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Evaluation of Quantitative Phytochemicals, Liver Enzymes and Histological Changes in Isoniazid Induced Hepatotoxicity in Adult Male Wistar Rats Treated with Aqueous Extracts of *Brysocarpus coccineus*

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Abstract

Isoniazid induced hepatotoxicity is a major concern in patients taking anti tuberculosis treatment and prophylaxis. It can result in elevated serum liver enzymes and hepatic failure. The aim of the study was to evaluate the phytochemicals and ameliorative effects of aqueous extracts of *Brysocarpus coccineus* on serum liver enzymes in isoniazid (INH) induced hepatotoxicity in adult male Wistar rats. Thirty six (36) adult male Wistar rats were divided into six groups of six rats each and were 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). At the end of the experiments, the Wistar rats were sacrificed and sera obtained for liver enzymes assay, whereas the liver tissue was also harvested and used for histological studies. Tanins, saponins, alkaloids and flavonoids were quantitatively present at 2.29%, 18.05%, 23.24% and 18.99%, respectively. There was an increase in the serum AST and ALT in the isoniazid treated group, which was reversed by livolin forte and the aqueous extracts at a dose of 200 mg/kg, however the extracts increased the serum levels of AST and ALT at higher doses, which was however not significant ($p > 0.05$) when compared to the controls. There was evidence of a reduction in hepatocytes

damage in the extract treated groups when compared to the Isoniazid untreated group. In conclusion, aqueous extracts of *B. coccineus* shows hepatoprotective effects at 200 mg/kg in isoniazid hepatotoxicity in adult male Wistar rats.

Keywords

Brysocarpus coccineus, Isoniazid, Hepatotoxicity, Phytochemicals

1. Introduction

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 Tuberculosis (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 [1] [2]. 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% [2].

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 (CYP2E1) into hepatotoxic intermediates (Ching, 2011). Acetylhydrazine can be hydrolysed to hydrazine which may further induce CYP2E1, increasing the production of toxic metabolites. 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. 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 [3].

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 [4] [5].

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*" [6]. Kilba people in Adamawa State call it *"mblakiki"*. *Byrsocarpus coccineus* have been shown to be useful in oropharngeal, dermatological, urogenital tract and haematological problems [7] [8].

2. Materials and Methods

2.1. Animal Grouping and Drug Administration

A total of 36 adult male Wistar rats weighing 100 g - 150 g 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.* The study lasted for a period of thirty (30) days. Grouping and administration was done as follows:

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. **Group IV**—Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 200 mg/kg p.o for 30 days. **Group V**—Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 400 mg/kg p.o for 30 days. **Group VI**—Isoniazid (27 mg/kg/day), p.o. and *Byrsocarpus coccineus* 800 mg/kg p.o for 30 days. Ethical approval was obtained from the ethical committee of Ahmadu Bello University, Zaria on animal handling, consistent with standard animal welfare guideline.

2.2. Sample Collection

Plant collection and identification

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.

Preparation of Aqueous Extract of B. coccineus

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˚C. The extract was decanted 24 hours later. The filtrate was then evaporated to dryness in an oven at 40˚C to yield a dark brown powder (Akindele and Adeyemi, 2006). A portion of the extract was reconstituted in distilled waters 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.

Blood sample 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 the assessment of liver enzymes; alanine amino transferase, alkaline phosphatase and aspartate amino transferase. Liver tissues were harvested also and used for histological study.

Liver tissue collection

Liver tissue was harvested on the 30th 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% [9]. Each of these steps lasted three (3) minutes and the tissues were then washed in running tap water. The tissue were 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 were mounted with cover slips using a mounting media. The tissues were viewed under a light microscope and the photomicrographs taken.

2.3. 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 4 h. 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 [10].

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 [11].

Saponin determination

The method used was that of [12]. 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 re-extracted 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 while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried 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 300 ml 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 [13].

2.4. 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.

Determination of serum aspartate aminotransferase (*AST*)

This was estimated by method as described by [14]. Exactly, 1000 µL of the reagent will be added to 100 µL of the samples and then mixed and incubated at 37˚C for 1 min. The change in absorbance of the sample was measured per minute spectrophotometerically at the wavelength of 590.

Determination of serum alanine aminotransferase (*ALT*)

This was estimated by the method as described by [14]. Exactly, 1000 µL of the reagent was added to 100 µL of the samples and then mixed and incubated at 37˚C for 1 min. The change in absorbance of the sample was measured per minute.

Determination of serum alkaline phosphatase (*ALP*)

This was estimated by method as described by [15]. Exactly, 0.5 mL of the reagent was added to 0.05 mL (500 µL) of the samples and then mixed and

incubated at 37˚C for 10 min. The change in absorbance of the sample was measured per minute.

3. Results and Discussion

The result of this present study from Table 1 shows the percentages of tannins, saponin, alkaloids and flavonoids; 2.29%, 18.05%, 23.24% and 18.99% respectively, with the highest percentage present as alkaloids. Antioxidant property of plant products are mainly mediated by their content of phenolic compounds the examples of which include flavonoids, phenolic acids, tannins and phenolic diterpenes [16] [17] [18]. These could have played a part in the ameliorative action of the extract at 200 mg/kg. Phytochemicals are the chemicals extracted from plants. These chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [19].

Serum liver enzymes are important markers of hepatotoxicity, with serum Alanine aminotransferase being more specific for acute hepatocyte injury while aspartate aminotransferase is located in the cells lining the bile ducts [20]. In Table 2, the serum aspartate aminotransferase (AST) was non-significantly increased ($P > 0.05$) in the group treated with isoniazid indicating a possible acute injury to the hepatic bile ducts, this increase was reversed by treatment with

Table 1. Result of Phytochemical studies (Quantitative) of *Brysocarpus coccineus* (*Conaraceae*) aqueous extracts.

Phytochemicals	Percentage (%)
Tannins	2.29
Saponin	18.05
Alkaloid	23.24
Flavonoids	18.99

Table 2. Serum Aspartate amino transferase (AST) after 30 days of treatment with *B. coccineus* aqueous extracts in Isoniazid induced hepatotoxicity in adult male Wistar rats.

Grouping (Treatment)	Serum AST level (IU/L)
Normal control (N.S 1 ml/kg)	50.38 ± 4.41
Isoniazid (27 mg/kg)	50.83 ± 4.21
Livolin Forte (20 mg/kg)	44.67 ± 4.00
B. coccineus (200 mg/kg)	45.00 ± 2.61
B. coccineus (400 mg/kg)	52.67 ± 5.32
B. coccineus (800 mg/kg)	56.00 ± 2.19

NS = Normal saline, *B. coccineus* = *Byrsocarpus coccineus*.

livolin forte which is a standard curative drug for treatment of drug induced hepatotoxicity and oxidative stress [21]. The decrease in the serum level of AST in the group treated with the extract at 200 mg/kg and the dose dependent increase in the AST levels in the groups treated with 400 and 800 mg/kg of the aqueous extracts *B. coccineus* may indicate that the extracts reverses the hepatotoxic effects of isoniazid at the low dose while potentiating the toxic effects at higher doses. This could have resulted from a possible metabolites formed during metabolism of extract which tends to have a deleterious effect on the hepatocytes.

In Table 3, there was a non-significant increase in serum ALT level in the Isoniazid treated group when compared to the normal control. This was however reversed in the groups treated with Livolin forte and 200 mg/kg *B. coccineus* suggesting a possible ameliorative property of the extract on hepatocytes damage. The presence of flavonoids in the extract could also have contributed indirectly through the formation of uric acid from polymerization of flavonoids. Uric acid is known to play a vital role as an antioxidant, and could have in this study ameliorated or obliterated lipid peroxidation hence aiding in the restoration of hepatocytes integrity.

Table 4 shows the result of serum alkaline phosphatase (ALP). There was a

Table 3. Serum Alanine amino transferase (ALT) after 30 days of treatment with *B. coccineus* aqueous extracts in Isoniazid induced hepatotoxicity in adult male Wistar rats.

Grouping (Treatment)	Serum ALT level (IU/L)
Normal control (N.S 1 ml/kg)	54.17 ± 3.97
Isoniazid (27 mg/kg)	55.83 ± 5.11
Livolin Forte (20 mg/kg)	48.50 ± 5.54
B. coccineus (200 mg/kg)	50.83 ± 3.37
B. coccineus (400 mg/kg)	57.00 ± 6.03
B. coccineus (800 mg/kg)	59.83 ± 2.79

NS = Normal saline, *B. coccineus* = *Byrsocarpus coccineus*.

Table 4. Serum Alkaline Phosphatase (ALP) after 30 days of treatment with *B. coccineus* aqueous extracts in Isoniazid induced hepatotoxicity in adult male Wistar rats.

Grouping (Treatment)	Serum ALP level (IU/L)
Normal control (N.S 1 ml/kg)	84.17 ± 7.93
Isoniazid (27 mg/kg)	81.17 ± 1.84
Livolin Forte (20 mg/kg)	82.83 ± 5.57
B. coccineus (200 mg/kg)	95.17 ± 6.07
B. coccineus (400 mg/kg)	96.33 ± 3.43
B. coccineus (800 mg/kg)	107.17 ± 5.98 [a]

NS = Normal saline, *B. coccineus* = *Byrsocarpus coccineus*, superscript = statistical significance at ($P < 0.05$).

statistically significant ($P < 0.05$) increase in serum ALP in the 800 mg/kg extract treated group when compared to the control and the Isoniazid treated group. This significant increase could have resulted from the overwhelming of the hepatocytes due to the increased dosage of the extract. This could also have been from a possible deleterious metabolite formation from high dose administration of the extract, resulting in pathologies like lipid peroxidation of the hepatocytes, hence the leakage of the liver enzymes into circulation.

4. Results of Histological Studies after 30 Days of Treatment with *B. coccineus* Aqueous Extracts in Isoniazid Induced Hepatotoxicity in Adult Male Wistar Rats

Figure 1 shows the central vein and hepatocytes of normal liver tissue treated with distilled water, which served as the control. There was however evidence of sinusoidal dilatation in Figure 2 and Figure 3 but no ballooning of the hepatocytes, Figures 4-6 show absent ballooning of hepatocytes and/or sinusoidal dilatations. Histologic studies have been proven to be very important in depicting drug induced liver injury and the degree of this injury by showing disruptions in the basic liver architecture which corroborate with the biochemical changes observed. In this study, there were no visible indicators of hepatic tissue damage; which include balloon degeneration of hepatocytes, bridging necrosis, sinusoidal dilatation, fatty deposits, or vacuolated cytoplasm in all the treated groups; these features are usually seen in hepatitis induced by isoniazid. The liver parenchymal cells architecture appears intact with the central vein and sinusoids. This result could be due to the dosage administered or possibly the duration of drug administration which didn't allow for obvious features of hepatitis. Swelling with non-lipid cytoplasmic vacuolation of diffusely distributed hepatocytes is seen consistently after mild acute and sub-acute liver injury [22]. However, in this study there was no cytoplasmic vacuolation observed from the treated groups, this could be due to the duration of drug administration.

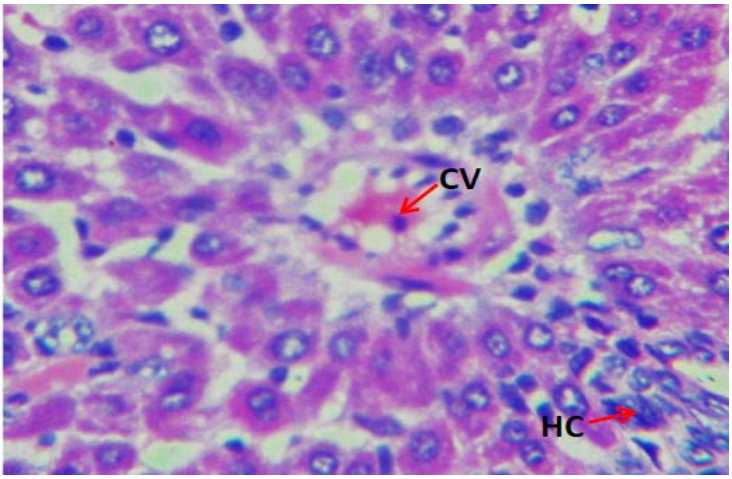

Figure 1. The photomicrograph sections of the liver from Wistar rats treated orally with distilled water for 30 days (H and E, ×400. CV = central vein, HC = hepatocyte).

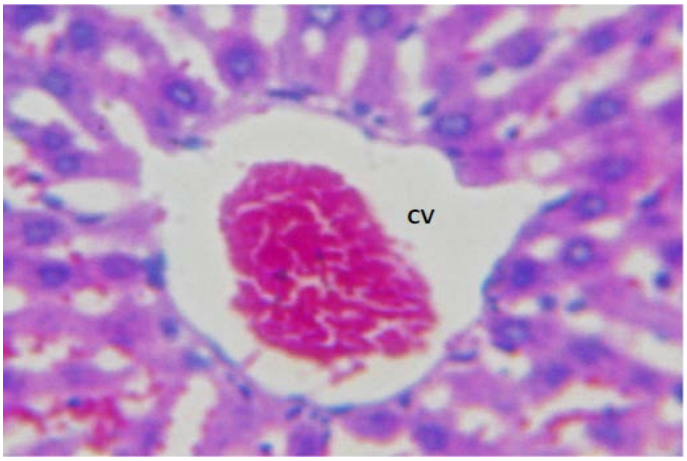

Figure 2. The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid for 30 days (H and E, ×400. H and E, ×400. CV = central vein, HC = hepatocyte).

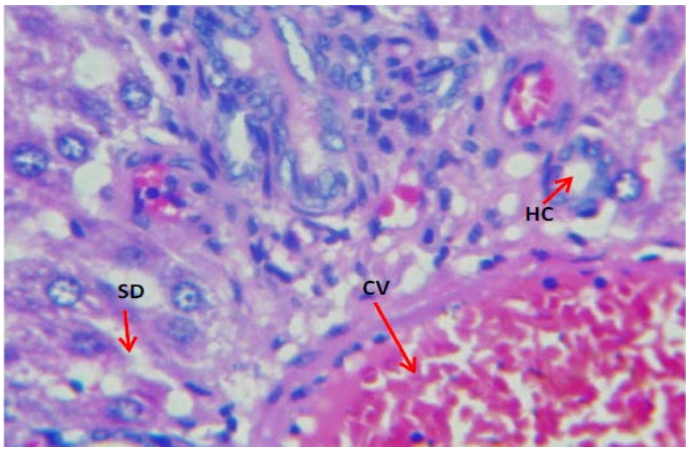

Figure 3. The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid and Livolin forte for 30 days (H and E, ×400. H and E, ×400. CV = central vein, HC = hepatocyte).

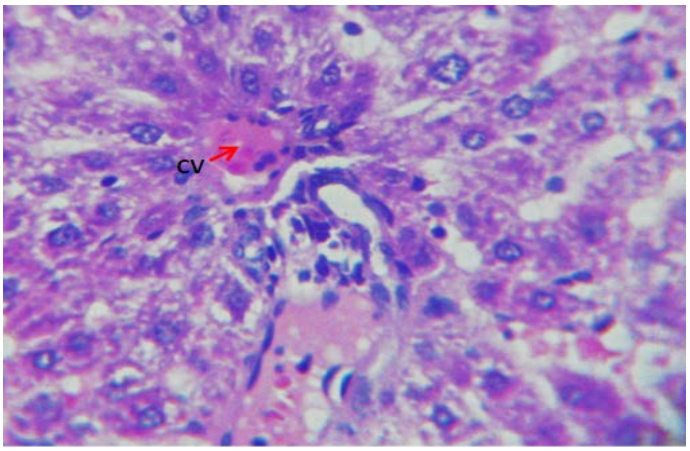

Figure 4. The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid and *B. coccineus* (200 mg/kg) for 30 days (H and E, ×400. CV = central vein, HC = hepatocyte).

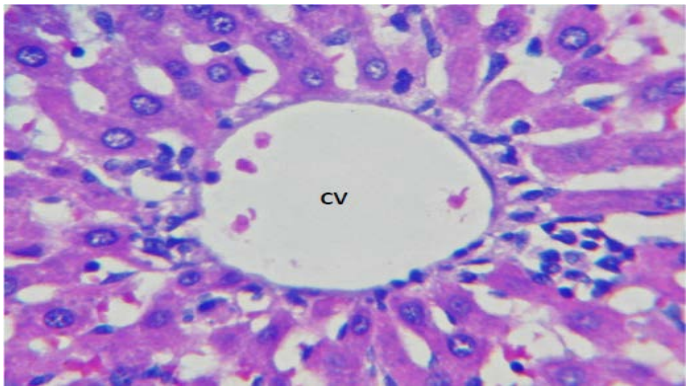

Figure 5. The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid and *B. coccineus* (400 mg/kg) for 30 days (H and E, ×400. CV = central vein, HC = hepatocyte).

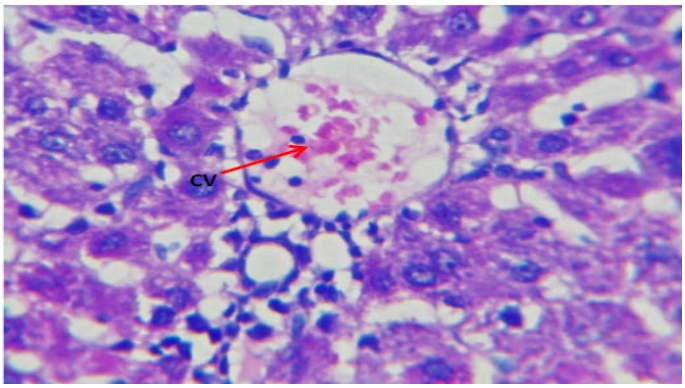

Figure 6. The photomicrograph sections of the liver from Wistar rats treated orally with Isoniazid and *B. coccineus* (800 mg/kg) for 30 days (H and E, ×400. CV = central vein, HC = hepatocyte).

5. Conclusion

Brysocarpus coccineus contains some polyphenolic constituents, which are known to enable an extract with antioxidant potentials. However, this study showed that *Brysocarpus coccineus* at a high dose is hepatotoxic when administered orally for a period of thirty days.

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