AMELIORATIVE EFFECTS OF AQUEOUS EXTRACTS OF *Brysocarpus coccineus* ON ISONIAZID INDUCED OXIDATIVE STRESS AND HEPATOTOXICITY IN ADULT MALE WISTAR RATS

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

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DECLARATION

I, Kashini ANDREW, declare that the work in this dissertation entitled: **"Ameliorative Effects of Aqueous Extracts of *Brysocarpus coccineus* on Isoniazid Induced Oxidative Stress and Hepatotoxicity in Adult Male Wistar Rats"** has been carried out by me in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Dr. Fatima A. Dawud and Dr. Jamilu Yau. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at this or any other institution.

_____ _____

Kashini Andrew M.B.B.S (A.B.U) Date

`

CERTIFICATION

This dissertation entitled AMELIORATIVE EFFECTS OF AQUEOUS EXTRACTS OF *Brysocarpus coccineus* ON ISONIAZID INDUCED OXIDATIVE STRESS AND HEPATOTOXICITY IN ADULT MALE WISTAR RATS by Kashini ANDREW, meets the regulations governing the award of a Master of Science degree in Human Physiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEGEMENT

I wish to express my gratitude to Almighty God for giving me the strength and wisdom to successfully complete this research work.

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I wish to express my profound gratitude to my colleagues, especially Emmanuel N. Solomon, Garkuwa Usman and Rukayya Adebisi. My deepest appreciation and love go to my parents Mr and Mrs Jonathan Andrew, thank you for being there and for your patience throughout my academic sojourn.

DEDICATION

To everyone that seeks academic excellence, through hard work and perseverance.

ABSTRACT

Tuberculosis is the most common cause of death from an infectious agent, it is an important cause of morbidity and mortality in Africa and Nigeria. Isoniazid (INH) is one of the effective drugs used in its treatment and prevention with the inadvertent complications of oxidative stress and hepatotoxicity. The aim of the current study was to determine the antioxidant and hepatoprotective effects of *Brysocarpus coccineus* aqueous extracts on isoniazid induced oxidative stress and hepatotoxicty. Adult male Wistar rats were divided into six groups of five rats each and treated orally for 30 days as follows: Group I: 1 ml/kg of distilled water; group II: Isoniazid (27 mg/kg); group III: Isoniazid (27 mg/kg) + Livolin forte (20 mg/kg); group IV: Isoniazid (27 mg/kg) + *B. coccineus* (200 mg/kg); group V: Isoniazid (27 mg/kg) + *B. coccineus* (400 mg/kg); group VI: Isoniazid (27 mg/kg) + *B. coccineus* (800 mg/kg). Afterwards rats were sacrificed and serum was collected for oxidative stress and hepatotoxicity biomarker assays, liver tissue was also collected for Histology. There was a significant ($p \leq 0.05$) increase in serum MDA levels in the group treated with isoniazid alone compared to the control, this was reversed in the groups treated with livolin forte and the extract in a dose dependent manner which was not significant ($p \leq 0.05$), there was also a decrease in the serum SOD and CAT in the groups treated with Isoniazid and livolin forte and all the groups treated with the extract compared to the control. There was an increase in the serum AST and ALT in the isoniazid group which was reversed by livolin forte and the aqueous extracts at a dose of 200mg/kg, however the extracts increased the serum levels of AST and ALT at higher doses this was not significant ($p \leq 0.05$) when compared to the controls. Serum total protein and albumin was increased in the isoniazid treated group, and decreased in the group treated with livolin forte and the extracts at 200mg/kg, it was however increased at high doses which was not significant ($p \leq 0.05$). The extracts caused statistically significant ($p \leq 0.05$) increases in the serum total and conjugated bilirubin compared

to the control and livolin treated group. The liver histology revealed sinusoidal dilatation and central veins congestion in the isoniazid treated group which was not evident in the groups treated with the extract at 400 and 800 mg/kg. In conclusion, aqueous extracts of *B.coccineus* showed anti-oxidant and hepatoprotective effects at a low dose in isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.

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ABBREVIATIONS

ABUTH	Ahmadu Bello University Teaching Hospital
AFB	Acid Fast Bacilli
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline Phosphatase
CAT	Catalase
DNA	Deoxyribonucleic acid
DOTS	Directly Observed Therapy Short Course
ELISA	Enzyme linked immunosorbent assay
FMOH	Federal Ministry of Health
GPx	Glutathione peroxidase
HIV	Human Immunodeficiency Virus
INH	Isoniazid
iNO	Inducible Nitrogen oxide
IPT	Isoniazid Preventive Therapy
MDA	Malondialdehyde
MTB	*Mycobacterium Tuberculosis*
NAT2	N-acetyl Transferase 2
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TB	Tuberculosis
WHO	World Health Organisation

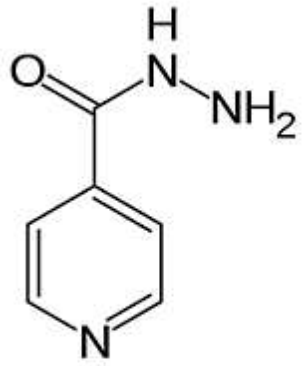
CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Tuberculosis is caused by *mycobacterium tuberculosis*; the mycobacteria are rod-shaped, gram positive, aerobic bacteria that are acid fast. Transmission of tuberculosis is via inhalation of infective aerosol droplets from an untreated case of tuberculosis (Wania, 2013). Tuberculosis (TB) is the most common cause of death from an infectious agent worldwide, which is after human immunodeficiency virus (HIV) (Corbel *et al*., 2003). The WHO Global TB Control Report estimated that 9 million new cases of tuberculosis were diagnosed in 2013; 6.1 million of them were reported to the World Health Organization in 2014 this included 1.1 people living with HIV (WHO, 2014).

In 1952 three companies; Hoffman LaRoche, E.R. Squibb & Sons, and Bayer simultaneously discovered isoniazid based on earlier observations by Chorine that the nicotinamide group had activity against the tubercle bacilli (Chorine, 1945). The discovery of isoniazid was celebrated because it was cheap and very effective in treating tuberculosis (Zhang, 2003); it was initially thought to be safe until the early 1970s when several cases of isoniazid induced hepatotoxicity were reported (Farrel *et al*., 1994). Isoniazid is a prodrug, the hydrazide of isonicotinic acid;mycobacterial catalase-peroxidase converts it into an active metabolite which inhibits the biosynthesis of mycolic acids that are attached to a unique polysaccharide, arabino galactan, to form part of the mycobacterial cell wall. Isoniazid also inhibits mycobacterial catalase-peroxidase (the isoniazid-activating enzyme), which may increase the likelihood of damage to the mycobacteria from reactive oxygen species (Zhang Ying *et al*., 2005; Shi *et al*., 2007).



Isoniazid

Figure 1.0 Chemical structure of Isoniazid (Barry and O'Conner, 2007).

There are numerous reports of isoniazid induced oxidative stress and hepatotoxicity, most are due to toxins formed as metabolites during the handling of the drug by the liver; more so slow acetylators have been shown to be more susceptible to this form of toxicity (Garg and Tandon, 2003; Huang, 2014).

Byrsocarpus coccineus Schum.and *Thonn*. (Connaraceae), a scandent shrub widely distributed in tropical Africa, is used in ethnomedicine to manage many illnesses. *Byrsocarpus coccineus schumach & Thonn.* belongs to the Equipseptopsida C. Agardh Class of flora, the Family Connaraceae and genus *Byrsocarpus schumach.* It is noted for its pain relieving effects in Ivory Coast where it is used to relief sore throat, muscular and rheumatic pains (Burkill, 1985). In the southern part of Chad republic the root sap is used for the treatment of earache while the leaves are employed as remedy for swelling (Olowokudejo, 2008).

1.2 Statement of Research Problem.

Isoniazid is the most important component of the Directly Observed Therapy Short Course (DOTS) for treatment of Tuberculosis and Isoniazid Preventive Therapy (IPT) (WHO 2010, 2011 and FMOH 2014); however toxicity has been reported in up to 10%–20% of subjects taking isoniazid, most common toxic effects are oxidative stress, neurotoxicity, acute pancreatitis, hepatotoxicity and hepatitis (Kucers *et al*., 1997; Farrah *et al.*, 2015).

1.3 Justification

Most of the administered Isoniazid is acetylated by the hepatic enzyme *N*-acetyltransferase 2 and then hydrolyzed, yielding isonicotinic acid and acetylhydrazine; these metabolites are activated into reactive oxygen species by *cytochrome* P-450. Acetylhydrazine and hydrazine have been shown to generate electrophilic intermediates and radicals capable of causing liver damage and Rifampicin, a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of isoniazid by enhancing the production these toxic metabolites (Kalra *et al*., 2007; Ching, 2011). Since prolonged oral administration of aqueous extracts of *Brysocarpus coccineus* has been shown to have in-vivo antioxidant and hepatoprotective effects (Akindele *et al.*, 2010), there is the need to carry out this study to identify any potential antioxidant and hepatoprotective effects aqueous extracts of *Brysocarpus coccineus* on isoniazid induced oxidative stress and hepatotoxicity for possible use as an antioxidant and hepatoprotective supplements in patients taking isoniazid.

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1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of this study is to determine the ameliorative effects of aqueous extracts of *Brysocarpus coccineus* on isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.

1.4.2 Objectives of the study

1. To evaluate the effects of aqueous extracts of *Brysocarpus coccineus* on oxidative stress biomarkers (Catalase, Superoxide dismutase and Malondialdehyde) in isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.
2. To evaluate the effects of aqueous extracts of *Brysocarpus coccineus* on liver enzymes (ALT, AST and ALP) in isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.
3. To evaluate the effects of aqueous extracts of *Brysocarpus coccineus* on the serum total proteins and albumin in isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.
4. To evaluate the effects of aqueous extracts of *Brysocarpus coccineus* on the serum total and conjugated (Direct) bilirubin in isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats

5. To evaluate the effects of aqueous extracts of *Brysocarpus coccineus* on the liver histology of isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.

1.5 Hypothesis

Aqueous extracts of *Brysocarpus coccineus* has ameliorative and hepatoprotective effects on isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. *Brysocarpus coccineus* (Connaraceae)

Traditional medicine practice is an important source of livelihood and major source of income to about 200,000 traditional medicine practitioners in Nigeria; most of them rely on medicinal plants as their primary source of medicines. In many countries modern medicine has replaced traditional medicine with many synthetic products but almost 30% of pharmaceutical preparations are still obtained directly from plants. A large number of studies have been carried out on herbal traditional medicines and significant amount of laboratory data have been generated on their efficiency (Gamaniel *et al.,* 1997; Wazi *et al.,* 2012).

Byrsocarpus coccineus Schum. and *Thonn*. (Connaraceae), a scandent shrub widely distributed in tropical Africa, is used in ethnomedicine to manage many illnesses. In Northern Nigeria, it is referred to by the Hausas as "*Tsamiyar kasa or kimbar maharba."*The Fulani people call it *"wangarabubi or yangara-bubihi",* while the Bassange people call it *"Kogi"*. In the southern part of Nigeria the Yoruba people call it *"Oke abolo"* or *"Mybo-apepea"* (Dalziel, 1937). Kilba

people in Adamawa State call it *''mblakiki''*. *Byrsocarpus coccineus* have been shown to be useful in oropharngeal, dermatological, urogenital tract and haematological problems (Amos *et al*., 2002; Akindele and Adeyemi, 2006).

Table 1.0: Taxonomy of *Brysocarpus coccineus*

Class	Equipseptopsida C. Agardh
Family	Connaraceae
Genus	*Byrsocarpus schumach*
Species	*Byrsocarpus coccineus Schum*. and *Thonn*.

The aqueous leaf extract of *B. coccineus* is relatively safe when administered orally; prolonged administration has revealed effects which suggest that the extract may possess hepatoprotective and in vivo antioxidant properties (Akindele *et al.,* 2010). The anti-inflammatory effect of the aqueous leaf extract of *Byrsocarpus coccineus* was found to inhibited edema formation in all four rats' models used justifying its use in treating arthritis in traditional medicine (Akindele and Adeyemi, 2007). The aqueous leaf extracts of *Byrsocarpus coccineus* Schum and Thonn. (Connaraceae) was also investigated for antipyretic activity in rats and rabbits using yeast,

amphetamine and lipopolysaccharide induced pyrexia models. In both models, the extract produced direct reduction of elevated temperature post therapy suggesting a possible antipyretic effect (Akindele and Adeyemi, 2007).

Studies with Ethylacetate leaf extract of *Byrsocarpus coccineus* found it to be relatively safe and also potentiate the delivery of pregnant rats on day 21 with no significant change in hepatic and renal functions and this supports the traditional use of this plant to induce labour at term (Amos *et al.*, 2002).

The antidiarrhoeal activity of the aqueous leaf extract of *Byrsocarpus coccineus* was evaluated on normal and castor oil-induced intestinal transit, castor oil-induced diarrhoea, enteropooling and gastric emptying. The extract produced a decrease in propulsion in the castor oil-induced intestinal transit in mice. In a dose dependent manner, it delayed the onset of diarrhoea, produced a significant decrease in the frequency of defaecation, severity of diarrhoea and protected the mice treated with castor oil. The results suggested that *Byrsocarpus coccineus* possesses antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion, mediated through alpha 2 adrenoceptors, and also inhibition of fluid secretion (Akindele and Adeyemi, 2006).

Phytochemical studies showed the presence of saponins in ethylacetate and methanol extracts of *Brysocarpus coccineus* leaves but absent in hexane extracts of the plants, while tannins, were present only in ethylacetate extracts of the leaves; both tannins and saponins were found to be present in the aqueous extracts of the plant (Akindele and Adeyemi, 2006; Hammid *et al.*, 2011).



Figure 2.1.*Byrsocarpus coccineus Schum*. and *Thonn*. (Connaraceae) (Snapped by the researcher)

2.2. Role of isoniazid in tuberculosis

Isoniazid (INH) and rifampicin are the backbone of TB treatment. Isoniazid is the first line Antituberculous Therapy agent and was introduced in 1952.Isoniazid is a prodrug, the hydrazide of isonicotinic acid; mycobacterial catalase-peroxidase converts it into an active metabolite which inhibits the biosynthesis of mycolic acids that are attached to a unique polysaccharide, arabino galactan, to form part of the mycobacterial cell wall (Farrah *et al.*, 2015).

2.2.1 Mechanism of action of isoniazid (INH)

Isoniazid (INH) is bactericidal and acts by inhibiting synthetic pathways of mycolic acid and metabolized by hepatic N-acetyltransferase 2. Isoniazid has infrequent major adverse effects, including hepatitis, peripheral neuropathy, neurotoxicity, hypersensitivity reactions (fever, skin eruptions or hematologic alterations along with lupus-like syndrome that is reversible on discontinuation of INH. The most notable adverse effect is hepatitis. The estimated rate of clinical hepatitis in patients given INH alone is approximately 0.6% (Wallace and Griffiths, 2009; Farrah *et al.*, 2015).

2.2.2 Clinical uses of isoniazid in tuberculosis

Isoniazid is a component of the Directly Observed Therapy Short Course (DOTS) for treatment of Tuberculosis and Isoniazid Preventive Therapy (IPT). In Regimen I and Regimen II of DOTS, Isoniazid is given orally in combination with Rifampicin, Pyrazinamide and ethambutol for six months and twelve months respectively; while in the IPT guidelines Isoniazid (5mg/kg) is given orally alone for six months (WHO, 2010 and 2011; FMOH, 2014).Randomized controlled trials have demonstrated that a course of Isoniazid Preventive Therapy(IPT) reduces the incidence of Tuberculosis in HIV-negative populations at risk of developing active disease and in HIV infected patients; IPT also reduced reactivation of latent TB infection, both in industrialized countries as well as in developing countries (Ayele *et al.*, 2015).

2.3. Free radicals and oxidative stress

2.3.1 Free radicals

Free radicals are atoms or molecules that have unpaired electrons, usually unstable and highly reactive. In biological systems, oxygen based radicals and nitrogen based radicals are the two types of free radicals. Oxygen free radicals, such as superoxide, hydroxyl radicals, and peroxyl radicals, with the addition of non-radicals, such as hydrogen peroxide, hypochlorous acid and ozone, are known as reactive oxygen species (ROS), which are generated during the metabolism process of oxygen (Apel and Hirt, 2004). Reactive nitrogen species (RNS), including nitrogen based radicals and non-radicals, such as nitrogen dioxide, nitric oxide radicals and peroxynitrite, are derived from nitric oxide and superoxide via inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, respectively. Due to their special chemical characteristics, ROS/RNS can initiate lipidperoxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury (Apel and Hirt, 2004). However, since the body is able to remove ROS/RNS to a certain degree, these reactive species are not necessarily a threat to the body under physiological conditions. The generation of ROS is a natural part of aerobic life, which is responsible for the manifestation of cellular functions including signal transduction pathways, defense against invading microorganisms and gene expression to the promotion of growth or death (Apel and Hirt, 2004; Li *et al*., 2015).

2.3.2. Oxidative stress and anti-oxidants

Oxidative/Nitrosative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen and nitrogen species as well as decreased production of antioxidants. Oxidative stress, in specific physiological conditions is actually useful; For

example, it could strengthen biological defense mechanisms during appropriate physical exercise and ischemia, and induce apoptosis to prepare the birth canal for delivery. However, this is confined to particular situations, and in most other cases, large levels of ROS and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury (Han *et al.,* 2001; Li *et al*., 2013).

ROS can be classified as either free radicals or non-radicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When two free radicals share their unpaired electrons, non-radical forms are created. The three major ROS that are of physiological significance are superoxide anion (O^{2-}**.**), hydroxyl radical (**.**OH) and hydrogen peroxide (H_2O_2) (Esra *et al.,* 2012).

Exogenous ROS can be produced as a result of smoking as well as exposure to pollutants, ozone, hyperoxia, heavy metals or radiation. Cigarette smoke contains many oxidants, free radicals and organic compounds, such as superoxide and nitric oxide. Short-term exposure to ozone also causes the release of inflammatory mediators, such as MPO, eosinophil cationic proteins, lactate dehydrogenase and albumin (Al-Dalaen and Al-Qtaitat, 2014).

An important marker of oxidative stress is malodialdehyde (MDA) a marker of lipid peroxidation. This occurs in areas where polyunsaturated fatty acid side chains are prevalent. These chains react with O_2 creating the peroxyl radical, which can obtain H^+ from another fatty acid, creating a continuous reaction (Li *et al*., 201).

The major enzymatic antioxidants are superoxide dismutases (SODs), catalase, glutathione peroxidases (GSH-Pxs) and glutathione-S-transferase (GSTs).Nonenzymatic Antioxidants include; Vitamin E (α-Tocopherol), Vitamin C (Ascorbic Acid), Carotenoids (β-Carotene) Thiol Antioxidants and Melatonin (Li *et al*., 2013).

2.4. Oxidative stress and Drug Induced Hepatotoxicity.

2.4.1 Oxidative stress and hepatotoxicity

The liver is a major organ attacked by reactive species (Li *et al.,* 2015). Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS, regulating on PPARγ, which is mainly related to the liver fatty acid oxidation gene expression. Moreover, Kupffer cells, hepatic stellate cells and endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules. A variety of cytokines like TNF-α can be produced in Kupffer cells induced by oxidative stress, which might increase inflammation and apoptosis. With regard to hepatic stellate cells, the proliferation and collagen synthesis of hepatic stellate cells is triggered by lipid peroxidation caused by oxidative stress (Wu and Cederbaum, 2009; Sakaguchi *et al.,* 2011; Cichoz-Lach and Michalak, 2014).

In mammals, a sophisticated antioxidant system has been developed to maintain the redox homeostasis in the liver (Figure 2.2). When the ROS is excessive, the homeostasis will be disturbed, resulting in oxidative stress, which plays a critical role in liver diseases and other chronic and degenerative disorders. The oxidative stress not only triggers hepatic damage by inducing irretrievable alteration of lipids, proteins and DNA contents and more importantly, modulating pathways that control normal biological functions. Since these pathways regulate genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation; oxidative stress is regarded as one of the pathological mechanisms that results in initiation and progression of various liver diseases, such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatitis (Feng *et al.,* 2011; Li *et al.,* 2014 and 2015).

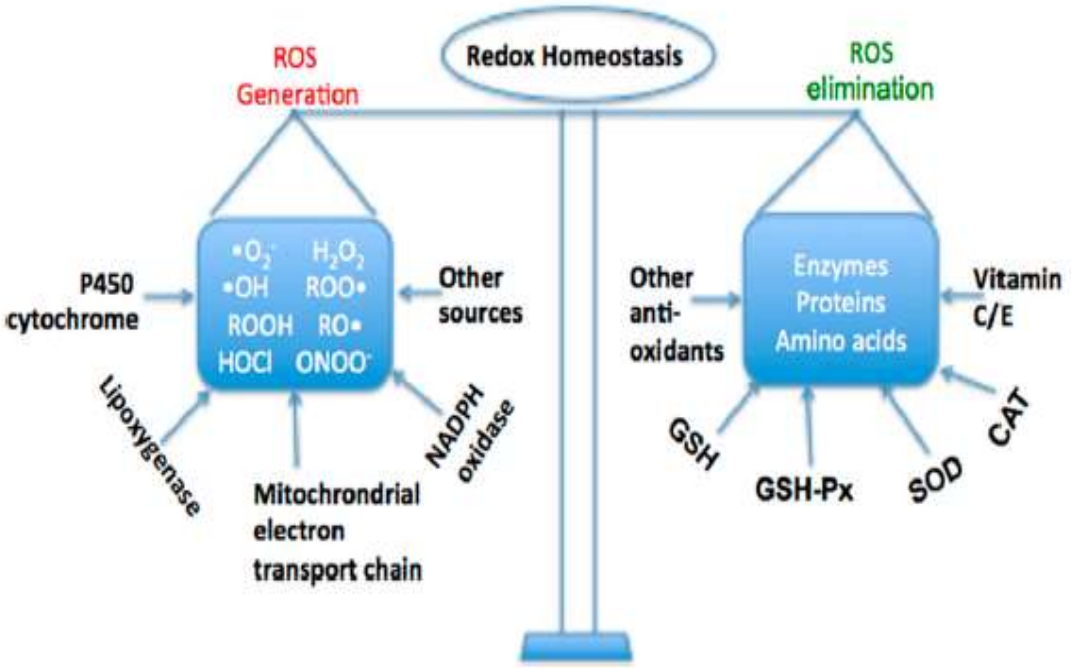


Figure 2.2 The redox homeostasis in the Liver (Li *et al.*, 2015)

The general mechanism scheme of oxidative stress induced by various factors on liverdisease is concluded in Figure 2.2. Moreover, systemic oxidative stress arising during liver disease can also cause damage to extra-hepatic organs, such as brain impairment and kidney failure. It was suggested systemic oxidative stress might be a significant “first hit”, acting synergistically with

ammonia to induce brain edema in chronic liver failure (Bosoi *et al.*, 2012; Palma *et al.*, 2014; Wang *et al.*, 2015).

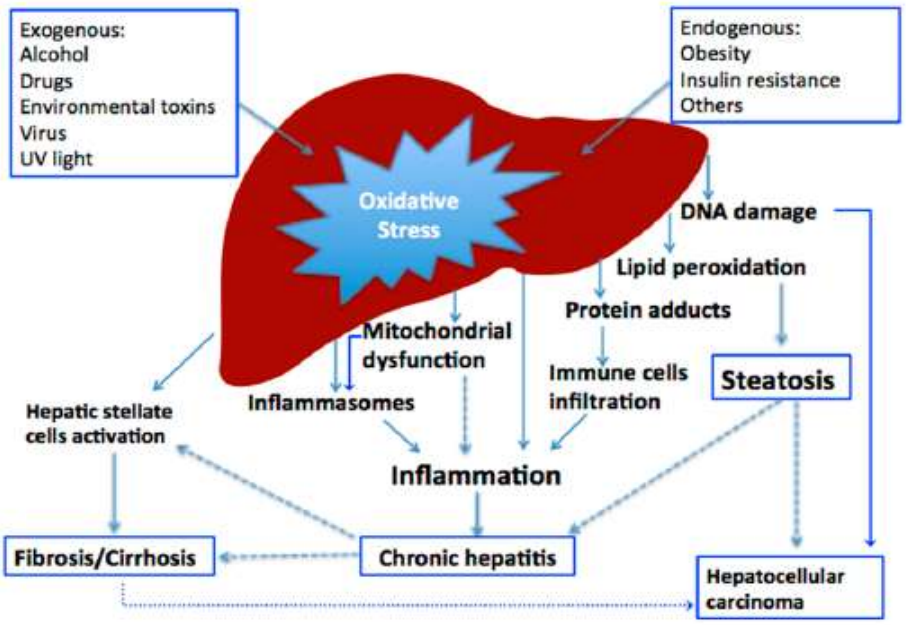


Figure 2.3 The general scheme of oxidative stress induced by various factors on liver disease (Li *et al.*, 2015).

Both enzymatic and non-enzymatic antioxidant system are essential for cellular response inorder to deal with oxidative stress under physiological condition. Therefore, antioxidant enzyme such as CAT, SOD, and GSH-Px and non-enzymatic electron receptors such as GSH are affected and used as indexes to evaluate the level of oxidative stress. Notably, erythroid 2-related factor 2 (Nrf2) is a major regulator of cellular redox balance. Under physiological condition, Nrf2 binds to kelch-like ECH-associated protein-1 (Keap1) in the cytoplasm and the ones remaining are inactivated and easily to be degraded. Under oxidative stress, however, Nrf2 dissociates form

Keap1 by Keap1 modification or Nrf2 phosphorylation and are thus activated. The activated Nrf2 translocates into the nucleus and interacts with antioxidant response element (ARE), promoting the expression of cytoprotective target genes including antioxidant enzymes and phase II detoxifying enzymes (Zhang *et al.*, 2013; Dey and Lesmanan, 2013; Tang *et al.*, 2014; Li *et al.*, 2015).

2.4.2 Drug induced liver injury

The liver plays an array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Sharma *et al.,* 1991).

Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes when administered within therapeutic ranges, may injure the liver. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests (Ostapowich *et al.,* 2002, Pandit *et al.,* 2012).

Drug induced liver injury is responsible for 5% of all hospital admissions and 50 % of all acute liver failures. More than 75 % of cases of idiosyncratic drug reactions result in liver transplantation or death (Pandit *et al.,* 2012).

2.4.3 Anti TB-Drug Induced Liver Injury

Anti-Tuberculosis drug-induced liver injury (ATDILI) is the most prevalent drug-induced hepatotoxicity in Taiwan, China, South Africa and many other areas, which may both threaten patients' health and hinder the treatment of TB. Hence attempts at ameliorating this potentially grave drug-induced liver injury (DILI) are crucial. The three common first-line drugs for TB are isoniazid, rifampicin and pyrazinamide, which have the potential to induce liver damage (Huang, 2007 and 2014).

Anti-tuberculosis drug-induced liver injury (ATDILI) ranges from mild to severe forms, and can even be fatal. The incidence of ATDILI depends on different anti-tuberculosis regimens, definitions of liver injury, and ethnic populations. Generally, 10 - 20% of patients may have elevation of serum aminotransferase during administration of these drugs. Approximately 1% of patients may develop overt hepatitis, defined as symptomatic hepatotoxicity with jaundice, and significant elevation of serum aminotransferase. The mortality rate of patients with overt hepatitis is estimated to be around 10% (Huang, 2014).

2.4.4 Isoniazid induced liver injury

Isoniazid is primarily cleared through acetylation by N-acetyltransferase 2 (NAT2) in the liver, resulting in acetylisonazid which is then hydrolysed to isonicotinic acid and monoacetylhydrazine (MAH). INH also undergoes hydrolysis catalysed by isoniazid hydrolase and forms hydrazine, which is then metabolised to MAH by NAT2. MAH can be acetylated to diacetylhydrazine which is non-toxic, or oxidised by cytochrome P4502E1 (CYP2E1) into hepatotoxic intermediates (Ryan *et al.*, 1985, Ching, 2011).

Acetylhydrazine can be hydrolysed to hydrazine which may further induce CYP2E1, increasing the production of toxic metabolites as shown in figure 2.3 below. Hydrazine, MAH and

isonicotinic acid are potentially hepatotoxic metabolites of INH and accumulation of these reactive metabolites in patients may cause serious adverse drug reactions. The enzyme responsible for INH hydrolysis has still not been well characterized. It is usually described as an amidase but in a recent report found that hydrazine formation from INH in rat hepatocytes could be inhibited by treatment with bis-p-nitrophenylphosphate, which was originally described as a carboxylesterase inhibitor. There is increasing evidence that the hydrolysis of INH is catalyzed by a carboxylesterase but the precise isoform responsible is still unclear (Yamada *et al.*, 2009). In human liver, two carboxylesterase isoforms CES1A1 and CES2 are expressed at detectable levels and show different substrate specificities (Yang *et al.*, 2009; Ching, 2011).

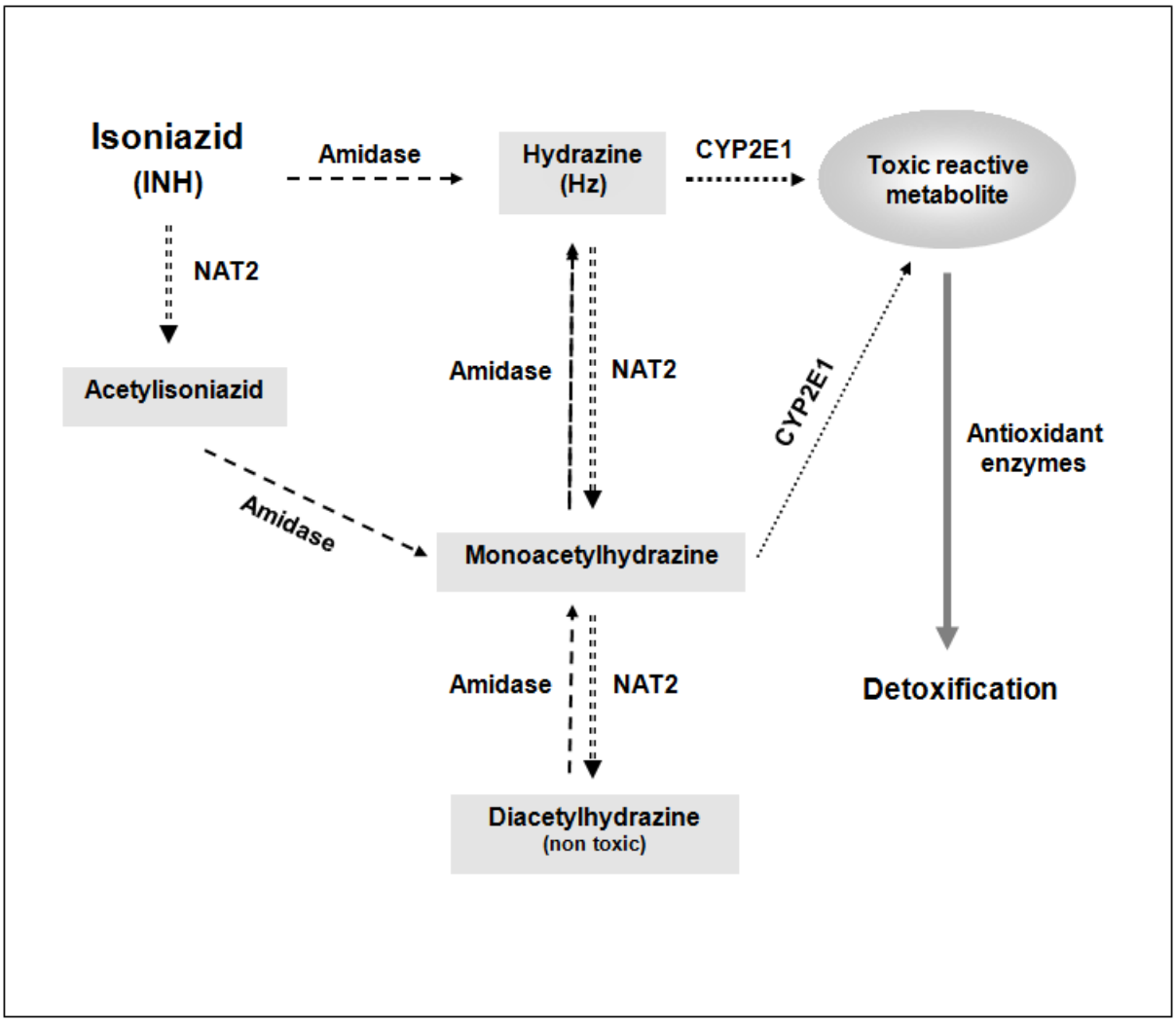


Figure 2.3 Metabolic Pathways of Isoniazid (Ching, 2011)

In 1969, INH received a black box warning from the US Food and Drug Administration due to its association with high incidence of adverse drug reactions, particularly hepatocyte injury. Various metabolites of INH have been suggested as hepatotoxic, including hydrazine, monoacetyl hydrazine (MAH), acetylisoniazid and isonicotinic acid. Administration of acetylhydrazine or acetylisoniazid in rats leads to the production of reactive alkylating species and covalent binding to liver proteins, causing hepatocyte injury manifesting as hepatocyte

necrosis, sinusoidal dilatation and infiltration by inflammatory cells. NAT2, the major known enzyme involved in the metabolic pathway of INH and metabolites is suggested to play an important role in INH-induced hepatotoxicity, particularly the NAT2 slow acetylator genotypes/phenotypes (Ching, 2011).

2.5 Liver Enzymes and other Biomarkers of Hepatotoxicity

The liver is the primary site of drug metabolism; other tissues, such as kidney, brain, skin, blood, lung and gastrointestinal mucosa, also contribute (Krishna and Klotz, 1994). The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea. In conjunction with the spleen it is involved in the destruction of spent red blood cells and the reclamation of their constituents. It is responsible for synthesizing and secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors (Burkitt *et al*., 1993). It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from non-carbohydrate sources such as amino acids (gluconeogenesis). Measurement of liver enzymes remains the most practical tool to diagnose liver damage, and includes mainly alanine aminotransferase ALT), an enzyme present in hepatocytes, and alkaline phosphatase (ALP), an enzyme in the cells lining the biliary ducts of the liver. Based on elevation of, and ratio between elevations of, these enzymes, hepatocellular, cholestatic or mixed liver injury is diagnosed (Benichou, 1990).

2.5.1 Alkaline phosphatase (ALP)

ALP transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines (Gowda *et al.*, 2009). ALP is present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. It performs lipid transportation in the intestine and calcification in bone. The serum ALP activity is mainly from the liver with 50% contributed by bone (Mauro *et al*., 2006). Normal serum ALP is 41 to 133U/L. Drug-induced liver injury may present with a cholestatic pattern (preferential increase in ALP or ALT/ALP ratio < 2), although the degree of ALP alteration is variable and may be accompanied by hyperbilirubinemia (Velayudham and Farrell, 2003)

2.5.2 Alanine aminotransferase (ALT)

ALT is found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body. ALT is purely cytoplasmic catalyzing the transamination reaction (Mauro *et al*., 2006). Normal serum ALT is 7-56 U/ L (James *et al*., 2006). Any type of liver cell injury can reasonably increases ALT levels. Elevated values up to 300 U/L are considered nonspecific. Marked elevations of ALT levels greater than 500 U/L observed most often in persons with diseases that affect primarily hepatocytes such as viral hepatitis, ischemic liver injury (shock liver) and toxin-induced liver damage. Despite the association between greatly elevated ALT levels and its specificity to hepatocellular diseases, the absolute peak of the ALT elevation does not correlate with the extent of liver cell damage (Kallei *et al*., 1964). Moreover increased ALT level was associated with reduced insulin sensitivity, adiponectin and glucose tolerance as well as increased free fatty acids and triglycerides (James *et al*., 2006).

2.5.3 Aspartate aminotransferase (AST)

AST catalyzes transamination reaction. It exists in two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasmic form. AST is found in highest concentration in heart compared with other tissues of the body such as liver, skeletal muscle and kidney (Mauro *et al*., 2006). Normal serum AST is 0 to 35U/L (Diana, 2007) and elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also in chronic liver diseases like liver tissue degeneration and necrosis. About 80% of AST activity of the liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme. However the ratio of mitochondrial AST to total AST activity has diagnostic importance in identifying the liver cell necrotic type condition and alcoholic hepatitis. In alcoholic hepatitis an AST/ALT ratio of greater than 2 is characteristic (Panteghini *et al*., 1983).

2.5.4 Serum total and direct (Conjugated) bilirubin

Bilirubin is the catabolic product of haemoglobin produced within the reticuloendothelial system, released in unconjugated form which enters into the liver, converted to conjugated forms bilirubin mono and diglucuronides by the enzyme UDP-glucuronyltransferase (Mauro *et al.*, 2006). Normal serum total bilirubin varies from 2 to 21µmol/L. The indirect (unconjugated) bilirubin level is less than 12µmol/L and direct (conjugated) bilirubin less than 8µmol/L. The serum bilirubin levels more than 17µmol/L suggest liver diseases and levels above 24µmol/L indicate abnormal laboratory liver tests (Diana, 2007). The occurrence of unconjugated hyperbilirubinemia is due to over production of bilirubin, decreased hepatic uptake or

conjugation or both. It is observed in genetic defect of UDP-glucuronyltransferase causing Gilbert's syndrome, Crigler-Najjar syndrome and reabsorption of large hematomas and ineffective erythropoiesis. In viral hepatitis, hepatocellular damage, toxic or ischemic liver injury higher levels of serum conjugated bilirubin is seen (Gowda *et al.,* 2009). Hyperbilirubinemia in acute viral hepatitis is directly proportional to the degree of histological injury of hepatocytes and the longer course of the disease. It has been observed that the decrease of conjugated serum bilirubin is a bimodal fashion when the biliary obstruction is resolved. Parenchymal liver diseases or incomplete extrahepatic obstruction due to biliary canaliculi give lower serum bilirubin value than those occur with malignant obstruction of common bile duct but the level remains normal in infiltrative diseases like tumours and granuloma. Drugs such as rifampicin and isoniazid have been shown to cause isolated rise in serum unconjugated bilirubin (Limdi and Hyde, 2003; Gowda *et al.,* 2009).

2.5.5 Serum total protein and albumin.

Serum protein consists of majorly albumin, globulins and fibrinogen; with albumin forming more than 50% of the total serum protein. Human albumin is an essential protein found in human plasma and accounts for about 50%-60% of plasma proteins (Kratz and Elsadek, 2012). Each molecule is composed of three units, or domains, which function together to give albumin its unique binding properties. Albumin is primarily responsible for 75%-80% of plasma's normal colloid oncotic pressure (Rathod *et al.,* 2016). Albumin is synthesized in the liver and its serum level may be decreased in disease such as chronic liver disease and cirrhosis. The rate of synthesis is constant in normal individuals at 150 to 250 mg/kg/day (Rathod *et al.,* 2016). Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in

the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure (known also as colloid osmotic pressure) of plasma because smaller animals (for example rats) function at a lower blood pressure, they need less oncotic pressure to balance this, and thus need less albumin to maintain proper fluid distribution (Fasona *et al.*, 2005).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Chemicals and drugs

Isoniazid tablets (300mg) and Livolin Forte® (Phosphatidylcholine, vitamins B complex and E) capsules were obtained from the Pharmacy Department of Ahmadu Bello University Teaching Hospital (ABUTH). MDA, SOD and CAT assays kits were obtained from Northwest Life Sciences Specialities, with product specifications; Product NWK-MDA01, Product NWK-SOD02 and Product NWK-CAT0, Vancouver WA, Canada.

The Serum Liver Enzymes (ALT, AST and ALP) Assay Kits, were obtained from Agape Diagnostic Laboratories, India. The BCG (Bromocresol Green) Albumin Assay Kit was obtained from Sigma – Aldrich USA, while Bilirubin Liquid Assay kit was from Randox Laboratories, UK

3.1.2 Instruments and Devices

Routine laboratory equipment were used including a Bench Centrifuge Hawksley (RegNo: 891481 Ser. No. 07. 4. 26.®), Weighing balance, Weighing balance (Salter, HoMedics Group Ltd. Production no. IB-1066-1011-03) and metal cages.

3.2. Collection of plant materials

The plant was obtained from Nimbia Forest Reserve, Jema'a LGA; Kaduna State, Nigeria. Plant identification was done at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria and a specimen Voucher (Voucher Number : 926) was collected for further reference.

3.3 Preparation of plant extract

Fresh leaves of *B. coccineus* were air-dried until a constant weight was obtained. Aqueous extraction was done at the Basic Research Laboratory of the National Research Institute for Chemical Technology, Bassawa Zaria. The dried material was macerated in distilled water (30 g in 3 L) and refrigerated at 4 ^{0}C. The extract was decanted 24 hours later. The filtrate was then evaporated to dryness in an oven at 40^{0}C to yield a dark brown powder (Akindele and Adeyemi, 2006). A portion of the extract was reconstituted in distilled water and used for phytochemical analysis. The dried extract was weighed and reconstituted in distilled water (pH = 6.8), just before administration to experimental animals, to obtain a concentration of 10 mg/ml to enable administration of appropriate volumes based on the dose to be administered to the different treatment groups.

3.4. Experimental animals

A total of 36 adult male Wistar rats weighing 80g – 150g were purchased at the Experimental Animal House of the Department of Human Physiology, Ahmadu Bello University Zaria. Animals were maintained under normal laboratory conditions. Animals were maintained on *pellets* of growers mash and given access to water *ad libitum.*

3.5 Methodology

3.5.1 Acute Toxicity Studies of the aqueous extracts of *Brysocarpus Coccineus (Conaraceae)*

The LD50 determination for aqueous extracts of *B. Coccineus*was conducted using the method of Lorke (1983). The evaluation was done in two phases. In phase I three groups of three rats each (n=3), were treated orally with 10, 100 and 1000 mg/kg of the aqueous extracts dissolved in distilled water respectively. A fourth group of three rats received distilled water and served as control. The rats were observed for clinical signs and symptoms of toxicity within 24 hours and death within 72 hours. Based on the results of phase I, fresh rats three in number (n=3) were each be treated with 1600, 2900 and 5000 mg/kg of the aqueous extracts of *B. Coccineus*. A fourth rat received distilled water and served as control. Clinical signs and symptoms of toxic effects and mortality were then observed within 24 hours and death within 72 hours. The LD50 was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in phase II.

3.5.2 Phytochemical analysis

A portion of the extract was subjected to series of phytochemical screening using standard protocols as described below

3.5.2.1 Test for Carbohydrates

A few drops of the molisch reagent were added to a clear solution of the extract (0.5 mg) dissolved in water. This was followed by the addition of 1.0 ml of concentrated Sulphuric acid down the side of the test tube, the mixture was allowed to stand for 2 minutes and then it was

diluted with 5.0 ml of water. The appearance of a red colour at the interphase of the two layers confirms the presence of carbohydrates (Brain and Tuner, 1975).

3.5.2.2 Barfoed's Test

To a clear solution of the extract dissolved in water in a test tube, 1.0 ml of Barfoeds reagent was added and heated in a water bath for 2 minutes. A red precipitate of Cu_2O was taken as a positive test for the presence of sugars (Trease and Evans, 1997).

3.5.2.3 Test for Tannins

About 0.5 mg of the extract was boiled with 10.0 ml of water, cooled and filtered. To 1,0 ml of the filtrate a few drops of lead acetonate was added, and the formation of a white precipitate indicated the presence of tannins (Trease and Evans, 1997). To another portion of the filtrate, a few drops of 1% Iron II Chloride solution was added, the presence of blue – black, green or blue – green precipitate was an evidence for the presence of tannins (Harbone, 1973).

3.5.2.4 Test for Saponins

About 0.5 mg of the extract was shaken with water in a test tube and then warmed in a water bath. Persistent frothing indicates the presence of saponins. A small quantity of each extract portion was boiled with 10 ml of water. This was filtered and to the filtrate, 2.5 ml equal volumes mixture of Fehlings solution A and B was added. The appearance of a brick red precipitate indicates he presence of Saponin Glycosides (Sofowora, 2008).

3.5.2.5 Test for Flavonoids (Shinoda's Test)

A little portion of the extracts was dissolved in ethanol this was further warmed and filtered. Three to Four pieces of Magnesium chips was added to the filtrate, followed by the addition of few drops of concentrated Hydrochloric Acid. The presence of pink coloration was an indication of the presence of Flavonoids (Trease and Evans, 1997).

3.5.2.6 Test for Glycosides (General Test)

A small quantity of each extract was boiled with 25ml of 2.5M Tetraoxosulphate (VI) acid. This was cooled and neutralized with 20 % potassium hydroxide and then boiled 5.0 ml of a mixture of equal volume of Fehlings solution A and B. Formation of a brick red precipitate shows the presence of glycosides (Trease and Evans, 1997).

3.5.2.7 Test for Alkaloids

Little portion of the extract was stired with 5.0 mls of 1% aqueous hydrochloric acid (HCl) on a water bath and later filtered, 3ml of the filtrate was divided into 3 portions of 1.0 ml each. The first portion was treated with few drops of Dragendoffs reagent to give an orange red precipitate as an evidence of alkaloid. To the second 1 ml portion, Mayers reagent was added to give a buff coloured precipitate as an evidence of alkaloid. Lastly, the third portion was treated with wagners reagent to give a dark brown precipitate. All three colours confirm the presence of alkaloid (Brain and Tuner, 1975).

3.5.2.8 Quantitative Phytochemical analysis

Alkaloid determination

Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Flavonoid determination

Ten grams of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipai- Abyazan, 1994).

Saponin determination:

The method used was that of Obadoni and Ochuko (2001). Twenty grams of samples powder was put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue reextracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered whilethe ether layer was discarded. The purificationprocess was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of

5% aqueous sodiumchloride. The remaining solution was heated in a waterbath. After evaporation the samples weredried in the oven to a constant weight and the saponin content was calculated as percentage.

Determination of tannins

Powdered extract (0.5 g) was extracted with 300ml of diethyl ether for 20 hours at room temperature. The residue was boiled for 2 h with 100 ml of distilled water, and then allowed to cool, and was filtered. The extract was adjusted to a volume of 100 ml in a volumetric flask. The content of tannins in the extract was determined colorimetrically using Folin–Denis reagent, and by measuring absorbance of the blue complex at 760 nm, using tannic acid solution as a standard Solution (Chanwitheesuk *et al.*, 2005).

3.5.3 Drug preparation

Isoniazid tablets (300mg each) were dissolved in distilled water to make a solution of 10mg/ml and administered as an aqueous suspension. The drug suspension was continuously agitated during administration in order to deliver the drug homogeneously to the animals. Livolin Forte was dissolved in Tween 80 to make a solution of 10mg/ml which was continuously stirred during administration in order to deliver the drug homogeneously. Administration of isoniazid was done in the mornings at 0900hrs while Livolin forte and aqueous extracts of *B. Coccineus* were administered in the evenings at 1600hrs.

3.5.4 Experimental design

The rats were randomly assigned into six groups consisting of six animals each (n = 6)

Group I: Distilled water for 30 days (control) per oral (p.o).

Group II - Isoniazid (27 mg/kg/day), p.o. for 30 days which served as toxic control

Group III - Isoniazid (27 mg/kg/day), p.o and Livolin® 20 mg/kg p.o for 30 days which served as positive control (Akindele, *et al*., 2010)

Group IV - Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 200 mg/kg p.o for 30 days (Akindele, *et al*., 2010).

Group V - Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 400 mg/kg p.o for 30 days (Akindele, *et al*., 2010).

Group VI - Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 800 mg/kg p.o for 30 days (Akindele, *et al*., 2010).

3.6 Data Collection

At the end of the experiment, after overnight fasting, the animals were euthanized and dissected; blood was collected through cardiac puncture, centrifuged at 3000 rpm and used for measurement of oxidative stress and hepatotoxicity biomarkers.

3.6.1 Assay of antioxidant enzyme activities.

3.6.1.1 Malondialdehyde (MDA) Concentration

The level of thiobarbituric-acid reactive substance, malondialdehyde (MDA), as an index of lipid peroxidation was evaluated. Quantitative measurement of lipid peroxidation of MDA was determined using NWLSSTM MDA assay kit (Northwest Life Sciences Specialities, Product NWK-MDA01, Vancouver WA, and Specificity: Malondialdehyde, sensitivity: 0.08 μM). The principle is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA adducts that absorbed strongly at 532 nm (Janero, 1990).

3.6.1.2 Catalase Activity

Catalase (CAT) activity was assessed using NWLSS CAT activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL). Catalase enzyme activity was measured based on the principle of catalase consumption of H_2O_2 substrate at 240 nm (Beers and Sizer, 1952).

3.6.1.3 Superoxide Dismutase Activity

Activity of SOD in the rat serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit was based on the principle of superoxide inhibition of autooxidation of hematoxylin as described by (Martin *et al.*, 1987).

3.6.2 Liver enzymes analysis

The serum liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined from the serum, using Agape Diagnostic kits, India.

3.6.2.1 Determination of serum aspartate aminotransferase (AST)

This was estimated by method as described by, Bergmeyer and Walefeld (1978). Exactly, 1000µL of the reagent was added to 100µL of the samples and then mixed and incubated at 37ºC for 1 min. The change in absorbance of the sample was measured per minute spectrophotometerically at the wavelength of 590.

3.6.2.2 Determination of serum alanine aminotransferase (ALT)

This was estimated by the method as described by, Bergmeyer and Walefeld (1978). Exactly,1000µL of the reagent was added to 100µL of the samples and then mixed and incubated at 37ºC for 1 min. The change in absorbance of the sample was measured per minute.

3.6.2.3 Determination of serum alkaline phosphatase (ALP)

This was estimated by method as described by, Bowers and Mc Comb (1966). Exactly, 0.5 mL of the reagent was added to 0.05 mL (500µL) of the samples and then mixed and incubated at 37ºC for 10 min. The change in absorbance of the sample was measured per minute.

3.6.3. Determination of total proteins and serum albumin

3.6.3.1 Determination of Serum total protein

This was assayed by the direct biuret method described by Layne (1957). Exactly,0.9 ml Biuret Reagent was added to 0.3 ml of sample in a microcentrifuge tubeand mixed thoroughly by

vortexing. After waiting for 5 minutes the Absorbance at 540 nm was measured against a blank containing Biuret Reagent and distilled water, but no protein.

3.6.3.2 Determination of Serum albumin

This was assayed using the BCG (Bromocresol Green) Albumin Assay Kit from Sigma – Aldrich USA based on the principle developed by Doumas and Watson (1971). Exactly,20 mL each of diluted standards, Blank, and diluted samples were transfered to appropriately labeled tubes. Then 1,000 mL of Reagent was added and tapped lightly to mix, this was incubated for 5 minutes at room temperature and the absorbance measured at 620 nm (A620).

3.6.4 Determination of serum total bilirubin and conjugated bilirubin

These was determined using the Bilirubin Liquid Assay kit from Randox Laboratories, UK based on the Colorimetric principle described by Jendrassik and Grof (1938). Direct bilirubin reacts with sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin was determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

3.6.5 Histological studies of liver tissue

Liver tissue was harvested on the 30^{th} day from the experimental groups and used for histological study in the Department of Histopathology, Faculty of Medicine; Ahmadu Bello University Teaching Hospital, Zaria. The method of H and E staining technique was used which involved hydrating the tissue sections in descending grades of alcohol from 100%, 95%, 90% and finally 70% (Laporta *et al*., 2013). Each of these steps lasted three (3) minutes and the tissues were then washed in running tap water. The tissue was stained with haematoxylin for twenty five (25) minutes, washed with water and then differentiated in acid alcohol. The tissue were then counter

stained with eosin and blued in Scott water. The tissues were hydrated with ascending grades of alcohol and cleared in xylene for three (3) changes in five (5) minutes each. The tissues was mounted with cover slips using a mounting media. The tissues were viewed under a light microscope and the photomicrographs taken.

3.7 Statistical analyses

Data obtained was expressed as mean (±SEM). The results were analyzed using one-way analysis of variance (ANOVA), followed by Tukeys *post-hoc* test to compare the level of significance between groups using SPSS version 20.0, values of $p \leq 0.05$ were considered significant (Duncan *et al*., 1977).

CHAPTER FOUR

4.0 RESULTS

4.1 The results of toxicity studies of aqueous extracts of *Brysocarpus coccineus (connaraceae)*

There was no mortality in both phase I and II studies, hence the LD 50 was greater than 5000 mg/kg bw

Table 4.1 Acute lethal effects of aqueous extracts of *Brysocarpus coccineus (connaraceae)* administered orally to adult male Wistar rats.

Experiment	**Dose (mg/kg bw)**	**Number of Dead rats after 24hrs**	**Treated rats after 24hrs**
Phase I	10	0/3	0/3
	1000	0/3	0/3
	10000	0/3	0/3
Control	0	0/3	0/3
Phase II	1600	0/1	0/1
	2900	0/1	0/1
	5000	0/1	0/1

4.2 The results of phytochemical studies of *Brysocarpus coccineus (connaraceae).*

Both qualitative and quantitative assays showed high levels of Alkaloids, Flavonoids and sugars.

Table 4.2 Qualitative phytochemical screening of aqueous extracts of *Brysocarpus coccineus*

CONSTITUENT	TEST	INFERENCE
Carbohydrates	Molisch test	++
Sugar	Aniline test	+++
Tannins	Iron (III) Chloride	++
Saponin Glycoside	Fehlings Solution	++
Alkaloids	Dragendoorffs	++
Flavonoids	Shnodas	+++
Sterolds	Lieberman buchard	_
Cardiac Glycoside	Lega's	+
Emodol	Borntrager's	_

Table 4.3 Quantitative Phytochemical studies of *Brysocarpus Coccineus (Conaraceae*) aqueous extracts

Phytochemical	**Percentage (%)**
Tannins	2.29
Saponin	18.05
Alkaloid	23.24
Flavonoid	18.99

4.3 The effects of aqueous extracts of *Brysocarpus coccineus* on serum Malondialdehyde level.

The serum level of malondialdehyde in rats treated with Isoniazid (1.38 ± 0.12) was increased significantly compared to the control group (1.18 ± 0.15). It was reduced in rats treated livolin forte and the aqueous extracts of *B.coccineus*at 200 mg/kg (1.33 ± 0.14), 400 mg/kg (1.35 ± 0.11) and 800mg/kg (1.32 ± 0.16) compared to the control groups; which was not significant.

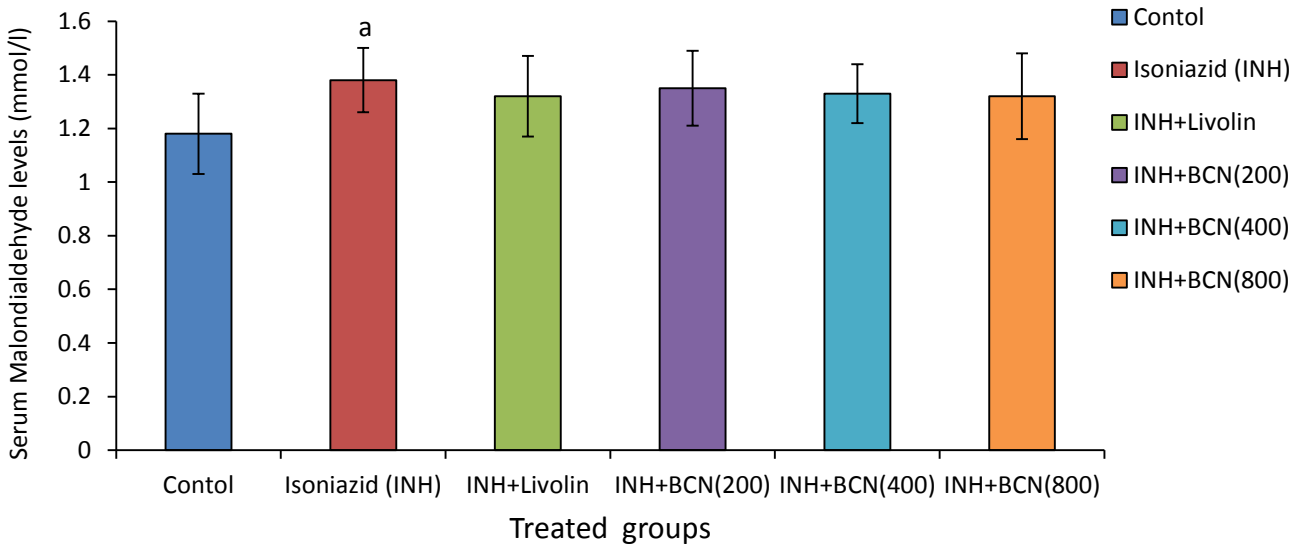


Figure 4.1: Serum Malondialdehyde levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.4. The effects of aqueous extracts of *Brysocarpus coccineus* on serum Superoxide dismutase level.

The serum levels of Superoxide dismutase (SOD) was reduced in the group treated with INH (2.13 ± 0.29) compared to the control (2.28 ± 0.17), however it was not statistically significant. There was also a decrease in SOD level in the group treated with livolin forte compared to the control. The serum SOD was increased only in the group treated with the aqueous extracts of *B. coccinneus* at 200 mg/kg (2.17 ± 0.21) compared to the isoniazid group (2.13 ± 0.29) and the livolin forte group (2.00 ± 0.24), however this increase was not significant.

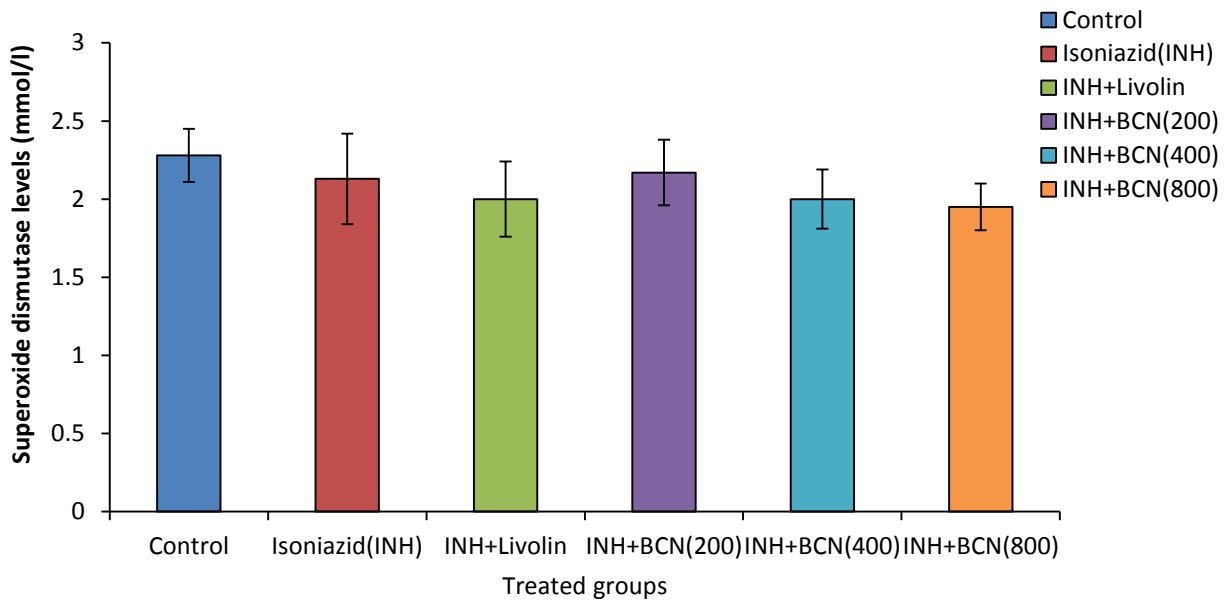


Figure 4.2: Serum Superoxide Dismutases levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.5 The effects of aqueous extracts of *Brysocarpus coccineus* on serum Catalase level.

In Figure 4.3 there was reduction in serum levels of catalase level in the group treated with isoniazid (48.17 ± 1.84) and the livolin forte (48.33 ± 4.50) groups compared to the normal (51.33 ± 3.39), however it was not statistically significant. There was a general reduction in the serum catalase in the treated groups compared to the normal control (51.33 ± 3.39), this was significant in the group treated with *B.coccineus* 200 mg/kg (46.0 ± 1.79), when compared with the normal control (51.3 ± 3.39).

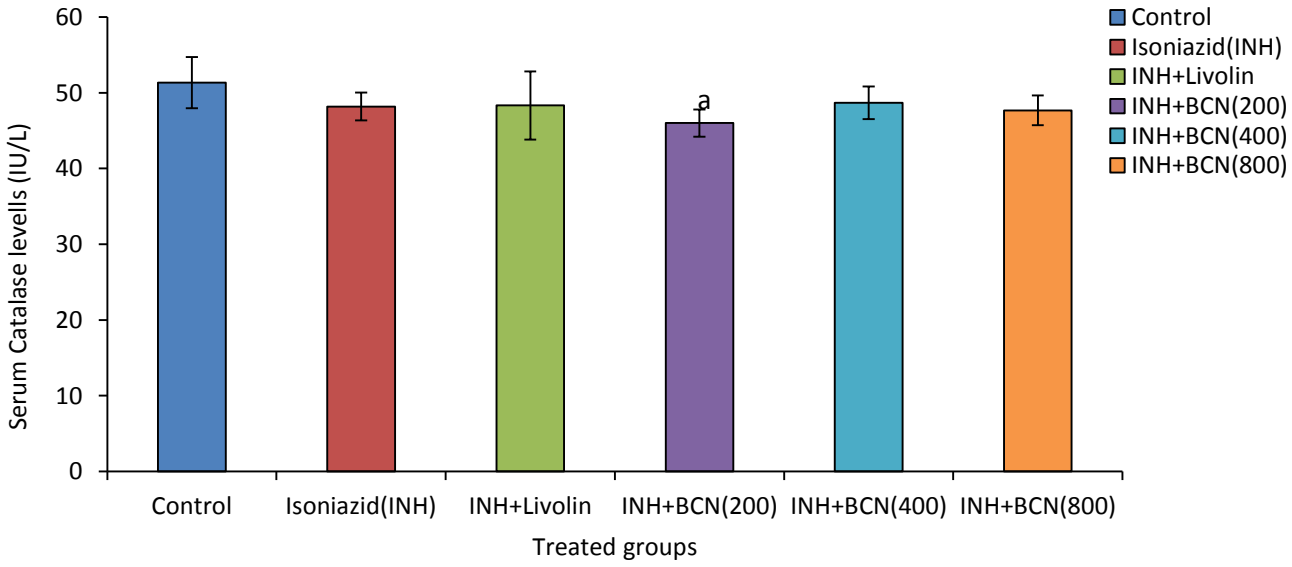


Figure 4.3: Serum Catalase levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.6 The effects of aqueous extracts of *Brysocarpus coccineus* on serum Aspartate Aminotransferase (AST) level.

Figure 4.4 shows an insignificant increase in the levels of aspartate aminotransferase in the isoniazid treated group (50.83 ± 4.21) compared to the control (44.67 ± 4.00). There was a decrease in serum AST levels in the group treated with livolin forte (44.67 ± 6.41) and the extracts at 200 mg/kg (45.0 ± 2.61) when both are compared to the control (50.38 ± 4.41), however this difference was not significant. Progressive, insignificant increase in the serum levels of aspartate aminotransferase levels was seen in groups treated with the aqueous extracts of *B. coccineus* at 400 and 800 mg/kg when compared with the control group,this increase was greatest in the group that received *B. coccineus* 800 mg/kg (56.00 ± 2.19)

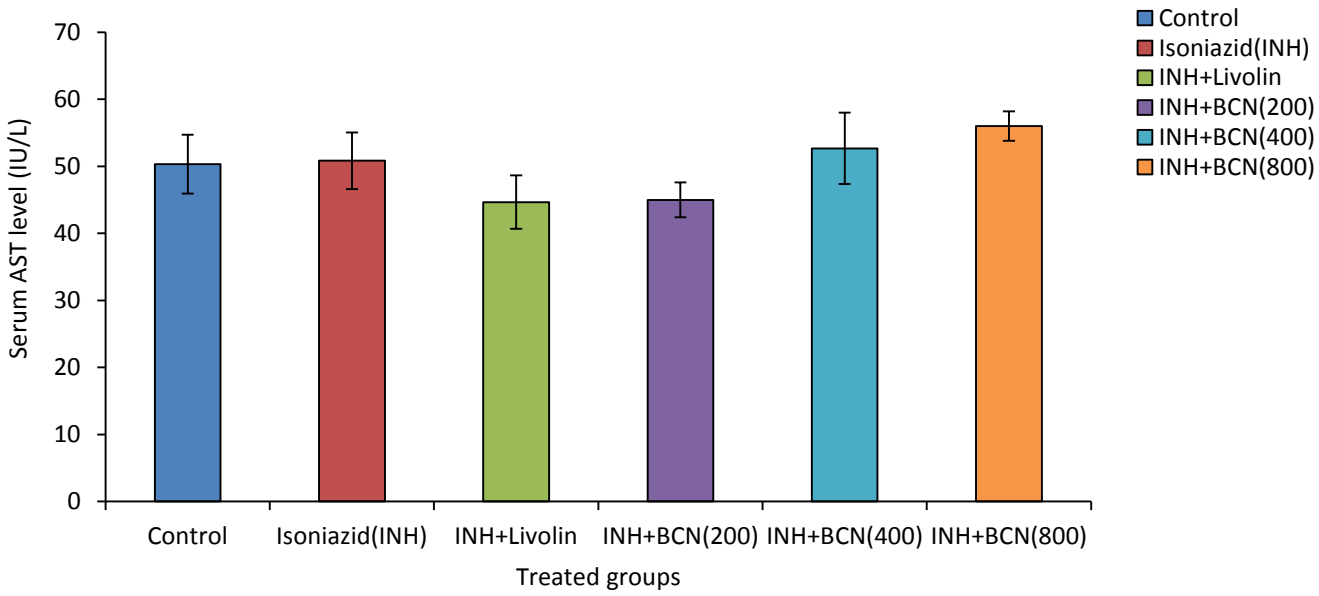

70
60
50
40
30
20
10
0
Serum AST level (IU/L)
Control
Isoniazid(INH)
INH+Livolin
INH+BCN(200)
INH+BCN(400)
INH+BCN(800)
Treated groups
Control
Isoniazid(INH)
INH+Livolin
INH+BCN(200)
INH+BCN(400)
INH+BCN(800)

Figure 4.4: Serum Aspartate Aminotransferase (AST) levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.7. The effects of aqueous extracts of *Brysocarpus coccineus* on serum Alanine Aminotransferase (ALT) level.

There was a slight increase in the serum alanine aminotransferase in the Isoniazid (55.83 ± 5.11) group compared to the control group (54.17 ± 3.97) which was not significant. There was however a decrease in serum ALT level in the group treated with *B.Coccineus* 200 mg/kg (50.83 ± 3.37) and the group treated with livolin forte (48.83 ±5.54) when compared to the control (54.17 ± 3.97) but it was not significant. Progressive, insignificant increase in the serum levels of aspartate aminotransferase levels was observed in groups treated with the extracts at 400 and 800

mg/kg when compared with the control group this increase was greatest in the group that received *B. coccineus* 800 mg/kg (59.83 ± 2.79).

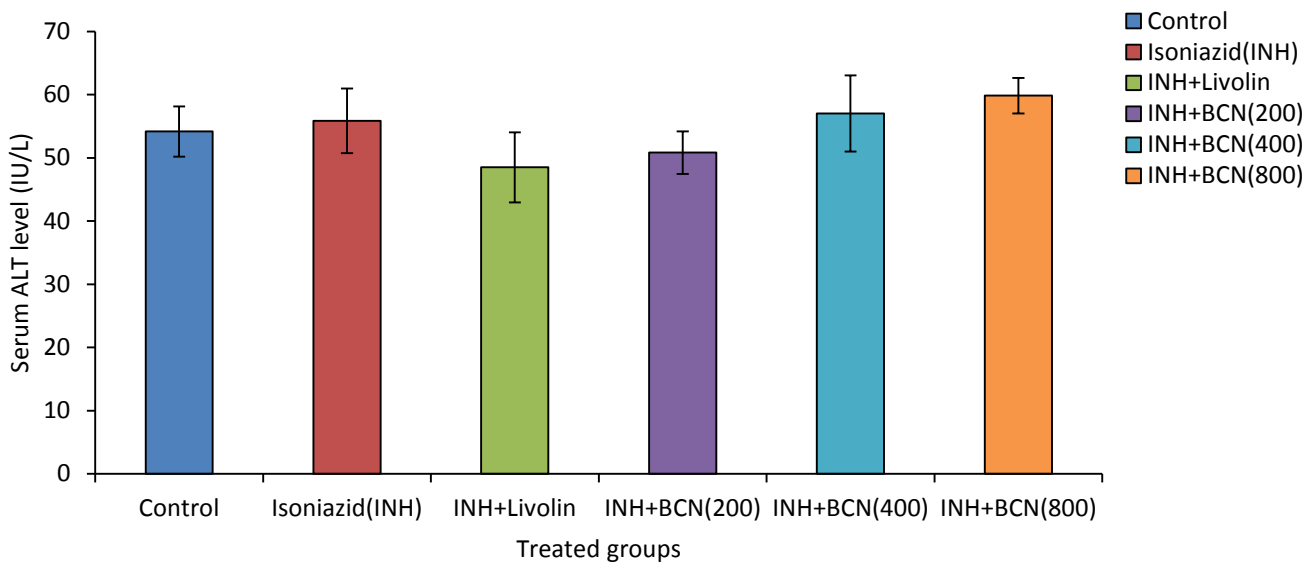


Figure 4.5: Serum Alanine Aminotransferase (ALT) levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.8. The effects of aqueous extracts of *Brysocarpus coccineus* on serum Alkaline Phosphatase (ALP) level.

Figure 4.6 shows a slight increase in the serum level of alkaline phosphatase in the isoniazid group (81.17 ± 1.84) compared to the normal, however it was not significant. There was also a decrease in the level of alkaline phosphatase in the group treated with livolin forte (82.83 ± 5.57) compared to the control, with an increase in the groups treated with the extracts at 200, 400 and 800 mg/kg (107.17 ± 5.98) compared to the control. The increase in the group treated with *B.Coccineus* 800 mg/kg (107.17 ± 5.98) was significant.

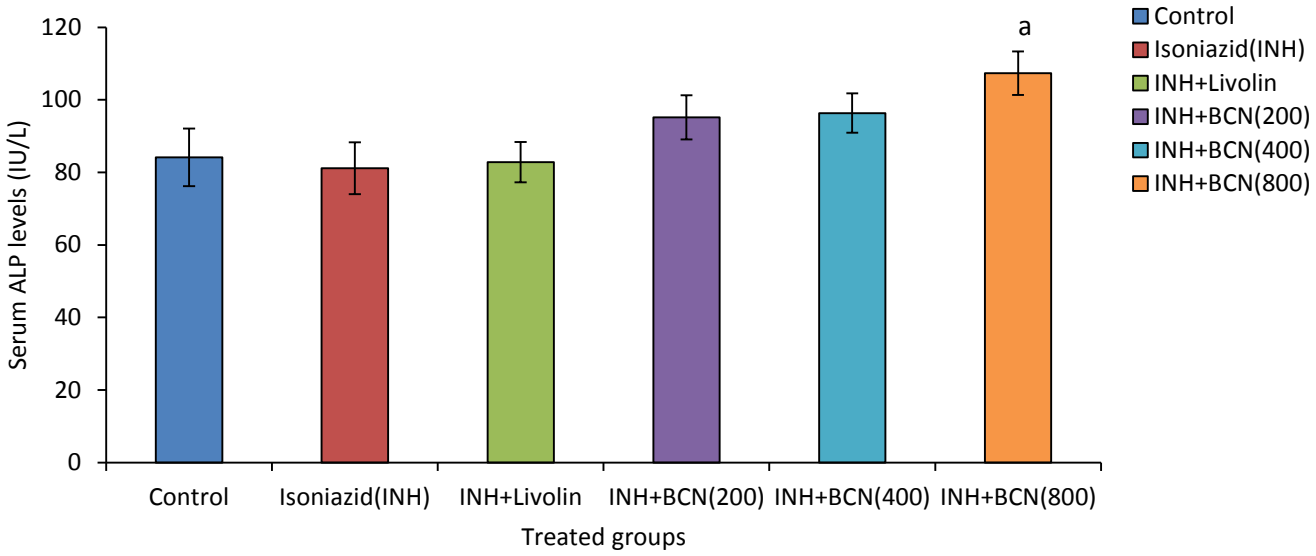


Figure 4.6: Serum Alkaline Phosphatase (ALP) levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.9 The effects of aqueous extracts of *Brysocarpus coccineus* on Serum Total Protein

Figure 4.7 shows a statistically significant ($p < 0.05$) increase in the serum total protein in the groups treated with isoniazid (66.50 ± 0.50) compared to the control group (66.17 ± 0.39), this increase was reversed in the group treated with livolin forte (66.00 ± 0.45) however it was not statistically significant. There was an increase in serum total protein in groups treated with *B. Coccineus* 400 mg/kg (66.3 ± 0.65) and 800 mg/kg (66.5 ± 0.63) compared to the control (66.17 ± 0.39), this statistically significant in the *B. Coccineus* 800 mg/kg.

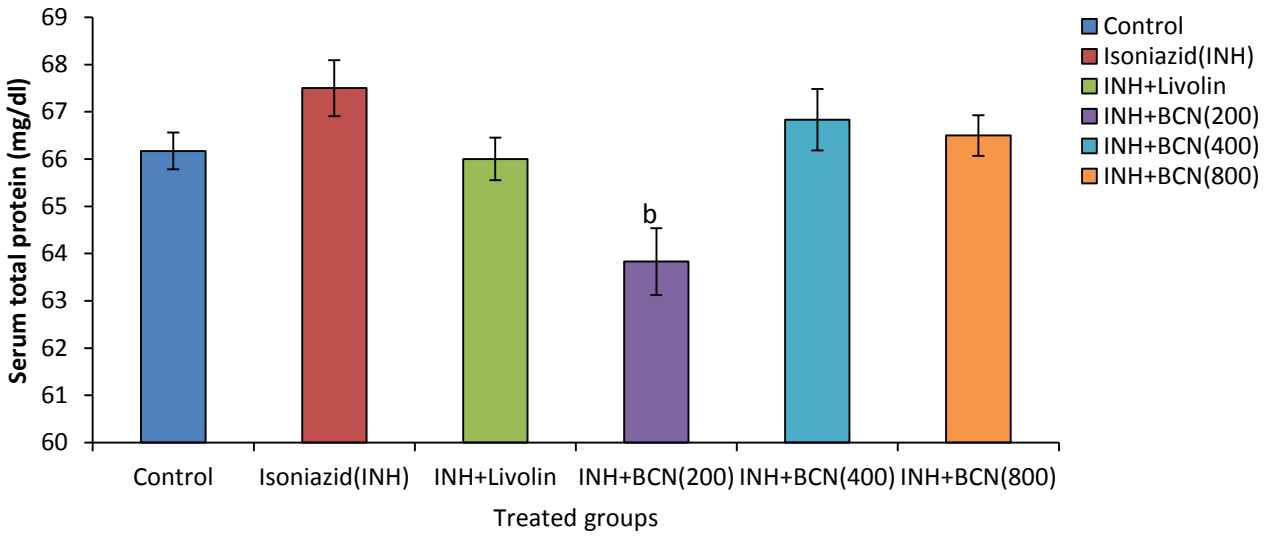


Figure 4.7: Serum Total Protein levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.10 The effects of aqueous extracts of *Brysocarpus coccineus* on Serum Albumin

Figure 4.8 shows an increase in the serum albumin in the group treated with isoniazid (40.50 ± 3.39) compared to the control (35.67 ± 5.28) which was not significant, with a decrease in the serum albumin in the group treated with livolin forte (36.33 ± 3.08). The figure also shows an increase in the serum albumin in the groups treated with the *B. coccineus* extracts at 400 mg/kg (34.67 ± 5.28) and 800 mg/kg (36.83 ± 2.31) when compared to the control (35.67 ± 5.28) however it was not statistically significant.

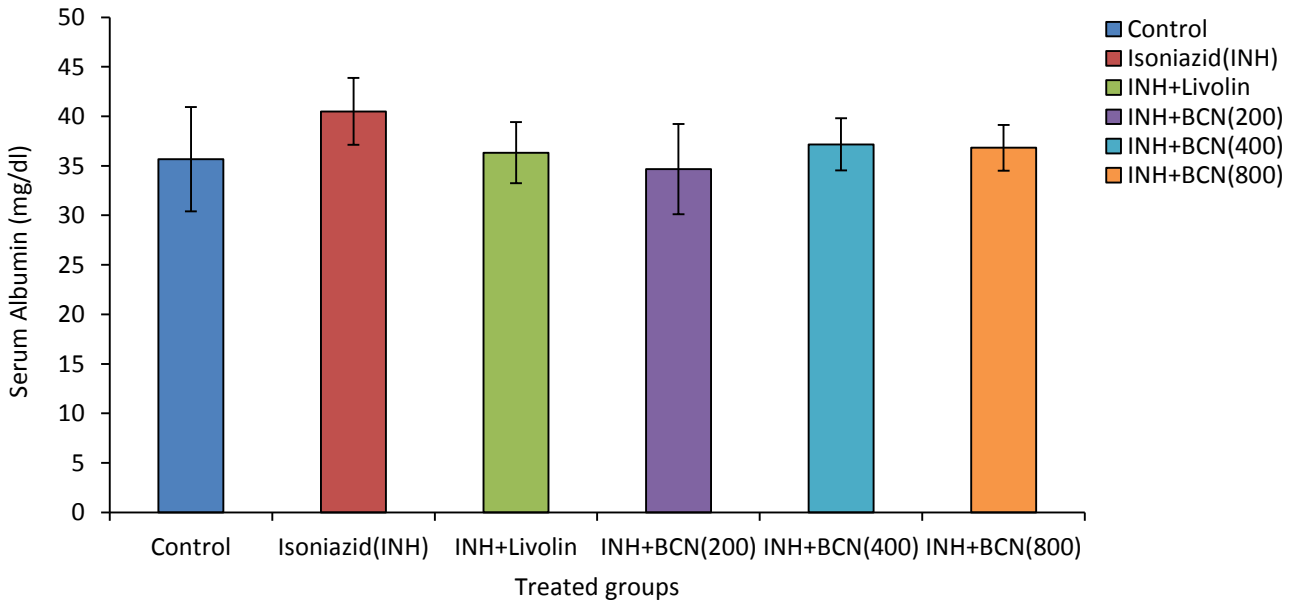


Figure 4.8: Serum Albumin levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.11 The effects of aqueous extracts of *Brysocarpus coccineus* on Serum Total Bilirubin levels.

Figure 4.9 shows an increase in the serum total bilirubin in the groups treated with isoniazid (10.39 ± 1.83) compared with to the control (9.80 ± 0.93) however it was not significant, there was also a reduction in the total bilirubin in the group treated with livolin forte compared to the control. There was an increase in the serum total bilirubin levels in groups treated with the aqueous extract of *B. coccineus* at 200 and 800 mg/kg compared to the control group (9.8 ± 0.93) which was not significant.

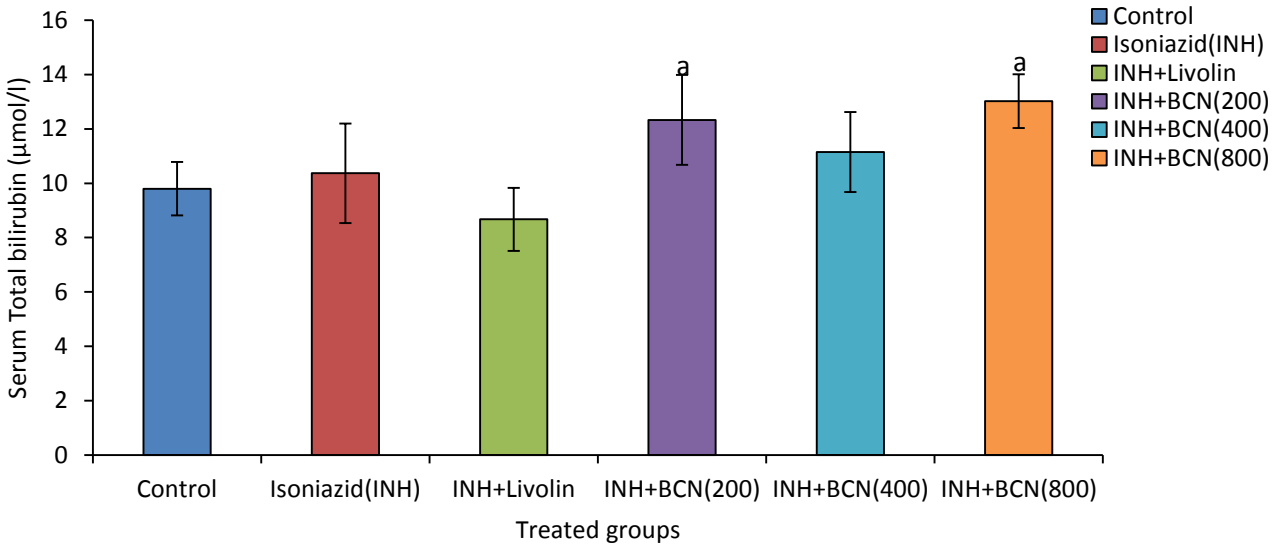


Figure 4.9: Serum Total Bilirubin levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.12. The effects of aqueous extracts of *Brysocarpus coccineus* on Serum Conjugated (Direct) Bilirubin levels.

Figure 4.10 shows an increase in serum direct bilirubin in the isoniazid group (5.40 ± 0.35) compared to the control (4.4 ± 0.35) however it was not significant, there was also an increase in the serum direct bilirubin in the group treated with livolin forte (5.83 ± 0.83) compared to the control which was also not significant ($P< 0.05$). The figure also shows an increase in serum conjugated bilirubin in the groups treated with *B.coccineus* 200mg/kg(9.05 ± 0.72), 400mg/kg (9.40 ± 1.80) and 800mg/kg (9.33 ± 0.76) when compared to the normal control (4.4 ± 0.35). The increase was dose dependent and statistically significant ($P< 0.05$).

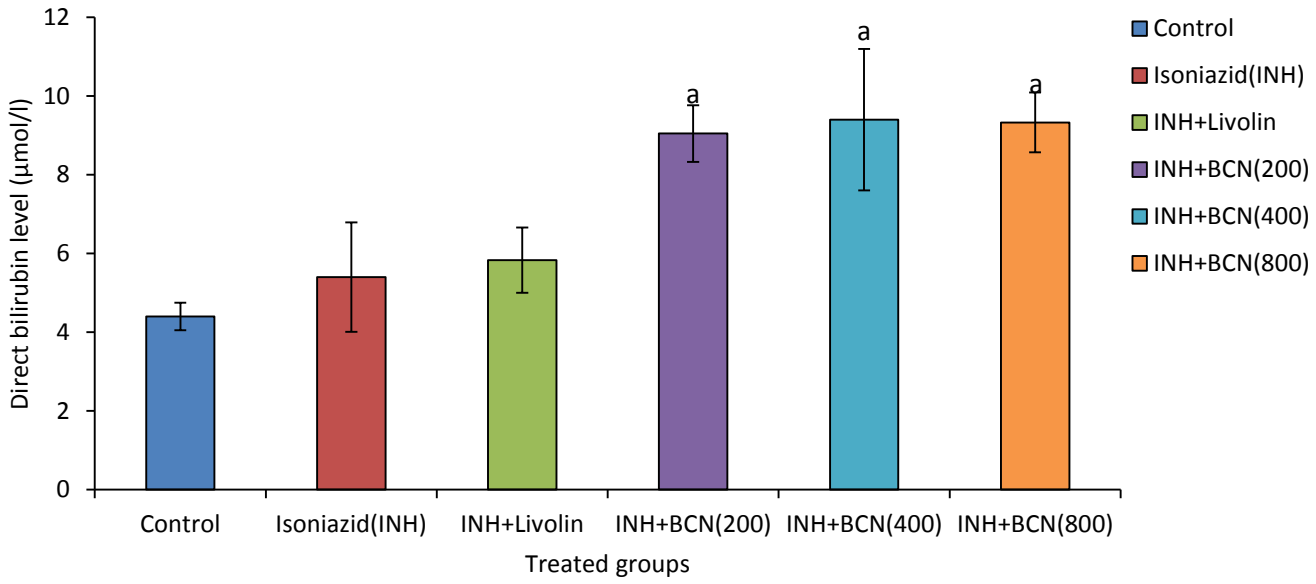


Figure 4.10: Serum Conjugated (Direct) Bilirubin levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

4.13. The effects of aqueous extracts of *Brysocarpus coccineus* on the Histology of the Liver in isoniazid induced oxidative stress and hepatotoxic.

Plates I: Photomicrograph sections of the liver after 30 days of oral administration of Distilled water 1ml/kg.

In this slide, the hepatocytes are normal, no balloon degeneration or vacuolation. No fatty inclusions or bridging necrosis all of which are evident in isoniazid induced hepatotoxicty. The central veins also show no features of congestion.

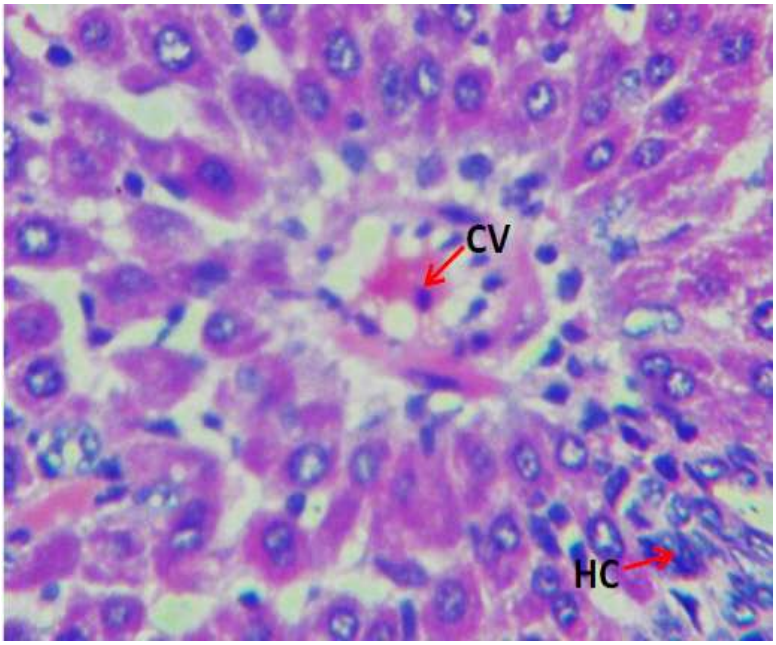


Plate I: The photomicrograph sections of the liver from Wistar rats treated orally with distilled water for 30 days (H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

Plate II: Photomicrograph sections of the liver after 30 days of oral administration of Isoniazid (27mg/kg).

In this plate there are signs of degeneration of the parenchymal cells evident by sinusoidal dilation and congestion of the central vein. There was also depletion of hepatocytes when compared to the control group implying loss of hepatocytes from isoniazid induced hepatotoxicity, however no vacoulation or bridging necrosis.



Plate II: The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid for 30 days (H and E, x400.H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

Plate III: Photomicrograph sections of the liver after 30 days of oral administration of Isoniazid (27mg/kg) and Livolin (20mg/kg)

In this plate there are signs of degeneration of the parenchymal cells evident by sinusoidal dilation and congestion of the central vein. There was also depletion of hepatocytes when compared to the control group implying loss of hepatocytes from isoniazid induced hepatotoxicity, however no vacoulation or bridging necrosis. This implies that there was no reversal of the toxicity induced by isoniazid following treatment with livolin.

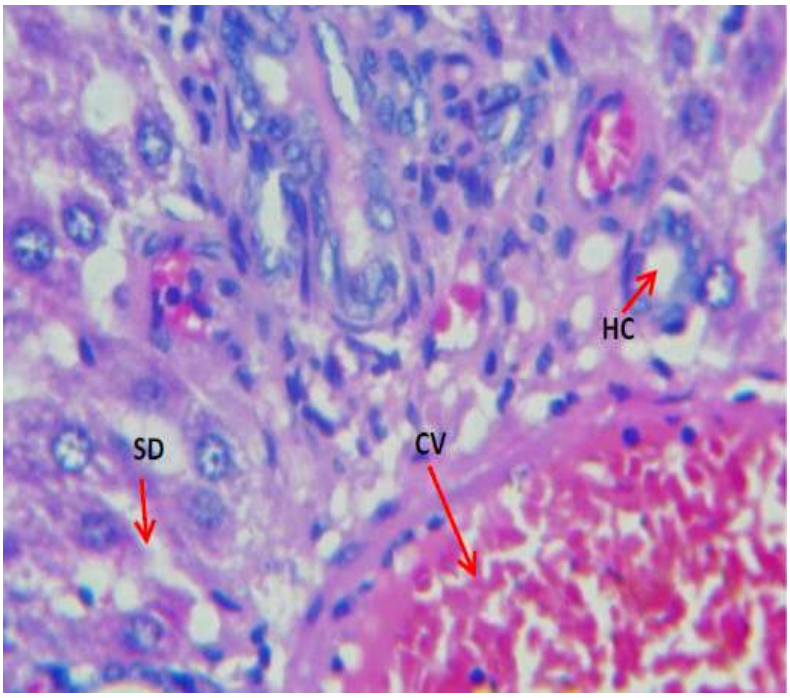


Plate III: The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid and Livolin forte for 30 days (H and E, x400.H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

Plate IV: Photomicrograph sections of the liver after 30 days of oral administration of Isoniazid (27mg/kg) and *B. coccineus* (200mg/kg)

This plate shows features of parenchymal cells degeneration as evident by sinusoidal dilation and congestion of the central vein. There was also depletion of hepatocytes when compared to the control group implying loss of hepatocytes from isoniazid induced hepatotoxicity, however no vacoulation or bridging necrosis. This implies that there was no reversal of the toxicity induced by isoniazid following treatment with the extracts at 200mg/kg.

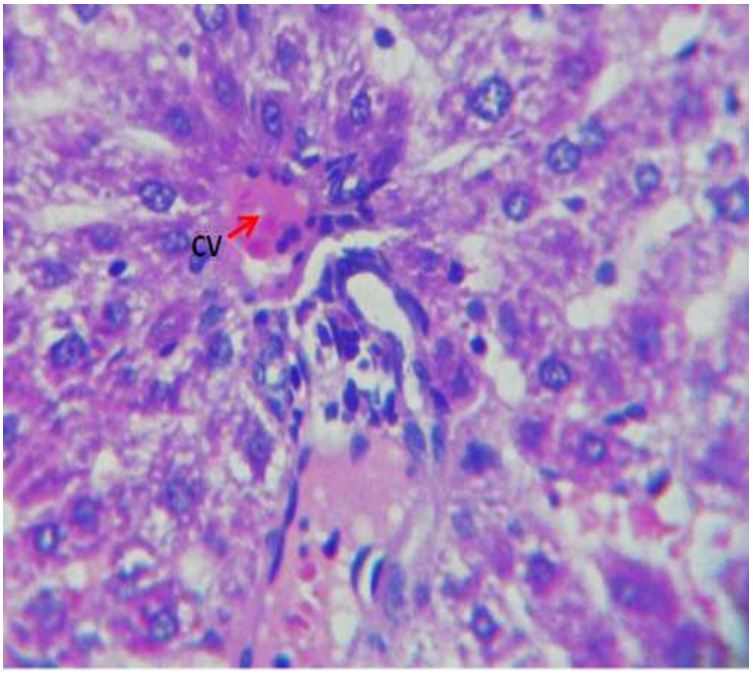


Plate IV: The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazidand *B.coccineus* (200mg/kg) for 30 days (H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

Plate V: Photomicrograph sections of the liver after 30 days of oral administration of Isoniazid (27mg/kg) and *B. coccineus* (400mg/kg)

This plate shows mild sinusoidal dilation with no congestion of the central vein. There was also no evidence of depletion of hepatocytes when compared to the control group implying no loss of hepatocytes. This implies a reversal of the toxicity induced by isoniazid following treatment with the extracts at 400mg/kg.

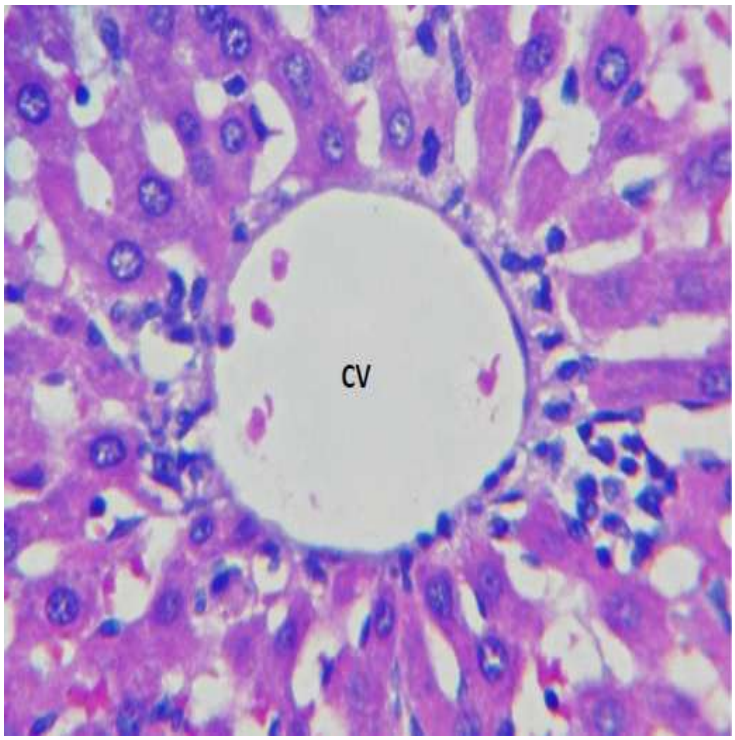


Plate V: The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazidand *B.coccineus* (400mg/kg) for 30 days (H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

Plate VI: Photomicrograph sections of the liver after 30 days of oral administration of Isoniazid (27mg/kg) and *B. coccineus* (600mg/kg)

This plate shows mild sinusoidal dilation with no congestion of the central vein. There was also no evidence of depletion of hepatocytes when compared to the control group implying no loss of hepatocytes. This implies a reversal of the toxicity induced by isoniazid following treatment with the extracts at 800mg/kg.

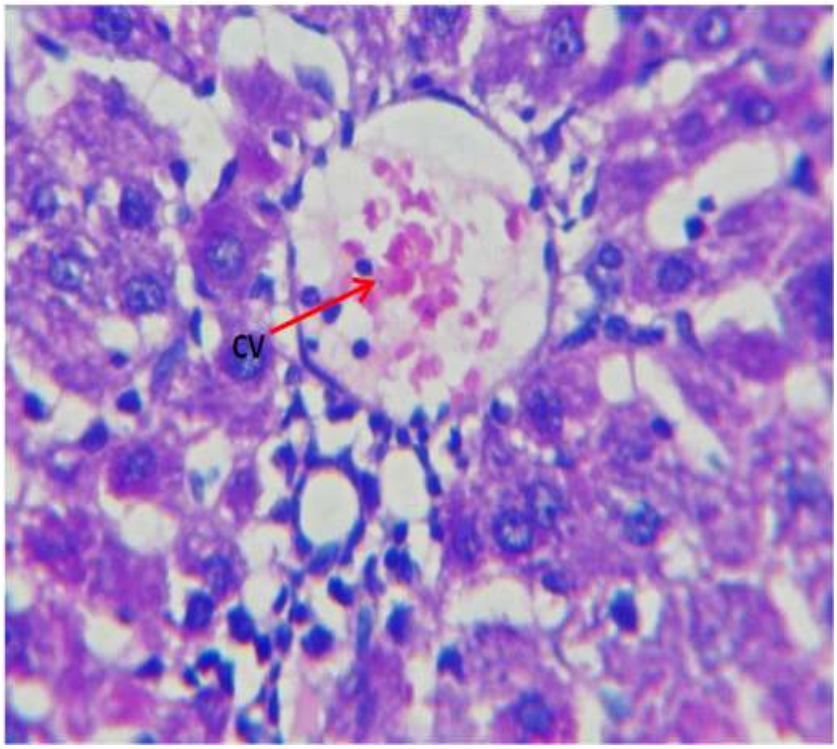


Plate VI: The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazidand *B.coccineus* (800mg/kg) for 30 days (H and E, x400.CV= central vein, HC = hepatocyte, SD = sinusoidal dilatation)

CHAPTER FIVE

5.0 DISCUSSIONS

In this study isoniazid induced oxidative stress as evident by the significant increase in serum malondialdehyde a marker of lipid peroxidation in the isoniazid treated groups, while *B.coccineus* aqueous extracts caused a decrease in the serum malondialdeyde levels in a dose dependent manner in the groups treated with the extracts, this could be due to the high levels of alkaloids and flavonoids in the aqueous extracts; these polyphenolic bioactive molecules are capable of donating electrons to reactive oxygen species generated by cytochrome p450 from isonicotinic acid and acetylhydrazine thereby inactivating them, flavonoids also have the capacity to activate antioxidant enzymes such as gluthatione-S-transferase, thereby combating oxidative stress. Rice-evans *et al*., (1995) also demonstrated that flavonoids and alkaloids may function as scavengers mopping up reactive oxygen species, however the insignificant decrease of the lipid peroxidation biomarker (MDA) in the groups treated with *B.coccineus* may be due to

the presence of other electrophilic metabolites such as hydrazine which have been implicated in other pathways of isoniazid induced hepatotoxicity where it has been shown to cause irreversible cellular damage (Tostmann *et al.,* 2007). The effect of the aqueous extracts of *B.coccineus* was also comparable to that of livolin forte a standard antioxidant agent used to treat oxidative stress and drug induced hepatotoxicity (Olukiran *et al.*, 2014).

The serum level of the antioxidant enzyme superoxide dismutase (SOD) was decreased in the groups treated with isoniazid and in all the groups treated with the extract and this may be due to consumption of the enzymes in the setting of oxidative stress. Isoniazid was found to also cause a decrease in serum levels of Catalase (CAT) when compared to the normal control, there was also a decrease in the serum levels of catalase in all the treated groups which may be due to the use up of the enzyme in combating oxidative stress.

Serum liver enzymes are important markers of hepatotoxicity, with serum Alanine aminotransferase being more specific for acute hepatocyte injury (Benichou, 1990). In the present study the serum aspartate aminotransferase (AST) was increased in the group treated with isoniazid indicating an acute injury to the hepatocytes, this increase was reversed by treatment with livolin forte which is a standard curative drug for treatment of drug induced hepatotoxicity and oxidative stress (Olukiran *et al.*, 2014). The decrease in the serum level of AST in the group treated with the extract at 200mg/kg and the dose dependent increase in the AST levels in the groups treated with 400 and 800mg/kg of the aqueous extracts *B.coccineus* may indicate that the extracts at the low dose inactivates reactive oxygen species which are capable of causing hepatic parenchymal cell wall injury, thereby ameliorating the hepatotoxic effects of isoniazid while potentiating the toxic effects at higher doses. The reversal of raised

serum alanine aminotransferase (ALT) level by both livolin forte and *B.coccineus* extracts at 200mg/kg with livolin forte producing more reduction indicating a possible acute hepatocyte injury which was reversed by the treatment with the low dose of the extracts. The serum alkaline phosphatase levels has a different pattern from that of ALT and ALT, here there was a dose dependent increase in its serum levels in the group treated with the extracts pointing towards a worsening of bile duct injury induced by isoniazid. This results show that the aqueous extracts of *B.coccineus* at 200mg/kg may protect against isoniazid induced hepatotoxicity in adult male Wistar rats at a low dose.

The serum total protein and serum albumin were increased in the group treated with isoniazid pointing towards a possible acute inflammatory response triggering the release of acute phase proteins, this was however reversed by the extract at a low dose, while higher doses of the extracts at 400 and 800mg/kg potentiated the inflammatory response with a high level of serum total protein and albumin.

In this study the rise in serum total bilirubin in the groups treated with the extracts when compared to the control group points either towards a potentiation of bile duct injury or an inhibition of hepatic conjugation of bile; while the dose dependent significant rise in the serum conjugated bilirubin in the group treated with the extracts points towards a worsening of the isoniazid induced bile duct injury by the extracts.

In this study the liver parenchymal cell architecture was distorted by isoniazid treatment as evident by sinusoidal dilatation, congestion of the central vein but no balloon degeneration of hepatocytes, bridging necrosis, fatty deposits, or vacuolated cytoplasm usually present in isoniazid induced hepatotoxicity indicating cell wall damage and loss of parenchymal cells with recruitment of inflammatory cells. These changes were not reversed by treatment with livolin

and the extracts at 200mg/kg, however at high doses there was no sinusoidal dilatation or congestion of the central vein implying hepatoprotection from these toxic metabolites by either mopping up of the reactive species generated by isoniazid during its metabolism in the liver.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Brysocarpus Coccineus aqueous extracts was found to cause a dose dependent decrease in MDA levels in adult male Wistar rats with Isoniazid induced oxidative stress and hepatotoxicity, however it caused an increase in SOD levels at 200 mg/kg compared to the isoniazid treated groups and a significant decrease in CAT levels at 200 mg/kg. *Brysocarpus Coccineus* also caused a decrease in the serum ALT and AST at a dose of 200 mg/kg, however it caused a dose dependent increase in serum ALP levels even though it was not significant. The aqueous extracts

of *B. Coccineus* also caused an increase in the serum total proteins and albumin at 400 mg/kg and 800mg/kg when compared to the control.

The extract also caused an increase in serum total bilirubin at 200 mg/kg (12.33 ± 1.16) and 800 mg/kg (13.02 ± 0.99), which was statistically significant; there was also an increase in serum direct bilirubin in a dose dependent manner from 200 mg/kg (9.05 ± 0.72), 400 mg/kg (9.40 ± 1.80) and 800 mg/kg (9.33 ± 0.76).

6.2 Conclusion

Aqueous extracts of *Brysocarpus coccineus* caused a dose dependent decrease in serum malondialdehyde levels, while it caused a decrease in serum superoxide dismutase and catalase levels. *Brysocarpus coccineus* may have antioxidant and hepatoprotective activity against isoniazid induced hepatotoxicity at a low dose.

6.3 Recommendations

i. Detailed investigation should be carried out on the long term administration of low doses of aqueous extracts *B.Coccineus* in Isoniazid treated tuberculosis model of Wistar rats.
ii. Further investigation should be carried out on the effect of long-term administration of *B.Coccineus* on serum liver enzymes and serum proteins in Wistar rats.

6.4 Contributions of the study to knowledge

1) Oral administration of Isoniazid at 27mg/kg bw for 30 days caused lipid peroxidation in adult male wistar rats.
2) Oral administration of aqueous extracts of *B.Coccineus* caused a decrease in lipid peroxidation as evidenced by decreased serum MDA in isoniazid induced oxidative stress in a dose dependent manner from 200 mg/kg (1.35 ± 0.21), 400 mg/kg (1.33 ± 0.11) and 800 mg/kg (1.32 ± 0.23).
3) Oral administration of aqueous extracts of *B.Coccineus* caused a decrease in serum ALT and AST at 200 mg/kg in adult male Wistar rats with isoniazid induced oxidative stress and hepatotoxicity.

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APPENDICES

APPENDIX I: Serum oxidative stress biomarkers after 30 days of treatment with *B. Coccineus* aqueous extracts in Isoniazid induced oxidative stress and hepatotoxicity.

Treatment Groups	MDA (µmol/L)	SOD (µmol/L)	CAT(IU/L)
Control (1 mg/kg Normal saline)	1.18 ± 0.15	2.28 ± 0.17	51.33 ± 3.39
Isoniazid (INH) (27mg/kg)	1.38 ± 0.12[a]	2.13 ± 0.29	48.17 ± 1.84
Livolin forte (20 mg/kg)	1.32 ± 0.15	2.00 ± 0.24	48.33± 4.50
***B. Coccineus* (200 mg/kg)**	1.35 ± 0.14	2.17 ± 0.21	46.00 ±1.79[a]

***B. Coccineus*(400 mg/kg)**	1.33 ± 0.11	2.00 ± 0.19	48.67 ± 2.16
***B. Coccineus* (800 mg/kg)**	1.32 ± 0.16	1.95 ±0.15	47.67 ± 1.97

± = SEM; Means with different superscripts within columns are statistically significant ($P \leq 0.05$). MDA= Malondialdehyde, SOD= Superoxide Dismutase, CAT = Catalase
a,b= significantly different

APPENDIX II: Serum Liver enzymes after 30 days of treatment with *B. Coccineus* aqueous extracts in Isoniazid induced oxidative stress and hepatotoxicity.

Treatment Groups	**AST (IU/L)**	**ALT(IU/L)**	**ALP(IU/L)**
Control (1 mg/kg Normal saline)	50.38 ± 4.41	54.17 ± 3.97	84.17 ± 7.93
Isoniazid (INH) (27mg/kg)	50.83 ± 4.21	55.83 ± 5.11	81.17 ± 1.84
Livolin forte (20 mg/kg)	44.67 ± 4.00	48.50 ± 5.54	82.83 ± 5.57
***B. Coccineus* (200 mg/kg)**	45.00 ± 2.61	50.83 ± 3.37	95.17 ±6.07

***B. Coccineus* (400 mg/kg)**	52.67± 5.32	57.00 ± 6.03	96.33 ± 5.43
***B. Coccineus* (800 mg/kg)**	56.00 ± 2.19	59.83 ±2.79	107.17 ± 5.98[a]

± = SEM; Means with different superscripts within columns are statistically significant ($P \leq 0.05$). ALT= Alanine amino transferase, AST= Aspartate amino transferase, ALP= Alkaline phosphatase

a,b= significantly different

APPENDIX III: Serum Total Protein and Albumin after 30 days of treatment with *B. Coccineus* aqueous extracts in Isoniazid induced oxidative stress and hepatotoxicity.

Treatment Groups	**Total Protein (mg/dl)**	**Serum Albumin (mg/dl)**
Control (1 mg/kg Normal saline)	66.17 ± 0.39	35.67 ± 5.28

Isoniazid (INH) (27mg/kg)	67.50 ± 0.50[a]	40.50 ± 3.39
Livolin forte (20 mg/kg)	66.00 ± 0.45	36.33± 3.08
***B. Coccineus* (200 mg/kg)**	63.83 ± 0.71	34.67 ± 4.55
***B. Coccineus* (400 mg/kg)**	66.83 ± 0.65[b]	37.17 ± 2.64
***B. Coccineus* (800 mg/kg)**	66.50 ± 0.43	36.83 ± 2.31

± = SEM; Means with different superscripts within columns are statistically significant ($P \leq 0.05$).
a,b= significantly different.

APPENDIX IV: Serum Total Bilirubin and Direct Bilirubin after 30 days of treatment with *B. Coccineus* aqueous extracts in Isoniazid induced oxidative stress and hepatotoxicity.

Treatment Groups	Total Bilirubin (µmol/l)	Direct Bilirubin(µmol/l)
Control (1 mg/kg Normal saline)	9.80 ± 0.93	4.40 ± 0.35
Isoniazid (INH) (27mg/kg)	10.39 ± 1.83	5.40 ± 1.39
Livolin forte (20 mg/kg)	8.67 ± 1.16	5.83 ± 0.83
***B. Coccineus* (200 mg/kg)**	12.33 ± 1.16[a]	9.05 ± 0.72
***B. Coccineus* (400 mg/kg)**	11.15 ± 1.47	9.40 ± 1.80
***B. Coccineus* (800 mg/kg)**	13.02 ± 0.99[a]	9.33 ± 0.76

± = SEM; Means with different superscripts within columns are statistically significant ($P \leq 0.05$).
a,b= significantly different.