, arthritis, oedma, withlow and cold. People of Nsukka area in the South East part of Nigeria, use the plant to support Orthopaedic patients for pain relieve after bone fracture (Udegbunam, Kene, Asuzu, Oyiga, Udegbunam and Nwaehujor, 2013).

Strerculia species are rich in alkaloids, saponins, flavoniod and glycosides, which show a wide array of biological activities such as antimicrobial, antifungi, insecticidal, cytotoxic, antioxidant and anti inflammatory activities (El-Sherei, Ragheb, Kassaem, Marzouk, Mosharrafa *et al.*, 2016). *Sterculia tragacantha* is sometimes a deciduous shrub growing 5-12 metres tall, but more often it becomes a tree up to 25 metres tall with exceptional specimen to 40 metres. The crown tends to be fairly small and sparely branched (Barwick, 2004). The tree is valued especially for the gum obtained from its trunk, which has a range of applications. The young leaves are sometimes an important local food (Enoch, Achigan-Dako, Magaret, Pasquini, 2009).

The liver is the largest internal organ of the human body, weighing approximately 1.5kg. Embryogically, it develops from the foregut and it spans the upper right and part of left abdominal quadrants. Anatomically, the liver consist of four lobes: Two larger ones (right and left) and two smaller ones (quadrate and caudate) (Andrian, 2020). Histologically, it has a complex microscopic structure that can be viewed from several different angles and components, namely; the parenchyma, which is represented by the hepatocytes. The stroma, which is a continuation of the surrounding capsule of Glisson. It constist of connective tissues and contains the

vessels. The capsule is also covered by a layer of mesothelia, arising from the peritoneum covering the liver. The connective tissue of the stroma is type III Collogen (reticulin), which forms a meshwork that provides integrity for the hepatocytes and sinusoids. The Sinusoids, which are capillaries travelling between hepatocytes. While the Spaces of Disse (perisinusoidal spaces), which are located between the hepatocytes and the sinusoids. (Andrian, 2020).

The liver receives its blood supply from two major sources namely: the hepatic artery which delivers oxygenated blood from the general circulation, the second being the hepatic portal vein delivers deoxygenated blood from the small intestine containing nutrient. However, the blood flows through the liver tissue to the hepatic cells, where many metabolic functions take place. (Andrian, 2020). The blood drains out of the liver via the hepatic vein, also the liver tissue is not vascularised with a capillary network as with most other organs but consist of blood filled sinusoids surrounding the hepatic cells. (Schmidt-Arras, Rose-John, 2016).

The kupffer cells are the resident macrophages of the liver, it comprises of the largest population of resident tissue macrophages in the body, this was first postulated by Karl Wilhelin Von Kupffer in 1876 as "Sternzellen" (star cells or stellate cells). Kupffer cells were first thought to be a part of the endothelium of the liver blood vessels. It was not until 1898 that Tadeusz Browlecz correctly identified them as macrophages (Wan, Benkdane, Alons, Lotersztajn, 2014). Kupffer cells plays a critical role in the innate immune response, their localization in the hepatic sinusoid allows them to efficiently phagocytise pathogens entering from the portal or arterial circulation. Kupffer cells also serve as a first line of defence against particulates and immune reactive material passing from the gastrointestinal tract via the portal circulation and may be considered as a final component in gut derived function. (Andrian, 2020). Kupffer cells thus play a major anti-inflammatory role by preventing the movement of these gut-derived immune reactive substances from travelling past the hepatic sinusoid. Kupffer cells are also highly poised for clearance of particle as well as dead and dying erythrocytes and cells in the hepatic parenchymal, from the systemic circulation. Kupffer cells thus comprised the major phagocytic activity of what was classical termed the reticular-endothelial system and now more properly called the mononuclear phagocytic system (Wan *et al.*, 2014).
However, kupffer cells as well as other cells of the innate immune system, including natural killer, natural killer-T cells and dendritic cells, reside within the sinusoid. The close proximity of kupffer cells to parenchymal and non-parenchymal within the liver support the ability of kupffer cells to regulate hepatic function, both in health and disease. In a healthy liver, the kupffer cell exhibit what has been termed "tolerogenic" phenotype. This tolerance is necessary to prevent underestimated immune response in the face of incoming immune material into the hepatic sinusoid, including gut-derived materials also antigen present on dead or dying cells as they are cleared from the circulations. (Bleriot, Dupuis, Jouvion, Eberl, Disson, (2015).

Moreover, more than five hundred vital function have been identified in the liver (Andrian, 2020). The liver regulates most chemical level in the blood and excretes a product called bile. This helps carry away waste product from the liver, all the blood leaving the stomach and the intestine passes through the liver. The liver processes this blood and breaks down, balances and create the nutrient and also metabolises drugs into form that are easier to use for the rest of the body or that are nontoxic. (Sato, Nakashima, Nishiyama, Kinoshita, 2014). These however, could be described based on the following functions: Synthesis; the liver hepatocyte produces bile which helps in carrying away of waste and break down fat in the small intestine during digestion, the liver is the site where bulk of lipoproteins are synthesised, it is the major site for converting excess carbohydrate and proteins into fatty acid and triglyceride, which are then exported and stored in adipose tissue, also aid in the synthesis of large quantities of cholesterol and phospholipid. (Moore 2018). However, the liver hepatocytes also produces proteins such as plasma albumins, lipoproteins, glycoproteins, prothrombin, fibrinogen and production of cholesterol and transport protein to help carry fat through the body. Hence, the liver aid in the regulation of blood clothing. (Allen, Gurrin 2008). However, the liver synthesises and stores around 100g of glycogen via glycogenesis, the formation of glycogen from glucose. When needed, the liver releases glucose into the blood performing glycogenolysis, the breakdown into glucose (Chikwana, Khanna, Baskaran, 2013). The liver is also responsible for gluconeogenesis, which is the synthesis of glucose from certain amino acids, lactate or glycerol. Adipose and liver cells produce glycerol by breakdown of fat, which the liver used for gluconeogenesis. (Jensen, Rustard, Kolnes and Lai, 2011).

Based on metabolism, deamination and detoxification; the liver helps in the conversion of excess glucose into glycogen for storage (glycogen can later be converted back to glucose for energy) and to balance, and make glucose as needed. (Jensen *et al*., 2011). One of its functions is that, it regulate blood level of amino acids which form the building blocks of proteins. The liver converts poisonous ammonia to urea (urea is an end product of protein metabolism and is excreted in urine. In storage, liver stores and modifies vitamins such as Vit. A, D and K. (Bowen, Warren, Davis, 2002).

In glycogen metabolism in the liver; a meal containing carbohydrate or protein is eaten and digested, blood glucose levels rise, and the pancreas secrete insulin. Blood glucose from the portal vein enters liver cells. (hepatocytes). Insulin acts on the hapatocytes to stimulate the reaction of several enzymes, including glycogen synthase. Glucose molecules are added to the chains of glycogen as long as both insulin and glucose remain plentiful. (Chikwana *et al*., 2013). In this postprandial or fed state, the liver takes in more glucose from the blood than it releases.

After a meal has been digested and glucose levels begin to fall, insulin secretion is reduced, and glycogen synthesis stops. When it is needed for energy, glycogen is broken down and converted again to glucose. (Jensen *et al*., 2011). Glycogen phosphorylase is the primary enzyme of glycogen

breakdown. For next 8-12 hours, glucose derives from liver glycogen is the primary sources of blood glucose used by the rest of the body for fuel (Giorgis, Veggiotti, 2013).

Glucagon, another hormone produced by the pancreas, in many respects serves as a countersignal to insulin. in response to insulin level being below the normal (when blood levels of glucose begin to fall below the normal range), glucagon is secreted in increasing amounts and stimulates both glycogenolysis (the breakdown of glycogen) and gluconeogenesis (the production of glucose from other sources) (Jensen *et al*., 2011).

1.2 Statement of the problem

It is worthy of note that, the ingestion and use of most medicinal plant as therapeutic agents ha cause change (toxicity) on the liver of people. It is on this back drop that, the effect of the use if *Sterculia tragacentha* as therapeutic agent will be checked on the biochemical parameters and histological parameters and histology of the liver of *sparague dawley* rats.

Sterculia species has received a lot of attention due to its claims of potentials health benefit in the treatment of various ailments. Although, *S. tragacantha* has been used over the years by traditional heaters for the treatment of arthritis, oedema, dysentery, gout without and cold.

However, little or no study has been done on the histological and biochemical effects of agueous leaf extract of *sterculia* on the liver of *sparague dawley* rats.

1.3 Aim and objectives of the study

The aim of this study is on the therapeutic assessment of aqueous crude leaf extract of *Sterculia tragacantha* (Bitter Tree) on biochemical parameters and histology of the liver of Sprague dawley rats.

Objectives of the study:
The objectives are to:
1) Determine mean lethal dose (LD50) of the plant extract.
2) Measure liver function test (LFT) parameters after administration of aqueous crude leaf extract of *S. tragacantha*.
3) Evaluate the histological effect of the extract on the liver using haematoxylin and eosin (H & E) stain.
4) Ascertain the effect of the extract on liver glycogen using periodic acid schiff (PAS) stain.
5) Determine the effect of the extract on collagen fiber using van Gieson method.

1.4 Justification of the study

This study intends to reveal the biochemical parameters and histological effects following the administration of aqueous leaf extract of *Sterculia tragacantha* on the liver of *Sprague dawley* rat. Firstly, the study will determine how safe the extract could be in the histological change of the extract when administered for different purposes; secondly, the study is to determine the dose that will give the least toxic effect on clinical chemistry of liver parameter and histological component of the cell, also, this study is to determine if there be any effect on glucose metabolism, as well as if found to be safe, it will help in ameliorating health challenges.

1.5 Scope of the study

The study is limited to the determination of LD50 and measurement of clinical chemistry liver tests such as: Total bilirubin, conjugated bilirubin, aspartate transaminase, alanine transaminanse and alkaline phosphatase.

The histological tissue processing with paraffin wax embedding method will be limited to light microscopic study after staining with, haematoxylin and eosin, periodic acid Schiff and van gieson stains.

2. Materials and Method

2.1 Plant material and preparation of crude leaf extract

Sterculia tragacantha leaves were obtained from Nto-Otong Village, Abak, Akwa Ibom State. It was Identified and authenticated in Department of Plant and Ecological Studies, University of Calabar, Calabar, with herbariun number (BOT/HERB/UCC/050).

2.2 Preparation of crude plant extract

The leaves were air-dried, pulverized into powder, weighed and macerated in distilled water. After 72 hours, it was filtered and concentrated to dryness in a water bath at 45^{O}C. It was preserved at 4^{O}C until required for use.

2.3 Experimental animal

Twenty nine Albino rats weighing 100-137g were used. The Albino rats were purchased and kept in the Animal house of the Department of Pharmacology and Toxicology, University of Uyo, to acclimatise for two weeks. They were housed in standard cages and provided with food and water ad libitum.

2.4 Ethical approval

The ethical approval was gotten from Animal and Research ethics committee of faculty of basic medical sciences, University of Calabar.

2.5 Lethal testing (LD50)

The LD50 for the *S. tragacantha* leave extract was performed using Locke's method, prior to the commencement of the experiment:

The median lethal dose (LD50) of *S. tragacantha* aqueous leaf extract was estimated using thirty-two (32) *Sprague Dawley* rats. Lorke method (1983) was employed. The rats were obtained and maintained under standard environmental conditions and had free access to feed and water ad libitum. They were acclimatized for 14 days in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy before the commencement of research, and

handled as approved by the Animal Ethics Committee, University of Calabar.

However, the research was carried out in Phases.

In phase one, twelve (12) Sprague Dawley rats were fasted for 18 hours, thereafter they were weighed and grouped into three (3) groups of four (4) rats each weighing 150-180g. Each group received an arbitrary dosage of 1, 000, 3, 000 and 5, 000mg/kg of *S. tragacantha* leaf extract intra-peritoneally (i. p) respectively and were observed for physical signs of toxicity and mortality within 24 hours.100% mortality was recorded within 24 hours. Based on the value of phase one, phase two was conducted.

In phase two, twenty (20) *Sprague Dawley* rats were equally fasted for 18 hours. Thereafter, they were weighed and grouped into five (5) groups of four (4) rats each weighing 150-180g. Each group of four received 100, 200, 300, 400 and 500mg/kg of *S. tragacantha* leaf extract intraperitoneally (i. p.) respectively and were equally observed for physical signs of toxicity and mortality within 24 hours. Groups administered with 100, 200, 300 and 400mg/kg of extract recorded 0% mortality, while group administered with 500mg/kg recorded 100% mortality within 24 hours. Based on these records, LD50 was calculated as follows:

That is LD50 =
Where a = maximum dose producing 0% mortality= 400mg/kg
b = minimum dose producing 100% mortality= 500mg/kg
Hence, LD50 = √400 X 500 = √200, 000 = 447.21mg/kg
Based on this result 10%, 20% and 30% of the LD50 were calculated for this research work.
That is: Low dose (LD)----------10% of LD50 = 44.72mg/kg bw.
Medium dose (MD)-------20 % of LD50 = 89.44mg/kg bw.
High dose (LD)----------30% of LD50 = 134.16mg/kg bw.

2.6 Experimental design

The albino rats were randomly divided into 4 groups of 5 animals per group based on diet, orally for 14days.
Control: Given distilled water with feed only.
Low Dose: 44.72mg/kg body weight.
Medium Dose: 89.44mg/kg body weight.
High Dose: 134.16mg/kg body weight.

2.7 Termination of experiment

The animals were sacrificed by chloroform inhalation method. Blood specimens were obtained by cardiac puncture into plain sample containers. The liver tissues were removed and fixed in 10% neutral buffered formalin, grossed and, Histologically, processed (dehydration, clearing and paraffin wax embedding) by paraffin wax embedding technique and then microtomed with rotary microtome (Leica). The staining was done with haematoxylin and eosin, periodic acid schiff (PAS) and van gieson technique. Moreover, mounting was done with Dibutylpthalate Xylene (DPX). Followed by microscopy and photomicrography. Data analysis was done using Student t-test (SPSS Version 20). P-Value ≤ 0.05 was statistically significant.

3. Results

3.1 Mean lethal dose (LD50)

The LD50 value of the aqueous crude leaf extract of *S. tragacantha* was 447.2mg/kg of the extract. Base on this LD50 of 447.21mg/kg the low dose (10% of LD50) was 44.72mg/kg the medium dose (20% of LD50) 89.44mg/kg, while the high dose (30% of LD50) was 134.16mg/kg (Table 1).

3.2 Clinical Chemistry Results

The serum level of total bilirubin at negative control was 2.20±0.17, Low dose was 3.13±1.10, medium dose 3.00±0.40 and high dose being 1.93±0.47, which was statistically non significant. However, the P-value was 2.568 and F=0.127 respectively. The serum levels of conjugated bilirubin at negative control was 1.50±0.26, low dose was 1.27±0.38, medium dose 1.37±0.30 and high dose 1.27±0.15, which was statistically non significant. The p-value was 0.728 and F=0.444. Serum levels of aspartate transaminase at negative control was 113.67±2.52, low dose was 136.33±26.69, medium dose was 130.67±15.50 and high dose 134.00±22.61, which was statistically non significant with p-value 0.500 and F=0.860. Moreso, the serum levels of alanine transaminase at negative control was 83.00±5.57, low dose was 83.33±19.55, medium dose was 100.67±13.65 and high dose being 77.00±18.08, which was statistically non significant with a p-value of 0.326 and F=1.348 respectively (Table 2)

Table 2: Clinical chemistry result

Test	Neg. cont.	LD	MD	HD	F	P. value	Comments
TB	2.20±**0.17**	3.13±**1.10**	3.00±**0.40**	**1.93±0.47**	2.568	0.127	NS
CB	1.50±**0.26**	1.27±**0.38**	1.37±**0.30**	**1.27±0.15**	0.444	0.728	NS
AST	113.67±**2.52**	136.33±**26.69**	130.67±**15.50**	**134.00±22.61**	0.860	0.500	NS
ALT	83.00± 5.57	83.33±**19.55**	100.67±**13.65**	**77.00±18.08**	1.348	0.326	NS
ALP	524.33±185.58	654.33±**138.35**	569.67±**174.25**	**611.67±241.06**	0.263	0.850	NS

3.3 Photomicrographs description

Plate 1:
Photomicrograph of control liver showing the central vein and portal traid areas.

Plate1A is liver with normal central vein (V) hepatocytes (H) and sinusoid (S) Plate1B is liver showing partial tract with normal hepatic portal vein (P), hepatic artery (A) and bile canaliculi (B) heamatoxylin and eosin x100 magnification.

Plate 2:
Photomicrograph of low dose showing the central vein and portal tract areas:
Plate2A is liver with normal central vein (V) hepatocyte (H) and sinusoid (S) heamatoxylin and eosin x 100 magnification
Plate2B: Liver is showing portal tract with hepatic portal vein (P) hepatic artery (A) and bile canaliculi (B) heamatoxylin and eosin x100 magnification.

Plate 3:
Photomicrograph of medium dose showing normal central vein and portal vein (P) areas. Plate3 (A) liver with normal central vein filled with blood, scanty leucocytes (1) normal hepatocytes (H) and normal sinusoid (S) heamatoxylin and eosin x100 magnification.
Plate 3 (B) is liver showing portal tract with normal hepatic portal vein (P) hepatic artery (A) and bile canaliculi (B). There is scanty inflammatory cells (I) heamatoxylin and eosin x100 magnification.

Plate 4:
Photomicrograph of high dose showing central vein and enlarge thick-walled hepatic portal vein areas.
Plate4 (A) is liver with normal central vein filled with blood, scanty leucocytes (I), normal sinusoid (S) heamatoxylin and eosin x100 magnification.
Plate4 (B) Is liver showing enlarged thick walled hepatic portal vein (P), normal hepatic artery (A), and bile canaliculi (B). There is a small portal tract (T). heamatoxylin and eosin x100 magnification.

Plate 5
Plates 5: Photomicrograph of control liver showing the central vein. (V) with intact wall of well-stained fine network of collagen fibre (C). Van gieson x100 magnification.

Plate 6
Plates 6: Photomicrograph of middle dose liver showing the central vein. (V) with intact wall of well-stained thick collagen fibre (C). Van gieson x 100 magnification.

Plate 7
Plate 7: Photomicrograph of high dose liver showing two central veins (V) with intact wall of well-stained fine network of collagen fibres (C). The infiltrated leucocytes (L) is surrounded by hyaline (Hy) material and fine collagen fibres (C). Van Gieson x100 magnification.

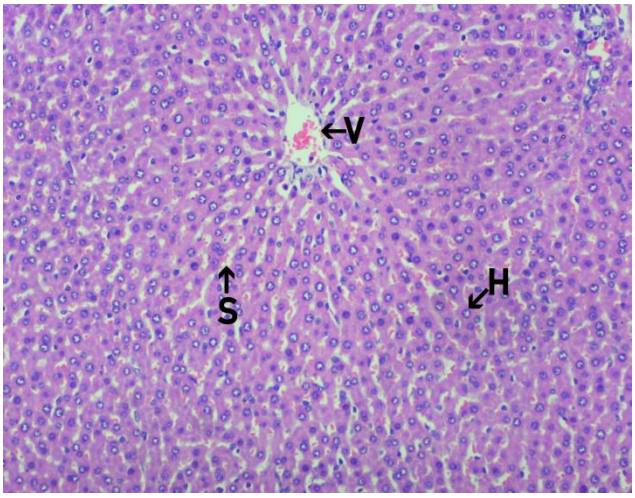
Plate 8
Plates 8: Photomicrograph of control liver showing high staining intensity (HS) of glycogen, two prominent components of the portal traid, portal vein. (P) and bile canaliculi (B). Periodic acid schiff x 100 magnification.

Plate 9
Plates 9: Photomicrograph of liver low dose showing both high staining intensity (HS) and medium staining intensities of the parenchyma. The central vein (V) is shown. Periodic acid schiff x 100 magnification.

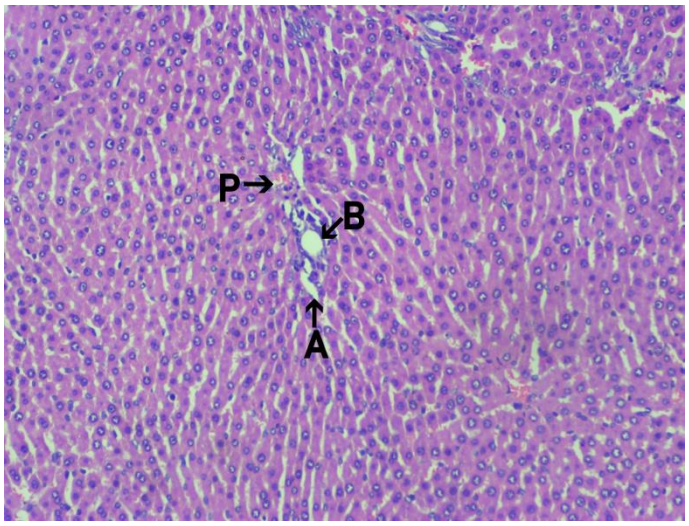
Plate 10:
Photomicrograph of low dose showing both high and medium starring intensity.
Plate 5 (A) is liver with both high and medium staining intensities of the central vein (V) is shown periodic acid schiff x100 magnification.

Plate 11
Photomicrograph of medium dose showing high and low staining intensitity (HS & LS) of the central vein (V) is shown. Periodic acid x100 magnification

Plate 6 (B) Photomicrograph of high dose showing medium staining (MS) intensity of the central vein (V) is shown. Periodic acid is schiff x100 magnification.



A: Central vein area



B: Portal tract area

Plate 1: Photomicrograph of control liver showing the central vein and portal triad areas. Plate 1A is liver with normal central vein (V), hepatocyte (H) and sinusoid (S). **Plate 1B is l**iver showing portal tract with normal hepatic portal vein (P), hepatic artery (A) and bile canaliculi (B). Haematoxylin and eosin x100 magnification.

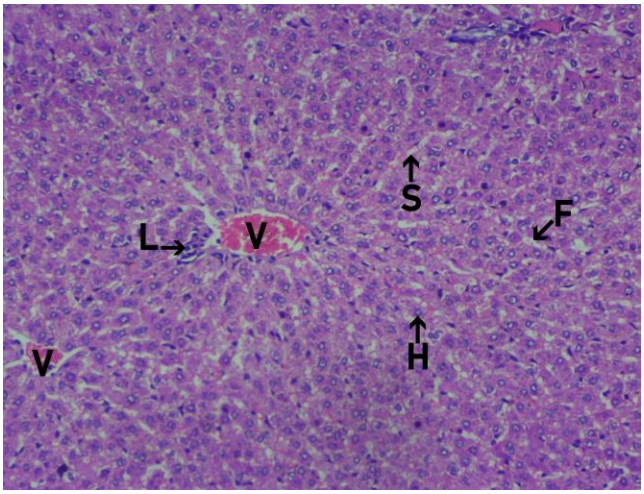


Plate 2A: Central vein

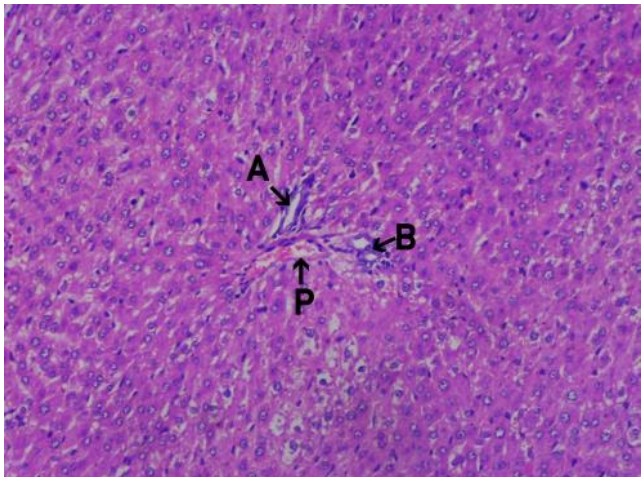


Plate 2B: Portal tract

Plate 2: Phtomicrograph of low dose showing the central vein and portal tract area. Plat (2A) is liver with normal central vein (V) filled with blood and scanty leucocytes (L), normal hepatocytes (H) mild fatty degeneration and normal sinusoid (S) Plate (2B) is liver with normal hepatic portal vein (P), hepatic artery (A) and bile canaliculi (B). Haematoxyline and eosin x 100 magnifications.

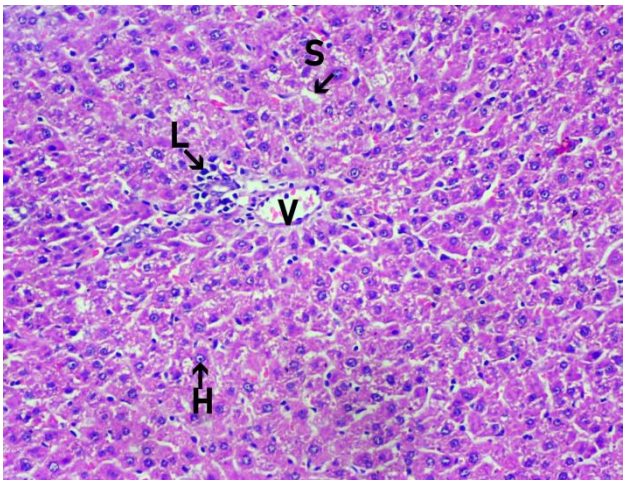


Plate 3A: Central vein

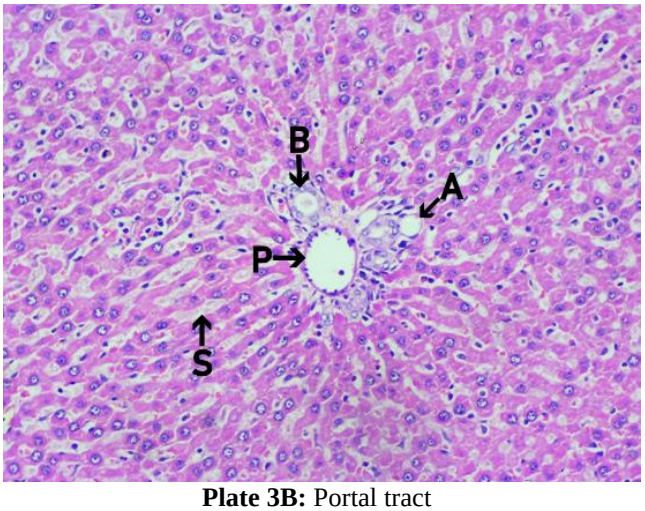


Plate 3B: Portal tract

Plates 3: Photomicrograph of medium dose showing the central vein with portal tract area. Plate (3A) is liver with normal central vein (V) filled with blood scanty leucocytes (L), normal hepatocytes (H), and normal sinusoid (S). Plate (3B) is liver with normal hepatic portal vein (P), hepatic artery (A) and bile canaliculi (B). There is scanty inflammation cells (1). Haematoxylin and eosin x100 magnification

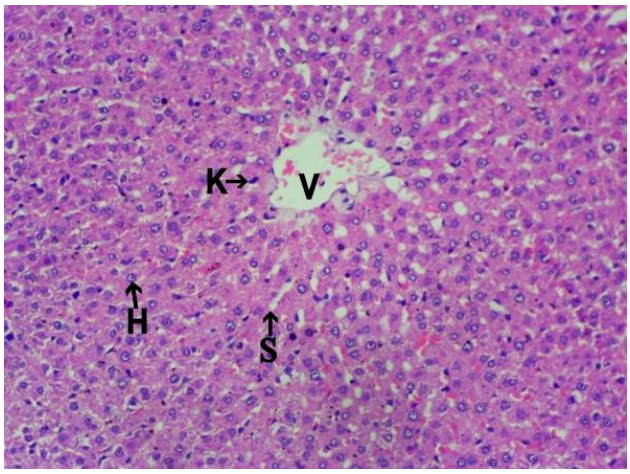


Plate 4A: Central Vein

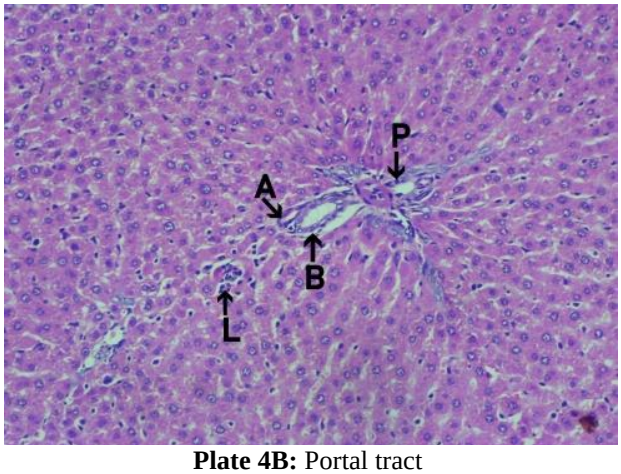


Plate 4B: Portal tract

Plates 4: Photomicrograph of High dose. Showing the central vein and enlarge portal tract area. Plate (4A) is liver with normal central vein (V) filled with blood scanty

leucocytes (L), normal hepatocytes (H). Plate (4B) is liver showing enlarge portal tract with enlarge thick walled hepatic portal vein (P), normal hepatic artery (A), bile canaliculi (B). There is smaller portal tract (T). Haematoxylin and eosin x100 magnification.

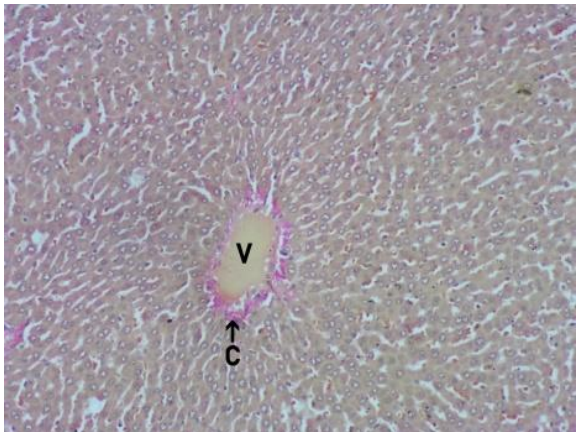


Plate 5

Plates 5: Photomicrograph of control liver showing the central vein. (V) with intact wall of well-stained fine network of collagen fibre (C). Van gieson x100 magnification.

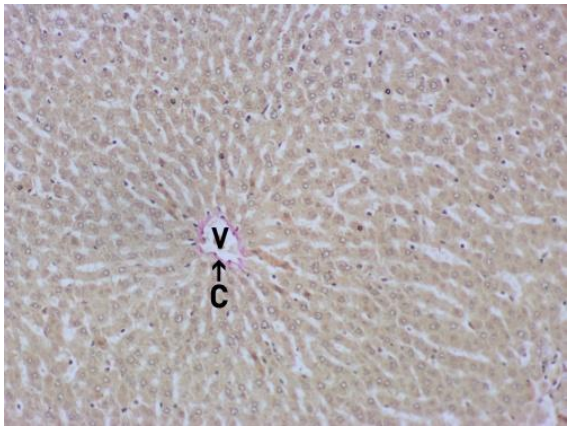


Plate 6

Plates 6: Photomicrograph of control liver of low dose showing the central vein. (V) with intact wall of well-stained fine collagen fibres (C). Van gieson x100 magnification.

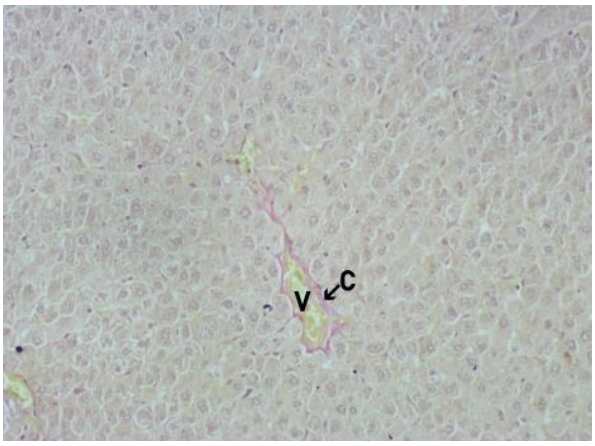


Plate 7

Plates 7: Photomicrograph of middle dose liver showing the central vein. (V) with intact wall of well-stained thick collagen fibre (C). Van gieson x 100 magnification.

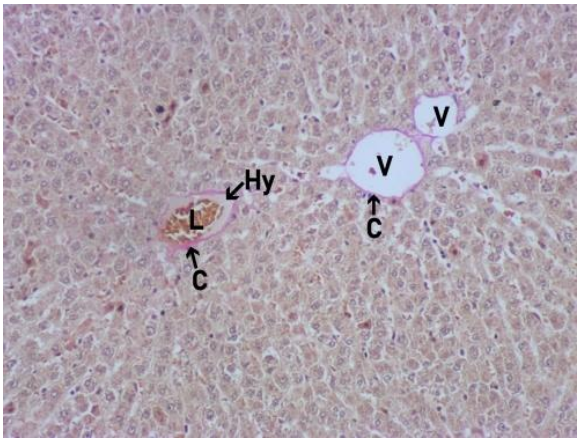


Plate 8

Plate 8: Photomicrograph of high dose liver showing two central veins (V) with intact wall of well-stained fine network of collagen fibres (C). The infiltrated leucocytes (L) is surrounded by hyaline (Hy) material and fine collagen fibres (C). Van Gieson x100 magnification.

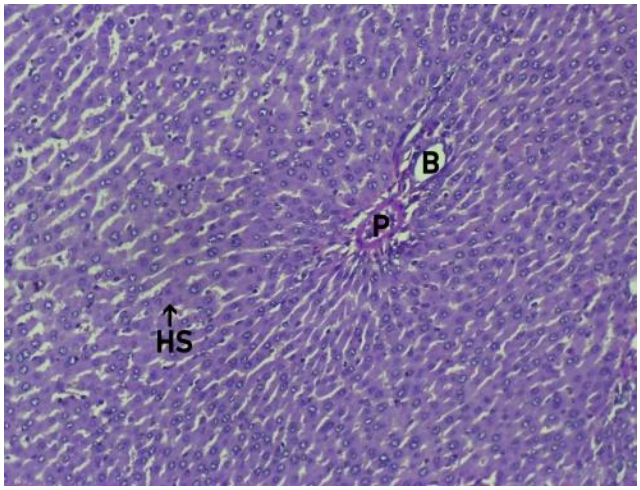


Plate 9

Plates 9: Photomicrograph of control liver showing high staining intensity (HS) of glycogen, two prominent components of the portal traid, portal vein. (P) and bile canaliculi (B). Periodic acid schiff x 100 magnification.

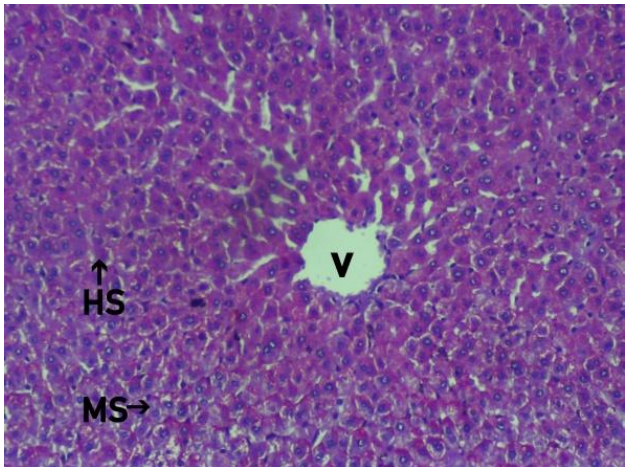


Plate 10

Plates 10: Photomicrograph of liver low dose showing both high staining intensity (HS) and medium staining intensities of the parenchyma. The central vein (V) is shown. Periodic acid schiff x 100 magnification.

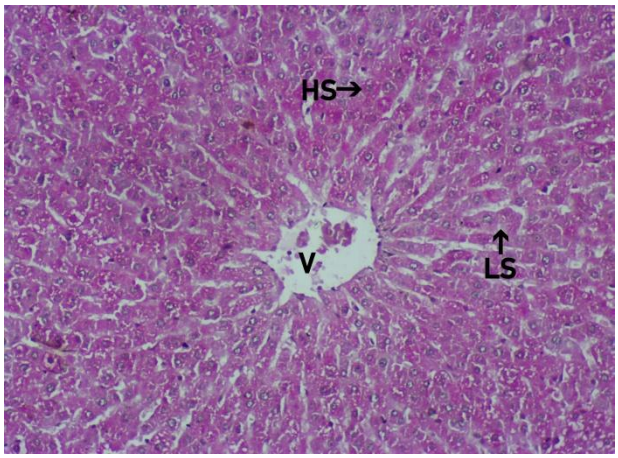


Plate 11

Plate 11: Photomicrograph of liver medium dose showing both high and low staining (HS) and low staining in intensities of the parenchyma. The central vein (v) is shown. Periodic acid schiff.

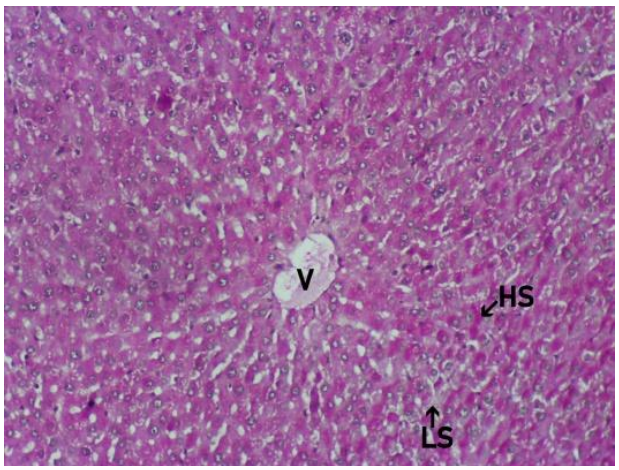


Plate 12

Plate12: Photomicrograph Liver of high dose showing both high intensity (HS) and low staining intensities of the parenchyma. The central vein (V) is shown. Periodic acid Schiff x100 magnification.

4. Discussion, Conclusion And Recommendation

4.1 Discussion

The extract was found to be moderately toxic based on the LD50. The result from this study shows that there is no significant differences between the test and the control result. Hence, no changes seen on the serum levels of the liver function parameters (total bilirubin, conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) were observed. This depict that, the *S. tragacantha* extract doesn't cause any effect on the liver of albino rats. However, this study is in contrast with a study done on the effect of biochemical parameters on *Sterculia setigera* stembark extract of wista rats, which showed low levels of liver enzymes parameters (Moses *et al.*, 2016).

Moreover, the results from this study showed that medium dose and higher dose of *S. tragacantha* extract might cause mild inflamation characterised by mild inlamatory cells to the portal triad area. The presence of t-lymphocytes as a result of the release of chemokines particularly, interleukin-10, TGF-beta and IFN-gammar are thought to be involve in the development of chronic liver disease and T-cells dysfunction. This study is in line with a study on B-cells responses and cytokine production (*Monica et al.*, 2016).

On Periodic Acid Schiff, the presence of flavonoid in the extract, helps in the promotion of glucose storage and utilization in the liver. Hence, no significant changes in glycogen storage, this is in line with a study on flavonoid and their anti-diabetic effect; cellular mechanisms and effects to improve blood sugar levels (Raghad *et al.*, 2019).

4.2 Conclusion

In this study, low dose of *S. tragacantha* did not affect serum levels of liver enzymes therefore, *S. tragacantha* is safer at low dose and doesn't cause changes in the histological morphology and the biochemical parameters on the liver of albino rats.
Also, in this study, *S. tragacantha* did not cause any morphological changes on the glycogen of the liver of albino rats.

4.3 Recommendations

From the finding of this study, administration of 44.721kg/body weight at low dose is recommended, since no effect was observed. Also, if administered in a long period of time, may have a significant effect on the liver of albino rats.

4.4 Contribution to knowledge

1) In this study, the extract of *S. tragacantha* was found to be moderately toxic based on the LD50.

2) This study has shown that. *S. tragacantha* is safer at low dose.
3) Also, that at high dose of administration, *S. tragacantha* might lead to mild inflammation when administered.
4) In glycogen, the presence of flavonoid in the extract, helps in the promotion of glucose storage and utilization in the liver. Hence, this study showed no significant changes in glycogen storage,
5) The results shown, indicates that the serum levels of total bilirubin, conjugated bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were not altered by the extract of *S. tragacantha*.

References

[1] Achigan-Dako, Margaret, W (2009) Traditional vegetables in Benin. *Darwin Initiative; International Foundation for Science.*
[2] Adjanahun, E. M., Ahyi, R. A., Ake-assi, L., Elewede, J. E., Fadoju, S. O., Gbile, ZO, Goudote E, Johanson CLA, Eita A, Morakunyo O, Ojewole JAO, Olatunji AO, Sofowora E. A. (1991). Traditional Medicine and pharmacopoeia: Contribution to ethnobotanical floristic studies in Western Nigeria. *Organization of African Unity Scientific, Technical and Research Commission, Lagos, Nigeria*: pg no
[3] Adrian Rad (2020). Liver Histology. *Kenhub*
[4] Allen KJ, Gurrin L. C. (2008). *Constant in CC, Iron overlow-related disease in HFE Hereditary Hemochromatosis.* N Engl. J. Med; 358.221-30.
[5] Almagoul AX, Bashir AK, Farouk A, Salih AKM (1985). Antimicrobial activity of certain Sudanese plants used in folkoric medicine: Screening of antibacterial activity (4). *Fitoterapia*, 56 (6): 331-337.
[6] Babalola T., Adelakun E., Wang Y., Shode F (2012). Anti-TB activity of sterculia setigera Del., leaves (sterculiceae). *J Pharmacogen Phytochem*.1: 19-26.
[7] Barwick M. (2004) Tropical and Subtropical Trees-A Worldwide Encyclopedic Guide. *Thames & Hudson, London*.
[8] El-Sherei MM., Ragheb AY., Kassem M., Marzouk M., Mosharrafa S., Saleh N., (2016). Phytochemistry, biological activities and economical uses of the genus Sterculia and the related genera: a review. *Asian Pac J Trop Dis*; 6: 492-501.
[9] Facciola. S. (1998) Cornucopia II. *Kampong Publications, California*. ISBN: 0-9628087-25
[10] Feng D (1942). Sterculia Scaphigera (Pantai-hai or Tungtahai) a Chinese laxative drug. *J Chin Med*.61: 9-11
[11] Galla NR *et al., (*2012). Chemicals, amino acid and fatty acid composition of sterculia urens L. Seed. *Food Hydrocoll* 28: 320-324.
[12] Garba M. H., Sherifat M. L., AbdulMajeed A. O., Hafsa L. M., Awal A. B., Sa'adu, A. A., & Lekene, B. J., (2018). Hepato-protective potentials of *sterculia setigera* stem-bark extract on acetaminophen induced hepatotoxicity in Albino albino rats. *Journal of Medicinal Plants Research*, **12** (29), 557-562.
[13] Global Burden Of Disease (GBD) (2015). Disease and Injury Incidence and Prevalence, Collaborations (8 October 2016). Global, Regional and National Incidence, Prevalence and Years Lived With Disability for 310 Diseases Injuries, 1990-2015; a Systematic Analysis for the Global Burden of Disease Study 2015. *Lancet*.388 (10053): 1545-1602.
[14] Green RM, Flamm S. (2002). *AGA Technique Review on the Evaluation of Liver Chemistry Tests*. *Gastroenterology*: 123: 1367-84 doi: 10.1053/gast.2002.306061.
[15] Histology of the Liver. https: //vivo/colostate. edu/ hbooks/ pathphys/ digestion/ liver/ histology. html*Viva Pathophysiology*
[16] Hossain MM *et al., (*2013). Evaluation of Analgesic Activity of Sterculia Villosa Roxb. (Sterculiaceae) Bark in Swiss Albino Mice. *Dhaka Univ. J Pharm Sci*.12: 125-129.
[17] Huang Y *et al*., (2012). Chinese Medicinal Herbs for Sore Throat. *Cochrane Database Syst Rev*.3: CD004877
[18] Igolie JO, Igwe IC, Igoli NP (2003). Traditional Medicine Practices Among the Igede People of Nigeria part II. *Journal of Herbs, Spices and Medicinal Plants***10** (4): 1-10
[19] Igolie JO, Ogaji OG, To-ayiin, Igoli NP (2005). Traditional Medicine Practices Among the Igede people of Nigeria part II. *African Journal of Traditional and Complimentary alternative medicine***2** (2): 134-152
[20] Katade SR., Misar AV., Mujumdar AM., Phalgune UD., Wakharkar RD., Deshpande NR (2009). CNS Depressant Activity of Ethanol Extract of Sterculia Gutata Seeds in Mice. *Indian J Pharm Sci.7*1: 445-447.
[21] Khalid, Hassan (2016). Hepatoprotective Activity of Ethanolic and Ethyl Acetate Extracts of Sterculia Setige Against Carbon Tetrachloride Induced Hepatoxicity in Albino Rats. *Mediterranean Journal of Biosciences. Mediterr. j. bio.,* **1** (3).
[22] Khan AS (2017). Antipyretic and Analgesic Activity of Some Economically Important Woody Plants. Medically Important Trees. *Cham Switzerland*.159-185
[23] Kriew M, Ahmed A. (2009). *The Asymptomatic Outpatient with Abnormal Liver Function Tests.* din. Liver Dis.8; 13 (2): 167-77.
[24] Lazo M, Selvin E, Clark, JM. (2008) *Clinical Implications of Short Term Variability in Liver Function Test Result.* Ann Intern. Med.148: 348-52.
[25] Manjumdar AM, Naik DG, Waghole RJ., Kulkarni DK., Kumbhojkar MS (2000). Pharmacological Studies on Sterculia Foetida Leaves. *Pharm Biol*.38: 13-17
[26] Moshi MJ *et al*., (2007). Brine Shrimp Toxicity Evaluation Of Some Tanzanian Plants Used Traditionally For The Treatment Of Fungal Infections. *Afr J Tradit Complement Altem Med.4*: 219-225
[27] O' Brien CB. (2009). *The Hospitalized Patient with Abnormal Liver Function Tests.* clin. liver Dis.13 (2): 179-92.
[28] Raja T et al., (2014). Evaluation of Anticonvulsant Effect of Sterculia Foetida (Pinari) in Pentylenetetratrozole (ptz) and Mes Induced Convulsions in Albino Rats. *World J. Pharm Sci* 3: 1898-1907.

[29] Ruffo, C. K.: Birnie, A. & Tengnas, B. (2002) Edible wild plants of Tanzania. Regional Land Management Unit; *Nairobi*. ISBN: 9966-896-60-0
[30] Souso J *et al*., (2012). Gastroprotective Activity of Sterculia Striata A. St. Hil. & Naudin (Malvaceae) in Rodents. *Z. Natuforsch* C.67: 163-171.
[31] Teschke R., Wolff A, Frenzel C, Schulze J, Eickhoff A, (2012). Herbal hepatotoxicity: A tabular compilation of reported cases. Liver International **32** (10): 1543-1556
[32] Tor-Anyiin T el., (2011). Phytochemical and Antimicrobial Studies on Stem Bark Extract of Sterculia Setigera, Dek,, *Afr J Biotechnol*.10: 11011-11015
[33] Udegbunam RI, Asuzu UI, Kene RO, Oyiga CT, Udegbunam SO, and Nwaehujor CC. (2013). Anti-Inflammatory and Antioxidant Effects of Sterculia Tragacantha Fractions in mice. *African Journal of Biotechnology*. Vol **12** (6), pp.592-597.
[34] Udegbunam RI, Asuzu UI, Kene RO, Oyiga CT, Udegbunam SO, and Nwaehujor CC. (2013). Evaluation of Local Aesthetics Efficacy of the Crude Extract of Sterculia Tragacantha Using West African Dwarf Goats. *Sokoto J vet Sci*; 11: 13-21
[35] Wang R-F *et al., (*2013). Two Cerebrosides Isolated from the Seeds of Sterculia Lychnophora and their Neuroprotective Effects. *Molecules.1*8: 1181-1187
[36] Wilkie (2006). Phylogenic Relationships Within the sub Family Sterculiodeae (Malvaceae/sterculiaceae-sterculiaeae) Using the Chloroplast Gene ndhF. *Syst Bot*.31: 160-170
[37] Williams R, Aspinall R, Bellis M (2004). *Addressing Liver Disease in UK: a Blue Print for Attaining Excellence in Health Care and Reducing Premature Mortality From Life Style Issues of Excess Consumption of Alcohol Obesity and Viral Hepatitis Cancet*; 384: 1953-97 doi: 1.0.0106/80140-6736 (14).1838-9. Cross Red. Pub. Med. Google Scholar.

Appendix 1

Procedure of Haematoxyline and eosin staining

1) Clean the section to distilled water
2) Then stain nuclei with alum haematoxylin (Mayer's) to fix the tissue, for about 5 minutes.
3) Rinse the stain with smoothly running tap water.
4) Using the differentiator, 0.3% acid alcohol and note the endpoint i. e the correct endpoint is after bluing up, the background becomes colored.
5) Rinse the stain in smoothly running tap water
6) Rinse the stain in SCOH's tape water substitute which shortens the time for the correct endpoint.
7) Rise with running tap water
8) Flood the smear with eosin for 2 minutes and since eosin is highly soluble in water, use enough quantity of it. The over stained eosin can be removed or washed off with running tap water.
9) Dehydrate the smear, clear and mount using a clean cover slip.

Appendix 2

Procedure for periodic acid schiff (for GLYCOGEN)

1) Deparaffinze and hydrate to water
2) Oxidize in 0.5% periodic acid solution for 5 minutes.
3) Rinse in distilled water
4) Place in schiff reagent for 15 minutes (sections become light pink colour during this step).
5) Wash in lukewarm tap water for 5 minutes (immediately sections turn dark pink colour).
6) Counterstained in Mayer's haematoxylin for 1 minutes.
7) Wash in tap water for 5 minutes
8) Dehydrate and coverslip using a synthetic mounting medium.

Appendix 3

Procedure for Van Gieson's technique

1) Deparaffinze and hydrate to distilled water.
2) Weigert's working haematoxylin for 10 minutes Weigert's working solution is prepared by mixing equal parts of Weigert's iron haematoxylin A H 26350-02 and Weigert's iron haematoxylin B. (H26350-03).
3) Wash in distilled water
4) Stain 1-3 minutes in Van Gieson's solution (H26350-01).
5) Dehydrate in 95% alcohol, absolute alcohol, two changes each clear in 2 changes Xylene (H23400).
6) Mount with permount (H17986-01) or DPX (H13510).