



EFFECT OF AQUEOUS EXTRACT OF *Bryocarpus coccineus* (Connaraceae) ON OXIDATIVE STRESS BIOMARKERS IN ISONIAZID INDUCED OXIDATIVE STRESS IN ADULT MALE WISTAR RATS

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ABSTRACT

The study was aimed at evaluating the effect of aqueous extract of Bryocarpus coccineus (Connaraceae) on oxidative stress biomarkers in isoniazid induced oxidative stress in adult male Wistar rats. Adult male Wistar rats (n = 36), weighing 80g-150g were divided into six groups of six rats each and treated as follows: Group I received 1ml/kg normal saline; group II: received 27mg/kg isoniazid (INH); group III: received 27mg/kg isoniazid (INH) + 20mg/kg Livolin forte; group IV: received 27mg/kg isoniazid (INH) + 200mg/kg B. coccineus; group V: received 27mg/kg isoniazid (INH) + 400mg/kg B. coccineus; group VI: received 27mg/kg isoniazid (INH) + 800mg/kg B. coccineus. Administration was done orally twice a day for a period of 30days; isoniazid was administered at 0900hrs while livolin forte and B. coccineus were administered at 1800hrs daily. At the end of the experiment, the male Wistar rats were sacrificed and the blood was collected for assay of oxidative stress biomarkers. The results obtained showed a statistically significant ($p \leq 0.05$) increase in serum Malondialdehyde in the Isoniazid group and an insignificant ($p \leq 0.05$) decrease in serum Malondialdehyde levels in the groups treated with aqueous extracts of B. coccineus, the extracts also caused an insignificant ($p \leq 0.05$) increase in both the serum Superoxide dismutase at 200mg/kg and serum Catalase at 400mg/kg when compared to the control and Livolin forte. The studies showed that aqueous extract of B. coccineus has anti-oxidant effects in isoniazid induced oxidative stress in adult male Wistar rats.

Keywords: Isoniazid, Oxidative stress, *Bryocarpus coccineus*, anti-oxidants, Livolin forte.

INTRODUCTION:

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; however, this is confined to particular situations and in most other cases, large levels of Reactive oxygen species (ROS) and oxidative stress will induce cell death through necrotic and/or apoptotic mechanisms, leading to cellular and tissue injury (Li *et al.*, 2015). Due to their special chemical characteristics, ROS can initiate lipid peroxidation, cause DNA strand breaks, and indiscriminately oxidize virtually all molecules in biological membranes and tissues, resulting in injury (Apel and Hirt, 2004).

Isoniazid (INH) is the first line Antituberculosis therapy agent and was introduced in 1952. INH 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).

Isoniazid is a component of the Directly Observed Therapy Short Course (DOTS) for treatment of

Tuberculosis and Isoniazid Preventive Therapy (IPT). Isoniazid is a prodrug, mycobacterial catalase-peroxidase converts it into an active metabolite which inhibits the biosynthesis of mycolic acids—long, branched lipids that are attached to a unique polysaccharide, arabinogalactan, to form part of the mycobacterial cell wall (Zhang Ying *et al.*, 2005; Shi *et al.*, 2007; FMOH 2014).

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 (Garget *et al.*, 2003; Huang, 2014). Oxidative stress has also been shown to be an important mechanism of hepatotoxicity caused by isoniazid in young rats (Sodhi *et al.*, 1997 and 1998).

Most of the administered Isoniazid is acetylated (by the hepatic enzyme *N*-acetyltransferase 2) and then hydrolyzed, yielding isonicotinic acid and acetylhydrazine; monoacetylhydrazine can be activated to a toxic species by *cytochrome* P-450. Acetylhydrazine and hydrazine are the implicated hepatotoxins generated by the cytochromes which have been shown to generate electrophilic intermediates and radicals capable of causing liver damage in animals (Kalra *et al.*, 2007).

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*. In South west Nigeria, the plant is locally known as *Amuje Wewe* and has been used to treat Jaundice, hemorrhoids, venereal diseases and tumors (Olowokudejo, 2008; Soladoye *et al.*, 2010). In Northern Nigeria, it is referred to by the Hausas as "*Tsamiyar kasa or kimbar maharba*." The Fulani people call it "*wangarabubi or yangarabubih*" and it is commonly used to augment labour (Dalziel, 1937).

Materials and methods: Isoniazid tablets 300mg and Livolin Forte® (Phosphatidylcholine, vitamins B complex and E) capsules of analytical grade were obtained from the Pharmacy Department of Ahmadu Bello University Teaching Hospital (ABUTH).

Preparation of aqueous extracts of *B. coccineus* and acute toxicity studies:

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 deposited.

Aqueous extraction was done using air dried leaves at the Basic Research Laboratory of the National Research Institute for Chemical Technology, Bassawa Zaria according to standard methods described by Akindele and Adeyemi, 2006. A portion of the extract was reconstituted in distilled water and subjected to a series of phytochemical screening using standard protocols as described by Santhi and Sengottuvel (2016). The dried extract was weighed and reconstituted in distilled water (pH = 6.8), just before administration to experimental animals, to obtain a concentration of 10mg/ml. The LD50 determination for aqueous extracts of *B. coccineus* was conducted using modified method of Lorke (1983). No mortality was recorded in both Phase I and phase II studies.

Drug preparation:

Isoniazid tablets (300mg each) were dissolved in distilled water to make a solution of 10mg/ml and administered as an aqueous suspension. Livolin Forte was dissolved in Tween 80 to make a solution of 10mg/ml which was continuously agitated 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

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*. The study was conducted in accordance with the guidelines of the Research Policy of the Ahmadu Bello University Zaria (ABU, 2010).

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

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

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

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

Data Collection

At the end of the experiment, after overnight fasting, the animals were anaesthetized and dissected; blood was collected through cardiac puncture, centrifuged and used for assessment of oxidative stress biomarkers.

Assay of Antioxidant Enzyme Activities.

Malondialdehyde 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 NWLSS 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).

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 H₂O₂ substrate at 240 nm (Beers and Sizer, 1952).

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).

Statistical Analysis:

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

RESULTS

The serum level of malondialdehyde in rats treated with Isoniazid (1.38 ± 0.12) was increased significantly (p ≤ 0.05) 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 800 mg/kg (1.32 ± 0.16) compared to the control groups; this was not significant ($p \leq 0.05$) (figure 1).

In figure 2, the serum activity 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 ($p < 0.05$). 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. coccineus* 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.

In Figure 3, there was reduction in serum activity of Catalase 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 ($p \leq 0.05$) in the group treated with *B. coccineus* 200 mg/kg (46.0 ± 1.79), when compared with the normal control (51.3 ± 3.39).

Table 1.0 Result of Phytochemical studies (Quantitative) of *Bryocarpus coccineus* (Conaraceae) aqueous extracts

Phytochemical	Percentage (%)
Tannins	2.29
Saponins	18.05
Alkaloids	23.24
Flavonoids	18.99

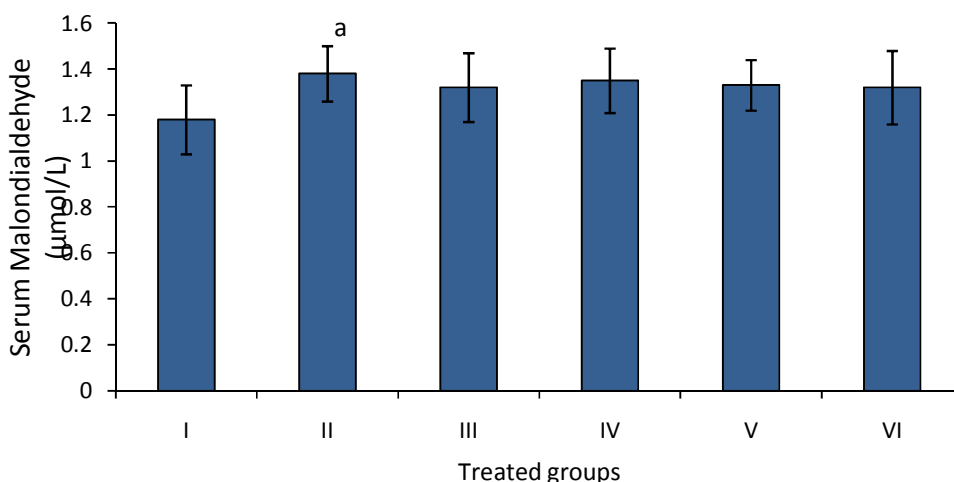


Figure 1.0: Serum Malondialdehyde levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Bryocarpus coccineus* aqueous extracts.

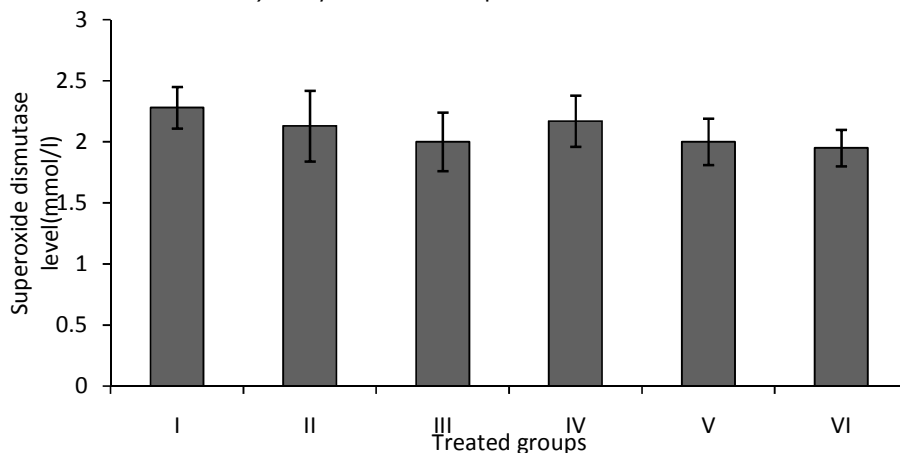


Figure 2.0: Serum Superoxide Dismutase levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Bryocarpus coccineus* aqueous extracts.

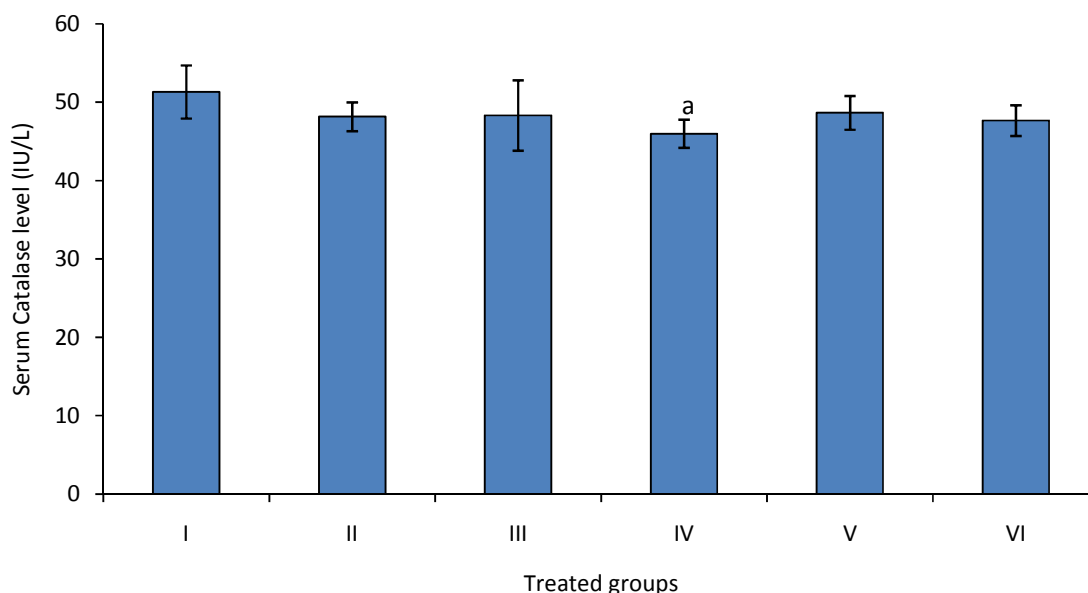


Figure 3.0: Serum Catalase levels in Isoniazid induced oxidative stress and hepatotoxicity in adult male Wistar rats treated with *Brysocarpus coccineus* aqueous extracts.

DISCUSSION

In Figure 1, the marker of lipid peroxidation, MDA was increased significantly in the group treated with isoniazid which is known to cause oxidative stress (Sodhi *et al.*, 1997 and 1998); however this increase was reduced in the group treated with livolin forte and in the groups treated with the aqueous extracts of *B. coccineus* in a dose dependent manner even though it was not significant this could be due to the documented antioxidant effects of *B. coccineus*. This finding is in consonance with that of Akindele *et al.*, (2010) who showed that the aqueous extracts of *B. coccineus* caused a reduction in the serum Malondialdehyde (MDA) level in adult male Wistar rats with hepatotoxicity induced by carbon tetrachloride (CCl₄). The decreased serum level of the antioxidant enzyme superoxide dismutase (SOD) in Figure 2, in both the groups treated with Isoniazid and also the extract may be due to consumption of the enzymes in the setting of oxidative stress, and was not in keeping with the results of Akindele *et al.*, 2010.

Isoniazid was found to also cause a decrease in serum levels of Catalase (CAT) (Figure 3) 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, this result is not in keeping with the results of Akindele *et al.*, 2010 who showed a dose dependent increase in serum SOD levels in both carbon tetrachloride and alcohol sucrose models of oxidative stress and hepatotoxicity; the difference may be due to the different models used to induce oxidative stress. The presence of high levels of alkaloid, flavonoids and tannins in the aqueous extract of *B. coccineus* (Tables 1) may explain the antioxidant activity of the extracts as Rice-evans *et al.*, in 1995 demonstrated that these bioactive metabolites may function as scavengers mopping up reactive oxygen species; also 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 (with its various metabolites) which have been implicated in other pathways of isoniazid induced oxidative stress and toxicity where it has been shown to cause irreversible cellular damage (Tostmann *et al.*, 2007). This shows that the aqueous extract of *B. coccineus* has antioxidant effects in the isoniazid induced oxidative stress animal model.

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