RESTORATIVE AND INHIBITORY HISTO-QUANTITATIVE EFFECTS OF LIVERCARE (Liv-52) ON ACETAMINOPHEN INDUCED LIVER TOXICITY IN ADULT ALBINO RATS (*RATTUS NORVEGICUS*)

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The Inhibitory and Restorative Histo-Stereological Study on Effects of live care (Liv-52) on Acetaminophen Induced Liver Toxicity in Adult Albino Rats (*Rattus Norvegicus*)

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A Thesis Submitted in Partial Fulfilment for the Degree of Master of Science in Human Anatomy in the Jomo Kenyatta University of Agriculture and Technology

2020

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

This thesis is dedicated to my wife Mrs. Viola Rono, my daughters Candy, Lynne and my mother Mrs. Sally Too.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALT Aspartate aminotransferase

ALT Alanine aminotransferase

APAP Acetaminophen

APC Antigen presenting cells

Bwt Body weight

Ca+- Calcium ion

COX-2 cyclooxygenase

CYP 450 Cytochrome p450

DMSO Dimethyl sulfoxide

ECM Extracellular matrix

GSH Glutathione

GST-S Glutathione tranferase

HC Hepatocytes cell

HSC Hepatic stellate cell

IL Interleukin

JKUAT Jomo Kenyatta University of Agriculture and Technology

KC Kupffer cells

Kg Kilogram

LIV52 Liver-care

LPS Lipopolysaccharides

NAPQI N-acetyl-para-benzoquinone imine

NCD Non-communicable diseases

PCM Paracetamol

PIH Paracetamol induced hepatotoxicity

SAFARI Small Animal Facility for Research and Innovation

WIM Water immersion method

DEFINITION OF TERMS

Acetaminophen Also known as Paracetamol or Panadol is a medication used to treat pain and fever .its typically used for mild to moderate pain relief

Histomophological The use of microscope to study the cellular structures, distribution, cellular patterning and interconnecting stromal tissues in the extracellular matrices.

Histostereology Is the study of microscopic anatomy of the cell and tissue in three dimensional quantification of two dimension cross section to extract quantitative information

Histo-morphometry This is a three-dimensional measurement of microscopic structures important to obtain reliable quantitative data that enables calculation of volumes and volume ratio, the area of samples, the number of particles per unit volume, particle size, unit volume, length and weight.

Liv-52 (Liver care) This is Polyherbal Indian medicine from Himalaya company known to be hepatoprotective

Morphometric It is the process of taking dimensional measurement of a structure either internally or externally to determine the actual volume or densities

Stereology Is the three dimensional quantification of cellular structures of two dimension cross section of material or tissue to extract quantitative information

ABSTRACT

Acetaminophen commonly known as Paracetamol has been associated with liver toxicity and is a probable contributor to the rising cases of liver failure. On the other hand, Liver care abbreviated as (Liv-52), polyherbal medicine from Himalaya Indian Company is being used to reverse the acetaminophen induced liver toxicity effects. Though studies have showed continued application of Liv -52, in management of liver toxicities arising from Paracetamol usage, there is paucity of data on the histo-stereological inhibitory and restorative effects of Liv-52. At the same time, data on restorative and inhibitory effects of Liv -52 is dose dependent. The current study aimed at evaluating the histo- stereological restorative and inhibitory effects of varied doses Liv52 on Paracetamol induced liver toxicity. A static-case- controlled-experimental study design was adopted. A total of 60 adult Albino rats weighing between 150-170 grams were used in the study. These 60 rats were randomly assigned into two main study groups of 10 controls and 50 experimental. To evaluate the restorative and inhibitory effects of Liv-52, the 50 rats in the experimental category were assigned into two study groups; 25 restorative and 25 rats for inhibitory. To evaluate effective inhibitory and restorative doses of Liv-52, the 25 rats in each of the two study groups were further divided into five groups of 5 rats each as follows: 100mg/kgbwt-5rats; 200mg/kgbwt -5 rats; 300mg/kdbwt-5rats; and 500mg/kgbwt-5rats (v) 5 rats- positive control. All animals were humanely sacrificed with Uethatol on day 21 and all livers harvested. The liver were then fixed with 5% zenkers solution and routinely processed for both light microscopy and stereological analysis. The sections for stereology were analyzed using stepinizer software, where volume densities and total parenchymal and stromal tissues were determined using cavarieli point counting method. The data was entered into excel sheet analyzed through SPPS version 25 and statistically tested using one way analysis of variance (ANOVA) for the group means and p-value of less than 0.05 were taken to be significant. Both the histo-morphological and the stereological finding of the study have showed that liver care (Liv-52) has both inhibitory and restorative effects to the liver-induced hepatocellular toxicity from acetaminophen. The most critical inhibitory and restorative doses of LIV-52 were between 300mg - 500mg/kg/bwt. The most effective way to protect the liver is concurrent admisntration of acetaminophen with Liver care (Liv-52). In conclusion, the present study demonstrated that Liv-52 is hepatoprotective and hepatorestorative. It is therefore recommended that Liver care (Liv-52) may be used in prevention of acute liver toxicity or in combination with acetaminophen to prevent liver damage.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Description of acetaminophen structure and its hepatocellular toxicity induction mechanism

Acetaminophen also known as Paracetamol or Panadol is an N-acetyl- para-aminophenol (APAP) pharmaceutical drug classified as antipyretic and aniline analgesic used to treat or manage all kinds of pain and to control fevers of a wide range based on its safety and its effectiveness as anti-inflammatory medicine (Tittarelli, *et al.* 2017). It is majorly metabolized in the liver and it has been shown that its prolonged use and overdose may cause liver injury. Liver care also known as Liv-52 is a herbal formulation from Himalaya company is used to treat liver toxicity especially caused by drug (Maji, *et al*.,2013) .

Liver, whose the main cellular building blocs are the hepatocytes and key physiological role entails metabolism, excretion and detoxification, is shown to suffer dysfunctional toxicity from acetaminophen and this would lead to liver failure following acute or prolong use of acetaminophen. The injurious effect of acetaminophen to the liver histo-cytoarchitecture are associated with oxidative metabolites N-acetyl-para-benzoquinone imine (NAPQI) that makes hepatocytes to undergo oxidative stress leading to bursting of hepatocellular mitochondria, generating oxygen radicals and nitrogen ion that later leads to necrosis to these hepatocelluar cells of the liver, with eventual lead to liver damage (Majee, *et al*, 2013).

1.2 Description of livercare (Liv-52) and components and postulated hepatocellular restorative mechanism

Liver-care (Liv 52) has gained a popular combination therapy with Acetamenophen as it has been shown to restore or reverse the functional and the biochemical status of the

liver following the acetaminophen injurious effects (Dubey, *et al.*, 1977; Girish, Koner *et al*., 2009; Sapakal, *et al*., 2008). Though this data exist there is paucity of data on the Livercare histo-qualitative restorative and inhibitory effects following its concurrent adminstration with Acetaminophen or when administered after the acute overdose or prolonged used on liver histo-cytomorphology as well as structural histostereology.

Livercare (**Liv -52**) is available in either syrup or capsule and is compose of 6 ingredients namely; Capparis spinosa 17 mg, Cichorium intybus 17 mg, Solanum nigrum 8 mg, Cassia occidentalis 4 mg , Terminalia arjuna 8 mg , Achillea millefolium 4 mg, Tamarix gallica 4 mg. its mode of reversal and inhibition of alcohol induced hepatotoxicity has been shown to reduce liver enzymes alanine transaminase (ALT), alanine aminotransferase (AST) and alkaline phosphatase (ALP) due to Paracetamol toxicity and other related liver toxicant. This component in Liv-52 have biochemical, physiological and antioxidant effects that are hepatoprotective to the liver cells. Liv-52 (**Liver Care**) a multi-herbal formula clinically proven safe and effective in protecting the liver and maintaining optimum liver function (Maji *et al.*, 2013). Liv-52 was first introduced in 1955 by The Himalaya Drug Company, it is a blend of several botanicals proven to protect the liver against harmful toxins found in food, water and air (Ghosh, *et al.*, 2014). It is sold in a preparation of 250 mg, 500mg and 125mg suspension for children, and readily available in chemist.

1.3 Comparative histo-morphological structure of human and rat's liver

A comparative analysis of gross and histo-morphological structure of the rat and human liver share common features in terms of gross morphology and histological organization in both classical function and hexagonal arrangement except the number of lobes (Yaghi, 2017). The human liver is a compact solid organ with two major lobes (right and left) and two minor lobes (caudate lobe and quadrate lobe), while rats liver consists of six lobes of different size namely: the left lateral lobe, right lateral lobe, left medial, right medial, caudate, and quadrate lobe (Stan & Gheorghe, 2018; Vdoviaková *et al*., 2016). The parenchyma of the liver divided into small units called lobules with layers of connective

tissue separating them (Bhunchet & Wake, 1998). These hepatic lobules are the structural unit of the liver. The lobule of both rats and human consists of a hexagonal arrangement of plates named as hepatocytes, radiating outward from a central vein. The portal triad that is a triangular area is comprised of a bile duct and a terminal branch of the hepatic artery and the portal vein (Fawcett, 1994). The lateral branches of these vessels are confluent with the thin-walled hepatic sinusoids that are present between the branching hepatic plates/cords (Fawcett, 1994). Liver sinusoids provide large surface area for the exchange of metabolites between blood and hepatocytes as sinusoids have their endothelium that lacks the basal membrane. The sub-endothelial space called space of Disse or per sinusoidal space, separates endothelium from the hepatocytes plates (Grisham, 1962).The liver cells consist of: Hepatocytes, Biliary epithelium, Hepatic stellate (Ito) cells, Kupffer cells (resident Macrophages), Liver-associated lymphocytes, Nerves and connective tissue cells. Constitutes parenchymal and liver stromal tissues.

1.4 Acetaminophen doses and metabolic pathway leading to hepatocellular toxicity

The recommended human doses of Acetaminophen is 1-4gm per day. Using more than 4gm is regarded as an overdose while using about 4 grams for more than two weeks is termed as prolonged use and these can cause accumulated liver toxicity and may lead to liver failure. Acetaminophen has been documented as among 25 most poisonous drugs of liver worldwide, either used as singly or in combination (Penna & Buchanan, 1991).

The metabolic pathway of acetaminophen in the liver occurs in three processes namely sulphonation , gluronudation and oxidization that occur in main cell of the liver hepatocytes (Tittarelli *et al.*, 2017). Nearly about 85% of Paracetamol undergo conjugation into sulphate and gluronated conjugate which is actively eliminated in the urine, while 15% is oxidized by CYP- 450 into N-acetyl-para-benzoquinone imine (Penna & Buchanan, 1991), which is later conjugated by glutathione peroxidase into cysteine and mercupturate metabolite which is nontoxic in the body (Tittarelli et al., 2017). However, when gluronidation and sulphonation is saturated due to overdose or prolonged use of acetaminophen then rate limiting step is shifted to oxidation, which may

leads to depletion of glutathione peroxidase enzymes (which is an anti- oxidizing agent) leading to accumulation of N-acetyl-para-benzoquinone imine (NAPQ1) which cause accumulation of oxygen radicals, depletion of glutathione peroxidase and protein arylation. This subsequently causes calcium derangement and cytosolic disturbances that is the primary cause of injury to the liver cells (Penna& Buchanan, 1991), further the accumulated NAPQ1 react with sulply dry1 compound found in hepatocytes (Afroz *et al.*, 2014).There after Hepatocytes undergo oxidative stress leading to bursting of hepatocyte mitochondria, generation of oxygen radicals and accumulation nitrogen ions that leads to cell death (Reza, *et al.*,2016). At same time Hepatocellular necrosis activates kupffer cell (KC) which are major source of inflammatory mediators including cytokines, chemokine's, nitric oxide, eicosanoids and proteolytic enzymes(Sprague & Rats, 2015). Kupffer cells also produce mediators that induce production of anti-oxidant agent glutathione and production of interleukin (IL)-10 and Interleukin (IL -18), hence depletion of KC due to hepatocellur necrosis increase susceptibility of the liver injury. This whole process leads to liver failure and increase liver related morbidity and mortalities. Other studies have shown that Acetaminophen causes extensive vascular degenerative changes, sinusoidal dilation, central vein congestion, and central lobular necrosis

1.5 Problem Statement

Liver failure is currently among the leading causes of mortality among many people in their economically productive age worldwide (Majee *et al* 2017, WHO 2017). On the other hand, studies have shown increasing usage of acetaminophen by communities across the globe in the management of all types of pain could be due to its easy accessibility as well as its affordability as an analgesic and antipyretic medicine (Verma & Kaplowitz, 2009) . This exposure of acetaminophen has consequently been associated with the rising cases of liver failure due to its associated hepatocellular toxicity induction mechanism following its prolonged usage or its high dose application. Acetaminophen is therefore being regarded as silent killer due to its progressive hepatocellular effects over time in someone's life before it is discovered at its late stages (Kaplowitz, 2011). Further, Liver care **(Liv-52),** a poly-herbal formulation from India has been shown to have both

protective and restorative physiological properties to the liver in some physiological studies that have been done to evaluate the levels of liver enzymes using liver functional tests (LFTs) (Ghosh *et a*l., 2014). Though physiological studies have shown liver care is able to restore the liver enzymes following hepatocellular toxicity from acetaminophen, there is paucity of data on its histo-morphological and stereological effects when either concurrently applied with Paracetamol or when administered after the liver induced hepatotoxicity. Moreover data on the most critical restorative and inhibitory doses of Liv-52 is also generally lacking.

1.6 Justification and Significant of the study

The lack of scientific data repository that describes the histo-stereological inhibitory and restorative effects of Liver care (**Liv-52**) on acetaminophen induced liver toxicity is a major setback in guiding the community and health care workers on the rational application of Liver care (**Liv-52**) in countering the arising cases of liver failure associated by the prolonged use or high acute doses of acetaminophen. As such Liver failure due the heavy use of acetaminophen is likely to become worse if mitigation products like Liv-52 that have physiologically shown signs of hope into countering the arising liver toxicities are not well studied to establish their actual restorative and inhibitory effects. The increasing trends of liver associated morbidity and mortality will then continue to increase with increasing usage of Paracetamol as it is currently implicated to be a major causative factor of liver toxicity. Therefore, there is a need to determine the restorative and inhibitory histo-stereological effects of liver from usage of Liver care (**Liv-52)** due to Paracetamol toxicity. This histostereological data generated will form the basis of advising the health care provider on the usage of Livercare (**Liv-52)** as a combination therapy to reduce the burden of liver related mortalities that may be arising from Paracetamol induced hepatotoxicity.

1.7 Research question

What are the histo-stereological and histo-morphological restorative and inhibitory effects of Liver-care (**Liv-52**) in restoring the histo- cyto- archicture of the liver of adult albino rats following Paracetamol induced liver toxicity?

1.8 Broad objective

To evaluate the restorative and inhibitory histo-morphological and stereological effects of ***Liv-52*** on the acetaminophen induced liver toxicity in adult albino rats (*Rattus norvegicus*).

1.8.1 Specific objectives

1. To evaluate the restorative gross morphometric effects of Livercare (Liv-**52**) in the gross features of the adult liver of albino rats following acetaminophen induced liver toxicity.
2. To evaluate the histo-morphological injurious effects that may occur on the liver histo-cyto-archicture following acetaminophen induced hepatocellular toxicity in the adult albino rats.
3. To establish the restorative histo-stereological effects of Livercare (**Liv-52)** on the liver histo-cyto-archicture following acetaminophen induced hepatocellular toxicity in the adult albino rats.
4. To establish the inhibitory histo-stereological effects on liver tissues following concurrent administration of livercare **(Liv52)** with doses of acetaminophen in the adult albino rats.

1.9 Hypothesis null (HO)

There is no significant difference in histo-morphology and histo-stereological features of the liver in the liver care (Liv-52) treated group compared with the control groups following the Paracetamol induced liver toxicity in albino rats

1.10 The study model assumptions

In carrying out this study it was assumed that the adoption of adult albino rats (*Rattus Norvegicus)* animal model would replicate similar effects to what would happen in humans based on the documented close association of this rat species (*Rattus Norvegicus*) to humans in terms of their biological and functional mechanism features when exposed to chemical agents.

1.11 Study limitations

Two animals died during the course of experiment at day 6 and 8 in the positive control group and another in negative control group of Liv-52 and 0.5% DMSO group

1.12 Study delimitations

To overcome these challenges the following delimitation measures were applied:-

(i). For the rats that died at the course of the experimentation in this study, their study groups were noted as per dosage and the time of exposure. Postmortems were conducted to establish the cause of death then repeat experiments on those that died were done after the main experiment was completed.

(ii). A pilot study was done to test the study protocol and to minimize causes of errors as much as possible.

CHAPTER TWO

LITERATURE REVIEW

2.1 Acetaminophen structure and mode of action in inducing hepatocellular toxicity

Paracetamol (acetaminophen) is classified under class of an analgesic and antipyretic (Benista & Nowak, 2014 , Graham & Davies, 2013). It has similar properties like of Non-Steroidal Anti-Inflammatory drugs (NSAIDs) and resembles particularly the cyclooxygenase (COX-2) selective inhibitors, however does not possess any anti-inflammatory activity (Kingsley Ogemdi, 2019). When used in recommended doses, Paracetamol does not induce typical NSAIDs side effects such as gastrointestinal disturbances (Graham & Davies, 2013; Kingsley Ogemdi, 2019). Paracetamol suppresses prostaglandin production which is similar NSAIDs by inhibits COX-1 and COX-2, through metabolism by the peroxidase function of these isoenzymes inhibition of phenoxyl radical formation (Graham & Davies, 2013). Paracetamol have both central and peripheral effects (Zealand, 2008).

Oral dose administration of Paracetamol is rapidly absorbed by small intestine due to its lipid solubility .Then about 50 and 60% is converted to its main and pharmacologically inactive glucuronidated and sulfated conjugates which can be eliminated in urine (Benista & Nowak, 2014; Graham & Davies, 2013). In liver microsomes, a small percentage of Paracetamol (5-10%) is converted by cytochrome P450 isoforms (CYP2E1, CYP2A6) into a reactive metabolite, N-acetyl-para-benzo-quinone imine (NAPQI), which primarily related to Paracetamol hepatotoxicity (Bailey *et al.*, 2014; Chen *et al.*, 2015) .About 2% of Paracetamol is excreted in urine unchanged .The cellular damages caused by NAPQI are directly related to the dose of Paracetamol consumed. In the case of non-toxic consumption, NAPQI is rapidly conjugated by hepatic glutathione, through glucuronidation and sulfonation reactions, to form mercaptate and cysteine complexes, that are eliminated with urine (Bjõrnsson, 2016; Boyd & Bereczky, 1966). Once Paracetamol has been ingested at higher doses or in prolong period of time , the majority

of the drug is metabolized by CYP2E1 pathway resulting in glutathione depletion, by activation of GST-S-transferases, and with the build-up of NAPQI at toxic concentrations leading to hepatotoxicity then liver failure (Benista & Nowak, 2014).

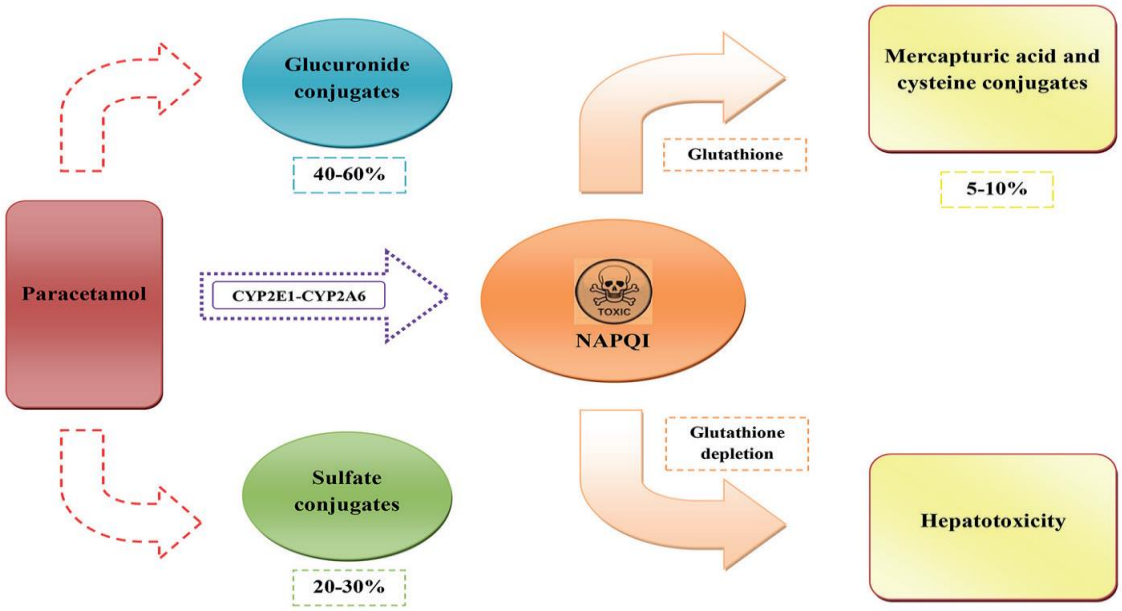

Figure 2.1: Flow chart diagram illustrating how Paracetamol metabolism occurs in the liver (adapted text book of clinical Pharmacology and Therapeutic by Karen Hodson)

The end metabolite of Paracetamol NAPQ1 is main toxic agent which can cause the calcium ion (ca+) derangement, moreover other research shows that accumulation of this metabolite injures liver cells (Afroz *et al.*, 2014; Iyanda & Adeniyi, 2011). A study done by Joulideh Pouh (2016) on broiler chicks show that after 25 minute of Paracetamol ingestion of 65mg/kg/bwt dose, shows increased in liver enzymes ALT, AST, ASP and Ammonia serum level, this was an indication of how the liver parenchymal cell are damage, moreover it also indicates that other organ may as well be affected like kidney and bone tissues.

2.2 Liver care (Liv-52) components and postulated restorative mechanism to liver histo-cyto-archictures

Liver-care is available in preparation of 125mg/5ml and 250mg/5ml in syrup in the market and capsule dosage 250mg and 500mg .The drug is metabolized in the liver and excreted in the kidney, but no data has shown the active ingredient, the drug has shown to be hepatoprotective and widely used for treatment of acute liver toxicity and liver failure (Sapakal *et al*., 2008)

Due to high burden of morbidity and mortality associated with liver failure, Livercare (**Liv-52**) medicine has shown to reverse the physiological effects of the Paracetamol toxicity and associated liver failure (Dhawan & Devinde, 1994). The previous studies have shown that when Livercare (**Liv-52**) is administered at 206.6mg/kg bwt for 16 days there is restorative effect archived, evidence by restored liver protein and normal liver enzymes ,other studies documented that Livercare (**Liv-52**) has antiperixodative and antiviral effects (Maji *et al*., 2013) which restored physical activities of studied animals which was noted by weight gain, activeness, drinking and food consumption (Sapakal *et al*., 2008).

2.3 The patterns of hepatocellular toxicity metabolic pathways associated with Acetaminophen liver failure

Paracetamol is metabolized in the liver (Graham & Davies, 2013),which is the major used due to it analgesic and anti-pyretic effects , however it has been rated as the second most cause of liver failure after alcohol when either used in overdose or within therapeutic dose for prolong period of time(Ben-shachar *et al.*, 2012). Due to its availability , people get it easily over the counter when either prescribed or non-prescribed (Guzy *et al.*, 2004). Despite extensively use of Paracetamol it is a potential cause of liver failure (Tittarelli *et al*., 2017), Paracetamol overdose is associated with 56000 emergency and 4000 fatalities each year in causality department in USA. Nonetheless in 2008-2009 Paracetamol caused 90 -155 deaths in UK. The research shows that when Paracetamol is used together with

alcohol it causes synergistic effects leading to severe liver failure (Kolios, *et al.* 2007). Other medication like acetylsalicylic acid, codeine, and oxycodone has been implicated as the cause of liver injuries.

Studies have shown that Paracetamol adult human dose is 1-4gm per day, while 50-75mg/kg bwt/ day in infant , exceeding recommended dose may cause metabolic as well as changes in histo-architecture of a liver (Tittarelli *et al*., 2017). These doses have been shown to be exceeded by person leading to devastating outcome to liver function. Paracetamol maximum daily dose of 3 g/day (for up to 2 days) or at a daily dose of 1 g/day (for up to 25 days) does not appear to be associated with acute hepatic decompensation (Yaghi, 2017). Acetaminophen at a dose less than 2 gm/day is a reasonably safe option. A policy has been adopted in United Kingdom to reduce the dispensing dose into 16 tablets, this limits the package sizes which limit the consequences. The optimal dose of Paracetamol may cause slow progression of liver damage, while exceeding the dose of 4gm per day causes acute liver toxicity and major liver histo-archirtecture changes (Mahmood *et al*., 2014) .In the European countries, Paracetamol is the major cause of liver transplant, about 2 to 3 persons require liver transplant in USA (Ilic *et al*., 2010) every day either due to chronic liver failure . And due to this the Paracetamol packaging has been introduce to limited dosage. In animal model Paracetamol of 750-1000mg/kgbwt causes hepatic toxicity when administered in single dose for 7 days (Mahmood *et al*., 2014). Hepatocytes is the major liver cell which carry most of function, and most of this cells are in found in the first zone of hepatic acinus (Pandit, Sachdeva, & Bafna, 2012) about 15 % of this cell compose of other organelles namely smooth and rough endoplasmic reticulum, mitochondria, ribosomes, Golgi complex cytoskeleton (Bjõrnsson, 2016). Elevation of cytosolic calcium ion (ca+) causes the bursting of mitochondria hence rendering the cells inactive and death (Kheradpezhouh, *et al* .,2009).With time the number of hepatocytes decreases and become hypertrophy contrary to other studies which indicate cells number increases, but other organelles like rough and smooth reticulum reduced due bursting of mitochondria (Mahmood *et al*., 2014). Due to reduce hepatocyte, metabolism and other vital function of liver is

compromise. Moreover the biliary tree epithelium is also affected. Others cell that are involved in injury response is KC which is also reactivated and further causes liver injuries (Kolios *et al.*, 2007). KCs are the first cells to be exposed to materials absorbed from the gastrointestinal tract in zone one of hepatic acinus. KC has ability to eliminate micro-organisms, detoxify endotoxins, degenerated cells and reactivate the immune complexes which is an important physiological changes in liver (Tittarelli *et al.*, 2017). Due to that, KCs function as antigen presenting cell (APC), participate as tumour surveillance and restoration of liver cells. However other studies documented that KC and lipopolysaccharide (LPS) interaction causes liver injury which include toxinaemia and ischemia reperfusion by KC proliferating locally and also major production of immune mediators which are potent injury to other liver parenchyma and stromal liver tissue (Kolios *et al.*, 2007).

Hepatic stellate cells also called Ito or fat-storing cells are supporting liver by hepatic fibro- genesis and matrix production .The extracellular matrix (ECM) is important in the regulation and modulation of hepatic function. About 5-10 % of the liver architecture is extracellular matrix (ECM) and this form the basis of fatty liver. The detoxification process involves multiple phases where the final products are highly water-soluble conjugates of the parent compound that are easier to eliminate (Ferrari *et al*, 2007; Nab *et al,* 2006). Damage to the liver cells can severely hamper an organ ability to do its metabolism.

2.4 The comparative gross morphology of the liver in humans and rats

The liver in both human and rats is the biggest accessory gland of the gastrointestinal tract, it is located in the upper right hypochondriac region of the abdomen in human while in rats are located in all or part of sub-diaphragmatic region (Stan & Gheorghe, 2018; Tajiri & Shimizu, 2017). It provides metabolic exocrine and endocrine function in the body (Stan & Gheorghe, 2018). Its principle function is production of bile, metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage and control of blood homeostasis by secretion of clotting factors and serum

proteins such as Albumin (Stan & Gheorghe, 2018). It has been identified to play more than 500 essential roles (Ney, , *et al.*2017).The liver receives 75% of blood from portal circulation and 25% form hepatic circulation then all drain into inferior venacave to the right atrium of the heart .The liver has fibrous capsule called glisson capsule, which encloses the portal triad, bile duct and hepatic artery .In rats the liver represent about 5% of total weight while in human it is 2.5% .the adult liver weight of the rats is 250gm – 300gm ,while the gross measurement of fresh livers the transverse diameter is about 7.5-8.0 cm, superior inferior is about 3.8-4.2cm and anterior posterior is about 2.2-2.5cm (Stan & Gheorghe, 2018).

The liver is organized into liver units called the liver lobules ,the lobules are separated by connective tissue called interlobular septa ,the hexagonal functional unit that are compose of hepatocytes ,blood flows from periphery of the lobules towards the central vein where they then flow to hepatic vein .The interlobular vein are less define in human being than rats and other animals, on other hand the liver acinus are divided into three zones with majority of hepatocytes are found in the first zones followed by second zone and third zone .The first zone is next to perilobular vessels, while the third zone is next to central vein. The first zone receives nutrient and toxin cause of portal vein followed by second zone and third zone (Yaghi, 2017). In case of hepatotoxicity due to Paracetamol, the hepatocytes in the first zone is the last to die, but the first to regenerate, while the hepatocytes in the third zone is the last to receives nutrient and toxin, but first region to show features of necrosis in case of Paracetamol hepatotoxicity of the liver (Maji *et al*., 2013). The portal lobule is the area where the interlobular lobule collect biles, it's a triangular in shape with bile duct at the center and central vein at the edge

2.5 The comparative histo-morphological structure of the human and rat liver

In both human and rats the hepatocytes are the main cellular component of the liver parenchyma and comprises of 60% of total liver cells, they have large encromatic nuclei (Malarkey *et al.*, 2005). Bile canaliculi lie between adjacent hepatocytes (.Malarkey *et al.*, 2005). The sinusoid is an area where hepatocyte and plasma exchange large substances,

its line with fenestrated endothelia next to the Sinusoidal endothelial cells (SECs), the hepatocyte and sinusoid is separated by space called perisinusidal space or space of disse, the hepatocyte extend microvilli into this space. The reticular fibers support hepatocyte in the space of disse .Therefore the ratio of interstisum and parenchymal of liver is small hence the liver is vulnerable to injuries (Kolios *et al.*, 2007). Kupffer cells are resident macrophages, they are triangular, small shaped migrating cell within sinusoid spaces (Yaghi, 2017), They acts as part of immune system by phagocyting and degrading foreign material (Kolios *et al.*, 2007). They proliferate and enlarges in response to hepatocytes damage and bacterial toxin. The hepatic stellate cell (HSC) also known as ito cells or parisunosuidal cell are located at space of disse and stores and metabolize vitamin A, they produce connective tissue of interlobular septa and biliary epithelium. Both cell make up a total of 3-20% of total liver cells. All these cells plays a crucial role on liver hemostasis and pathogenesis progression of liver diseases.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study location/ area:

The research part on animal experimentation was carried out in SAFARI (Small Animal facility for Research and Innovation) while the part on histology and stereological analysis was carried in histology laboratory department of human anatomy Jomo Kenyatta University of agriculture and technology (JKUAT) located in in Juja town of Kiambu County, Kenya

3.2 Study design

A laboratory static- controlled-experimental study design was adopted.

3.3 Study sample/ subject:

The study sample included total of 60 adult albino rats of the species *Rattus norvegicus* derived from a pure colony. These 60 rats were sourced from small animal facility for research and innovation(SAFARI) in the school of biomedical sciences of Jomo Kenyatta University of Agriculture and Technology (JKUAT).The use of these albino rat was guided by the following known facts; (i) they have a relatively short gestational span, making it easier to get study subjects or a pure bleed colony (ii) Low cost of maintaining the animals , (iii) Are plentiful, (iv)Considerable amount of the reproductive data on the rat is already available,(v) They are relatively small and easy to care for and handle during an experiment (vi) they are relatively resilient in terms of withstanding a wide range of study medicines (Bailey *et al.*, 2014; Pritchett & Corning, 2016). By appearance, both the male and female albino rats have red eyes and white fur resembling the 'Japanese hooded rats', hence essentially genetically identical from a common ancestor. (Pritchett & Corning, 2016). They were the first mammalian species domesticated for scientific research. They live about 2-3.5 years (average 3 years). They develop rapidly during

infancy and become sexually mature at about 4-5 weeks in females and at around post-natal dates 45-48 in males (Pallav and Sengupta, 2013). Male rats are usually larger than females and are about 9 to 11 inches long.

3.4 Site for Specimen processing for light microscopy and stereology

Specimen processing for light microscopy and histo-steriological was done in laboratory at college of health sciences (COHES) complex, in the Department of human anatomy of JKUAT.

3.5 Sample size determination and groupings

3.5.1 Sampling

The sampled size was drawn from modified Resource equation method" of which there was no previous research done to determine the standard deviation (Arifin, *et al*, 2017)

$n=DF/K+1$

N= n+k

n- Number of animals per group

DF-Error of degree of freedom

K-Number of groups

N=Total number of subjects

DF range from 10 to 20 to obtain minimum and maximum number of each group

Sample size in restorative was calculated as follows;

k=6

=20/6+1

=3.333+1

=4.3

n=5 rats in each group

=5x6

n=30

For the two category restorative and inhibitory group total of 60 was arrived at;

Simple random sampling with replacement was used to assign the albino rats into groups so that each group has a representative sample. This is to ensure that each experimental unit has a known, often equal, probability of receiving a given treatment in the various treatment combinations.

3.5.2 Animal grouping

The 60 rats were randomly assigned into two main study group of 10 control and 50 experimental groups.

i). **The Control group (10 Rats)**

(a). Control restorative .subgroup1: 5 albino rats which received water and feeds (Mice Pellets UNGA® feeds, Kenya)*ad libitum* for 21 days where they were humanely sacrifice after experiment (Larson *et al.*, 2003)

(b)Control inhibitory subgroup 2: 5 albino rats received PCM 1500MG for induction for 5 days, water and feeds *ad libitum* for 21 days then they were humanely sacrificed after experiment in 21st day (Larson *et al*., 2003)

ii**) The experimental groups (50rats)**

Group 2; The restorative group of 25 albino rats ;Received high dose of Paracetamol (1500mg/kgbwt) for five days for induction of hepatotoxicity then was randomly assign groups as follows:

Subgroup 2a; consisted of 5 Albino rats that received Paracetamol high dose for 5 days then continue with 0. 5%DMSO only group for the remaining 16 days:

Subgroup 2b consisted of 5 Albino rats that received high dose of Paracetamol (1500mg/kgbwt) through gastric lavage for 5 days then Liv-52 100mg for duration of the remaining 16 days for treatment, (**Table 3.1**)

Subgroup 2c consisted of 5 Albino rats that received high dose of Paracetamol (1500mg/kgbwt) through gastric lavage for 5 days then Liv-52 200mg for duration of the remaining 16 days for treatment (**Table 3.1)**

Subgroup 2d consisted of 5 Albino rats that received high dose of Paracetamol (1500mg/kgbwt) through gastric lavage for 5 days then Liv-52 300mg for duration of the remaining 16 days for treatment **(Table 3.1).**

Subgroup 2e consisted of 5 Albino rats that received high dose of Paracetamol (1500mg/kgbwt) through gastric lavage for 5 days then Liv-52 500mg for duration of the remaining 16 days for treatment **(Table 3.1).**

All rats were humanely sacrifice at 21day after the end of experimentation period as described previously by (Mahmood *et al.,* 2014).

Table 3.1: Shows how the rats in the restorative study groups were grouped.

Subgroup	PIH for 5 days	Liv-52 doses(HED)in 0.5% DMSO for 16 days
Subgroup2a -(5 rats)	1500mg/kgbwt	0.5%DMSO only
Subgroup2b -(5 rats)	1500mg/kgbwt	100mg/kgbwt
Subgroup2c -(5 rats)	1500mg/kgwt	200mg/kgwt
Subgroup2d- (5 rats)	1500mg/kbwt	300mg/kgwt
Subgroup2e- (5rats)	1500mg/kbwt	500mg/kgwt

Group 3: The Inhibitory group of 25 albino rats: These 25 rats was group were divided into 5 sub group of 5 rats each, the assigning of these subgroups was done randomly sampled based on the doses of Liv-52 applied as 1st dose, 2nd dose, 3th dose and 4th dose respectively. The rats in each category received high dose of Paracetamol (Paracetamol 1500mg concurrently with varying doses of Liv-52) for 21 days after which they all humanely sacrifice and liver harvested for histo-stereological analysis (**Table 3.2)**

Table 3.2: Shows how the rats in the inhibitory study group were organized into five subgroup of 5 rats each.

Subgroup	Paracetamol dose (HED) Liv52doses(HED)in 0.5%DMSO for 21 days
Subgroup3a, -(5 rats)	Liv52 +0.5%DMSO
Subgroup3b, -(5 rats)	1500mg/kbwt and 100mg/kgbwt
Subgroup3c, -(5 rats)	1500mg/kbwt and 200mg/kgbwt
Subgroup3d, -(5 rats)	1500mg/kbwt and 300mg/kgbwt
Subgroup 3e,-(5 rats)	1500mg/kbwt and 500mg/kgbwt

ANIMAL GROUPING

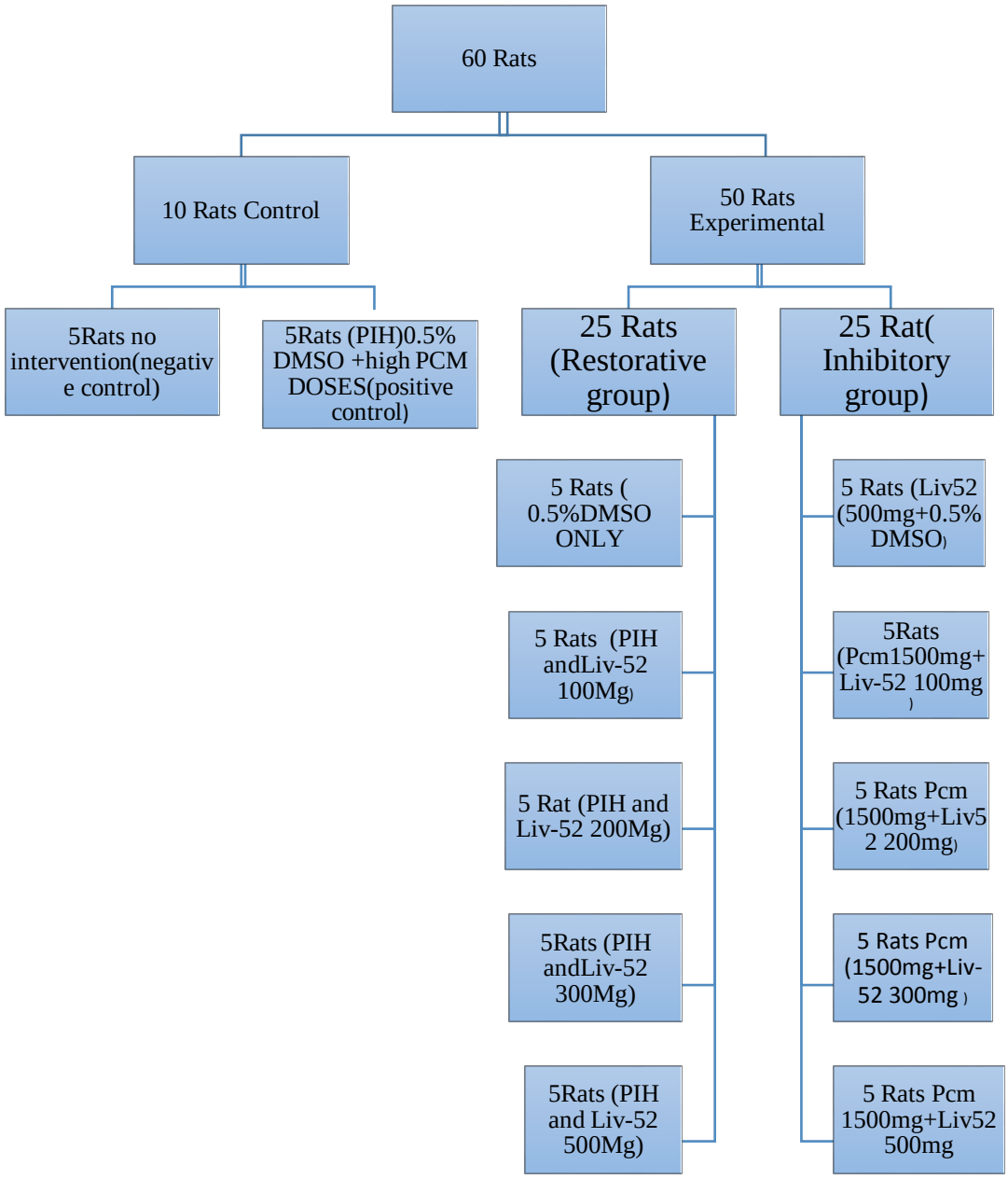

Figure 3.1: A flow chart diagram showing how the groupings of the 60 rats was done between the experimental, negative and positive control categories.

3.6 Animal feeding and weighing

The animals weight were taken between 00; 8: Am and 00; 9: Am in all study groups then feeding and water *ad libitium* was provided.

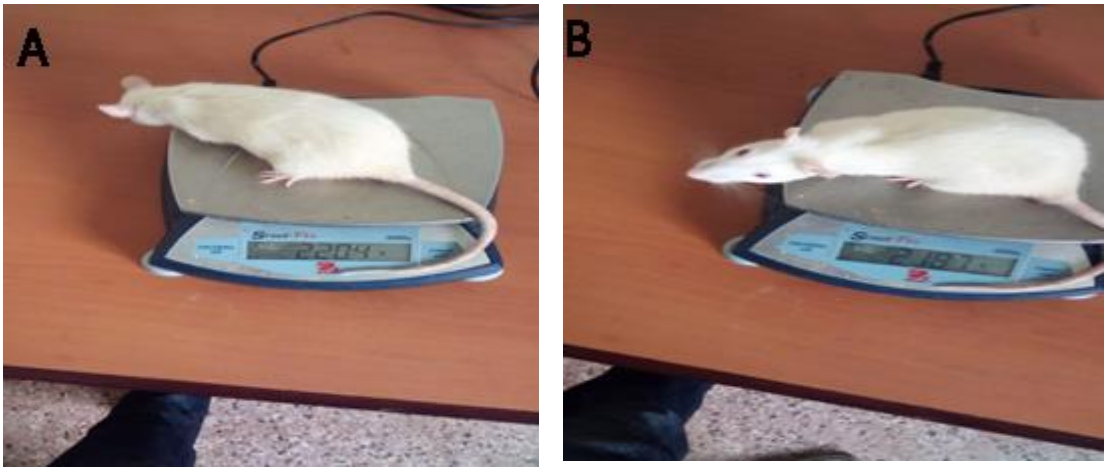

Figure 3.2: Showing how the daily weights of rats were taken using electronic weighing scale (scout pro model SPU4001) from japan

3.7 Acquisition of the Paracetamol and Liv-52 (liver care)

Paracetamol was acquired from *GSK* batch No 2087- 17 while Liv-52 was procured from Nairobi chemist batch no 30201-18 India

3.8 The method used in administering Paracetamol and Liv-52 using gastric gavage needle.

To induce hepatotoxicity Paracetamol dosage was administer between 10:00 am -13:00 pm daily for five days using gastric lavage needle gauge 18.

Procedure for gastric gavage

1. The rats was wrapped with the table cloth to avoid the animal from soiling the cloths of the researcher
2. Then, it was carefully held from the neck region using one hand
3. Then rat was rested against the body of the researcher with the animal mouth facing forward
4. Then gavage needle was gently inserted into the mouth of the animal turning it gently to circumvent the esophageal constrictions and the cardiac sphincter
5. Then the Paracetamol bolus was dropped right into the stomach
6. The gavage needle was then gently removed

3.8.1 Determination of the Pacetamol doses used in the experiment

To induce hepatoxicity Paracetamol dose of 750-1500mg/kbwt was used for 5 days restorative treatment was administered together with Liver care(**Liv-52**) for 21 days protocol adopt from (Kumar, *et al* 2015)

Animal equivalent dose=1500mg×rat weight =dose of the rat

E.g. if rats weigh 150grams

1500mg×150/1000=225mg per day

3.8.2 Administering of Paracetamol doses

Administering the doses of Paracetamol was done between 08:00 am and 10:00 am daily using gastric gauge.

Materials

1. Paracetamol
2. Gavages' needle gauge 18

3. 5ml syringe
4. 20 ml beaker for dilution
5. Syringes
6. Deionized water
7. 5abino rats
8. A table cloth

3.8.3 Determination of the Liv -52 dosages used in the experiment

The adult human dose of Liv-52 is 500mg taken 3-4 times a day(6-8 hourly),the dosage were converted to animal equivalent dose (Shin, *at el.*,2010)

Animal equivalent dose =human equivalent dose (Mg/kg) ×converting factor

Animal equivalent dose=100mg×rat weight in kg

Eg for Liv-52 100mg 400mg/60×6.2=41.12mgkbwt

For Liv-52 200mg 800mg/60×6.2=82.6mg/kgbwt

For Liv-52 300mg 1200/60×6.2=124mg/kgbwt

For Liv-52 500mg 2000mg/60×6.2=206.6mg/kgbwt

3.8.4 Administering of Liver care (Liv- 52) doses

To determine restoration effects, Liv-52 was administered from the 6^{th} day to 21^{th} day after Paracetamol induction. While for inhibitory effects, Liv-52 varying doses was administered concurrently with high dose of Paracetamol for 21 days .The Administration of Liv-52 doses was done between 10:00am and 13; 00 pm daily

Materials

1. Livercare (**Liv-52**)
2. Gavages' needle gauge 18
3. 5ml syringe
4. 20 ml beaker for dilution
5. Syringes
6. Deionized water
7. A table cloth

3.8.5 Dissolving and preparation of right dose concentrates of Paracetamol and Liv-52

PCM and Liv-52 was diluted with 0.5% DMSO, all the volumes were administered in a standard volume of 2- 3mls (the standard allowable daily oral volume of a rat per day) (OECD, 2002)

3.9 Weighing of the rats

The daily body weight of the animals were taken using scalar weighing scale and amount of water and feds consumed was also recorded

3.10 Humane Sacrificing of the animals and harvesting of the liver tissues

Materials

1. Albino rats
2. Diethyl ether 75% or chloroform 10 mls
3. Cotton gauze or cotton wool
4. Bell or dissector jar
5. Physiological saline 0.85% concentration
6. mounting board

7. mounting pins
8. Pair of scissors
9. A pair of forceps (toothed)
10. Scalpel blade
11. Fixatives- 5% formalin solution for light microscopy
12. Drip set 2 in number
13. Hypodermic needle gauge 20
14. Gloves (surgical)
15. Magnifying glass
16. Ruler
17. Electronic weighing machine
18. Specimen collection bottles

3.10.1 Anaesthetizing and per fusing the animals

Procedure

1. The cotton gauze or cotton wool was soaked in diethyl ether or chloroform
2. Then Soaked cotton wool was introduced into the bell jar
3. And then rat was put into the bell jar and then wait for 10-15 minutes for the animal to be anaesthetized
4. There after animal was removed from the bell jar and mounted onto the board using mounting pins with dorsal side on the board
5. A pair of scissors and forceps was used to cut through the ventral medial side from the symphysis pubis to the sternal angle of the thoracic cage
6. Perfusion needle that was connected to the perfusion set to the left ventricle of the heart was inserted.
7. Then blood was cleared from the animal with physiological 0.5% saline (200mls of 0.85mol/l) through the left ventricle of the heart (saline flows by force of gravity from one of the drip set)

8. After sufficiently clearing the saline drip was removed (leave needle in position of the heart and introduce the desired fixative (glutaldehyde or formalin solution)
9. Then firmness of the tail was checked as a sign of effective fixation
10. Then drip was removed and the perfusion needle from the heart
11. Then liver excised, then it was Immerse it in a container with fresh fixative to Continue fixation for 12 hours

3.11 Assessing the gross morphometric of the liver (liver thickness, width and length

Immediately the liver was resected, it was cleaned using 5% normal saline, then the lengths, widths and thickness was taken using a ruler and a caliper .For the estimation of percentage liver body ratio for the all groups, the following formula was used.

Percentage liver body ratio =liver weight÷ terminal weight in grams ×100%.

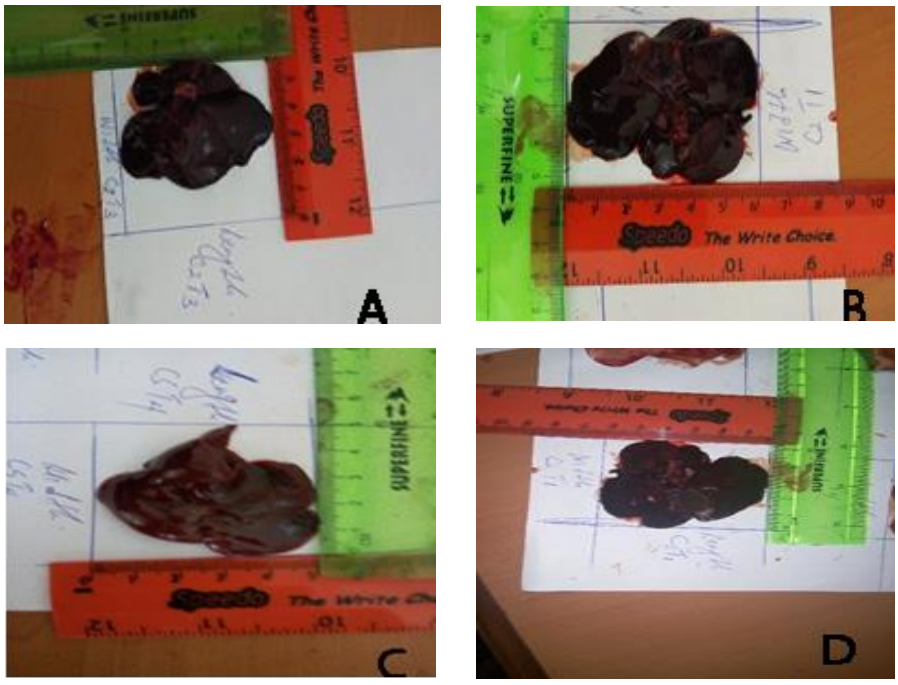

Figure 3.3: Images showing how the gross morphometric measurement of the fresh liver were taken after harvesting using plastic ruler manufactured in Kenya. (Haco Company LTD-Nairobi)

3.11.1 Evaluation of total liver volume using Archimedes principle

After the dissection and removal of the liver and the liver volume was estimated using the water immersion method the Archimedes principle (Cavazzini, 2018) .where the liver was placed on the calibrated beaker full of 5% normal saline and saline displaced by the liver was recorded as actual liver volumes. This methods was compared to the other methods and the mean standard deviation (± SD) of the measurements were calculated

3.11.2 Routine processing of livers tissues for light microscopy:

To determine histo-morphological changes of the liver in both restorative and inhibitory groups, all the liver tissues was processed for light microscopy

3.11.3 Procedure for processing specimen for light microscopy

Materials

1. The specimens (liver)
2. Formalin solution (1 litre)
3. Paraffin wax
4. Glass slides and cover slips
5. DPX moutan
6. Haematoxylin and eosin
7. Glass staining square jars
8. Microtome knives
9. Rotary microtome
10. Heater and water bath container
11. Specimen bottles
12. Slide holders
13. Specimen (the fetal and maternal pancreas)
14. Distilled water
15. Formaldehyde 40% concentration

16. Xylene
17. Isopropyl alcohol
18. Glass ware for preparing dilutions
19. Wood blocs
20. Beakers
21. Dropper
22. Cedar wood oil
23. Toluene solution.

3.11.4 Procedure for liver tissue processing

1. The liver was immersed in the 37% formalin' solution for 24 hours
2. Then dehydration was done in an ascending grade of concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100 %(absolute) each for one hour.
3. It was then cleared by immersion with xylene for 12 hours.
4. The liver tissue was oriented in the longitudinal axis and cut into blocks where average of 20-26 blocks was obtained in every liver and where 8-12 blocks was picked using systematic simple uniform random sampling and coding was done by laboratory assistant.
5. Then it was embedded in paraffin wax on the wooden blocks
6. Then excess wax was trimmed off until the entire length of the liver tissue was exposed
7. Then Liver tissues was Cut into 5µm thick longitudinal sections from head to right lobe to left lobe with Leitz© sledge rotary microtome where 220-240 slide section was obtained from each block.
8. Then sections was floated in water at 37^0c to spread the tissue
9. Then sections was sticked onto glass slides using egg albumin, applied as thin film with a micro-dropper.
10. Then slides was dried in an oven at 37 ^{0}cfor 24 hours
11. Then slides was stained with heamytoxylin and Eosin
12. Then 20-24 slide section was picked for light microscopy

3.12 Processing liver tissue for histo-stereological Analysis

3.12.1 Preparation of tissues for stereology

1. The liver tissue for stereological analysis was removed,
2. Then liver tissue was place in formalin solution for 24 hours at room temperature (23^0c) to allow for proper fixation.
3. Then dehydrated using graduated alcohol:50%,60%,70%,80%,90%, and 100% each for one hour
4. Then it was cleared with xylene for 12 hours.
5. The liver tissue was then infiltrated with paraffin wax for 12 hours and embedded in paraffin wax, microtome sledge was used to cut the embedded tissue into thin section of 5µm
6. Each liver was then exhaustively sectioned into 5µm thick sections.

3.12.2 Staining of liver slides

Liver sections were stained with hematoxylin and eosin solution as described by(Ghosh *et al*., 2014)

Procedure for staining with hematoxylin and eosin

The glass slides that hold the paraffin sections of the liver tissue was placed in staining racks. The paraffin from the samples was cleared in three dips of xylene for 2 minutes per change.

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Procedure for staining with haematoxylin and eosin

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2. Hydration of the liver samples was done as follows.

i. Transferring the slides through three changes of 100% ethanol for 2 minute per change.

ii. Transferring to 95% ethanol for 2 minutes.

iii. Transferring to 70% ethanol for 2 minutes.

iv. Then slides were rinsed in running tap water at room temperature for at least 2 minute.

3. Then liver samples were dipped in haematoxyline solution for 3 minutes.

4. Then slides were placed under running tap water at room temperature for at least 5 min.

5. Then samples were stained in eosin Y solution for 2 minutes.

6. Dehydration of the samples was done as follows.

i. Dipping the slides in 70% ethanol about 20 times.

ii. Transferring to 95% ethanol for 2 minutes.

iii. Transfer through two changes of 100% ethanol for 2 minutes per change.

7. Then samples were cleared in three changes of xylene for 2 minutes per change.

8. A drop of Permount was placed over the tissue on each slide and a coverslip was added. Then allowed to dry

9. Then slides were ready for viewing using a light microscope

3.13.3 Determination of liver volumes and histological changes

3.13.3.1 Estimation of liver volume by cavarieli method

After the slides processing ,20 slides was picked in each liver using simple uniform random sampling, it was then stored in an oven at 37c for 12 hour then viewed under light microscope, the microscopic fields were selected randomly in each liver section. The microscope stage was then moved along the X and Y directions every time, till the entire section was studied, photos was taken using LABOMED ivu 3100 imaging camera softener with pixel pros. Results was entered on excel sheet.

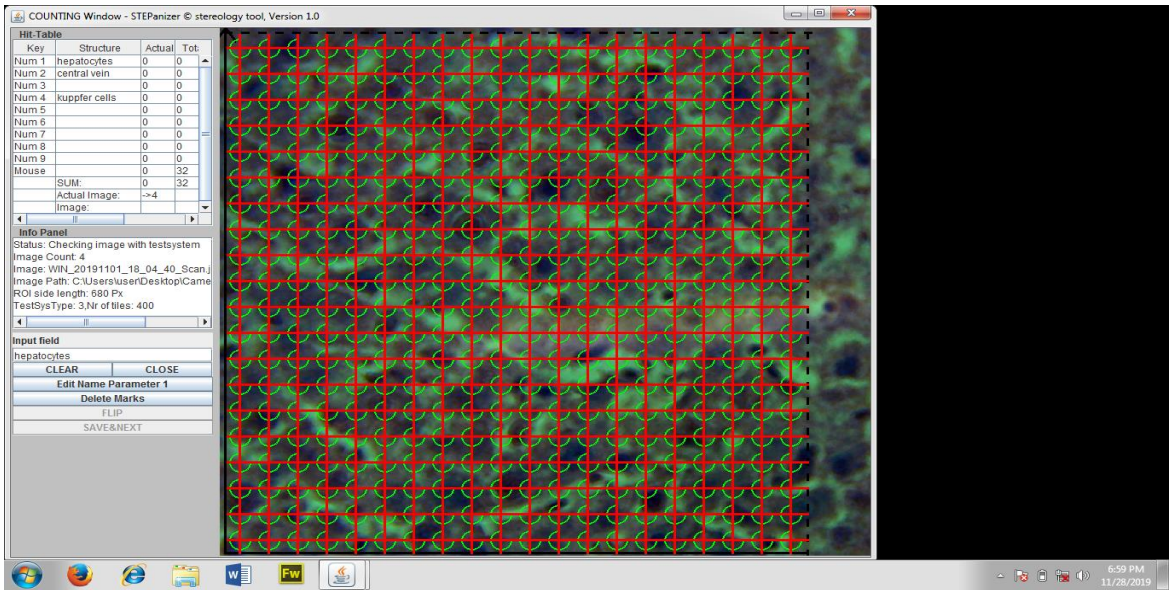


Figure 3.4: An image showing how the liver histology tissue sections were superimposed on a point-counting grid using stepanizer tool for stereological analysis.

3.13.3.2 Procedure for the determination of the total liver volume using Cavarieli principle

The images of the liver tissue were analyzed using quantitative stereological stepanizer software tool , the points that were over liver were marked ,.each point was counted and the volumes of the whole total liver volume was estimated by multiplying area of a point by thickness and interval of the section.

The following are the steps and the formula applied to determine the volume densities:-

1. Preparation of liver Cavarieli sections.

2. Selection of the spacing for the point probe was done.

3. The point probe was tossed randomly onto each section.

4. The points that hit the region of interest was counted using Stepanizer stereology tool.

5. All sections were processed keeping a tally of counts per section.

6. The shape factor was finally estimated and the calculation of the volume and the CE. (Coefficient of Error)

The volume of liver of liver sections was estimated using the following formula:

Volume = t x a/p ÷m ΣP

Where ("t", section thickness; "a/p", representing the area of each point on the point counting grid; "ΣP", total number of the points hitting the area of interest and m is the magnification)

3.13.3.3 Procedure for the determination of the numerical density of hepatocytes and kupffer cell.

To estimate the numerical volume density of the hepatocyte and kupffer cells, the systematical sampled sections were subsampled by systematic random sampling using the microscope's stage Vernier and images were at magnification of x100. The numerical volume density of the structures was estimated using Stepanizer a stereology software by the counting frame, the optical dissector counting rule was applied with counting in whole cell found inside the counting frame or allowance border excluding the forbidden one, the hepatocyte was track from reference line to look up section

Nv (Hc) $= \frac{\sum_{i-n}^{n} Q(Hep)}{\sum_{i-n}^{n} p(ref)} . \frac{p}{a-h}$

Where **Nv** = The number of hepatocytes displayed hitting the grid

P (ref) = The No, of points hitting the reference space, here whole liver sections.

P=Represent the total number of point

a=Represent the area of counting frame

h=Detonate the height of the dissector

The reference space comprise of all component of liver tissue connective tissue was avoided, the same process was done for the kupffer cells

3.13.4 Correction for tissue shrinkage during stereological analysis

All the stereological size estimators may be affected by shrinkage which may occur during histological processing of the tisues this include volume and density .The measurements were made to quantify shrinkage caused by fixation and histological procedures.The volume of removed fresh livers was calculated by displacement method (immersion method) and the liver volume which was obtained by cavarieli principle .Then shrinkage volume was then calculated as follows

Volume shrinkage = 1 – [volume after÷volume before)

3.14 Data management, analysis presentation

Data from the data sheet was put into similar soft copy data sheets in Microsoft excel then transferred to statistical package for social sciences (SPSS) software version 25.0 for analysis .The data was statistically evaluated by use of one-way analysis of variance (ANOVA) which compared the group means and group with had statistical significant ($P< 0.05$) were further tested with sheffe multiple comparison of liver volume and numerical volume densities for control group ,Paracetamol induced group and treatment group and the group that had significant ($P<0.05$) was tested using. Mann whitny U value was used to compare the means of the various stereological parameters of each group with controls. The parameters measured were expressed as mean ± CI (confidence intervals at 95%). The finding was presented using tables, histogram, and bar graph

3.15 Ethical approval

The ethical approval to carry out this study was obtained from Jomo Kenyatta animal research and ethics committee (Letter of approval attached as appendix IV) after undergoing a mandatory training in animal handling. In conducting the study all procedures were was conducted as per the protocol and the Guidelines for Care and Use of Laboratory Animals in Biomedical Research (Guidelines & Kenya, 2016) this guideline were adhered, and the study experimentation protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethical Committee (JKUAT AEC). The animals were only used once in the experiment. All animal were sacrificed using humane end points at the end of the study based on prescribed in Leary *et al.*, (2013) Protocol.

CHAPTER FOUR

RESULTS

4.1 The Gross morphometric findings of the liver

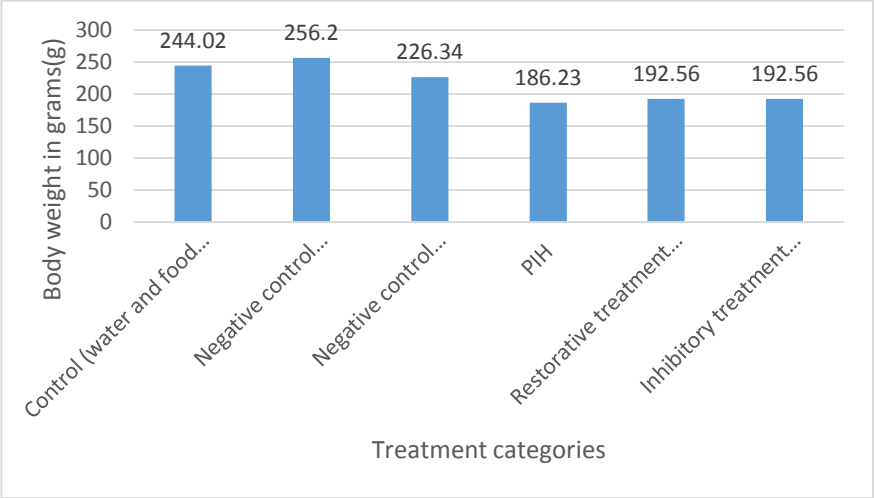
The study findings on the liver gross morphometric include the weight, length, width and thickness among the controls and treatment groups of restorative and inhibitory groups

4.1.1 Comparative mean terminal body weights (MTBW) and liver weights among the negative, positive controls and treatment groups.

It was observed that mean terminal body weights of the negative control (water *ad libitum* and feeds) groups was 244.02±0.12, 256.20±.06, that of the positive control (**Liv-52** plus 0.5% DMSO) was 226.23±.34 and PIH group 186.23±.46. It was observed that mean body weight for PIH group was reduced when it was compared with control groups, while the mean terminal body weight of restorative and inhibitory group (192.54±.03, 192.54±.62) was observed to be increasing when it was compared to PIH group **(Figure 4.1**).

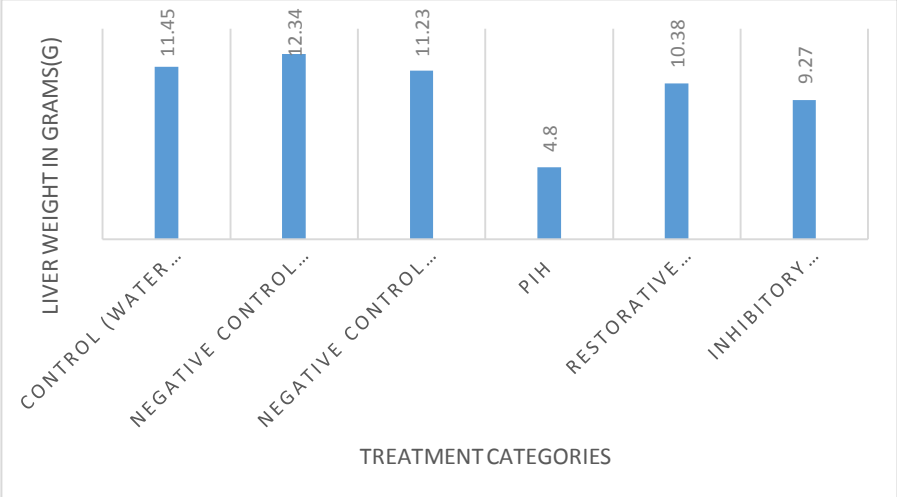
The mean liver weights for the negative control group (water *ad libitum* and feeds) was 12.34±.07 and (food water plus 0.5% DMSO) was 11.45±0.45 while the positive controls was (**Liv-52** plus 0.5% DMSO) 11.23±.23 and (PIH) 4.80±.08, while the mean terminal liver weight of restorative and inhibitory was (10.38±.06) and (9.27.±.24) respectively, which was observed to be increasing when it was compared to PIH group (**Figure 4.2**).

There was statistical significant different ($P<0.05$) in the means terminal body weights and liver weights in PIH group compared to controls groups, while there was no statistical significant ($P>0.05$) observed among control groups of no intervention, 0.5% of DMSO and Liv-52 500mg plus 0.5% DMSO.



Key; Values are presented as mean ± standard error of mean (n=5) PIH-Paracetamol induce hepatotoxicity, DMSO-dimethyl sulfoxide

Figure 4.1: Shows comparative mean terminal body weights trends among the control groups.



Key; Values are presented as mean ± standard error of mean (n=5) ***PIH****-Pcm induce hepatotoxicity, DMSO-dimethyl sulfoxide.*

Figure 4.2: Shows comparative means of the total liver weight trends of the treatment groups against control

4.1.2 The mean total liver weight (WIM) to the mean terminal total body weights (MTBW) ratio express in percentage in restorative group against the control.

Comparing the total liver weight and body weight ratio express as the percentages of total liver weight ratio to body weight ratio ,it was observed to be low in the positive control group (PIH group) 3.87%±0.028, while for **Liv-52** treatment groups was observed to be increasing with increasing dose of **Liv-52** 100mg (5.12%±0.13),**Liv-52** 200mg (5.50%±0.26),**Liv-52** 300mg (5.61%±.44) and **Liv-52** 500mg (5.26%±0.13) when it was compared to control group (5.31%±0.51). There was statistical significant difference ($P<0.05$) in PIH group as compared to control groups. However there were no statistical significant difference ($P>0.05$) among the group treated with varying doses of Liv-52 when was compared to control group and varying dosages (**Table 4.3).**

By comparing the liver volume (WIM),it was observed that, for PIH group was low (4.80±0.08) when it was compared to control group (12.02±.70) ,while the mean volume of **Liv-52** treated with varying dosages was observed to be normal with **Liv-52** 100mg (9.**55**±.07) **Liv-52** 200mg (10.825±.59),**Liv-52** 300mg (11.375±.53) and **Liv-52** 500mg (10.825±.84) when compared to that of the control group .The results indicated that there was significant difference ($P<0.05$) among PIH group when it was compared to control group ,while there was no statistical significant difference ($P>0.05$) among groups treated with varying doses of Liv-52 compared to control group, subsequently there was no significant different ($P>0.005$)among the groups treated with varying doses of liver care(**Liv-52**) (**Table 4.3)** .

Table 4.1: Shows comparative mean of total liver weight, percentage ratios of TLW to TBW and the mean liver volume in restorative groups against the control

	Restorative study groups according to doses					
Liver measurement	**Control group**	**PIH(1500mg/kg/bwt)**	**Liv-52 (100Mg/Kg/bwt)**	**Liv52 (200Mg/Kg/bwt)**	**Liv-52 (300Mg/Kg/bwt)**	**Liv-52 (500Mg/Kg/bwt)**
Mean TBW(g)	226.36±.23[a]	126.31±.23[b]	182.03±.32[a]	192.72±.23[a]	196.96±.23[a]	200.57±.02[a]
Mean LW (g)	12.02±.70[a]	4.80±0.08[b]	9.32±.35[a]	10.60±1.59[a]	11.050±.84[a]	10.550±.38[a]
Mean LBWR (%)	5.31%±0.51[a]	3.87%±0.08[b]	5.12%±0.1[a]	5.50%±0.26[a]	5.61%±.44[a]	5.26%±0.13[a]
Mean LVW (WIM)	12.22±1.63[a]	4.67±0.07[b]	9.55±.26[a]	10.82±1.53[a]	11.37±1.53[a]	10.82±.844[a]

*Key; All values are expressed as the mean ± the standard error of the mean (SEM).The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5) PIH;paracetamol induce hepatotoxicity,TBW-terminal body weight,.LW-liver weight ,LBWR-liver body weight ratio ,,: a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test, b indicates values that were significant different (p <0.05) from the PCM induced group using ANOVA in Tukey test on post hoc t –test,*Indicates significant different (p<0.05)* **.**

4.1.3. The liver gross morphometric finding in the restorative group against the control

On the gross morphometric findings on the liver sizes, it was observed that there was marked reduction on mean liver sizes for PIH group (positive control) with a width of (2.07±0.090, length (2.12±0.05) and thickness of (0.925±0.05) when it was compared with negative control group with width of (2.30±0.08) length (2.47±0.09) and thickness (1.12±0.09), there was noticeable similarities and increment on liver dimension of Liv-52 restorative group **Liv-52** 100mg width (2.27±0.050), length (2.22±0.050), thickness (1.07±0.095), Liv-52 200mg width (1.07±0.09), length (2.27±0.050), thickness (1.07±0.095), **Liv-52** 300mg width (2.22±0.095), length (2.35±0.057), thickness (1.35±0.05) and Liv-52 500mg(1.35±0.12) when it was compared to control group (**Table 4.4**) .There was statistical significant difference (P< 0.05) in the means liver dimension of

PIH group when it was compared to control group. However there was no significant difference (P>0.05) observed on means liver dimension among Liv-52 treatment groups with varying dosages and control group (**Table 4.3**).

Table 4.2: Shows comparative mean liver sizes of the restorative group against that of the control

	Restorative study groups according to doses					
Liver sizes	***Control***	***PIH(1500 mg/kg/bwt)***	***Liv52(100mg/kgbwt***	***Liv52(200mg/kgbwt***	***Liv52 (300mg/kgbwt***	***Liv52 (500mg/kgbwt***
Liver width	2.30±.08[a]	2.07±.09[b]	2.27±.05[a]	2.25±.05[a]	2.22±.09[a]	2.40±0.08[a]
Liver length	2.47±.09[a]	2.12±.05[b]	2.22±.05[a]	2.27±.05[a]	2.35±.05[a]	2.60±0.08[a]
Liver thickness	1.12±.09[a]	0.925±.05[b]	1.07±.09[a]	1.07±.09[a]	1.35±.05[a]	1.35±0.12[a]

*Note; All values are expressed as the mean ± the standard error of the mean (SEM).The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5) PIH-Paracetamol induced hepatotoxicity, a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test, b indicates values that were significant different (p <0.05) from the PCM group using ANOVA in Tukey test on post hoc t –test,*Indicates significant different (p<0.05*

4.1.4 Comparative mean ratio of the total liver weight to the terminal total body weights (MTBW) express in percentage in inhibitory group against that of the control.

The mean terminal weights of PIH group (200.33±0.26) were observed to be lower, where else in **Liv-52** inhibitory group **Liv-52** 100mg (231.50±0.23), **Liv-52** 200mg (234.24±0.65), **Liv-52** 300mg (242.82±0.26) and **Liv-52** 500mg (238.92±0.32) showed to be increasing with dosages. There was statistical significant differences ($P<0.05$) observed among PIH group compared to control group, while on other hand there was no significant differences ($P >0.05$) observed among Liver care (**Liv-52)** inhibitory groups and control group (**Table 4.5).**

In comparing the total liver weight and body weight ratio express as the percentage ,it was observed to be low in the positive control group(PIH) (3.21%±1.4) when it was compared to negative control group (5.35%±0.21), where else it was observed that, the mean liver weight to body weight ratio in percentage was increasing with **Liv-52** doses **Liv-52** 100mg (3.65%±0.68), **Liv-52** 200mg (3.72%±0.86), **Liv-52** 300mg (4.35%±0.84) and **Liv-52** 500mg (3.98%±0.45) which were noticed to be increasing with increasing dosages. There was statistical significant differences ($P<0.05$) for PCM induced group when it was compared to control group .However there was no statistical significant differences ($P>0.05$) among Liver care (**Liv-52**) inhibitory groups compared to control groups **(Table 4.5)**.

When comparing the mean liver volume (WIM) ,it was observed that PIH group was low (6.60±.96) as compared to control groups (12.23±0,85) , while **Liv-52** with PCM group was observed to be increasing **Liv-52** 100mg was (8.75±0.84), **Liv-52** 200mg (8.54±0.48), **Liv-52** 300mg (10.34±0.98) and **Liv-52** 500mg (9.54±0.67) with increasing doses . There was significant differences ($P<0.05$) among PIH group compared to control group, while on other hand there was no significant ($P >0.05$) of **Liv-52** inhibitory group compared to control (**Table 4.5).**

Table 4.3: Shows comparative means of terminal body weight, liver weight, liver weight to body weight ratio (percentage) and liver volumes between the inhibitory groups against the control

Gross measurement	Inhibitory study group according to doses					
	Control group	**PIH(1500mg /kg/bwt)**	**Liv52(100Mg/Kg/bwt)**	**Liv52(200Mg/Kg/bwt)**	**Liv52(300Mg/Kg/bwt)**	**Liv52(500Mg/Kg/bwt)**
Mean TBW(g)	232.33±.1[a]	200.31±.2[b]	231.50±.2[a]	234.24±.2[b]	242.82±.2[b]	238..97±.3[b]
Mean LW (g)	12.43±.9[a]	6.43±.9[b]	8.45±.6[bc]	8.68±.6[b]	10.52±.8[b]	9.45±.8[b]
Means LBWR (%)	5.35%±.2[a]	3.21%±1.4[b]	3.65%±.6[bc]	3.72%±.8[bc]	4.35%±.8[ab]	3.98%±.4[ab]
Mean LV (by DM in Mls)	12.23±.8[a]	6.6±.9[b]	8.54±.8[b]	8.75±.8[b]	10.34±.9[b]	9.54±.6[bc]

*Key; The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5, PIH-Paracetamol induced hepatotoxicity , LW- liver weight ,TBW-terminal body weight ,DM-Displacement method, a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test,b indicates values that were significant different (p <0.05) from the PCM group using ANOVA in Tukey test on post hoc t –test,*Indicates significant different (p<0.05)*

4.1.5 The comparative means of liver width, length and thickness between the inhibitory groups and that of the control

It was observed that the mean liver dimensions for PIH group were remarkably lower, width (1.90±0.98, length (2.04±0.89) and thickness of (0.921±0.12) as compared to control group, width (2.33±0.70) length (2.45±0.07) and thickness (1.63±0.21) ,while liver dimensions of the rats treated with PCM 1500mg plus Liv-52 was high **Liv-52** 100mg the width (2.22±.89), length (2.21±0.07) , thickness (1.70±0.64), PCM 1500mg plus Liv-52 200mg the width (2.32±0.94), length (2.23±.65),thickness (1.84±93),PCM 1500MG **Liv-52** plus 300mg the width (2.34±.95), length (2.34±053), thickness

(1.84±0.94) and PCM 1500mg plus **Liv52** 500mg the width was (2.33±.89), length (2.33±.78), thickness (1.35±0.12) ,however it was observed to be increasing with varying doses of **Liv52** and Pcm group . There was statistical significant differences ($P < 0.05$) of mean liver dimension for PCM induced when it was compared with control group, while on other hand there was no statistical significant difference ($P>0.05$) observed in varying dose of **Liv-52** plus Paracetamol and control group (**Table 4.6).**

Table 4.4: Shows comparative means of liver width, length and thickness between the inhibitory groups and that of the control

	Inhibitory study groups according to doses					
Liver sizes	**Control group**	**PIH(1500 mg/kg/bwt)**	**Pcm+Liv52 (1500Mg/kgbwt)**	**Pcm+Liv52(200 Mg/kgbwt)**	**Pcm+Liv52 300Mg/kgbwt**	**Pcm+Liv52 500Mg/kbwt**
Liver width	2.33±0.7[a]	*1.90±0.98[b]*	2.22±0.8[a]	*2.32±.9[a]*	2.34±.9[a]	2.33±.8[a]
Liver length	2.45±.07[a]	2.04±0.89[b]	2.21±.07[bc]	2.23±.6[a]	2.34±0.5[a]	2.33±.7[a]
Liver thickness	1.63±.2[a]	0.921±.12[b]	1.70±.6[a]	1.82±.9[a]	1.84±.9[a]	1.82±.9[a]

*Key: a- The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5) PIH- Paracetamol induced hepatotoxicity, a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test, b indicates values that were significant different (p <0.05) from the PCM group using ANOVA in Tukey test on post hoc t –test*Indicates significant different (p<0.05)*

4.2 The Histo-morphological findings

The comparative histo-morphology of the liver in experimental groups and control groups.

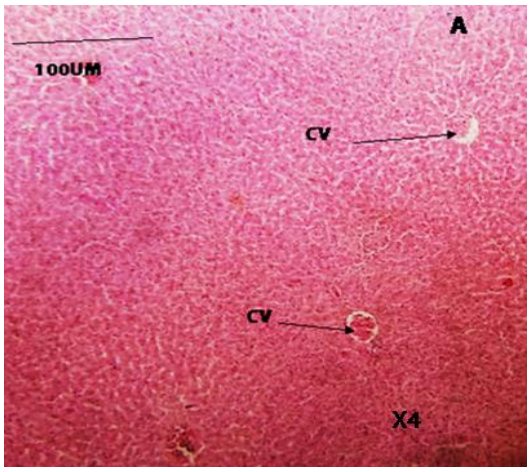
4.2.1 Liver histo-morphological findings among the control groups

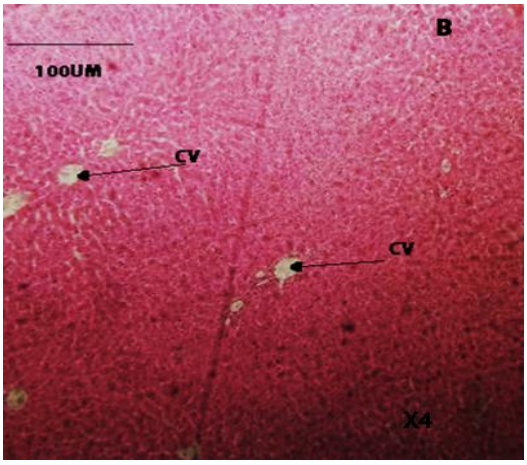
The comparative liver histo- morphology in the PIH (positive) group was different from that of the negative control group in that there was disrupted cell organization with areas of focal necrosis and disorganization of classical liver lobules in PIH group (**Figure 4.1 C**) while control groups (no intervention ,0.5% DMSO and Liv-52 plus 0.5% DMSO) showed normal liver cell arrangement with no areas of necrosis and normal of liver hepatic lobule (**Figure 4.1 A, B and D)** .

4.2.2 Liver histo-morphological findings between restorative groups and that of the control

The histo-morphological parenchymal features of PIH group showed prominent dilatations of sinusoidal capillaries with constricted central veins and kupffer cell infiltration (**Figure 4.2 A**) while that of the control and **Liv-52** (restorative group) varying doses showed well organized liver lobule without dilatation of sinusoids, areas of necrosis nor hemorrhages (**Figure 4. 2 B, C, D and E**).

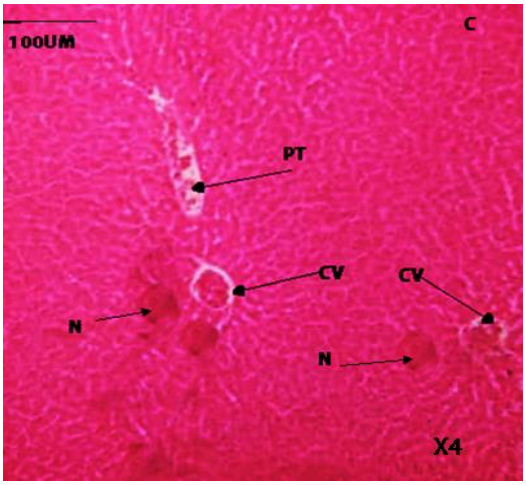
The histo-morphological stromal features of PIH group showed dilatation of sinusoids with many hypertrophied hepatocytes containing large cytoplasmic vacuoles with necrotic foci (Figure **4.3 A**). Additionally, there was hemorrhagic areas around para- central vein, dense cytoplasm with dark nuclei, suggestive of cellular degeneration. There was normal histo-morphology of the liver in the restorative treatment groups of **Liv-52** 100mg, 200mg, 300mg and 500mg (**Figure 4.3 C,D ,E and F)** in that there was no areas of hemorrhage, sinusoidal dilatation nor disruption of hepatic cord plates as well as diffuse or local necrotic areas, the same was seen among the control group (**Figure 4.3B).**

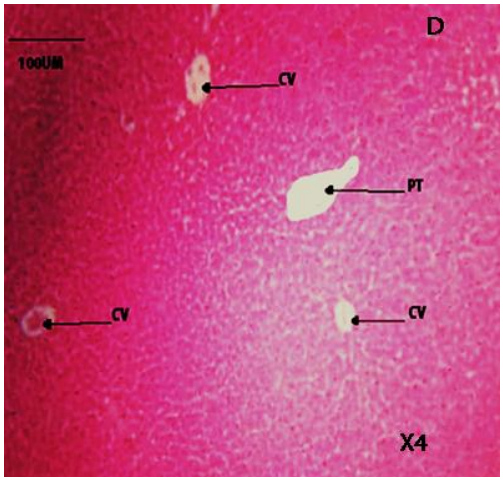




A: Control: showing normal portal triad (**PT)** normal central vein (**CV)**-well arrange hepatic lobule with no disruption with normal sunisoid (**S**), stain **H** and **E**

B Negative control; normal sinusoid(**S**) with normal central vein (**CV)**-central vein, well define hepatic lobule stain **H** and **E**

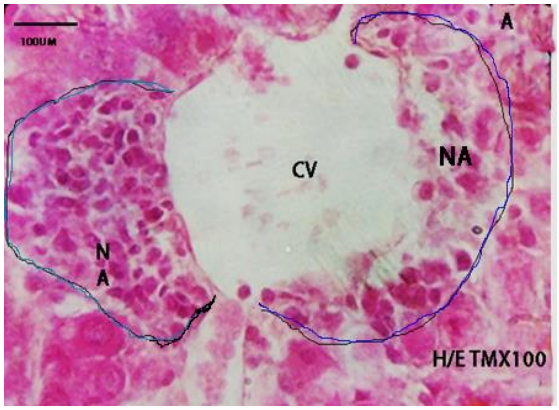


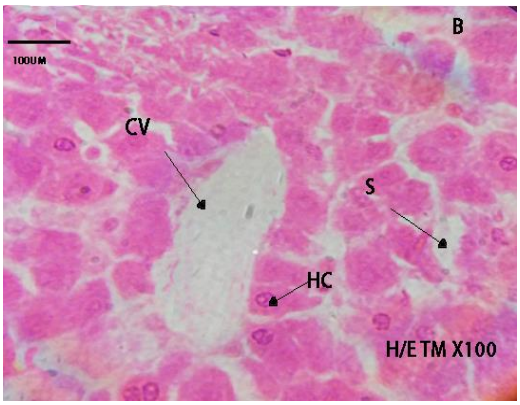


C; PIH induced group, foci necrotic region (N) disruption of hepatic lobule, stain **H** and **E**

D; Liv-52 + 5% DMSO group showing normal hepatocytes (HC) normal sinusoid (**S**) no disruption of hepatic lobule, stain **H** and **E**

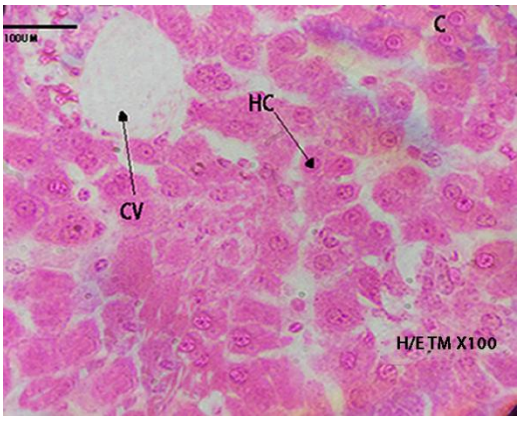
Figure 4.3: Photomicrographs showing liver histo-morphology among the control groups ;(A) No intervention; (B) 0.5% DMSO; (C) PIH and (D) Liv-52 and 0,5 %DMSO

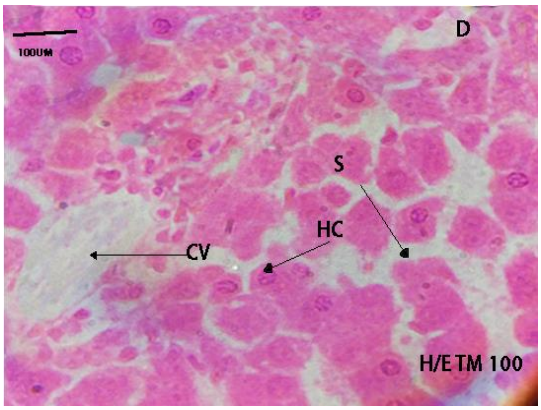




A; PCM induced group showing disarranged central vein (**CV**) foci necrotic region(**N**) dilated sinusoid (**S**) infiltration of kupffer cells(**KC),**stain **H** and **E**

B; **0,5% DMSO** showing normal hepatocytes cell**(HC)** normal sinusoid (**S**) and well define central vein (**CV),** ,stain **H**

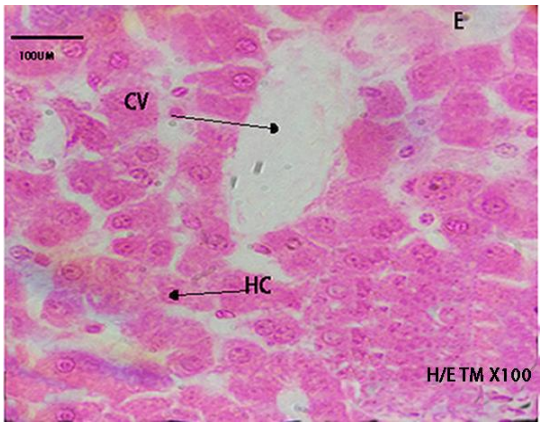


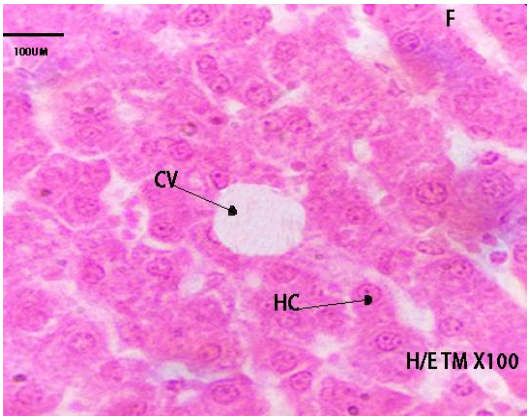


C; Restorative group liv52 100mg showing normal hepatocytes cell **(HC)** normal sinusoid (**S**) and well define central vein (**CV),** stain **H** and **E**

D ; Restorative liv52 200mg group showing normal hepatocyte cells (**HC)** with no disruption of central vein (**CV**) ,normal

Figure 4.4: Photomicrographs showing liver histo-morphological features in PIH group (A) and restorative group (B) DMSO 0.5%mg; (C) Liv-52 100mg and (D) Liv-52200MG.

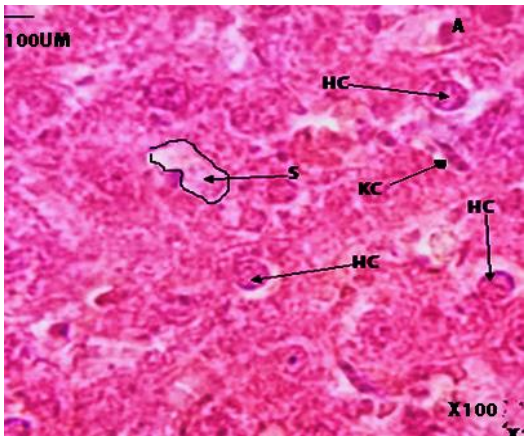


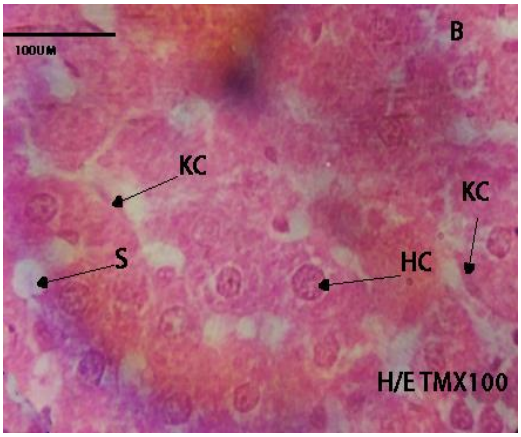


E;Restorative Liv-52 300mg group showing normal sinusoid**(S)** normal distribution of hepatocytes cells**(HC)** normal

F;Restorative Liv-52 500mg group showing normal sinusoid (**S)** normal distribution of hepatocytes cells**(HC) ,**normal central vein **(cv)**

Figure 4.5: Shows liver histo-morphological features among restorative groups (E) Liv-52 300mg and (F) Liv-52 500mg (stain with H and E)

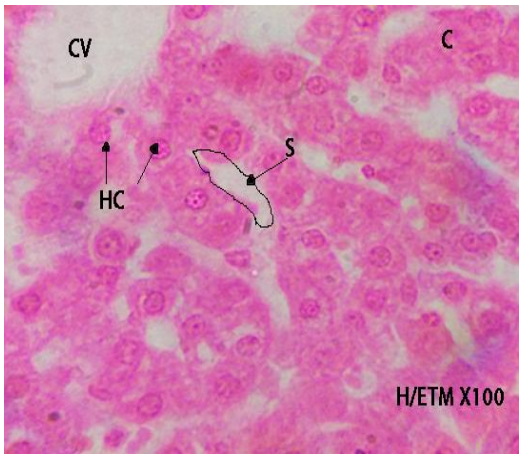




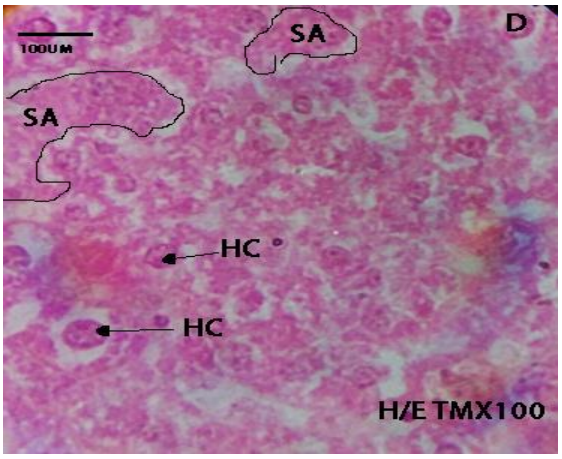
A; PIH group, showing deranged sinusoid (S) infiltration of kupffer cells (**KC)**, stain **H** and **E**

B; 0.5%DMSO, showing normal hepatocyte (**HC)** non dilated sinusoid **(S**) no area of diffuse or foci necrosis**, stain H** and **E**

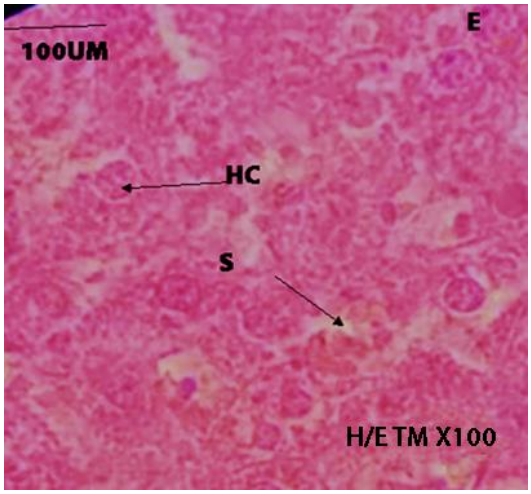
Figure 4.6: Shows liver stromal histo-morphological features between (A) PIH and (B) negative control (0.5% DMSO)



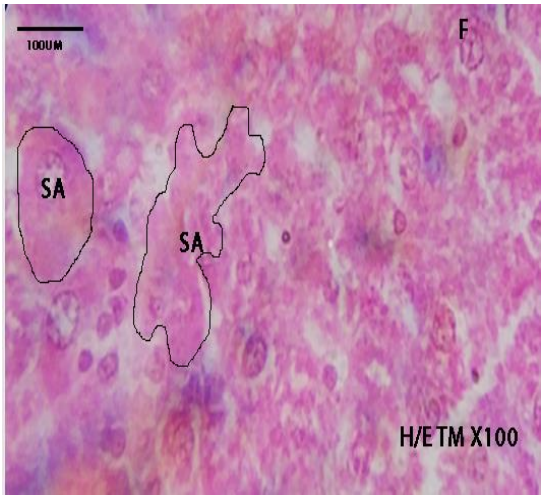
C; Restorative Liv52 100mg group, showing normal hepatocyte (**HC) non dilated sinusoid (S) no area of diffuse or foci necrosis, stain H** and **E**



D; Restorative Liv-52 200mg group showing normal sinusoid(**S)** normal distribution of hepatocytes cells**(HC)**no areas of diffuse or foci necrosis ,stain **H** and **E**



E;Restorative Liv52 300mg_group, showing normal hepatocyte (**HC) non dilated sinusoid (S) no area of diffuse or foci necrosis, stain H** and **E**



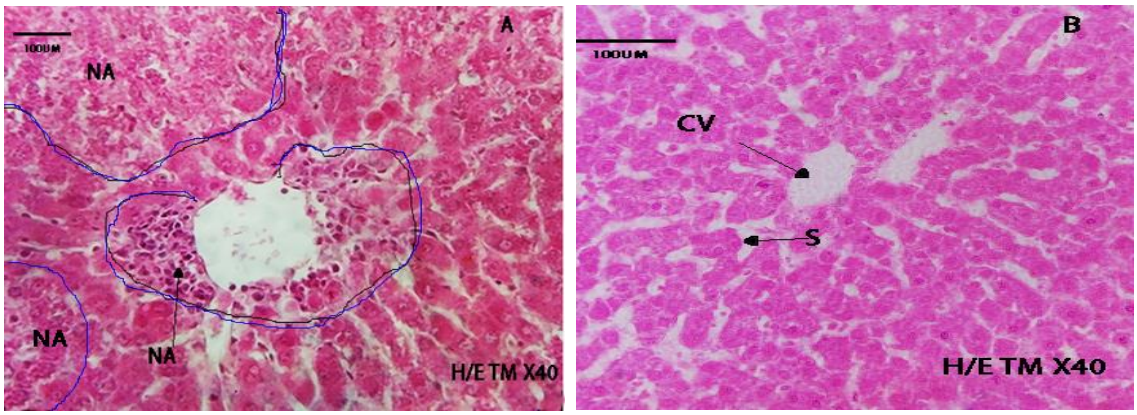
F;Restorative Liv52 500mg group, showing normal hepatocyte (**HC) non dilated sinusoid (S) no area of diffuse or foci necrosis, stain H** and **E**

Figure 4.7: Shows liver stromal histo-morphological features among restorative groups (C) Liv52 100mg; (D) Liv52 200mg; (E) Liv52 300mg and Liv52 500mg (stain with H and E)

4.2.3 Liver histo-morphological findings among the inhibitory groups and the controls

The liver histo-morphological features of the control group (**Liv-52** and 0.5% DMSO) and the inhibitory group (**Liv-52** 100mg and **Liv-52** 200mg **Liv-52** 300mg and **Liv-52** 500mg) showed similarities in that there was normal hepatocytes with no areas of necrosis, dilatation of sinusoids , disruption of central vein nor areas of diffuse or focal necrosis (**Figure 4.6 B,C,D** and **E**),while in PIH (control) group there was poor organization of the cells , central vein constriction with infiltration of kupffer cell (**Figure 4.6 A)**.

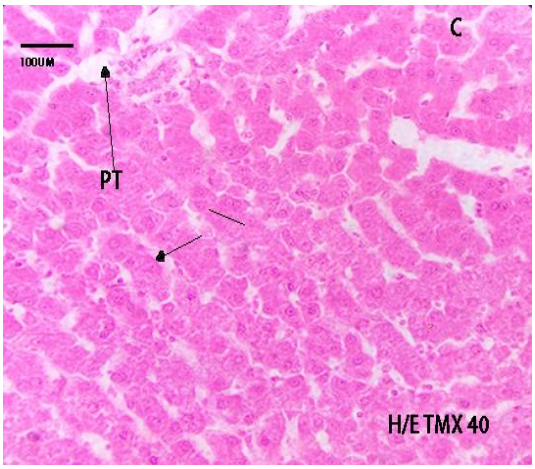
The histomorphology of the liver stroma among PIH group showed dilatation of sinusoids, few hypertrophied hepatocytes and liver cord disruption. (Figure **4.5 A**). Additionally, there was hemorrhagic areas around para- central vein and necrosis, on the other hand histomorphological features among the treatment groups showed normal liver cells with no dilatation of sinusoids, few infiltrations of kupffer cells, well-arranged liver cords with no areas of necrosis (**Figure 4.6 B, C, D** and **E).**



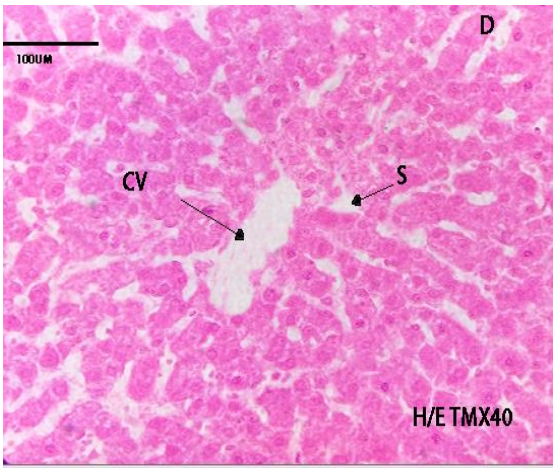
A;PIH showing (**HC) few** hepatocytes cells **–(S)**sinusoid, constricted central vein (**CV**) , stain **H** and **E**

B; Liv-52 **0.5% DMSO,** showing **HC**- hepatocytes cell,**S** –sinusoid, no necrotic foci(**NA**) ,stain **H** and **E**

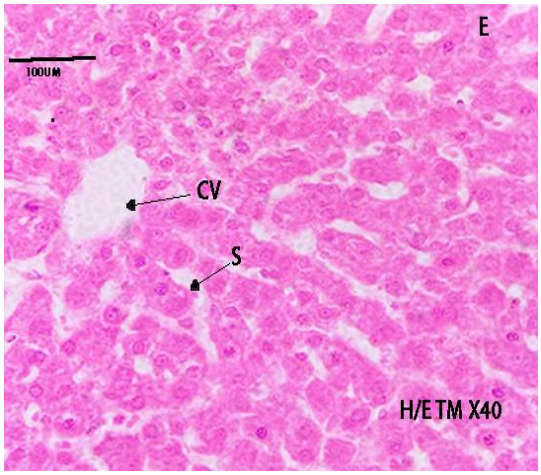
Figure 4.8: Shows liver histo-morphological features in; (A) PCM induced group; (B) Liv -52 and 0.5% DMSO (stain with H and E)



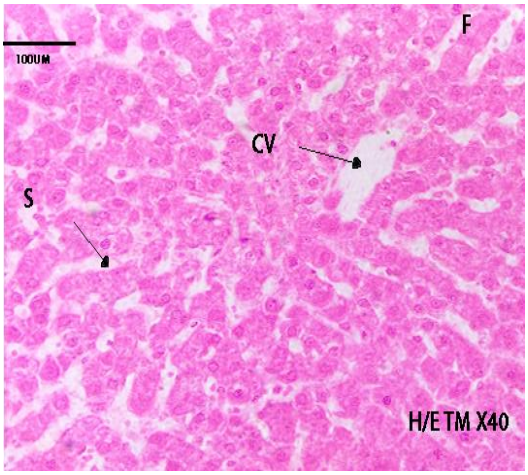
C; Liv-52 100mg showing normal hepatocyte cells **(HC)** normal sinusoid (**S)** no areas of hemorrhage, no areas of necrosis , stain **H** and



D; Liv-52 200mg showing normal hepatocytes cell (HC**)-** hepatocytes cell, sinusoid (**S),** ,stain **H** and **E**

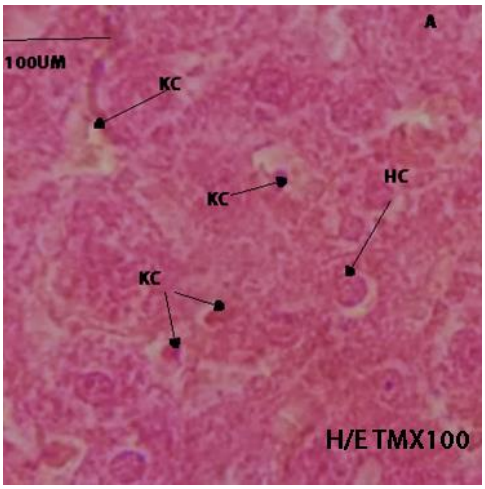


E; Liv-52 300mg showing normal hepatocytes cell(**HC)** normal sinusoid (**S)** stain **H** and E

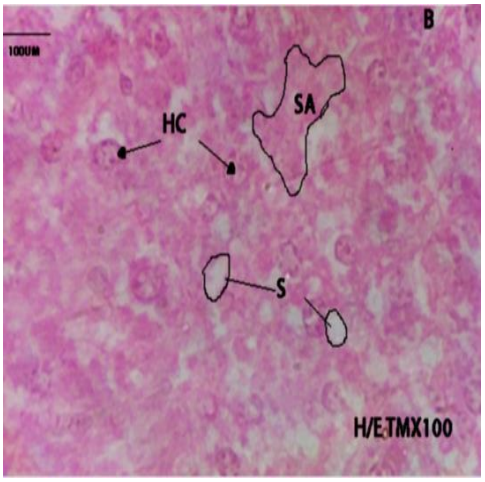


F;**Liv-52 500mg** showing **HC-** hepatocytes cell,**S** –sinusoid, mild necrotic foci(**NA**) ,stain **H** and **E**

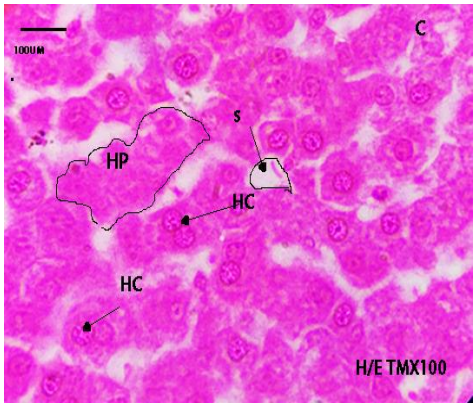
Figure 4.9: Shows liver histo-morphological features in :(C) Liv-52 100mg ;(D) Liv-52 200mg; (E) Liv-52 300mg and (F) Liv-52 500mg in inhibitory groups (stain with H and E)



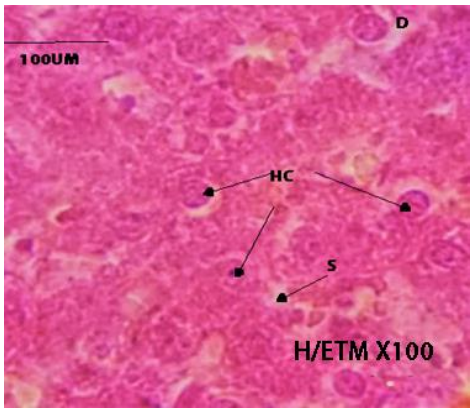
A; PIH showing (**HC) few** hepatocytes cell, infiltration of Kupffer (**KC**) ,stain **H** and **E**



B; Liv-52+0.5% DMSO showing (**HC) normal** hepatocytes cell,**S – normal** sinusoid, ,stain **H** and **E**

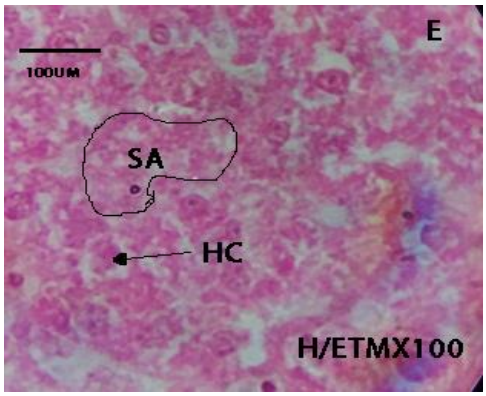


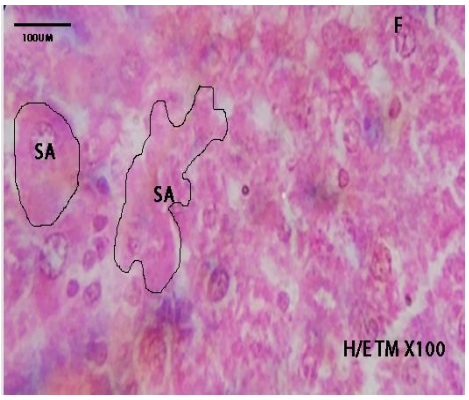
C;Liv-52 100mg showing (**HC) normal** hepatocytes cell,**S – normal** sinusoid, ,stain **H** and **E**



D;Liv-52 200mg showing (**HC) normal** hepatocytes cell,**S – normal** sinusoid, ,stain **H** and **E**

Figure 4.10: Shows liver stromal features among (A) Pcm induced, (B) Liv52 and 0.5% DMSO and the inhibitory groups (C) Liv-52 100mg, (D) Liv-52 200mg (stain with H and E)





E;Liv-52 300mg showing (**HC) normal** hepatocytes cell,**S – normal** sinusoid, ,stain **H** and **E**

F;Liv-52 500mg showing (**HC) normal** hepatocytes cell,**S – normal** sinusoid, ,stain **H** and **E**

Figure 4.11: Shows liver stromal features among the inhibitory groups (E) Liv-52 300mg and (F) Liv-52 500mg; (stain with H and E)

4.3 Histo-stereological findings

Comparative histo-stereology features of the liver in experimental and controls groups

4.3.1 Determination of coefficient error

Coefficient of Error (CE) was calculated in order to ensure precision of quantitative stereological parameters. It was calculated for both restorative and inhibitory effects group, as shown in the table. The lower the CE the higher confident hence the precision for the method applied was affirmed.

Table 4.5: Shows coefficient of error (CE) value for the whole liver volume in the restorative group.

	Restorative study group according to doses					
	CONTROL	**PIH**	**Liv-52 100mg**	**Liv52 200mg**	**Liv-52 300mg**	**Liv-52 500mg**
	0.0290	0.0317	0.0267	0.0299	0.0330	0.0324
	0.0309	0.0317	0.0272	0.0328	0.0288	0.0311
	0.0312	0.0340	0.0313	0.0302	0.0293	0.0354
	0.0297	0.0339	0.0317	0.0272	0.0298	0.0336
	0.0306	0.0341	0.0265	0.0269	0.0301	0.0323
Mean CE	0.03028	0.03308	0.02868	0.0294	0.0302	0.03295

KEY; The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5, CE-coefficient of error

Table 4.6: Shows coefficient of error (CE) value for the whole liver volume in the inhibitory groups.

	Inhibitory study groups according to doses					
	Control	**PIH**	**Pcm+Liv-52 100mg**	**Pcm+Liv-52 200mg**	**Pcm+Liv52 300mg**	**Pcm+Liv 52-500mg**
	0.019	0.0307	0.0247	0.0289	0.034	0.0304
	0.0209	0.0327	0.0272	0.0308	0.0284	0.0301
	0.0302	0.034	0.0313	0.0302	0.0283	0.0364
	0.0287	0.0329	0.0307	0.0282	0.0288	0.0346
	0.0316	0.0331	0.0265	0.0269	0.0341	0.0323
Mean CE	0,02608	0.0326	0.028080	0.0289	0.03072	0.03236

KEY; The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5, CE-coefficient of error

4.3.2 Comparative mean liver volumes among the restorative groups and the control by Cavarieli and water immersion method (WIM)

There was significant difference in the mean liver volumes of PIH by WIM (4.675±0.095) and cavarieli methods (4.13±0.313) compared to that of the control group (WIM) 11.225±.63 and cavarieli methods (11.77±1.33), (P<0.001) **(Table 4.8**) .On other hand there was slight increase in mean liver volume in Archimedes method and cavarieli method in treatment with varying doses of Liv-52 100mg, 200mg, 300mg and 500mg (6.370±.486,7.030±.337,7.55±.261 and 7490±.353 respectively).The results indicate that there was no significant difference (P>0.2) among PIH group and control group(**Table 4.8**)

Table 4.7: Shows comparative means of total liver volumes using (WIM) and cavarieli methods in restorative groups against the control

Stereological measurement	Restorative study group according to doses					
	Control group	**PIH**	**Liv-52(100mg/kgbwt)**	**Liv-52(200mg/kgbwt)**	**Liv-52(300mg/kgbwt)**	**Liv-52(500mg/kbwt)**
Means LV (*WIM)* Mls	12.22±.63[a]	4.67±.09[b]	9.550±.26[a]	10.825±.53[a]	11.375±.53[a]	10.825±.84[a]
Means LV by calverie methods (Mls)	11.77±.13[a]	4.13±.31[b]	9.37±.48[a]	10.03±0.33[a]	11..05±0.26[a]	10.70±0.35[a]
Shrinkage volume	0.11±.98a	0.26±.89	0.03±.76	0.08±1.25	0.03±.98	0.02±094

*KEY; The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5),PIH-paracetamol induced hepatocellular, LV- liver volume, DM-Displacement method, a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test, b indicates values that were significant different (p <0.05) from the PCM group using ANOVA in Tukey test on post hoc t –test,*Indicates significant different (p<0.05)*

4.3.3 The mean numerical volume densities of liver hepatocytes and kupffer cells among the restorative groups and the control

The mean numerical volume densities of the hepatocytes in PIH group was observed to be low (15345.00) while kupffer cells was high (2345.00) when it was compared with control group hepatocytes (22386.00) and kupffer cell (2345.00). On the other hand, the mean numerical volume densities of hepatocytes was observed to be increasing as the dose increases in **Liv-52** treatment group **Liv-52** 100mg (23876.00) **Liv-52** 200mg (25763.00), **Liv-52** 300mg (28643.00) and **Liv-52** 500mg (28764.00a), while kupffer cells was noted to be reducing as dose increases Liv-52 100mg (2173.00), **Liv-52** 200mg (2281.00), **Liv-52** 300mg (2034.00), and **Liv-52** (2245.00) when it was compared with control groups . There was significant difference in the mean numerical volumes of the hepatocytes and kupffer cells between PIH treatment group and that of **Liv-52** 300 mg and **Liv-52** 500 mg groups. Further, it was observed that the number of Hepatocytes in the **Liv-52** 300mg group was significantly different from that of the control group (**Table 4.10**).

Table 4.8: Shows comparative mean numerical volume densities (mg/ml) of hepatocytes and kupffer cells among the restorative groups and the control

Restorative study groups	Hepatocytes cells in (cells/mm3)	Kupffer cells(cell/mm3)
Control	22386.00	2356.00
PIH	15345.00	2345.00
Liv-52 100MG	23876.00	2173.00
Liv-52 200MG	25763.00	2281.00
Liv-52 300MG	28643.00a*	2034.00*
Liv -52 500MG	28764.00a	2245.00
Test statistic	24.267	16.448
P-value	<0.001	0.006

*Notes: Letter a in the superscript shows that there was a statistically significant difference with PIH at (P<0.05) using using Kruskall Wallis pairwise comparisons. * shows that the value was significantly different with that of the control at (p<0.05) using using Kruskall Wallis pairwise comparisons.*

4.3.4 Comparative mean liver volumes among the inhibitory groups and the control by Cavarieli and water immersion method (WIM)

The mean liver volume estimated via WIM and Cavarieli methods in the PIH group was observed to be lower 6.675±0.095, 6.12±0.31 respectively when it was compared to control group (12.23±1.63, 11.78±1.33).On other hand group that was treated with PCM 1500mg/kbwt concurrently with Liv-52 varying doses was observed to be increasing doses Liv-52 100mg, Liv-52 200mg, Liv-52 300mg and Liv-52 500mg recorded a mean liver volume (WIM) of (8.540±.264, 8.725±.53, 10.345±0.53, 9.546±0.84 respectively) cavareili method (8.38±0.48,8.02±0.33, 10.56±0.26, 9.46±0.35 respectively).There was statistical significant different (P<0.05) observed on PIH group when it was compared to control group .while there was no statistical significant different (P<0.05) observed with varying dosage of Liv-52 when it was compared to control group.

Table 4.9: Shows comparative mean liver volumes among the inhibitory group and that of the control using cavarieli and (WIM) method

			Inhibitory study group according doses			
Stereological Measurement	***Control group***	***PIH***	***Pcm+Liv-52 (100mg/kgbwt)***	***Pcm+Liv52 (200mg/kgbwt)***	***Pcm+Liv52 (300mg/kgbwt)***	***Pcm+Liv52 (500mg/kbwt)***
Means TLV (*WIM)* Mls)	12.23±1.6a	6.675±.09b	8.540±.26a	8.725±.53a	10.345±.53a	9.545±.84a
Means LV by calverie methods (ML)	11.78±1.3a	6.12±.31b	8.38±.48a	8.02±.33a	10.56±.26a	9.46±.35a
Shrinkage volume	0.045±1.23	0.083±.54	0.023±.987	0.091±1.234	0.019±.26	0.009±.28

*KEY; The test of significance was performed in rows. Values are presented as mean ± standard error of mean (n=5) LBR-liver Body Ratio,TLV-Terminal liver volume,TBW-terminal body weight ,DM-Displacement method, a indicates values that were significantly different (p <0.05) from the control using ANOVA in Tukey test on post hoc t –test, b indicates values that were significant different (p <0.05) from the PCM group using ANOVA in Tukey test on post hoc t –test,*Indicates significant different (p<0.05*

4.3.5 The comparative means volume densities of the hepatocyte and kupffer cell in inhibitory group

The mean numerical volume densities of the hepatocytes was observed to be low (13658.00) in the PIH induced group while the kupffer cells was high (3423.00) when it was compared to control group, where numerical volume densities of hepatocytes was high (23566.00) while for kupffer cell was observed to be low (2354.00). In **Liv-52** inhibitory group hepatocytes was increasing with increasing dose **Liv-52** 100mg (24426.00), **Liv-52** (24366.00), **Liv-52** (27362.00), and **Liv -52** (28438.00) when it was compared with control group. The number in the **Liv-52** 300MG and **Liv -52** 500MG was found to be significantly different with that in the PIH group. While the number of kupffer cells in the **Liv-52** 200 mg and **Liv-52** 300mg was found to be different significantly with that in the PIH group (**Table 4.11)**

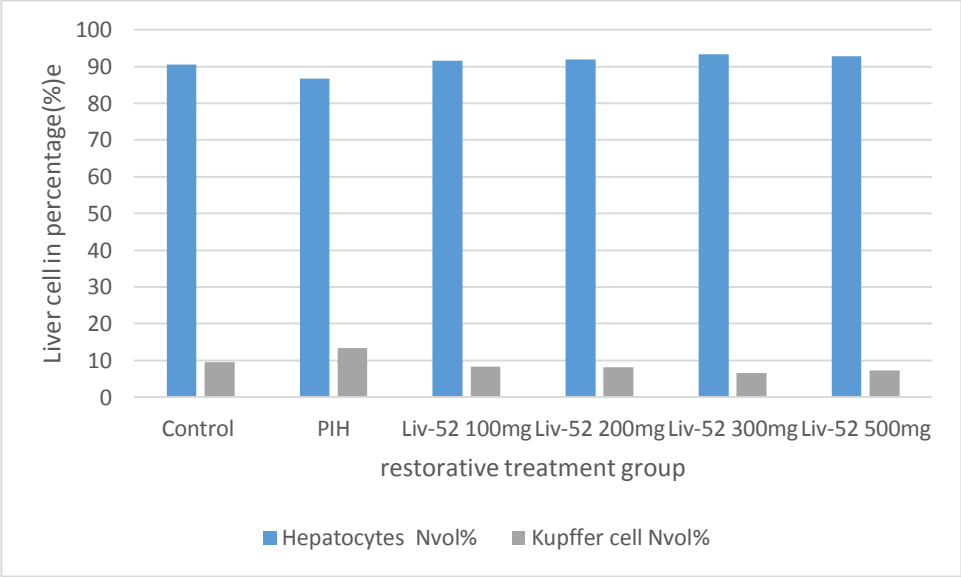
Table 4.10: Shows comparative mean numerical volume densities of the liver hepatocytes and kupffer cells in inhibitory groups and control group

	Numerical volumes densities	
Inhibitory group	**Hepatocytes cell(cellmm3)**	**kupffer cells(cell/mm3)**
Control	23566.00	2354.00
PIH	13658.00	3423.00
Liv-52 100MG	24426.00	2347.00
Liv-52 200MG	24366.00	2132.00a
Liv-52 300MG	27362.00a	1764.00a
Liv -52 500MG	28438.00a	2365.00
Test statistic	22.172	17.408
P-value	<0.001	0.004

*Notes: Letter a in the superscript shows that there was a statistically significant difference with PIH at (P<0.05) using using Kruskall Wallis pairwise comparisons. * shows that the value was significantly different with that of the control at (p<0.05) using using Kruskall Wallis pairwise comparisons.*

4.3.6 The percentage mean numerical volume densities of the liver hepatocytes and kupffer cells among the inhibitory group and the control

It was observed that the percentage mean numerical volume densities of hepatocytes in PIH group was low (86.74 %) when it was compared to control group (90.48%). On other hand the mean percentage volume densities for restorative group was noted to be increasing with Liver care dosage (**Liv-52**) 100mg (91.66%), **Liv-52** 200mg (91.87%), **Liv-52** 300mg (93.37%) and **Liv-52** 500mg (92.76%). The kupffer cells percentage mean numerical volume densities was seen to be high in PIH groups (13.26 %) when it was compared to control group (9.52%) but it was observed to be reducing with increasing dose of **Liv-52** 100mg (8.34%),**Liv-5**2 200mg (8.13%),**Liv-52**300mg (6.63%), and **Liv-52** 500mg (7.24%) (**Figure 4.13**).

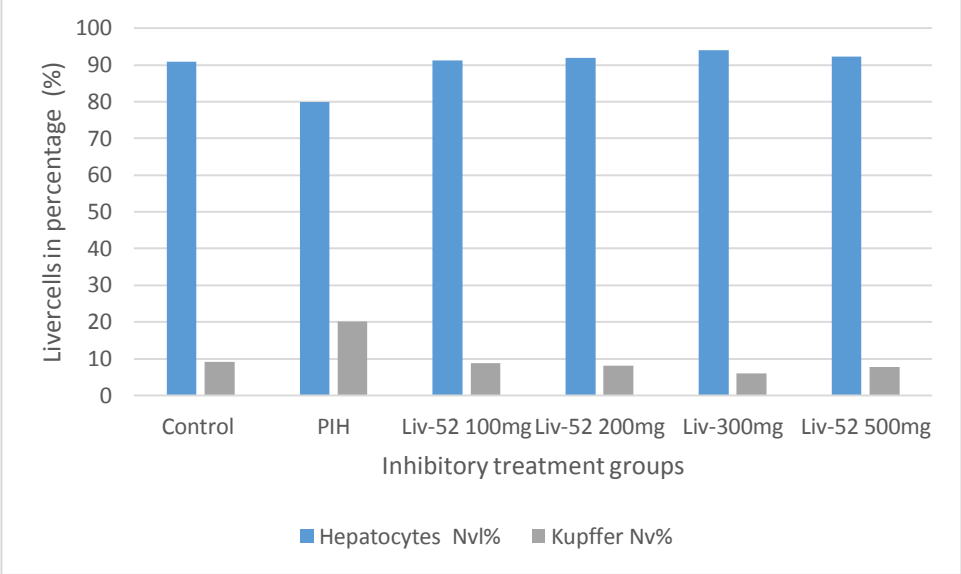


Key; Values are presented as mean ± standard error of mean (n=5) PIH-Paracetamol induce hepatotoxicity, Nv-numerical volume densities.

Figure 4.12: Shows percentage mean numerical volume densities of hepatocyte and kupffer cells in restorative groups against the control

4.3.7 The percentage mean liver volume densities among the inhibitory groups and the control

It was observed that the mean percentage numerical volume densities of hepatocytes was markedly reduced in PIH group (79.96%) when it was compared to that of the control group (90.92%). It was also observed that the mean percentage numerical volume densities in **Liv-52** inhibitory group was decreasing with increasing dose of **Liv-52** 100mg (91.23), **Liv-52** 200mg (91.95), **Liv-52** 300mg (93.94) and **Liv-52** 500mg (92.32) when it was compared to the control group. There was increasing values of mean percentage numerical volume densities for kupffer cells in PIH group (9.082%) when it was compared to the control group (9.082%), on other hand it was observed to be reducing among **Liv-52** and PIH groups **Liv-52** 100mg(8.77),**Liv-52** 200mg, (8.05) **Liv 52** 300mg(6.057)and **Liv-52** 500mg (7.68) (Figure **4.13**).



Key; Values are presented as mean ± standard error of mean (n=5) PIH-Paracetamol induce hepatotoxicity, Nv-numerical volume densities.

Figure 4.13: Shows percentage mean numerical volume densities of liver hepatocyte and kupffer cell in inhibitory groups and control

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Gross morphometric findings

5.1.1 The gross morphometric findings in the restorative groups

In this study it was observed that there was significant reduction in weight for the first 5 day of treatment when Paracetamol drug administered, however the weights was observed to increase when **Liv-52** treatment was introduced in varying dosages (**Figure 4.1**).There was statistical significant difference ($P<0.05$) in PIH group when it was compared with the control and the groups that received **Liv-52** varying doses. The same was observed by (Girish, Koner, *et al.,* 2009) when **Liv-52** was introduced in Paracetamol induced hepatotoxicity whereby physical wellbeing and feeding mode of the rats at the course of the experiment was noted (**Figure 4.1**). These also concur with same observation noted by Sandhir and Gill, (1999) and Afroz *et al., 2014).* Afroz *et al., (2014)* when they administered honey to reduce the effects of Paracetamol induced hepatotoxicity leading to reduction in weight for the group which did not receive honey treatment and these findings could be attributed to hepatotoxicity effects of Paracetamol while the group which received honey was observed to increase in weight trends. From the present study the morphometric and histo-cytoarchitecture findings suggested that hepatotoxic induced liver showed deranged liver width, length, thickness and volumes (**Table 4. 2).** The same results was seen by Mahmood *et al.,* (2014) and Girish, Koner, *et al*., (2009) in that the liver dimension was markedly reduced in Paracetamol induced hepatoxicity group , however, there was increase in liver dimensions when *Muntingia calabura* bark extract was introduced. In this study the groups treated with varying doses of **Liv-52** showed increase in liver dimensions which concur with study done by Sherif, *et al.* , (2017**)** but contrast with the study done by Girish, , *et al.,(*2009) which report increased liver volume and liver cells that could be due to deposition of fat causing fatty liver which occur after development of liver cirrhosis upon exposure to Paracetamol and alcohol .The same

protective effects of **Liv-52** was noted to be dose dependent (**Table 4.2**). There was increase in the liver sizes among **Liv- 52** (300mg) and **Liv-52** (500mg) when it was compared to **Liv-52** (100mg) and 200mg, this elude that the liver dimension was fully restored in 300mg and 500mg dose as compared to **Liv- 52** 100mg and **Liv 52** 200mg .The critical dose of **Liv-52** that restores and protect liver morphometric is between 300mg and 500mg.

5.1.2 The gross morphometric findings in inhibitory groups

The study findings on liver weight among the inhibitory groups did not have statistical significant different (P>0.05) when it was compared to control (Table **4.3**). The same observation was seen on the study done by Girish, Koner, et al., (2009) when they compared the liver weights on different agents including **Liv-52** that could have been due to **Liv-52** hepato-protection to the liver bio- physiological functional process .On other hand the liver length, width and thickness among the inhibitory groups was observed to have no significant different (P>0.05)when it was compared with the control group ,however there was significant difference (P<0.05) among the Liv-52 500mg and 100mg groups (**Table 4.4**). This study eluded that **Liv-52** has inhibitory effects to the Paracetamol hepatotoxicity which inhibit the end metabolite injury to the liver hepatocellular , same observation was noted by Saraswathy, *et al.* , (1998**)** , reported that **Liv-100** a generic of **Liv-52** has liver protection property in anti-tubarculous drugs induce hepatotoxicity when administered concurrently.

It was also observed that, the inhibition was dose dependant and observed with the dose of **Liv -52** 300 mg, 500mg compared to **Liv-52** 100mg and 200mg when they recorded increasing liver weights, length, width, thickness and volume.

5.2 Liver histo-morphological findings

5.2.1 Histo-morphological findings of the liver in the restorative group

The liver histomorphological findings in PIH group showed few dilated hepatocytes cells, hemorrhagic area and necrosis of liver at para-central vein (**Figure 4.1 A)**

This findings could be attributed to Paracetamol effects on the cytosolic ion of the hepatocytes leading to bursting of the cells , this agrees with the study done by Girish, *et al*., (2009) and Eesha *et al*., (2011).The Eesha *et al.*, (2011) observed Paracetamol high dose causes necrosis of the liver parenchymal which eventually leads to production of the liver enzymes disturbing physiological process of the liver while Girish, , *et al.*, 2009) observed that Paracetamol metabolites covalently bind to proteins, lipids or nucleic acids and produce oxidative stress by generating free oxygen radicals, depletion of glutathione and inducing lipid peroxidation, resulting in oxidative stress which affects mitochondrial function and inhibits movement of calcium from cytosol leading to death of hepatocytes and necrosis of surrounding liver parenchyma.

The current study showed that administration of varying doses of **Liv.52** exhibited a beneficial reversal and inhibitory effects of histo-morphological parameters as seen in (**Figure 4.2 C,D ,E** & **F; Figure 4.3 C,D, E** & **F**) same observation was observed by Dhawan, 1994 and Sandhir, 1999 exhibit same results, though the study used alcohol to induce hepatotoxicity but when **Liv-52** was introduced the histo-morphological features was observed to normalized. In the current study it was observed that **Liv.52** restore the cell of the liver parenchyma (**Figure 4.3 ,C,D, E & F,**) and stromal tissue(**Figure 4.4 C,D, E & F**) this observation agrees with study done by Sandhir, (1999**)** which indicate that **Liv-52** has hepato-protection properties. The mechanism behind the beneficial action of **Liv.52** in the present experimental study could be because of its potent antioxidant and other hepato-specific actions by six components formulation of **Liv-52** which was also cited by the study done by (Ghosh *et al.*, 2014; Maji *et al.*, 2013).

5.2.2 The liver histo-morphological findings in the inhibitory group

Liv-52 has shown to have inhibitory effects to paracetamol induced hepatotoxicity to the liver when both are administered concurrently. This study observes that when high dose of Paracetamol was given at same time with varying doses of Liv-**52**, there was similarities in the liver histo-morphological findings when compared with that of the control (**Figure 4. 8 C, D, E & F).** However**,** the liver histo-morphological findings of PIH group (Figure 4.8 **A)** showed few hypertrophied hepatocytes, dilated sinusoid and few kupffer cells infiltration, the same observation was reported by (Girish, Koner, *et al.*, 2009) . In addition, the study observed areas of hemorrhage and necrosis on the liver parenchyma. In the current study it was also observed that liver cells in the groups which received **Liv-52** 500mg and 300mg dose showed normal parenchymal and stromal histo-morphological features (**Figure 4.8 E & F**,) and **Figure 4.9 E & F**) similar to those of the control group (F**igure 4.8 B** and **4.9 B**). Moreover, **Liv-52** 200mg and 100mg showed few dilated hepatocyte and infiltration of kupffer cells with well-organized liver plates and no features of necrosis (**Figure 4.8 B; Figure 4.8 B**).

5.3 The stereological findings

5.3.1 Liver histo-stereological findings in restorative group

The study findings on mean total liver volume (cavarieli method) reduced among the PIH group (**Table 4.7)** compared with that of the control and the restorative group, this findings could be attributed to the effects of Paracetamol to the liver such as necrosis foci, dilated sinusoids, constricted central vein and disarrangement of parenchymal and stromal liver tissue (**Figure 4.8 A**). Study by Eesha et al., (2011, Essawy, *et al.*, (2017) and Dubey *et al*., (1977). Dubey et al., (1977) observed the same changes when alcohol was used to induce the liver hepatotoxicity and treated with **Liv-52.**

On other hand, the restorative group that received **Liv-52** for 16 days showed marked increase in total volume of the liver (cavarieli method), increasing hepatocytes and

reducing kupffer cells (**Table 4.8** and **Figure 4.12**) same observations were noted in a study done by Ghosh *et al*., (2014). These findings could be because of Liv-52 hepato-protection and restorative action. It was also noted that, the hepatocyte percentage restoration was high in **Liv-52** 500mg and 300mg (**Table4.8 and Figure 4.12**) compared to **Liv-52** 100mg and 200mg while the kupffer cells reduced as the dose increases **Table4.8 and Figure 4.12**). This could be attributed to **Liv-52** metabolic action inhibition of Paracetamol metabolites that could otherwise damage the liver hepatocytes and triggering of inflammatory cascade.

5.3.2 Liver Histo-stereological findings in the inhibitory group

The study found dose related effects on the mean total liver volume and the numerical volume densities following concurrent administration of Paracetamol and **Liv-52** (**Table 4.9**). It was observed that there was no difference in **Liv-52** 300mg and **Liv-52** 500mg doses (**Table 4.10**) , when it was compared to control group (**Table 4.10**).However, there was difference on mean total liver volumes and the numerical volume densities between the inhibitory groups treated with **Liv-52** 100mg and **Liv52** 200mg(**Table 4.10**) when compared with PIH group. The **Liv-52** 300mg and 500mg group showed increase in mean total liver volume and the numerical volume densities of the liver cells (**Table 4.10 and Figure 4.13),** this could be due to Livercare (**Liv-52)** inhibition property to the oxidative metabolites which are the main causes of free oxygen radical in the liver leading to hepatocytes and stromal injury which triggers the inflammatory process leading to increase in the number of kupffer cells (**Table 4.10 ; Figure 4.13).**

5.4 Conclusion

In conclusion the results of the study indicates that Paracetamol affects the liver gross morphometry and histo-morphology when used in high dosage and prolonged period of time.**Liv-52** may restore the histo-morphological structure of the liver in Paracetamol induced hepatotoxicity ,when either used in prolonged period of time or an overdose .On the other hand the **Liv-52** has showed to have inhibition property when both Paracetamol

and **Liv-52** is administered concurrently.in addition the study found that **Liv-52** has both histo-stereological restoration and inhibitory properties to the liver and most critical dose is Liv-52 300mg and 500mg.

5.5 Recommendation

The study recommends that

1. Paracetamol dose of 4gm per day in adults, 50-75mg/kgbwt in children and not for more than 6 days duration may be used.
2. The drug to be regulated to reduce the adverse side effects which may lead to liver toxicity hence liver failure.
3. That **Liv-52** may be safe when administered in treatment of acute hepatotoxicity and as well as chronic liver toxicity.
4. **Liv-52**, may be administered together with Paracetamol to counteract the effects of Paracetamol to the liver. The critical and effective dose of **Liv-52** is 300mg and 500mg.**Liv-52** has showed to have therapeutically approach to liver hepato-protection and hepato-inhibition.
5. Further investigation need to be done to ascertain effective component of **Liv-52** herbal formulation which leads to restoration and inhibition of liver toxicity.

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APPENDICES

Appendix I: A copy of Ethical approval

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
P. O. Box 62000-00200 Nairobi, Kenya Tel 0675870225 OR Extn 3209
Institutional Ethics Review Committee

April 19th, 2018

REF: JKU/2/4/896A

Kiplangat Rono Walter
Department of Human Anatomy.

Dear Mr. Kiplangat,

RE: RESTORATIVE HISTO-MORPHOMETRIC EFFECTS OF LIVARTHO ON ACETAMINOPHEN INDUCED LIVER TOXICITY IN ADULT ALBINO RATS

The JKUAT Institutional Ethics Review Committee has reviewed your responses to issues raised regarding your application to conduct the above mentioned study with you as the Principal Investigator.

The is to inform you that the IERC has approved your protocol. The approval period is from April 19th 2018 to April 19th 2019 and is subject to compliance with the following requirements:

a) Only approved documents (informed consent, study instruments, study protocol, etc.) will be used.
b) All changes (amendments, deviations, violations, etc.) must be submitted for review and approval by the JKUAT IERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the IERC immediately.
d) Any changes, anticipated or otherwise that may increase the risks to or affect the welfare of study participants and others or affect the integrity of the study must be reported immediately.
e) Should you require an extension of the approval period, kindly submit a request for extension 60 days prior to the expiry of the current approval period and attach supporting documentation.
f) Clearance for export of data or specimens must be obtained from the JKUAT IERC as well as the relevant government agencies for each consignment for export.
g) The IERC requires a copy of the final report for record to reduce chances for duplication of similar studies.

Should you require clarification, kindly contact the JKUAT IERC Secretariat.

Yours Sincerely,

DR. PATRICK MBINDYO
SECRETARY, IERC

J.K.U.A.T
DIRECTOR,
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Appendix II: Publication

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) e-ISSN: 2278-3008, p-ISSN: 2319-7676. Volume 14, Issue 4 Ser. I (Jul – Aug 2019), PP 44-51 www.Iosrjournals.Org DOI: 10.9790/3008-1404014451 www.iosrjournals.org 44 | Page

Histological and Morphometric Effects of Liv- 52 on Acetaminophen Induced Liver Toxicity in Adult Albino Rats Rono .k walter 1*, Kweri j Kariuki1, Kibe G. Kafanya1,Thuo Rueben 1, Kanyoni j.Mwangi 1 *(1.Department Of Human Anatomy,schoolof medicine (SOMED) College of Health Sciences(COHES)Jomo Kenyatta University of Agriculture and Technology Kenya(JKUAT)P.O.BOX 6200 Nairobi Kenya) Corresponding Author: Rono .k walter* ***Abstract:*** *Paracetamol a commonly used analgesic has been associated with liver toxicity and resultant alteration of its histomorphology with eventual liver malfunctions. This hepatotoxicity has been shown to be as a result of prolonged use or overdoses of paracetamol. On the other hand LIV52 also known as liver care, an herbal formulation has been shown to have restorative effects on the liver induced hepatotoxicity. However there is paucity of data on its restorative histo-morphological effects on the acute induced liver hepatotoxicity or its chronic usage. In addition, Data on the restorative effects of LIV52 on the liver induced hepatotoxicity when used in varied doses is also lacking. This study aimed at determining the histo- morphological and morphometric restorative effects of varied doses of LIV52 following the liver hepatotoxicity induced with paracetamol. The research was conducted on November 2017 to July 2018.A total 25 rats of 150gms-170gms was included in the study and paracetamol drug was used to induced hepatotoxicity . A total of 30 rats was divided into 6 group, control group, paracetamol induced group, liv52 100mg group, liv52200mg group, liv52300mg group and liv52500mg group Daily weighing and feeding of rats was done, control group received DMSO alone for same schedule. Other group was induced with paracetamol for 5 days thereafter treated with liv52 of varied dose,100,200,300,500mg/kgbwt/day for 16 days while one group did not received the treatment , all groups was sacrificed after the experiment , liver was removed and weighed ,and morphometric measurement was determine by use of a ruler and caliper, while liver*

volumes was determine using displacement method (Archimedes principle).all liver was processed and stained with H.E stain for histological examination and the liver stromal tissue, hepatocytes cell ,kupffer cell ,central vein and portal triad was assessed using light microscope. For morphometric assessment, for paracetamol induced group, the percentage liver body ratio reduced significantly ($p<0.05$) as compared to control group ($p>0.05$), while there was no significant different ($p>0.05$ with the treatment with varying doses of liv52. In light microscopy Paracetamol induced group, shows dilated sinusoid capillaries, necrosis of paracentral vein and areas of parenchymal necrosis ,as compared to normal control group which had normal liver, liv52 treated group shows the varying features of healed liver parenchymal when was treated with varying doses. Hence the present study conclude that liv52 (liver care) has dose depend hepatoprotective effects in a paracetamol induced hepatotoxicity. ***Key words:*** *hepatotoxicity, liver volume and morphological changes*

Date of Submission: 10-07-2019 Date of acceptance: 25-07-2019

Appendix III; Copy of thesis approval form

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
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REF: JKU/2/11/HSM301-4008/2016

1ST NOVEMBER, 2017

KIPLANGAT, WALTER RONO
C/o School of Medicine
JKUAT

Dear Mr. Kiplangat,

RE: APPROVAL OF RESEARCH PROPOSAL AND OF SUPERVISORS

Kindly note that your MSc. research proposal entitled: **"RESTORATIVE HISTO-MORPHOMETRIC EFFECTS OF LIVARTHO ON ACETAMINOPHEN INDUCED LIVER TOXICITY IN ADULT ALBINO RATS"** has been approved. The following are your approved supervisors:-

1. Dr. Joseph Kweri
2. Dr. Ruben Thuo
3. Dr. Kibe Kafaya

Yours sincerely

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES
Copy to: Dean, School of Medicine
/cm