HEPATOPROTECTIVE ACTIVITY OF LIV-52 ON RIFAMPICIN AND ISONIAZID INDUCED LIVER TOXICITY IN ADULT ALBINO RATS (*RATTUS NORVEGICUS)*

BY

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my entire family for their immense and constant source of encouragement, financial and mental support. That is, my late father, Isaac Newton Libamila, my late mother, Anne Emuria Libamila and a special feeling of gratitude to my three brothers, Clyde Libamila, Rowland Libamila and Carson Libamila. I also dedicate this work to my close and personal friend, Bruno Okal for his constant hours of proofreading and keeping me on truck.

ABSTRACT

Liver toxicity refers to injury to the liver due to drugs, chemical or environmental toxins. Rifampicin (RIF) and Isoniazid (INH) are two main medicinal drugs used as first line regimen in the treatment of Tuberculosis. These drugs have shown to induce hepatotoxicity upon administration. Liv-52, a polyherbal formulation has been shown to have clinical use in the treatment of several liver disorders in the recent years. However, there is deficiency in data regarding the histological and morphological effect of Liv-52 and the accurate dose required to prevent liver toxicity arising from INH and RIF induced liver toxicity. The broad objective was to evaluate the hepatoprotective activity of Liv-52 against of INH and RIF induced liver toxicity. The study was conducted at Zoology and Human Anatomy departments in Maseno University, Kisumu County, Kenya. Posttest-only experimental study design was adopted. Adult albino rats with an average weight of between 150g to 250g were used in the study. A total of 24 rats were randomly allocated into 4 groups each group containing 6 rats. The Albino rats were fed on normal rat pellets and water ad libitum. A negative control group with no intervention, an experimental group of; INH 50mg/kgbwt and RIF 50mg/kgbwt (positive control); INH 50mg/kgbwt, RIF 50mg/kgbwt and Liv-52 155mg/kgbwt(LD Liv-52); INH 50mg/kgbwt, RIF 50mg/kgbwt and Liv-52 207mg/kgbwt (HD Liv-52) orally daily. A 21 days, the albino rats were sacrificed humanely, liver harvested and weighed. Blood samples were taken from each group for estimation of liver serum biomarkers. Thereafter, the livers were processed and stained with Haematoxylin and Eosin for histological examination. Excel sheet was used to enter data which was later analyzed through SPPS version 25. One-way ANOVA with post hoc Bonferroni was used to compare the data obtained from experimental and control groups. Histomorphological data was described based on liver sections observed microscopically. Significance levels was P value less or equal to 0.05 ($p \leq 0.05$). The results was presented in images, tables, and figures. There was a significant (p value < 0.0001) decrease in gross morphometric measurements of the weight, length, width, thickness in positive at control compared to hepatoprotective groups. In the hepatoprotective group, there was a significant ($p \leq 0.0001$) increase in mean final body weight in HD Liv-52(207mg/kgbwt) and slight increase in LD Liv-52(155mg/kgbwt) groups at 236.31±.63 and 208.33±.83 respectively compared to 184.78±.78 in positive control(RIIH 50kg/kgbwt) group. Additionally, the mean liver weight in HD Liv-52 group was significantly ($p \leq 0.0001$) higher at 11.40±.21 compared to 9.197±.26 in positive control group. The means of the liver gross morphometric measures were observed to be increasing in both LD Liv-52 (155mg/kgbwt) and HD Liv-52 (207mg/kgbwt) hepatoprotective groups as compared to positive control (RIIH 50kg/kgbwt) group. That is length of 50.82±.23, width of 41.86±.23 and thickness of 0.39±.01 in LD Liv-52 and length of 54.25±.24, width of 44.68±17 and thickness of 0.50±.00 in HD Liv-52. The liver sections in positive control group showed deranged histomorphological features, partially deranged histomorphological features were observed in low dose Liv-52 at 155mg/kg/bwt group while those in high dose Liv-52 at 207mg/bwt showed normal histomorphological features. After administration of Liv-52, the three selected liver biochemical parameters (ALT, AST and ALP) were above their normal ranges. In conclusion, the gross morphometric and histomorphological changes on the liver among adult albino rats indicates that Liv-52 was able to prevent liver injury caused by rifampicin and isoniazid, therefore, its recommended that its pharmacokinetics and pharmacodynamics be evaluated for use in human patients on Rifampicin and Isoniazid treatment.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ALB	Albumin
ALT	Alanine Transaminase
AST	Aspartate Transaminase
DPX	Dibutylpthalate Polystyrene Xylene
HIV	Human immunodeficiency virus
HRP	Horseradish Peroxidase
INH	isoniazid
Liv-52	liver care
LTBI	Latent Tuberculosis Infection
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
OD	The optical density
RIF	Rifampicin
TP	Total protein
WHO	World Health Organization
RIIF	Rifampicin and Isoniazid Induced Toxicity
LD Liv-52	Low Dose Liv-52
HD Liv-52	High Dose Liv-52

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CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatotoxicity refers to as harm to the liver or disruption of hepatic function induced by exposure to xenobiotic such as alcohol, drugs, chlorinated solvents, food additives, peroxidized fatty acids, fungal toxins, environmental toxicants and radioactive isotopes (Gulati *et al.*, 2018). The liver is the major organ for drug elimination and metabolism (Choijamts *et al.*, 2018). Anti-tubercular drugs such as rifampicin (RIF) and isoniazid (INH), Nonsteroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, some anti-cancer drugs and general anesthetic drugs are among the drugs contributing to liver toxicity (Zeinab, 2012).

Rifampicin (RIF) and Isoniazid (INH) are the two main drugs being used to treat Tuberculosis (TB) for 4-6 months (Zeinab, 2012). INH is an anti-mycobacterial drug which has been applied clinically for about 70 years and is still currently being used for TB treatment. It is a bactericide that prevents the mycolic acids formation in the cell wall of bacterial (Tayal et al., 2007). The ability to cause liver damage and even eventual hepatic failure during INH and RIF's management forms a major challenge (Li *et al.,* 2015). Despite of hepatotoxicity caused by these drugs, they are still first line regimen in the treatment of TB because of their high level of efficacy.

The duration of manifestation of liver toxicity vary between 1-25 weeks with an average of 12 weeks. Elevation of liver biomarkers has been reported to occur as early as first week and as late as 9^{th} month (Arif *et al.,* 2022). The level of liver toxicity is high in cases of other risk factors such: high alcohol consumption, HIV/AIDS (Sankar *et al.*, 2015). Additionally, the liver is prone to several diseases including hepatitis, allergic reactions, hepatic encephalopathy and non-alcoholic fatty liver disease among others (Gulati *et al.*, 2018).

In Kenya, INH and RIF are widely used in the treatment of diseases such as tuberculosis for a period of between six to nine months depending on the type of tuberculosis. Mycobacterium Tuberculosis is treated for six months while extra-pulmonary tuberculosis is treated for nine month (WHO, 2021). Some of the side effects of the drugs include; nausea, vomiting, dark urine, numbness, chest congestion and occasional palpitations (Li *et al.*, 2015).

Hepatoprotective activity refers to as liver protection from Hepatotoxins or counteracting the alterations in the antiradical defense mechanisms (Wang *et al.*, 2008). Plant extracts can be the best source of such antioxidants and mediate hepatoprotective activity. Several chemical constituents such as coumarins, phenols, monoterpenes, alkaloids glycosides, and xanthenes are found in liver protective plants (Bhawna & Kumar, 2009).

Medicinal plants are vital alternative complimentary sources for hepatoprotective agents, and their safety and efficacy have been shown against liver toxicity (Shabbir *et al.*, 2020). In relation to the scarcity of reliable liver-protective drugs in modern medicine, hepatoprotective drugs obtained from plants seem to have attractive alternatives (Sankar *et al.*, 2015). Liv-52 is a manufactured polyherbal formulation by Himalaya Drug Company commonly used for the diagnosis or treatment of various liver disorders.

The aim of this study was to evaluate the hepatoprotective effect of Liv-52 which may help prevent liver toxicity caused by anti-tubercular drugs in the treatment of TB. This will facilitate compliance to the medication hence help eradication the chronic infection.

1.2 Statement of the problem

Liver failure is among the main mortality causes in human population worldwide, about 2.4 million deaths yearly occur due to liver disease (Wong et al., 2018). Isoniazid and Rifampicin are major anti-tuberculosis drugs used to treat TB. However, Isoniazid and Rifampicin are known to cause liver toxicity, which is potentially fatal because it leads to liver failure (Choijamts et al., 2018). About 5-28% of patients under these anti-TB drugs, develop liver toxicity. Therefore, there is need for an affordable solution with reduced side effect to prevent hepatotoxicity caused by these anti-tubercular drugs (Shabbir *et al.*, 2020).

Herbal formulations and nutritional supplements from plants have been used for treatment of various diseases affecting human population (Sandhir & Gill, 1999). Specifically, Liv-52, a polyherbal formulation has been shown to have clinical use to manage several liver disorders in the recent years *(*Sankar *et al.,* 2015*)*. However, there is paucity of data regarding hepatoprotective histomorphological effects of Liv-52 and the accurate dosage required to produce the hepatoprotective effect to the liver parenchymal cells following Isoniazid and Rifampicin induced liver toxicity.

1.3 Justification

Due to the rising cases of liver toxicity in the recent years, particularly drug induced liver toxicity (Gulati *et al.*, 2018), there is need for an amicable preventive therapy to counteract this effect without worsening the condition. It is of great importance to generate the scientific data about the application of Liv-52 to aid in prevention and treatment of liver toxicity. The choice of Liv-52 herbal formulation was guided by its accessibility, affordability and availability to most of the population. It is accessed from Western Cosmetics Company available across the country at an average of ten Kenyan shillings per tablet. The present study employs the use of adult albino rats which belong to *Rattus norvegicus* species. It is guided by the fact that they were the initial species of mammals to be tamed for scientific research and they also share

similar biological characteristics with humans therefore they will replicate same results (Rono *et al.*, 2021). Moreover, they possess a minimal gestational span, hence it's convenient to acquire subjects for study (Sengupta, 2013). The albino rats were obtained from Zoology department where they were bred till maturity.

1.4 Significance of the study.

The data generated from the present study will aid in the evaluation of pharmacokinetics and pharmacodynamics of Liv-52 for use in human patients on RIF and INH treatment.

This will guide the medical practitioners to concurrently administer isoniazid and rifampicin together with Liv-52 in the treatment of tuberculosis to mitigate the adverse effect of liver toxicity induced by the drugs.

Moreover, the study will also be helpful to the community as it will reduce economic, social and financial burden resulting from bedridden patients hence increasing productivity.

1.5 Objectives

1.5.1 Broad objective

To evaluate the hepatoprotective activity of Liv-52 against Rifampicin and Isoniazid induced liver toxicity in adult albino rats.

1.5.2 Specific objectives

1. To determine the gross morphometric and histomorphological changes of Liv-52 on rifampicin and isoniazid induced liver toxicity among adult albino rats
2. To determine the dosage of Liv-52 required to provide hepatoprotective effect following administration of Liv-52, Isoniazid and Rifampicin among albino rats
3. To assess the changes in liver bio-chemical parameters (Alanine Transferase, Aspartate Transaminase and Alkaline Phosphates) following administration of Liv-52, Isoniazid and Rifampicin among albino rats.

1.6 Hypotheses

1.6.1 Null hypotheses

Ho_1 Liv-52 does not induce gross morphometric and histomorphological changes when administered against rifampicin and Isoniazid induced liver toxicity.

Ho_2 High dose Liv-52 does not provide hepatoprotective effect following administration of Liv-52, Isoniazid and Rifampicin among albino rats

Ho_3 Liv-52 does not induce significant changes on liver bio-chemical parameters following administration with Isoniazid and Rifampicin among albino rats.

1.6.2Assumption of the study.

Adoption of the adult albino rats in the study would replicate the similar effect on human based on the documented close association in terms of biology, mechanism features and function.

CHAPTER TWO

LITERATURE REVIEW

2.1 Gross histo-morphological effects of Liv-52 on INH and RIF induced liver toxicity among albino rats`

The liver can be damaged by several insults including: infections e.g. hepatitis virus non-infectious causes e.g. alcohol, drugs. Some of the drugs inducing liver injury includes anti-tuberculosis drugs such Isoniazid, Rifampicin, pyrazinamide, anti-epileptic drugs e.g. phenytoin and others such as paracetamol (Sivakrishnan & Pharm., 2019).

The liver is a major organ of the body and has a number of functions. Glucose metabolism is one of the functions which maintains blood sugar within normal levels. The liver excretes wastes such as urea, ammonia and uric acid. It also excretes bile pigments which are bilirubin and biliverdin. In terms of synthetic functions, the liver synthesizes fatty acids, albumin and coagulation factors. Moreover, it acts as a storage organ for vitamins and iron (Gulati *et al.*, 2018).

Important liver structures of human and rat are similar in relation to gross morphology and histological structure except for the number of lobes. The rat's liver has 4 lobes including: right, left, caudate and middle lobes. The round ligament is attached to the deep notch formed by the middle lobe while the left and middle lobes form a single lobe. On the other hand, the liver of a human being is a solid organ consisting of: right and left major lobes, and caudate and quadrate lobes which are minor *(*Kogure *et al.,* 1999*)*. The general histological structure of the albino rat liver is similar to that of human beings (Benachi *et al.,* 2014). Portal areas, have hepatic triad, which has a branch of the hepatic artery, one or more small branches of the portal vein and a small bile duct, with lymph vessels.

Isoniazid is an anti-tubercular medication therapy which possesses both bactericidal and bacteriostatic properties. Isoniazid (inactive form) is metabolized by being converted into an active compound by a catalase-peroxidase bacterial enzyme. Hydrazine, a metabolite of isoniazid is converted into a toxic compound by cytochrome p-450, causing hepatotoxicity. This inhibits mycolic acid production which is necessary for the synthesis of mycobacterial cell wall. Hydrazine induces cell death due to oxidative stress (Humayun *et al.,* 2017). Rifampicin is attributed to aggravate hepatotoxicity by producing high activity of amidase and therefore producing large amounts of acetyl-hydrazine from isoniazid.

In a study where mice liver sections were examined using a microscope(normal control group), the hepatocytes revealed normal appearance of liver parenchyma made of the central vein, hepatocytes plate and tracts with bile ducts and portal veins. however, the slide section of liver of the rifampicin and isoniazid only treated group revealed liver parenchyma made up of hypertrophied liver cells, with mild to moderate degeneration of vacuoles, inclusive of mild necrosis, mixed infiltration of inflammatory cells throughout parenchyma of liver with fatty changes (Dubiwak *et al.,* 2021). Additionally, mucosal glands were observed to be arranged in compact form, having hepatocytes with vesicular nuclei and abundant eosinophilic cytoplasm. The glands are separated by thin strands of fibrous tissue and near the basement membrane they have abundant basophilic cytoplasm and few bundles of fibrous tissue (Mujahid *et al.,* 2013).

Hepatotoxicity resulting from Rifampicin and Isoniazid causes ballooning degeneration, focal hepatocyte necrosis with minimal cholestasis. In addition, on examination of histopathology of liver it was noted that animals showed multifocal lower grade of periportal mononuclear infiltration of cell after being exposed to INH. Histological lesions ranged from hepatocellar decomposition and vacuolation in the peri-central vein region to significant RER proliferation

(Mitchell *et al.*, 1976). Additionally, Rifampin was noted to cause hepatocellular injury and enhanced hepatotoxicity induced by other anti-tubercular medications (Prince *et al.*, 2002).

In pharmacological research by Parameswari *et al* (2013) where protective effect of Ficus religiosa leaves on the hepatocytes was studied under electron microscope, after hepatotoxicity was induced by rifampicin, paracetamol and isoniazid, the following was observed; changes in the nucleus shape with outer and inner membrane fused together and oval in shape. Condensed chromatin masses and appearance of hepatocytes was also noted (Parameswari, 2013). Reported hepatocytes' death with mild necrosis, mild-moderate vacuolar change accompanied by vacuolar degeneration, inflammatory infiltration, degeneration of fat throughout the liver parenchyma were seen in the slide sections of liver of mice treated with RIF and INH only (Shabbir *et al.*, 2020).

Histological assessment of the liver tissues in the hepatotoxic model rats exposed to Isoniazid and Rifampicin revealed marked changes at the peripheral regions, hepatocytes' necrosis and degeneration, granular cytoplasm, pycnotic nuclei appearance, and rise in intercellular spaces. In the central areas, similar focal changes were also observed (Mujahid *et al.*, 2013).

In the above mentioned studies, the focus is mainly on the effects of RIF and INH on the histoarchitecture on the liver. There is no evidence concerning histomorphological changes of Liv-52 on INH and RIF induced hepatotoxicity. This study aimed to address and solve this gap.

2.2 The dosage required to provide hepatoprotective effect following administration of Liv-52, Isoniazid and Rifampicin among albino rats

The Liv-52 is a herbal preparation introduced in 1955 from Himalayan Drug Company located in India for liver protection. It is recognized globally by health practitioners for its role in treatment of liver related diseases. It is available in either tablet or syrup form. Each formulation contains eight active medicinal herbs i.e. Capparis spinosa 32 mg, Mandur bhasma

32 mg, Cichorium intybus 32 mg, Solanum nigrum 32mg, Cassia occidentalis 16 mg, Terminalia arjuna 32 mg, Tamarix gallica 16 mg and Achillea millefolium 16 mg (Sankar *et al.,* 2015). It is readily available in chemists, in different formulations. Solid formulation consists of 125mg, 250 mg and 500mg and preparation for children.

These herbs have vital hepatoprotective effects and have been applied for many years for therapeutic approach to healthcare by preventing the increase of lipid peroxidation and the reduction of antioxidants in rat liver tissue. These herbs have a vast medicinal applications ranging from restoring the metabolic function of the liver in several etiological forms of jaundice such as infective and chronic active hepatitis to drug-induced hepatitis and alcohol induced hepatic damage. It has also been shown to raise appetite, cure the hepatitis and other hepatotoxic drug regimen. In addition, it is a supportive treatment in the process of hemodialysis and also an important adjuvant with hepatotoxic drugs (Choijamts *et al.*, 2018).

Liv-52 contains a series of hepatoprotective ingredients which have broad spectrum of hepatoprotective properties. They possess s anti-oxidative effect that hepatocytes from DNA damage caused by free radicles. Prevents oxidative stress and apoptotic tissue damage (Ganesh *et al.,* 2022). There is increase of level of liver enzymes in blood after tissue damage. Liv-52 suppresses increase in level of Malondialdehyde which is a marker of lipid peroxidation and Superoxide dismutase which removes intracellular free radicles (Abd-Elbaset *et al., 2017*). Previous study reveals that liv-52 increases levels of serum glycerol phosphate oxidase, reduced glutathione and Glutathione s Transferase Liv-52 ameliorates inflammatory reaction in damaged liver parenchyma by reducing hepatic Myeloperoxidase Iroanya *et al.,* 2014).

In a study by Shabbir *et al* (2020), rats were supplemented with Maytenus royleanus leaves extract at the dose of 200 mg/kg body weight. Upon histological examination of liver tissues in rats, normal structure of tissue with absence of inflammatory cells within the central areas

was observed. Moreover, at 400mg of similar extract, revealed peripheral changes, granular cytoplasm and reduced intercellular spaces in relation to the controlled hepatotoxic rats (Shabbir *et al.,* 2020).

In another study to examine the efficacy of heptoplus, a polyherbal formulation to supplement for rifampicin and isoniazid induced hepatotoxicity, the rats given 100 mg/kg of heptoplus inhibited oxidative liver damage and caused restoration of normal serum liver bio-markers levels. Upon supplementation with a dosage of 100 mg/kg of Heptoplus, the histopathological analysis revealed normal architecture of liver (Sankar *et al.,* 2015).

In study where a dosage of 400 mg/kg of Ensete ventricosum Cheesman Extract was administered against, Rifampicin and Isoniazid Induced liver toxicity in Swiss Albino rat, it significantly restored the normal liver histo-architecture in adult albino rats i.e. showed normal parenchyma of the liver consisting of hepatocytes with portal veins, central vein, bile ducts and hepatic vessels,. This was after the rats being subjected to doses of both INH and RIF orally for 30 days to induce liver damage (Dubiwak *et al.,* 2021).

As per the above reviewed literature, there is no established dosage of Liv-52 required to provide hepatoprotective effect to the liver upon INH and RIF induced liver toxicity. This study aimed to address this gap by establishing the accurate dose of Liv-52 required to restore the liver parenchyma to normal histo-architecture.

2.3 The changes in liver bio-chemical parameters following administration of Liv-52, Isoniazid and Rifampicin among albino rats.

Hepatic damage caused by rifampicin and isoniazid results in significant changes in bio-chemical parameters of liver function. This includes bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, and oxidative stress markers such as thiobarbituric acid reactive substances, superoxide dismutase, glutathione and catalase (Jnaneshwari *et al.,* 2014).

Increased levels of serum ALT, AST, and LDH shows damage and necrosis of hepatocytes. Total bilirubin is used to evaluate liver function. The elevated levels of serum bilirubin may be as a result of decreased hepatic clearance or impaired liver function (Bernier *et al.,* 2007). Evaluation of total protein and albumin levels are also used to assess the liver function. In hepatoxicity, normal synthesis of proteins by liver cells is impaired leading to hepatocelluar damage (Thapa & Walia, 2007). The normal range of total protein is 5.5 g/dl to 8 g/dl when frequently estimated. In severe liver damage, the level of blood plasma protein is reduced.

Alkaline phosphatase enzyme is found in liver. Increase in its level is caused by bile liver damage, flow obstruction or certain cancers. An increase in serum ALT is more specific for hepatocellular injury compared to increase in aspartate aminotransferase (AST) which may also signify abnormalities in heart, muscle or kidney. *(*Marcovina *et al.,* 2000*).*

In a study on hepatoprotective activity of corm of Ensete ventricosum (Welw.) Cheesman Extract against RIF and INH caused liver toxicity in Swiss Albino rat, after daily administration of RIF's dosage of 150 mg/kg and INH dosage of 75 mg/kg for 30 days, liver toxicity was observed by marked rise in the level of serum liver parameters, that is AST, ALT and ALP. Moreover, RIF and INH only treated group of mice showed rise in level of total bilirubin which is attributed to the use of both RIF and INH that led to an increased rate of prevention of removal of bile in liver, an rise in lipid enzymatic canalization of liver and cytochrome P450 taking part in the effect synergy of INH and RIF (Dubiwak *et al.,* 2021).

In another study on hepatoprotective effects of Adenanthera pavonina (Linn.) against anti-tubercular drugs-induced hepatotoxicity in rats, there was observed increase in serum glutamate pyruvate aminotransferase, serum glutamate oxalate aminotransferase, ALP, LDH, cholesterol, serum bilirubin and liver weight while decrease in final body weight, total protein and albumin levels in rats. This was after a group of rats were subjected to oral administration

of INH and RIF at doses of 50 mg/kg/day each for a period of 28 days. This pointed out to toxicity effects of RIF and INH on liver of rats. In another study, on treatment of Tuberculosis with RIF and INH, elevation in levels of serum bilirubin in rats was seen, whereas serum levels of albumin and total protein (TP) were significantly reduced showing hepatocellular damage. A decrease in proteins synthesis was seen upon intoxication of the liver with hepatotoxicants (Mujahid *et al.,* 2013).

The liver biochemical parameters are necessary to determine the level of hepatoprotective effect achieved by the Liv-52 herbal formulation. The parameters also measure the hepatotoxicity effect of INH and RIF the liver histo-architecture. The current study intends to base on the biochemical parameters to evaluate the effects of Liv-52 on the liver histo-architecture by comparing the biochemical parameters between the experimental group and control.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area/setting

The study was done at Maseno University located in Kisumu County situated at Maseno Kisumu – Busia Road. Breeding, weighing, handling and administration of drugs was done at the department of zoology in the school of biomedical sciences because of the well-established animal houses and expertise in handling the albino rats. The tissues were harvested and examined in the histology lab in the Department of human anatomy and blood sample examination was done in the Department of Clinical Studies, Faculty of Veterinary Medicine at University of Nairobi.

3.2 Study design.

This was be a posttest-only true experimental study design, whereby an intervention was made and results compared between control and experimental groups.

3.3 Study subject.

The study subject was adult Wister albino rat's species of (*Rattus Norveginicus*) from a pure bread were bred in a condition which is microbiologically controlled for all the experiments and control groups. The albino rats were bred in cages which hold a maximum of 6 rats per cage. The Wister albino rats had long ears, short tail compared to their body size with wide-sized head as also suggested by (Rono *et al.*, 2021).The Wister albino rats (*Rattus norveginicus*) were preferred because of the; share 90% of the genome with human being, they have a relatively high survival rate, big body size as compared to mice but easy to take care of them, they have a short gestational span (4 weeks) hence easier to find the study subjects, they are also resilient in withstanding most of the study medicine, male are always larger as compared

to females approximately 450-650grams and 350-450 grams respectively (Sengupta *et al*., 2013).

3.4 Sample size determination

This was derived from modified "Resource equation method" since there is no known standard deviation in previous researches. (Arifin & Zahiruddin, 2017).

$$\mathbf{N = n \ X \ k}$$

$$\mathbf{n = DF/k + 1}$$

n- Number of animals in a single group

DF-Error of degree of freedom (10-20)

k- Number of groups

Sample size was calculated as follows.

k=4

=20/4+1

=5+1

=6

N=Total number of subjects of each group

$$\mathbf{N = n \ X \ k}$$

n=6 X 4

= 24

3.5 Sampling method

In order to ensure each group has a representative sample, simple random sampling method with replacement was applied. This was aimed to achieve each experimental unit has an equal chance of getting treatment. (Extra rats that met the inclusion criteria were used as replacements)

3.6 Selection criteria

3.6.1 Inclusion criteria

- Healthy and pure breed albino rats in the cage
- Animals with average weight of 150-250g were included in this study.
- Animals between 6-8 weeks of age. (this is the estimated age to have attained maturity and achieved weight of 150-250g)

3.6.2 Exclusion criteria

- Sick animals in the cage

3.7 Animal grouping

The 24 rats were assigned randomly into 2 major study groups i.e. negative control group of 6 animals and experimental group of 18 animals.

3.7.1 Negative control group

There were no intervention in this group. The 6 albino rats received water and rat pellets ad-libitum for a period of twenty one days and thereafter sacrificed humanely.

3.7.2 Experimental group

The 18 animals in this group were randomly allocated into 3 sub- groups each consisting of 6 animals. In addition to the water and rat pellets ad-libitum, the animals received INH, RIF and Liv-52 according to the experimental design below.

Experimental design

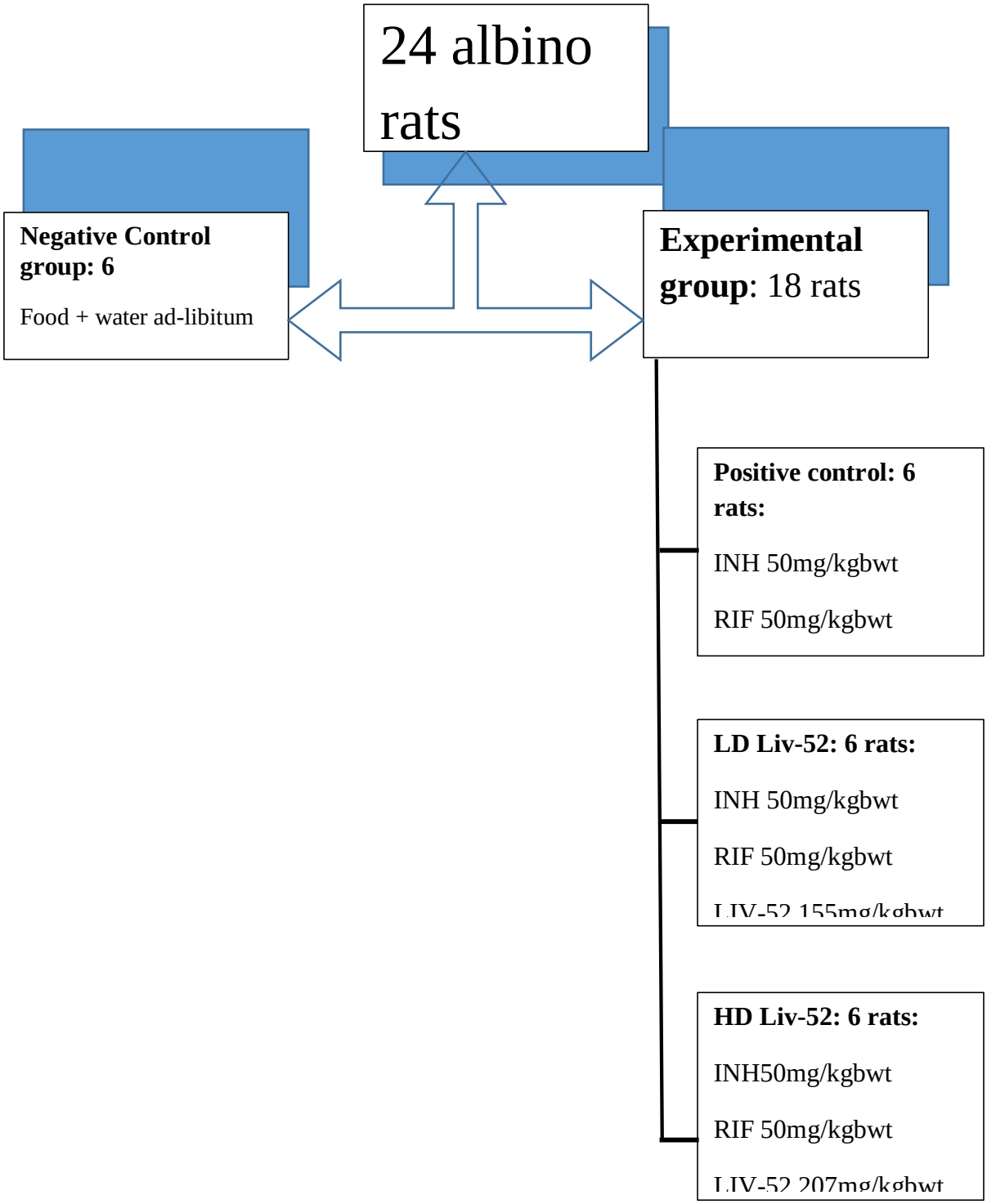

Figure 3.1: Flow chart on grouping of the albino rats

3.8 Feeding of rats

The rats were acclimatized for 1 week in an animal house (26±2°C) with 12h light and dark cycles. Acclimatization was done to avoid unwanted variations in data and potential complications. Animal feeds were obtained from Unga Feeds in Kisumu Town. The animals were fed with standard rodent pellets and water provided *ad libitum*. The albino rats were fed each morning at 0800 hours in their spacious polycarbonate cages.

Figure 3.2: Feeding of rats

3.9. Occupational safety and handling of animals

Principal investigator together with a trained research assistant handled the rats. Cleaning of the cages was done every morning before feeding and drug administration. The animals were also examined for their health status and head count taken. Occupational safety measures were applied while handling the animals i.e. wearing of gloves, lab coat and masks, use of spirit swabs in case of any scratch from the animals. Daily weights were obtained between 0800-0830hours. All procedures were performed in line with the Guide for the Care and Use of Laboratory Animals *(Albus, 2012).*

3.10 Acquisition of Isoniazid and Rifampicin

They were obtained from Kisumu County Referral Hospital. (Batch No. NRT2103A)

3.11 Determination of Isoniazid and Rifampicin dosage

3.11.1 Determination of Isoniazid dosage

To induce hepatotoxicity Isoniazid dosage of 50mg/kg/day was used for 21 days (Rana *et al.*, 2006).

Animal equivalent dose=50mg×rat weight

For example, if a rats=200grams

50mg×200/1000=10mg/day

3.11.2 Determination of Rifampicin dosage

To induce hepatotoxicity Rifampicin dosage of 50mg/kg/day was used for 21 days. *(Rana et al., 2006)*

Animal equivalent dose=50mg×rat weight

For example, if a rats=200grams

50mg×200/1000=10mg/day

3.12 Acquisition of Liv-52

The Liv-52 was purchased from Western Cosmetics. (Batch no. 722101195)

3.13 Determination of Liv-52 dosage

To provide hepatoprotective effect, liv-52 of adult human dose, which is 500mg, taken 6 or 8 hourly per day, was converted to animal equivalent dose.

Animal equivalent dose =human equivalent dose (Mg/kg) ×converting factor (6.2) (Shin et al., 2010).

E.g for liv-52 1500mg =1500/60 X 6.2 = 155mg/kg/day

For liv-52 2000mg = 2000/60 X 6.2 = 207mg/kg/day

3.14 Administration of Isoniazid and Rifampicin

Administering the doses of isoniazid was done daily using gastric gauge.

3.14.1 Materials for administration of Isoniazid and Rifampicin

Isoniazid, Rifampicin, 23 gauge 5ml syringe, gauge size 18 Gavages' needle, beaker 20 ml, deionized water, syringes, albino rats and tablecloth.

3.14.2 Procedure for administration of Isoniazid and Rifampicin

The drugs were given by use of gastric gavage needle i.e. the rats were wrapped with the tablecloth and then by one hand, held by the neck level by the help of the research assistant, the gavage tube was inserted into the mouth followed by pushing the 2mls drug solution into the stomach via the lavage. There after the gavage removed.

3.15 Administration of Liv-52

3.15.1 Materials for administration of Liv-52

Liv-52, 23 gauge 5ml syringe, gauge size 18 Gavages' needle, beaker 20 ml, deionized water, syringes, albino rats and tablecloth.

3.15.2 Procedure for administration of Liv-52

The drug were given by use of gastric gavage needle i.e. the rats were wrapped with the tablecloth and carefully held from the neck region using one hand. Then rested against the body of the researcher with the animal mouth facing forward. Then gavage needle gently inserted into the mouth of the animal turning it gently to circumvent the esophageal constrictions and

the cardiac sphincter. 2mls of drug solution was pushed into the stomach via the lavage and lastly the gavage needle then gently removed.

3.16 Humane sacrifice of animals and harvesting of the liver tissues

3.16.1 Materials for Humane sacrifice of animals and harvesting of the liver tissues

Albino rats, cotton gauze, 10 mls chloroform, 0.85% concentration of physiological saline, board for mounting, pins for mounting, a pair of toothed forceps, pair of scissors, 5% formalin solution, scalpel blade 25, 2 drip sets, surgical gloves, magnifying glass, gauge 20 hypodermic needle, weighing machine (electronic), specimen collection bottles and ruler.

Figure 3.3: Humane sacrifice of animals and harvesting of the liver tissues

3.16.2 Procedure

The cotton gauze was introduced into the bell jar after being soaked in chloroform. The rat was anaesthetized 10-15 minutes after the rat is put into the bell jar. After removing the rat from the bell jar, using mounting pins it was mounted onto the board on its dorsal side. The rat was then cut via the ventral medial side using a pair of scissors and forceps from the pubic symphysis to the manubrium. Through the left ventricle of the heart, the perfusion needle connected to the perfusion set was inserted. The blood cleared with 0.5% saline via the cardiac part of left ventricle (saline would flow by gravitational effect from drip sets). Saline drip removed, needle still in position thereafter introducing the fixative (formalin solution or glutaldehyde). The drip was removed from the heart together with the perfusion needle. The

liver was then excised, and immersed in a fixative-filled beaker to proceed for another twelve hours.

3.17 Blood sample collection

During the process of sacrificing the albino rats 4mls of blood sample was collected through the posterior vena cava.

3.17.1 Materials for blood collection

Surgical scissor, 21 - 25g needle, small glass rods, 1- 5ml syringe and blood sample collection tube.

3.17.2 Procedure for blood collection

After the albino rats were anaesthetized and the midline cut made, the liver was pushed anteriorly and the posterior vena cava seen between the kidneys. The syringe was inserted to collect the blood sample (Parasuraman et al., 2010). The blood sample were then tested for biochemical parameters within the groups.

Figure 3.4: Cardiac puncture in blood sample collection**.**

3.18 Bio-chemical parameters for assessing hepatoprotective activity of LIV-52

Biomarkers to assess liver toxicity following administration of Liv-52, Isoniazid and Rifampicin among albino rats. They include; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP).

Biochemical parameter	Normal ranges (µ/L)
ALT	14-30 µ/L
AST	45.7-80.8 µ/L
ALP	56.8-128 µ/L

Table 3.1: biochemical parameters of albino rats with their normal ranges

3.18.1 Principle of Liver biochemical parameters Analysis - AST, ALT and ALP

The analysis was done using AST/ALT/ALP ELISA KITs manufactured by Shenzen Mindray Bio-Medical Electronics Company Limited in China. The ELISA kits uses a method of Sandwich-ELISA. Samples or standards were adjoined to the befitting Microelisa stripplate wells and added to the proper antibody specific for AST/ALT/ALP pre-coated in the kits. A HRP-conjugated antibody designed for AST is adjoined to each Microelisa stripplate well and incubated. There is washing away of free component. The TMB substrate mixture is added to each well. The wells that have AST/ALT/ALP and HRP conjugated AST/ALT/ALP antibody showed blue in color then after the addition of the stop solution turned yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is equal to the concentration of AST/ALT/ALP. The concentration of AST/ALT/ALP/ in the samples was determined by making a comparison between the Optical Density of the samples to the standard curve.

3.18.2 Materials for Liver Assays Analysis

Closure plate membrane, sealed bags, microelisa stripplate standard, 4.5 ng/ml 0.5ml×1 bottle, standard diluent 1.5ml×1 bottle, HRP-Conjugate reagent 6ml×1 bottle, sample diluent 6ml×1 bottle, chromogen Solution A 6ml×1 bottle, chromogen Solution B 6ml×1 bottle, stop solution 6ml×1 bottle, wash solution, pre-coated plate, Rat ALT/AST/ALP Standards, Biotinylated antibody, enzyme conjugate, enzyme diluent, antibody diluent, standard diluent, sample diluent, washing buffer, color reagent A, color reagent B, Color Reagent C and Manual.

3.18.3 Procedure for Liver Assays Analysis

After preparing the reagents, samples and standards were added to the reaction at 37 for a period of 1hr and thirty minutes**.** The plate was washed, and the Biotinylated antibody working solution added, at 37 °C for 60 minutes. Then washed thrice and the Enzyme working solution added, at 37 degrees Celsius for 30 minutes. Thereafter, washed five times, and then the Colour Reagent solution added, at 37 degrees Celsius for 30 minutes. The Colour Reagent C was add. Microplate reader measured Optical Density values within 10 minutes. Then the factor content of specimens tested was calculate. The reagents from different lots was not mixed.

3.18.4 Interpretation of Liver Assays Analysis – AST, ALT and ALP Results

Known concentrations of Rat ALT Standard and its accompanying reading OD was plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Rat ALT in sample was calculated by plotting the sample's O.D. on the Y-axis. The original concentration was found by multiplying the dilution factor. The normal ranges are AST 0.05 ng/ml-3.2 ng/ml, ALT 30 pg/ml -1500 pg/ml, ALP, 0.2 ng/ml - 15 ng/ml while sensitivity results are AST 0.01 ng/ml, ALT 8 pg/ml and ALP 0.05 ng/ml

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

After resecting the liver, normal saline was used to clean it. Measure the widths, thickness and lengths using a caliper and a ruler. Percentage liver body ratio is equal to weight of the liver divide by weight in grams ×100%

3.19.1 Routine processing of liver tissues for light microscope

This was done using light microscope under 100X magnification.

3.19.2 Materials for routine processing of liver tissues for Haematoxylin and Eosin Staining

Liver**,** 1 litre of formalin solution, glass slides with cover slips, Dibutylpthalate Polystyrene Xylene (DPX), paraffin wax, square jars (glass staining), mouton Haematoxylin and eosin, microtome knives, container (water bath), heater, specimen bottles, **r**otary microtome, slide holders, liver, water (distilled), 40% formaldehyde, Isopropyl alcohol, Xylene, glass ware, Toluene solution, blocks (wood), dropper, beakers and cedar wood oil.

3.19.3 Procedure for routine processing of liver tissues for Haematoxylin and Eosin Staining

The liver was immersed in the 37% formalin solution for a period of 24 hours. Then in an increasing grade of alcohol concentration, dehydration is done (50%, 60%, 70%, 80%, 90%, 95% and 100 % (absolute) for 60 minutes period then by immersion with xylene for another 12 hours, it was cleared. The liver tissue was cut into blocks under orientation in longitudinal axis. It was then embedded in wax (paraffin) on the blocks (wooden). Excess wax was trimmed off and 220 slides were derived from each block by cutting the liver tissues into 5µm thick longitudinal sections with Leitz© sledge rotary microtome from head of right lobe to left lobe. Then, at 37^{0} c, sections were floated in water to spread the tissue. Using egg albumin the sections were stacked onto glass slides, with a micro-dropper, applied as thin film. Then for a

period of 24 hours, the slides were dried in an oven at 37'c.After staining the slides with Haematoxylin and Eosin, twenty-twenty four slide section were selected for light microscopy.

3.19.4 Photography of liver tissues for light microscope

The materials used include: BP Olympus microscope, digital camera of 32 megapixels, and glass slide**.** The histological slides are mounted on the stage then focus is enhancing by microscope adjustment. Then followed by magnification (X100) of the field appropriately. Photographs are taken and transferred to a laptop then later uploaded in Adobe fireworks programme for labelling.

3.20 Data management and analysis

The data was entered into excel sheet and then analysis done through SPSS version 25 (IBM). One-way ANOVA with post hoc Tukey was used to compare the data obtained from experimental and control groups. Significance levels was P value less than or equal to 0.05 ($p \leq 0.05$) at 95% confidence level. Histomorphological data was described based on liver sections observed microscopically.

3.21 Ethical approval

The proposal was initially presented and cleared by the School of Medicine, and then the School of Graduate Studies (SGS), Maseno University (SGS=MSC/SM/00023/020). Animal ethical approval was obtained from Institutional Scientific Ethical Review Committee (ISERC) of University of Eastern Africa Baraton (UEAB/ISERC/01/09/2023). The study research license was obtained from National Commission for Science Technology and Innovation (NACOSTI-P/23/23412).

3.22 Dissemination

The study findings was disseminated through presenting my final report to the faculty and students in school of Medicine, Maseno University, and publication.

CHAPTER FOUR

RESULTS

This chapter illustrates results obtained from the experimental study as per the specific objectives and are presented in tables and figures (mag. X100). There were no adverse effects, severe signs and symptoms nor any incident of mortality reported during entire study period.

4.1 Gross morphometric and histomorphological changes of Liv-52 on rifampicin and isoniazid induced liver toxicity among adult albino rats

4.1.1 Gross morphometric findings.

The liver gross morphometric measurements entail weight, length, width and thickness. There was no gross anatomical changes observed on the liver between the control and experimental groups. The liver was observed to have four lobes, dark brown in colour with a smooth texture.

4.1.1.1 Mean liver weight in comparison with mean final body weight between positive and negative controls.

A decrease (184.78±.78) in mean final body weight was observed in positive control group (RIIH 50kg/kgbwt), compared to 245.39±.57 in negative control group (Food+water). Similarly, there was a decrease (9.197±.26) in mean liver weight in positive control group as compared to negative control (11.86±.20 respectively). A statistical significant difference ($p \leq 0.0001$) between positive control group and negative control group as seen in Table 4.1.

4.1.1.2 Mean liver weight in comparison with mean terminal body weight between hepatoprotective groups.

In the hepatoprotective group, there was a significant ($p \leq 0.0001$) increase in mean final body weight in HD Liv-52(207mg/kgbwt) and slight increase in LD Liv-52(155mg/kgbwt) groups at 236.31±.63 and 208.33±.83 respectively compared to 184.78±.78 in positive control(RIIH 50kg/kgbwt) group. Additionally, the mean liver weight in HD Liv-52 group was significantly

($p \leq 0.0001$) higher at 11.40±.21 compared to 9.197±.26 in positive control group as seen in Table 4.1.

Table 14.1. Mean liver weight in comparison with mean final body weight among controls and hepatoprotective groups.

Liver Gross Dimensions	-VE control (Food+water)	+VE control (RIIH 50mg/ Kg/bwt)	LD Liv-52 (155mg/kg /bwt)	HD Liv-52 (207mg/kg /bwt)	df	F	sig.
MFBW (gms)	245.39±.57	184.78±.78	208.33±.83**	236.31±.63**	3	1500.181	0.0001
MLW (gms)	11.86±.20	9.197±.26	8.748±.38	11.40±.21**	3	37.841	0.0001

*KEY: All values are expressed and presented as the mean± the standard error of the mean (SEM); n=6. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks** represents significant ($p \leq 0.0001$), RIIH-Rifampicin and Isoniazid Induced Hepatotoxicity, +VE- Positive, -VE- Negative, HD- High Dose, LD- Low Dose, MFBW=Mean final body weight, MLW= Mean liver weight, df-degree of freedom, F- ANOVA value.*

Figure 14.1: Liver gross morphometric measurements (X100)

4.1.1.3 Comparative mean liver length, width and thickness between positive and negative control

A decrease in the mean of liver length, width and thickness was observed in positive control group compared to negative (Food+water) control. The length of 44.90±.40, width of 36.31±.19 and thickness of 0.53±.02 in positive (RIIH 50kg/kgbwt) control while length of 57.00±.53, width of 45.20±.33 and thickness of 0.35±.01 in negative control. There was statistically significant difference ($p \leq 0.0001$) in positive control compared to negative group as shown in Table 4.2.

4.1.1.4 Comparative mean liver length, width and thickness between positive control and hepatoprotective groups

The means of the liver gross morphometric measures were observed to be increasing in both LD Liv-52 (155mg/kgbwt) and HD Liv-52 (207mg/kgbwt) hepatoprotective groups as compared to positive control (RIIH 50kg/kgbwt) group. That is length of 50.82±.23, width of 41.86±.23 and thickness of 0.39±.01 in LD Liv-52 and length of 54.25±.24, width of 44.68±17 and thickness of 0.50±.00 in HD Liv-52. There was statistically significant difference ($p \leq 0.0001$) in HD Liv-52 when compared to positive control group as shown in Table 4.2.

Table 4.2: Comparative mean liver length, width and thickness between controls and hepatoprotective groups.

Liver Gross Dimensions	-VE control (Food+water)	+VE control (RIIH 50mg/ Kg/bwt)	LD Liv-52 (155mg/kg /bwt)	HD Liv-52 (207mg/kg /bwt)	df	F	sig.
Length (cm)	57.00±.53	44.90±.40	50.82±.23**	54.25±.24**	3	0.042	0.0001
Width (cm)	45.20±.33	36.31±.19	41.86±.23**	44.68±17**	3	0.148	0.0001
Thickness (cm)	0.53±.02	0.35±.01	0.39±.01	0.50±00**	3	0.064	0.0001

*KEY: All values are expressed and presented as the mean± the standard error of the mean (SEM); n=6. Data analyzed by one-way analysis of variance followed by post-hoc Bonferroni. Asterisks** represents significant ($p \leq 0.0001$), RIIH-Rifampicin and Isoniazid Induced Hepatotoxicity, +VE- Positive, -VE- Negative, HD- High Dose, LD- Low Dose, MFBW=Mean final body weight, MLW= Mean liver weight, df-degree of freedom, F- ANOVA value.*

4.1.2 Histomorphological findings.

In this subsection, the comparative histomorphological changes of Liv-52 on rifampicin and isoniazid induced liver toxicity was described.

4.1.2.1 Comparison histomorphological findings of liver between control groups.

A normal liver histomorphological features was observed in the negative control group (food and water ad-libitum), where the central vein, hepatocytes, hepatic triad and capillary sinusoid appeared normal as shown in Figure 4.2A. This was different from the positive control (RIIH 50kg/kgbwt) group that showed deranged histomorphological features, where areas of necrosis, dilatation of sinusoidal capillaries, disrupted central vein and inflamed Kupffer cells was observed as shown in figure 4.2B.

4.1.2.2 Comparison histomorphological features of liver between positive control and hepatoprotective groups

Liver sections in the positive control (RIIH 50kg/kgbwt) group showed deranged features, where areas of necrosis, dilatation of sinusoidal capillaries, disrupted central vein and inflamed Kupffer cells was observed as shown in figure 4.2C. This was slightly different from liver sections in the low dose Liv-52 (155mg/kgbwt) group which showed slight derangement, in that there were minimal areas of necrosis, central veins were minimally disruption and had moderately dilated sinusoids as shown in figure 4.2D. The histomorphological features in HD Liv-52 (207mg/kgbwt) appeared normal in that there was no histological derangement from the control group as shown in figure 4.3F.

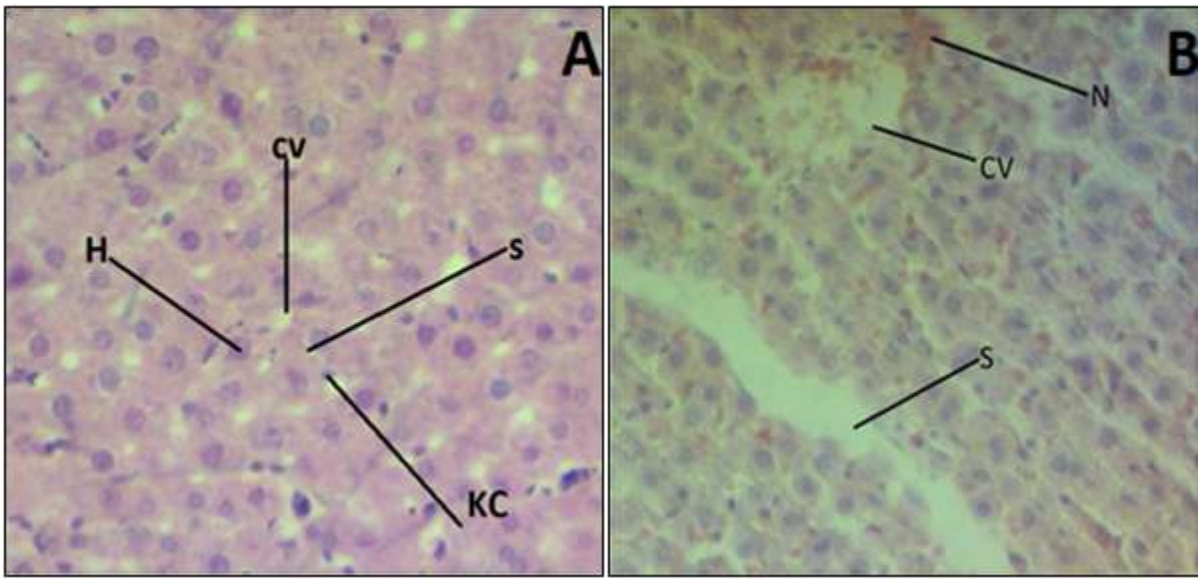

Figure 4.1: Photomicrograph A; negative control group, showing normal histological findings; normal central vein, hepatocytes, sinusoid and Kupffer cells. Photomicrograph B; positive control group showing disrupted central vein, dilated sinusoid and necrosis (X100).

KEY: A= negative control group, B = positive control (Rifampicin Isoniazid induced hepatotoxicity), CV= central vein, H=hepatocyte, KC= Kupffer cells N= Necrosis and S=sinusoid

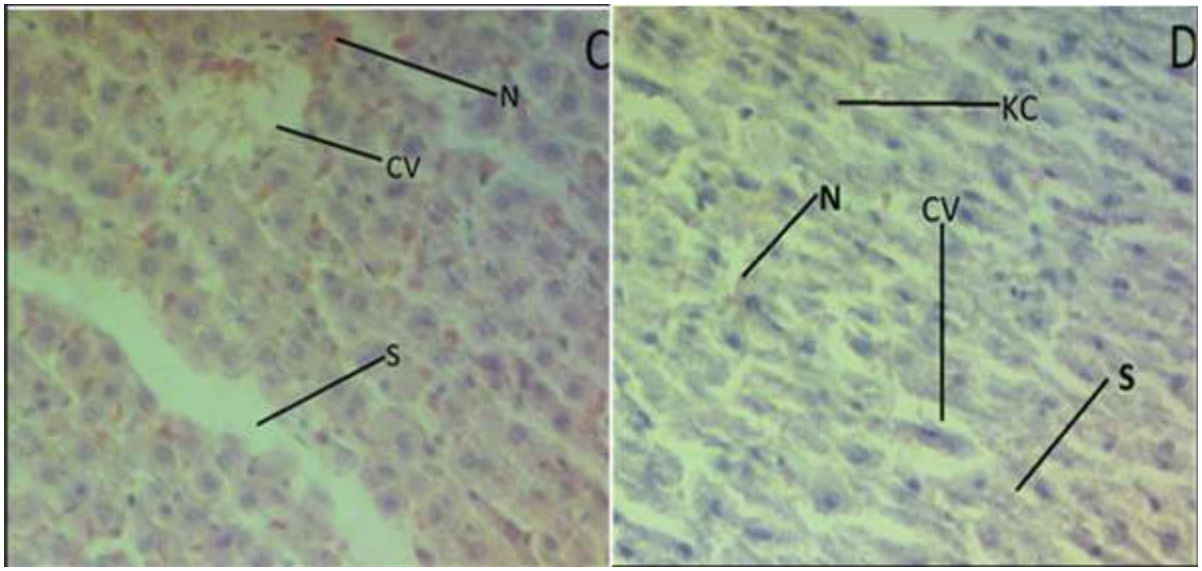

Figure 4.2: Photomicrograph C; positive control group (RIIH) showed deranged features, that is, areas of necrosis, dilatation of sinusoidal capillaries, deranged central vein and inflamed Kupffer cells. Photomicrograph D; Low Dose Liv-52 group; showed minim (X100).

KEY: C= control group, D =Rifampicin Isoniazid induced hepatotoxicity, CV= central vein, H=hepatocyte, KC= Kupffer cells N= Necrosis and S=sinusoid

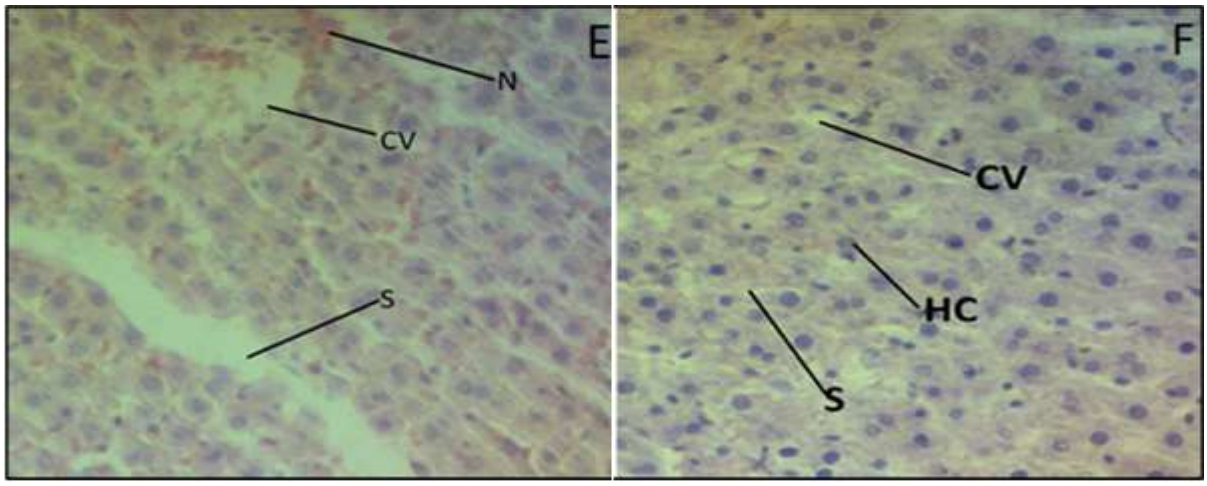

Figure 4.3: Photomicrograph E; positive control group (RIIH) showed deranged features; areas of necrosis, dilatation of sinusoidal capillaries, deranged central vein and inflamed Kupffer cells. Photomicrograph F; High Dose Liv-52 group; showing no derangement (X100).

KEY: E= control group, F =Rifampicin Isoniazid induced hepatotoxicity, CV= central vein, HC=hepatocyte, KC= Kupffer cells N= Necrosis and S=sinusoid

4.2 Dosage of Liv-52 required to provide hepatoprotective effect following administration of Liv-52, Isoniazid and Rifampicin among albino rats

4.2.1 Dosage determination.

The study established dosage required to provide hepatoprotective effect against Rifampicin and Isoniazid induced liver toxicity.

4.2.2. Low dose Liv-52

The low dose of Liv-52 was given at 155mg/kg body weight of albino rat. The liver sections in this group showed histomorphological features of minimal to moderate derangement. The central vein was slightly disrupted, capillary sinusoids minimally dilated, minimal areas of necrosis and mild hypertrophy of Kupffer cells as shown in Figure 4.3D.

4.2.3 High dose Liv-52

High dose of Liv-52 was administered at 207mg/kg body weight of albino rat. There were changes observed in histomorphological features in the liver sections compared to the positive control group. There were no derangement in the features that is, presence of numerous hepatocytes, no areas of necrosis and capillary sinusoids appeared normal with no dilatations as shown in Figure 4.3F.

4.3 Changes in liver bio-chemical parameters (ALT, AST and ALP) following administration of Liv-52, Isoniazid and Rifampicin among albino rats

The study sought to establish if there was mean difference or variations in the four groups. One- Way ANOVA was used followed by Turkey post hoc analysis to determine where there was a significant difference between the groups.

Table 4.3: Changes in liver bio-chemical parameters (ALT, AST and ALP) following administration of Liv-52, Isoniazid and Rifampicin among albino rats

Liver biochemical parameters	df	F	Sig. (P value)
ALT (+Ve control, -Ve control, LD Liv-52, HD Liv-52)	3	21.105	<0.0001*
ALP (+Ve control, -Ve control, LD Liv-52, HD Liv-52)	3	11.428	<0.0001*
AST (+Ve control, -Ve control, LD Liv-52, HD Liv-52)	3	53.808	<0.0001*

Key; *+Ve control group (RIIH 50mg/ Kg/bwt), -Ve control group (Food + water), LD Liv-52 (155mg/kg/bwt) HD Liv-52 ((207mg/kg/bwt)*

Tukey post hoc multiple analysis was done to establish where there was a statistical significant difference between the five groups (+ve control, -Ve control, Low dose and High dose of LIV 52.

Table 4.4: Statistical significant difference in ALT profile between the five groups by Tukey HSD post hoc analysis

Between groups		P value	95% Confidence interval	
			Lower Bound	Upper Bound
-VE CONTROL	+VE CONTROL	0.000*	-107.578	-44.888
	LD LIV-52	0.000*	-107.128	-44.438
	HD LIV-52	0.000*	-94.595	-31.905
+VE CONTROL	-VE CONTROL	0.000*	44.888	107.578
	LD LIV-52	1.000	-30.895	31.795
	HD LIV-52	0.658	-18.362	44.328
LD LIV-52	-VE CONTROL	0.000*	44.438	107.128
	+VE CONTROL	1.000	-31.795	30.895
	HD LIV-52	0.682	-18.812	43.878
HD LIV-52	-VE CONTROL	0.000*	31.905	94.595
	+VE CONTROL	0.658	-44.328	18.362
	LD LIV-52	0.682	-43.878	18.812

***statistically significant difference**

Table 4.5: Statistical significant difference in ALP profile between the five groups by Tukey HSD post hoc analysis

Between groups		P value	95% Confidence interval	
			Lower Bound	Upper Bound
-VE CONTROL	+VE CONTROL	0.002*	-69.881	-14.119
	LD LIV-52	0.000*	-81.281	-25.519
	HD LIV-52	0.249	-47.081	8.681
+VE CONTROL	-VE CONTROL	0.002*	14.119	69.881
	LD LIV-52	0.667	-39.281	16.481
	HD LIV-52	0.134	-5.081	50.681
LD LIV-52	-VE CONTROL	0.000*	25.519	81.281
	+VE CONTROL	0.667	-16.481	39.281
	HD LIV-52	0.013*	6.319	62.081
HD LIV-52	-VE CONTROL	0.249	-8.681	47.081
	+VE CONTROL	0.134	-50.681	5.081
	LD LIV-52	0.013*	-62.081	-6.319

***statistically significant difference**

Table 4.6: Statistical significant difference in AST profile between the five groups by Tukey HSD post hoc analysis

Between groups		P value	95% Confidence interval	
			Lower Bound	Upper Bound
-VE CONTROL	+VE CONTROL	0.000*	-89.803	-51.497
	LD LIV-52	0.000*	-93.986	-55.680
	HD LIV-52	0.000*	-85.653	-47.347
+VE CONTROL	-VE CONTROL	0.000*	51.497	89.803
	LD LIV-52	0.927	-23.336	14.970
	HD LIV-52	0.929	-15.003	23.303
LD LIV-52	-VE CONTROL	0.000*	55.680	93.986
	+VE CONTROL	0.927	-14.970	23.336
	HD LIV-52	0.623	-10.820	27.486
HD LIV-52			47.347	85.653
			-23.303	15.003
	LD LIV-52	0.623	-27.486	10.820

***statistically significant difference**

-VE CONTROL	0.000*
+VE CONTROL	0.929

CHAPTER FIVE

DISCUSSION

This chapter contains detailed discussion concerning the results in chapter four. The discussion has been done in in line with the specific objects, beginning from the first to the last.

5.1 Gross morphometric and histomorphological changes of Liv-52 on rifampicin and isoniazid induced liver toxicity among adult albino rats

5.1.1 Gross morphometric changes

Gross morphometric measurements of the liver include weight, length, width and thickness. The measurements are vital in determining the gross changes in the liver between controls and experimental groups (Buabeid *et al.*, 2022).

In this current study, the liver gross appearance was dark brown in colour with a smooth texture after administration of rifampicin and isoniazid (figure 4.1). These findings are in line with (Humayun *et al.,* 2017) who also observed similar features, which were attributed to the histological effects of Isoniazid induced liver toxicity on albino mice. The dark brown pigmentation on the liver could be attributed to damage to liver parenchyma upon introduction of rifampicin and isoniazid (Rono *et al.,* 2020; Shabbir *et al.*, 2020). However, a study by Shabbir *et al.,* (2020) observed that besides the change in the pigmentation, a significant decrease in liver weight and mean terminal body weight have been recorded when antitubercular is used to induce hepatotoxicity among Albino rats (table 4.1). The observation by Shabbir *et al.,* (2020) was consistent with the present study findings which recorded a decrease in mean final body weight in positive control (RIIH 50kg/kgbwt) group, compared to negative control (food and water ad-libitum) group.

Similarly, there was a significant decrease in mean liver weight in positive control (RIIH 50kg/kgbwt) group as compared to negative (food and water ad-libitum) control as shown in

table 4.2. The current study also recorded significant decrease in the length, width and thickness of the liver (table 4.2). These changes in the length, width, thickness of the liver and terminal body weight could be attributed to hepatotoxic injury to liver parenchyma induced by rifampicin and isoniazid (table 4.). Rono *et al.,* (2020) who observed a significant (p value < 0.05) decrease in liver weight upon introduction of high dose paracetamol to albino rats.

This study observed that, there was a significant increase in mean final body weight in high dose Liv-52 and a slight increase in low dose Liv-52 groups compared to positive control group (table 4.1). The mean liver weight in HD Liv-52 group was significantly higher compared to positive control group (table 4.2). The increase of measurements in hepatoprotective groups could be due to the hepatoprotective effect of Liv-52 which inhibits oxidation and lipid peroxidation. Rono *et al.,* (2020) and observed similar findings, that is significant decrease in liver morphometric findings when he introduced different dosage of Liv-52 upon paracetamol induced liver toxicity.

5.1.2 Histomorphological changes.

Histomorphological features of the liver include; central vein, capillary sinusoids, portal triad, lobules and Kupffer cells. The features are important in assessing injury to the liver histo-architecture (Buabeid *et al.,* 2022).

The present study observed normal liver histomorphological features (figure 4.2A) in negative control group (food and water ad-libitum). This observation was different from the positive control group (isoniazid and rifampicin) that showed deranged features where areas of necrosis, dilatation of sinusoidal capillaries, disrupted central vein and inflamed Kupffer cells was observed (figure 4.2B). This is attributed to necrosis caused by rifampicin and isoniazid which induces to injury to hepatocytes. These observations are in harmony with Zodape *et al.,* (2018) and Dubiwak *et al.,* (2021) who recorded deranged histological features on liver when he introduced Isoniazid and Rifampicin among albino rats.

However, this study observed that the histomorphological features recorded from LD Liv-52 (155mg/kgbwt) was suggestive of partial regeneration of liver architecture (figure 4.2D) while the HD Liv-52 (207mg/kgbwt)showed a fully regenerated and had features of normal liver architecture (figure 4.2F). The partial regeneration of liver architecture witnessed from LD Liv-52 (155mg/kgbwt) showed liver sections with minimal areas of focal necrosis, minimally disrupted central veins and moderately dilated sinusoids (figure 4.2D). Liver sections in HD Liv-52 (207mg/kgbwt) showed no derangement even when compared to the control group and had the following features; there were no areas necrosis, no hemorrhagic areas, normal hepatocytes and Kupffer cells (figure 4.2F). These features of normal liver findings observed in HD Liv 52 could be attributed to anti-oxidant activity found in Liv-52 which has been known to prevent injuries to tissues. This fully regenerated histomorphologically features described are consistent with Fulzele *et al.,* (2012); Sankar *et al.,* (2015); Cimen *et al.,* (2020) and Rono *et al*., (2020) who reported similar features when an antidote was given to reverse damages caused by a hepatotoxic agent (Fulzele *et al.,* 2012); (Sankar *et al.,* 2015); (Cimen *et al.,* 2020) and (Rono .k walter, 2020).

5.2 Dosage of Liv-52 required to provide hepatoprotective effect following administration of Liv-52, Isoniazid and Rifampicin among albino rats

Dose dependent difference was observed in regeneration capacity of the liver histomorphology between low and high dose Liv-52. The low dose of Liv-52 was given at 155mg/kg body weight of albino rat. The liver sections in this group showed histomorphological features of partial derangement. The central vein was slightly disrupted, capillary sinusoids minimally dilated, minimal areas of necrosis and mild hypertrophy of Kupffer cells. These findings are in harmony with Rono *et al.,* (2020) who administered 100mg per kilogram body weight of Liv-52 among albino rats in paracetamol induced liver toxicity.

On the other hand, high dose of Liv-52 which was administered at 207mg/kg body weight of albino rat showed normal histomorphological features in the liver sections. That is, no

derangement in the features that is, no areas of necrosis and capillary sinusoids appeared normal with no dilatations. A study by Paramwesi *et al.,* (2013) recorded a dosage of 10mg per kilogram body weight was effective to provide hepatotoxic activity against Rifampicin and Isoniazid induced liver toxicity.

5.3 Changes in liver bio-chemical parameters (ALT, AST and ALP) following administration of Liv-52, Isoniazid and Rifampicin among albino rats

The three (ALT, AST and ALP) selected liver biochemical parameters were determined to assess the hepatoprotective activity of Liv-52 against Rifampicin and Isoniazid induced liver toxicity in adult albino rats. In cases where liver is damaged, these enzymes are usually released into the blood stream (Buabeid et al., 2022). In the present study, all the three biochemical parameters; ALP, AST and ALT in negative control (water and food ad-libitum) group were within the normal ranges.

Contrary to this, the three parameters showed significant (p value < 0.0001) increase above their normal ranges in positive control (RIIH 50kg/kgbwt) group compared to negative control group. Dubiwak *et al.*, 2021 and Buabeid *et al.*, 2022 reported that AST, ALP and ALT levels were significantly high when Isoniazid and Rifampicin were administered to Albino rats (table 4.4 and 4.5). This further confirms that Isoniazid and Rifampicin used in this study was able to induce liver toxicity. In an effort to confer hepatoprotectivity, LD Liv-52 and HD Liv-52 was used and three liver profiles were evaluated to establish their hepatoprotective activity. This study observed that the three liver profiles in both LD Liv 52 and HD Liv 52 had deranged levels marked by levels above the reference ranges. The most likely explanation to this is that the markers of liver injury (ALT, AST and ALP) were still being released in circulation so, even as the liver was recovering due to hepatoprotective nature of Liv-52 these liver markers were dropping slowing and if the sample was collected more days after then the ranges would revert to normal. The study hypothesizes that if the three (ALT, AST and ALP) selected liver

biochemical parameters were determined serially days after the administration of Liv-52 then these markers would drop to normal ranges.

Previous studies have shown that Liv-52 has a clinical role in treatment of liver disorders but there is no clinical data concerning the dose of Liv-52 that inhibits hepatotoxicity induced by these antituberculosis drugs. This study gives evidence that Liv-52 (207mg/kgbwt) prevents alteration of liver parenchyma induced by Rifampicin and Isoniazid. The findings in this study gives insights to the scientific and clinical community concerning co-administration of Liv-52 with anti-TB therapy. This will prevent hepatotoxicity induction hence promoting normal functioning of the liver thereby alleviating patients suffering. Moreover, findings in this study are beneficial to the community as it will reduce economic, social and financial burden resulting from bedridden patients, thereby increasing productivity at large.

This research is subject to several limitations: We couldn't analyze gamma glutamyl transferase (GGT) which is a biochemical marker specific to the liver. However, we based on the three parameters ALT, AST and ALP. While ALT and ALP indicate damage specific to the liver, AST is non-specific and may indicate damage to other organs such as kidney, heart or liver.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

1. Liv-52 prevents gross morphometric and histomorphological changes on liver following administration of Isoniazid and Rifampicin among adult albino rats
2. High dose Liv-52 (207mg/kgbwt) provides hepatoprotective effect when liver toxicity induced by Isoniazid and Rifampicin among albino rats
3. The three selected liver parameters (ALT, AST and ALP) were persistently high above normal ranges even in the hepatoprotective groups, contrary to normal level which was expected.

6.2 Recommendations

1. Gross morphometric and histomorphological features be used to establish liver induced toxicity among adult albino rats
2. High dose Liv-52 (207mg/kgbwt) should be used to provide hepatoprotective effect of Liv-52 when liver toxicity is caused by Isoniazid and Rifampicin among albino rats
3. There is need to determine ALT, AST and ALP serially days after administering Liv-52 to ascertain reversion to normal ranges.

6.3 Recommendations for future study

There is need to clinically evaluate the pharmacodynamics and pharmacokinetics of high dose Liv-52 in human patients with liver damage attributed to Isoniazid and Rifampicin.

The current study proposes future researchers to embrace the use of GGT assess hepatoprotective effect of Liv-52 since its specific biomarker for liver functioning.

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APPENDICES

APPENDIX ONE: APPROVAL LETTER FROM SCHOOL OF GRADUATE STUDIES, MASENO UNIVERSITY

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES
Office of the Dean

Our Ref: MSC/SM/00023/2020

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 26th August 2022

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR HANS LWUNZA LIBAMILA — MSC/SM/00023/020

The above named is registered in the programme of Master of Science in Human Anatomy in the School of Medicine, Maseno University. This is to confirm that his research proposal titled **"Hepatoprotective Effect of LIV-52 on Rifampicin and Isoniazid Induced Liver Toxicity in Adult Albino Rats"** has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

APPENDIX TWO: ETHICAL APPROVAL LETTER FROM

OFFICE OF THE CHAIRPERSON
INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B0919012023 January 19, 2023

TO: Hans Lwunza Libamila
Department of Human Anatomy
Maseno University

Dear Hans,

RE: Hepatoprotective Activity of LIV-52 on Rifampicin and Isoniazid Induced Liver Toxicity in Adult Albino Rats

This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/09/01/2023. The approval period is 19th January, 2023 – 19th January, 2024.
This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Institutional Scientific Ethics Review Committee

University of Eastern Africa, Baraton
19 JAN 2023
Research Ethics Committee

APPENDIX THREE: PERMIT FROM NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **308370**

Date of Issue: **01/March/2023**

RESEARCH LICENSE

This is to Certify that Mr.. Hans Lwunza Libamila of Maseno University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisumu on the topic: Hpeatoprotective activity of LIV-52 on Rifampin and Isoniazid induced liver toxicity in adult albino rats for the period ending : 01/March/2024.

License No: **NACOSTI/P/23/23412**

308370

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

APPENDIX FOUR: DATA COLLECTION TOOLS

DATA CAPTURE SHEET FOR ALBINO RATS

INITIAL WEIGHT (MG)…….

DATE	WEIGHT (MG)	INH DOSE (mg/kgbwt)	RIF DOSE (mg/kgbwt)	LIV-52 DOSE (mg/kgbwt)	GENERAL CONDITION OF RAT

DATA CAPTURE SHEET FOR GROSS MORPHOLOGY ALBINO RAT LIVER

GROUP……...

DATE OF HARVESTING…….

FIXATIVE USED……...

No.	Gross appearance	thickness (mm)	Length (mm)	Width (mm)

DATA CAPTURE SHEET FOR BLOOD SAMPLE COLLETION

DATE OF COLLECTION……

GROUP…….

NO.	SITE	VOLUME (mls)