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Vol. 9(36), pp. 940-949, 25 September, 2015
DOI: 10.5897/JMPR2015.5911
Article Number: 52C93F355398
ISSN 1996-0875
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Journal of Medicinal Plants Research

Full Length Research Paper

Antioxidant and toxicological studies of ethanolic root extract of *Byrsocarpus coccineus*

Kossivi Dosseh, Amegnona Agbonon* and Messanvi Gbeassor

Laboratoire de Physiologie/Pharmacologie des substances naturelles, Faculté des Sciences, Université de Lomé, BP : 1515 Lomé, Togo.

Received 29 July, 2015; Accepted 8 September, 2015

***Byrsocarpus coccineus* (syn. *Rourea coccinea*) Schum. and Thonn. (Connaraceae) is used in traditional medicine to treat several ailments in which reactive oxygen species are involved. This study aims to investigate the *in vivo* antioxidative properties and moreover the toxicological potential of ethanolic root extract of *B. coccineus* (EEBc). Antioxidant activity was measured using ferric reducing antioxidant power (FRAP) and nitric oxide (NO) assays, respectively on serum and on bronchoalveolar lavage fluid and moreover by quantifying malondialdehyde (MDA) in rat model ovalbumin-induced airway inflammatory. Toxicological screening was performed using single oral administration at 5000 mg/kg and sub-chronic (4 weeks) administration at 400 and 800 mg/kg to rats. Results indicated that EEBc increases antioxidant potential in the blood. EEBc significantly reduced the NO level ($P < 0.05$) and MDA concentration ($P < 0.01$). The extract at a single dose did not produce the signs of toxicity or mortality during 14 days. The sub-chronic tests showed no alterations in animals. The results did not show any biochemical and hematological abnormalities. This study shows that EEBc may be used as natural antioxidant and may help to prevent pathological conditions related to oxidative stress.**

Key words: Antioxidant, malondialdehyde, toxicity, *Byrsocarpus coccineus*.

INTRODUCTION

Plants have been the source of natural products used, since earliest times, in non-conventional medicine known as traditional medicine through communities worldwide. Today, medicinal plants have continued to play an important role in the primary health care for more than 80% of people living in poor communities in the developing countries (Bennett and Brown, 2000; Nath et al., 2011). During the last decades, the use of medicinal plants in therapeutics has increased substantially (Castro et al., 2009; Lee et al., 2012) due to the increasing interests for natural substances. Among medicinal plants used in traditional medicine, some have antioxidant property and are used rightly or wrongly to prevent premature aging.

*Corresponding author. E-mail: aamegnona3@gmail.com. Tel: +228 22367582.
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Antioxidants have been reported to prevent oxidative damage caused by reactive oxygen species (ROS) which readily attack and induce damage to various biologic compounds, including proteins, lipids, sugars and DNA (Farber, 1994; Büyükokuroğlu et al., 2001; Kaur et al., 2006). These oxidative damages are considered as crucial etiological factor implicated in the initial phase of several chronic diseases, such as diabetes mellitus, pulmonary diseases, cancer, neurodegenerative diseases and also in the ageing process (Pong, 2003; Roussel, 2009). There is a growing interest of antioxidant considered as alternative opportunity to prevent chronic diseases. Plants rich in phenolic compounds like flavonoids have been demonstrated to have anti-inflammatory, anti-allergic, anti-viral, anti-aging, and anti-carcinogenic activities which can be attributed to their antioxidant properties (Agil et al., 2006).

Throughout popular knowledge, *Byrsocarpus coccineus* (syn. *Rourea coccinea*) Schum. and Thonn. (Connaraceae) is used in the traditional medicine in Togo (West Africa) to alleviate various diseases including dysmenorrhea, swellings, muscular and rheumatic pains, sore, wounds, hemorrhage, hypertension, primary and secondary sterility, abscess and anemia. Previous studies reported that the leave of *B. coccineus* have various pharmacological activities such as analgesic activities (Akindele and Adeyemi, 2006a), antidiarrhea activities (Akindele and Adeyemi, 2006b), antipyretic activities (Akindele and Adeyemi, 2007a), anti-inflammatory activities (Akindele and Adeyemi, 2007b), anxiolytic/sedative activities (Akindele and Adeyemi, 2010) and antidiabetic activity (Dada et al., 2013). Recently, we have shown that ethanolic root extract of *B. coccineus* (EEBc) has anti-inflammatory and *in vitro* antioxidant activities (Dosseh et al., 2014).

Although, the use of herbal medicine may be considered to be safe, some natural products are known to be toxic at high doses and others may have potential adverse effects after prolonged use. Many data concerning the safety of herbal medicine has been reported and frequently these reports are related to hepatotoxicity (Saad et al., 2006; Park et al., 2010) and nephrotoxicity (Cheng et al., 2006; Debelle et al., 2008). Hence, toxicological assessment of medicinal plants is required even if these plants are used many centuries ago. Despite intensive use in traditional medicine, the fruits of *B. coccineus* are known to be popularly highly toxic. Based on this consideration, toxicological studies had been undertaken on the leaves of *B.* coccineus (Akindele and Adeyemi, 2006b; Adeyemi et al., 2010; Akpan et al., 2012), however and according to our knowledge, any similar investigation had not been conducted on the root of this plant. Thus, this present study is aimed at investigating the antioxidant activity and toxicological screening of EEBc in rats. In addition, the content of total flavonoids was measured, in the extract in order to correlate them with antioxidant activity.

MATERIALS AND METHODS

Plant

Matured roots of *B. coccineus* were collected around the campus of University of Lomé in February 2013. The plant was authenticated by Dr. Kokou Kouami of the Botany Department (University of Lomé) and a voucher specimen has been deposited in the herbarium of the department under reference Number Togo 15075.

Ethanolic extract

The root bark was dried under air-conditioning and reduced into powder. The powder (100 g) was extracted with continuous agitation in ethanol 95° (1000 ml) at room temperature for 72 h based on traditional practice in which alcohol is used frequently. The filtrate was concentrated to dryness under vacuum in a rotary evaporator at 40°C and yielded a residue of 13.84% (w/w). The extract was stored at -4°C.

Experimental animals

Male and female Wistar rats weighing 130 to 160g were produced by the Department of Physiology/Pharmacology of University of Lomé. Animals were kept under ambient temperature, with a 12 h light and dark cycle and had free access to food and water. Before each experiment, the animals were fasted overnight with free access to water. All animal procedures were performed after approval from the Ethics Committee of the University of Lomé (Togo) and in accordance with the recommendations of the proper care and use of laboratory animals (No. SBM/UL/2015/SN 0005).

In vivo antioxidant activity

Serum ferric reducing antioxidant power (FRAP)

Animal were randomly divided into three groups of 5 animals (3 male and 2 female) each. The first group (control) received distilled water (10 ml/kg) and the second and third groups received, respectively EEBc at 400 and 800 mg/kg. The animals were daily treated orally (p.o.) for 21 days. At the end of EEBc administration, blood was collected from overnight fasted rats under anesthesia by retro-orbital bleeding into tubes without ethylenediaminetetraacetic acid (EDTA). Blood samples were then immediately centrifuged at 3000 rpm for 10 min and the serum was extracted and then stored at -20°C for further use.

The antioxidant power of serum was determined by measuring its ability to reduce Fe^{3+} into Fe^{2+} by FRAP assay (Nair et al., 2007). Briefly, 300 µl of a daily working reagent (prepared by mixing 25 mL of acetate buffer at 300 mM; 2.5 ml of Fe^{3+}-tripyridyl-s-triazine (Fe^{3+}-TPTZ) at 10 mM in 40 mM of HCl and 2.5 ml of $FeCl\text{-}6H_2O$ at 20 mM was mixed with 10 µl of serum sample and 30 µl of distilled water. The change in absorbance at 593 nm was measured against blank after 10 min of incubation. Aqueous solutions of $FeSO_4.7H_2O$ was used for calibration and antioxidant power was expressed as µM ($y = 0.000539x + 0.01227$; $r^2 = 0.999$).

Measurement of nitric oxide (NO) and malondialdehyde (MDA)

The assays for NO and MDA content were performed in bronchoalveolar lavage (BAL) fluid and lung tissue using ovalbumin (OVA)-induced airway inflammatory model in rats as described by Morris et al. (1989).

Sensitization, challenge and experimental protocol

Wistar rats (145 to 160 g) were divided into four groups (n = 5): non-sensitized or normal control group (NS), sensitized non-treated or OVA-control group (SNT), sensitized treated groups with EEBc at 400 mg/kg (ST400) and 800 mg/kg (ST800). The animals, except those of NS group were actively sensitized by an intraperitoneal injection of 10 mg/kg OVA (grade V; Sigma, St. Louis, MO, USA) mixed with 40 mg/kg aluminum hydroxide as adjuvant in normal saline (0.9%). Non-sensitized animals were injected with aluminum hydroxide (40 mg/kg) only. Sensitizations were performed 4 times at days 0, 3, 7 and 21. On days 24 to 27, SNT group and sensitized treated with EEBc groups under light ether anesthesia were challenged with intranasal instillations of 50 µl of 20% OVA in normal saline, while normal saline was administered to the NS group in a similar manner.

Rats in the EEBc-treated groups were administered orally with 10 ml/kg of 400 and 800 mg/kg 30 min prior OVA challenge, while the others were administered with normal saline. The rats were sacrificed on day 28. BAL was performed by cannulating the trachea with polyethylene catheter PE-240 (ID: 1.67 mm, OD: 2.41 mm) and infusing the lung with 5 ml of sterile 0.9% saline. BAL fluid was obtained by two aspirations via tracheal cannulation and recovery rate of BAL fluid was approximately 78 to 84% (Agbonon et al., 2005).

Measurement of NO production

The pulmonary production of NO was spectrophotometrically determined by assaying BAL fluid for nitrite using the Griess reagent (1% sulfanilic acid, 0.1% N-1 naphthylethylenediamine dihydrochloride, 2.5% phosphoric acid). Absorbance was measured at 570 nm and nitrite concentration was determined using sodium nitrite as a standard (Fermor et al., 2001).

Determination of lipid peroxidation

MDA concentration was determined as an indicator of lipid peroxidation. Whole lung samples were dissected out 24 h after the last OVA challenge and washed immediately with ice cold saline to remove as much blood as possible. They were weighed and 2 g of tissue were homogenized in 5 ml of a cold KCl solution (1.5%). The homogenate was centrifuged at 3000 rpm for 10 min. Subsequently, MDA content in the supernatants was measured according to the method described by Agbonon and Gbeassor (2009). Briefly, 200 µl of supernatant was exposed to 0.6 ml of phosphoric acid (1%) and 1 ml of thiobarbituric acids (1%) and the mixture was heated to 100°C for 50 min. At the end of the incubation period, the mixture was cooled in ice for 10 min and 2 ml of 1-butanol was added and the mixture was centrifuged as indicated earlier. After centrifuging, the supernatant was removed and the absorbance was read at 535 nm using an UV-visible recording spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, France). 1,1,3,3-tetramethoxypropane (MDA) was used as a standard to obtain the standard curve (0-60 nM; $y = 0.03860x + 0.04205$; $r^2 = 0.998$).

Total flavonoids content of the extract

Total flavonoids content in the extract was determined according to colorimetric method using Aluminum Chloride ($AlCl_3$) (Kim et al., 2003). The extract or standard (100 µl at 1 mg/ml) in ethanol was mixed with 0.4 ml of distilled water and 0.03 ml of 5% $NaNO_2$ solution. After 5 min, 0.02 ml of a 10% $AlCl_3$ solution was added. To the mixture was added 0.02 ml of 1 M Na_2CO_3 and 5 min later, 0.25 ml of distilled water was added. The solution was well stirred and the absorbance was read at 510 nm using UV-visible spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, France). Quercetin was used as a standard to obtain the standard curve (0 to 500 µg/ml; $y = 255.9x + 0.01850$; $r^2 = 0.998$). Result was expressed as µg quercetin equivalent per mg (µg QE/mg) of the extract.

Acute and subchronic toxicity

Acute toxicity test

Acute oral toxicity assay was performed using the limit test dose (Lorke, 1983; Bakoma et al., 2013). Five female Wistar rats were individually administered a single oral dose of 5000 mg/kg of extract (one after the other at a grace observation period of 24 h). The control group received water vehicle (10 ml/kg). Animals were observed individually for a period of 4 h for immediate signs of toxicity and mortality and at least once daily for 14 days for delayed mortality and toxic symptoms, such as changes in skin and fur, eyes, mucous membranes, convulsion, salivation, diarrhea, lethargy, sleep and coma. On the 15th day after administration, the survivor animals were weighed and sacrificed and then the vital organs including heart, lungs, livers, kidneys, spleen, and sex organs were grossly examined.

Sub-chronic toxicity test

Experimental design

Repeat-dose oral toxicity study was carried out according to Bakoma et al. (2013) and Diallo et al. (2010). Rats were randomly divided into three groups of 10 animals (5 males and 5 females) each. The first group (control) received distilled water (10 ml/kg); the second and third groups received, respectively EEBc at 400 and 800 mg/kg representing the pharmacological active doses in rats from our previous study (Dosseh et al., 2014). The animals were daily treated orally (p.o.) at the same time for 28 days. They were observed at least twice daily for morbidity and mortality. Body weights of the animals were evaluated weekly. On the 29th day, blood samples were collected from overnight fasted rats under anesthesia by retro-orbital bleeding into tubes with and without EDTA for hematological and biochemical analyses and the rats were sacrificed. The blood tubes without EDTA were centrifuged at 3000 rpm for 10 min and serum was separated and stored at -20°C for biochemical analyses. On the sacrificed rats, internal organs including liver, kidneys, spleen, heart, lung, ovaries and testicles were carefully collected and observed for macroscopic lesions or signs of apparent toxicity. Then, these organs were weighed to determine their relative weights [(organ weight/total body weight) ×

Table 1. Effect of ethanolic root extract of *B. coccineus* on the FRAP values in the blood of rats administered during 21 days.

Traitement groups	FRAP (µM)	
	Before treatment	After treatent
Control	447.98 ± 17.157	468.64 ± 15.04
400 mg/kg	444.52 ± 31.01	752.25 ± 83.47**
800 mg/kg	450.46 ± 21.83	769.44 ± 115.75

**Values are expressed as mean ± standard error of mean (SEM, n = 5); Rats were administered orally during 21 days by EEBc. ***P* < 0.01 when compared 400 mg/kg and 800 mg/kg treated groups to control after treatment (one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test). No statistical difference between the control and treated groups before treatment (*P* > 0.05).

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Blood analysis

White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and platelet count (PLT) were determined as hematological parameters using an automatic hematological analyzer (BC-2800, Mindray- China). The following marker enzymes were measured in the serum as biochemical indicators for liver injury/dysfunction: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), cholesterol and triglycerides. Kidney dysfunction was indicated by creatinine and blood urea levels. Glucose was assessed to evaluate pancreatic function. Standardized diagnostic kits purchased from Human GmbH. D-65205, Wiesbaden, Germany were used for spectrophotometric determination of the biochemical parameters.

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM). Data were analysed by one-way analysis of variance followed by Tukey post-hoc test. Results were considered to be significant at P < 0.05. All statistical analyses were carried out using GraphPad Prism 5.00 (GraphPad Software Inc., CA, USA).

RESULTS

Antioxidant activity of ethanolic root extract of *B. coccineus*

FRAP analysis

The FRAP assay in blood sera of rats treated with EEBc (400 and 800 mg/kg) showed increased levels of Fe^{2+} as compared to the control group. The initial FRAP values varied considerably after 21 daily gavage (P < 0.01) (Table 1).

Effect of ethanolic root extract of *B. coccineus* on NO and MDA level

The NO level in the BAL fluid was significantly increased (P < 0.05) in the SNT group (0.071 ± 0.015 µM) as compared to the NS group (0.020 ± 0,011 µM). EEBc at the dose of 800 mg/kg significantly restored the level of NO (0.021 ± 0.009 µM; P < 0.50) (Figure 1). The MDA level in the lung tissue was significantly increased (P < 0.001) in the SNT group as compared to SN group (36.30 ± 4.02 nM/ml vs. 15.60 ± 2.31 nM/ml). The MDA level was significantly decreased (P < 0.01) by EEBc at 400 (17.0 ± 2.52 nM/ml) and 800 mg/kg (16.7 ± 2.45 nM/ml) when compared with the SNT (Figure 2).

Total flavonoids content

The total flavonoids contents of EEBc were 414.16 ± 1.15 µg QE/mg of extract.

Acute toxicity study

After the rats were orally given a single dose of EEBc at 5000 mg/kg, no death of rats was recorded for a period of 4 h. Also any behavioral changes and lethargy was not observed in treated groups for 14 days post-treatment. Neither body weight nor internal organ weight in treated rats was significantly changed relative to that of the control group. No abnormality was found in organs at necropsy (data not shown).

Subchronic toxicity

Effect of extract on physical parameters

Daily oral administration of EEBc for 28 consecutive days did not induce any obvious symptom of toxicity in rat. No

Table 2. Mean relative organ weights of control and daily treated rats with ethanolic root extract of *B. coccineus* in sub-chronic oral toxicity test (g ± SEM per 100 g body weight).

Organs	Control	Extract doses (mg/kg/day b.w.)	
		400	800
Liver	3.49 ± 0.05	3.61 ± 0.14	3.47 ± 0.11
Spleen	0.28 ± 0.02	0.30 ± 0.02	0.30 ± 0.02
Kidneys	0.71 ± 0.04	0.77 ± 0.06	0.79 ± 0.05
Heart	0.42 ± 0.02	0.38 ± 0.01	0.37 ± 0.01
Lung	0.75 ± 0.02	0.68 ± 0.01	0.76 ± 0.02
Ovaries	0.10 ± 0.00	0.10 ± 0.01	0.11 ± 0.01
Testicles	1.50 ± 0.03	1.38 ± 0.01	1.38 ± 0.03

Values are expressed as mean ± standard error of mean (SEM, n = 5); No statistical difference between the control and treated groups ($P > 0.05$) (one-way ANOVA).

Table 3. Mean hematological value of control and daily treated rats with ethanolic root extract of *B. coccineus* in sub-chronic oral toxicity test.

Parameter	Control	Extract doses (mg/kg/day b.w.)	
		400	800
WBC (10^9/L)	9.48 ± 0.53	10.15 ± 0.63	9.50 ± 0.56
RBC (10^{12}/L)	6.96 ± 0.18	7.46 ± 0.09*	7.59± 0.11**
HGB (g/dl)	13.77 ± 0.36	14.48 ± 0.15	14.76± 0.21*
HCT (%)	40.90 ± 1.19	41.34 ± 0.84	41.84 ± 0.41
MCV (fL)	58.08 ± 1.08	56.40 ± 1.07	56.16 ± 1.06
MCH (pg)	19.05 ± 0.19	19.21 ± 0.36	18.92 ± 0.26
MCHC (g/dl)	33.48 ± 0.21	34.15 ± 0.18	33.49 ± 0.79
PLT (10^3/µl)	524.20 ± 25.12	535.50 ± 23.15	564.10 ± 24.50

Values are expressed as mean ± standard error of mean (SEM, n = 5); $^{**}P < 0.01$, $^{*}P < 0.05$ vs. control (one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test).

mortality was recorded during the 28 days. No differences in general behavior were observed between the groups of rats. The body weight in EEBc treated rats was normal in comparison with vehicle treated rats (Figure 3). The means relative organ weights show that there were no significant changes between control and treated groups (P > 0.05) (Table 2). At necropsy, no macroscopic change was observed in the internal organs in treated rats.

Effect of extract on hematological parameters

Hematological parameters of rats showed that there were no significant difference between control and treated groups (P > 0.05) except the RBC count (400 mg/kg and 800 mg/kg) and HGB (800 mg/kg), which were significantly increased in treated groups (Table 3).

Effect of extract on serum biochemical parameters

Single daily oral administration of EEBc at 400 and 800 mg/kg throughout the treatment period (28 days) did not cause any significant changes (P > 0.05) in serum levels of ALT, AST, PAL, creatinine, urea, TP, TB in treated animals compare to the OVA-control group. However, results showed significant decrease in cholesterol and triglycerides levels at the dose of 800 mg/kg (P < 0.05) (Table 4).

DISCUSSION

Growing interest for natural products from medicinal plants is evidence nowadays and many of these plants products are used for their antioxidant potential, but this practice may be expose to the toxicological risks. The

Table 4. Mean blood clinical chemistry value of control and daily treated rats with ethanolic root extract of *B. coccineus* in sub-chronic oral toxicity test.

Parameter	Control	Extract doses (mg/kg/day b.w.)	
		400	800
AST (U/L)	123.17 ± 4.73	120.28 ± 5.88	116.92 ± 4.71
ALT (U/L)	86.60 ± 8.53	80.68 ± 6.46	80.50 ± 4.48
ALP (U/L)	160.92 ± 5.69	144.00 ± 12.25	144.85 ± 9.77
Creatinine (mg/L)	7.00 ± 0.21	6.60 ± 0.22	6.50 ± 0.17
Urea (g/L)	1.01 ± 0.01	0.97 ± 0.02	0.96 ± 0.01
Total biluribin (mg/dl)	1.76 ± 0.15	1.73 ± 0.17	1.65 ± 0.11
Total protein (g/L)	59.52 ± 0.56	59.68 ± 0.31	59.76 ± 0.39
Glucose (g/dL)	0.76 ± 0.05	0.65 ± 0.04	0.64 ± 0.05
Triglycerides (g/L)	0.83 ± 0.02	0.80 ± 0.01	0.76 ± 0.01*
Total cholesterol (g/L)	0.96 ± 0.04	0.90 ± 0.02	0.85 ± 0.01*

Values are expressed as mean ± standard error of mean (SEM, n = 5); *P < 0.05 vs. control (one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test).

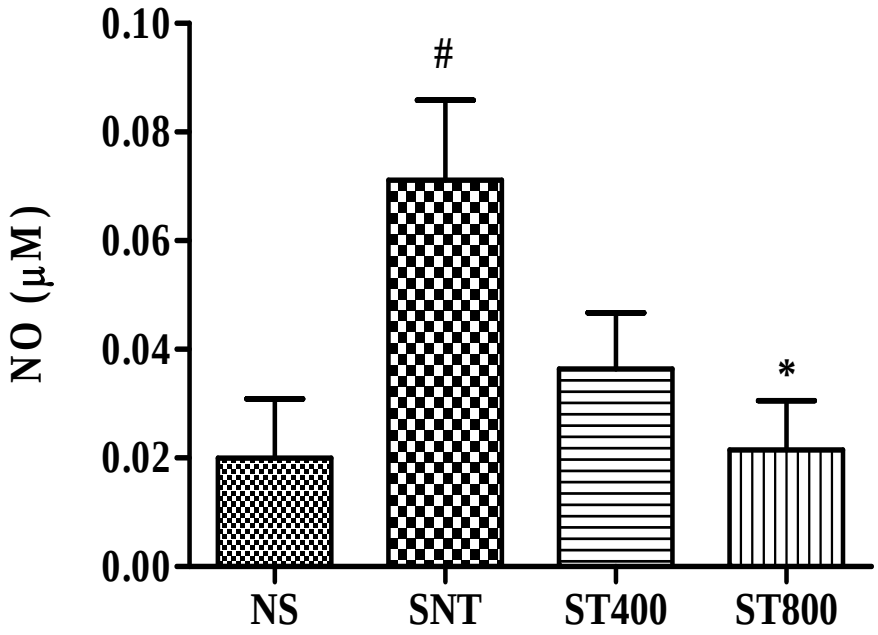


Figure 1. Effect of ethanolic root extract of *B. coccineus* c on NO in BAL. Data are expressed as Mean ± standard error of mean (SEM) n=5; NS = Non-sensitized group, SNT = Sensitized non-treated group with; ST400, ST800: Sensitized treated groups with EEBc at 400, 800 mg/kg, p.o.; $^{\#}P < 0.05$ compared with non-sensitize group; *P < 0.05 compared with sensitized non-treated group (one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test).

main objective of the present study was to investigate the antioxidant activity and the safety of EEBc.

Oxidative stress results from an imbalance caused by the excessive production of ROS or a reduction in the antioxidant defenses of the organism (Kaur et al., 2006). Generally, the reducing properties of antioxidant are associated with the presence of compounds which exert their action by breaking the free radical chain by donating

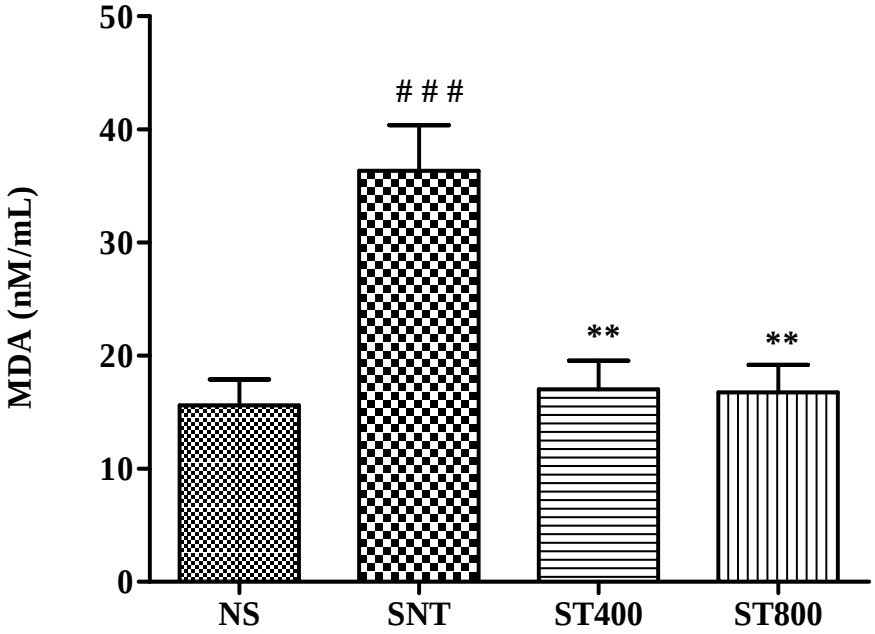


Figure 2. Effect of ethanolic root extract of *B. coccineus* on MDA in lung tissue. Data are expressed as Mean ± standard error of mean (SEM), n=5; NS = Non-sensitized group, SNT = Sensitized non-treated group with; ST400, ST800: Sensitized treated groups with EEBc at 400, 800 mg/kg, p.o.; ###P < 0.001 compared with non-sensitize group; **P < 0.01 compared with sensitized non-treated group (one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test).

a hydrogen atom (Duh et al., 1999). Our results suggest the possibility that EEBc is useful for prevention of the phenomenon of the oxidative stress. Increasing the serum antioxidant status has been suggested as a possible method of reducing the risk of many chronic diseases such as asthma.

In this study, OVA was used as an antigen in rats to provoke asthmatic symptoms (Schuster et al., 2000). NO plays a crucial role in the pathogenesis of airway inflammation in allergic asthma (Shin et al., 2012). At physiological concentration, NO functions as vasodilator, neurotransmitter and immune regulator (Valko et al., 2007; Shin et al., 2012). Excess of NO can react with superoxide radicals leading to peroxynitrite generation, a powerful oxidizing agent (Zhu and Li, 2012). In this study, EEBc significantly reduced the production of NO in BAL fluid and thus avoiding the damaging effect of excess NO production. MDA, the decomposition products of lipid peroxidation, reflects the severity of cell attack by free radicals (Feng et al., 2010; Du et al., 2013). MDA content is commonly regarded as a marker of oxidative stress and antioxidant status (Del Rio et al., 2005; Du et al., 2013). Pretreatment with EEBc showed a decrease in MDA level meaning that EEBc may effectively reduce oxidative burden during the inflammatory response to OVA.

Regarding the role of oxidative stress in the pathogenesis of inflammatory diseases, the *in vivo* antioxidant activities of EEBc in this study seem reasonable. The phenolic compounds included flavonoids present in EEBc may be responsible for *in vivo* anti-oxidant activities observed in this study. However, the involvement of other secondary metabolites present in the plant cannot be ruled out. These results confirm a previous antioxidant activity of EEBc, using the *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical model (Dosseh et al., 2014).

Many investigations on *B. coccineus* have reported that the plant has numerous pharmacological properties (Akindele and Adeyemi, 2007b; Dada et al., 2013). It is necessary to evaluate the toxicity of this plant to determine its safety for human use. In oral acute toxicity, the LD_{50} of the extract is above 5000 mg/kg. Thus, referring to the Hodge and Stemer scale (Hodge and Sterner, 1943), the orally administered EEBc could be considered practically non-toxic. This result supports the

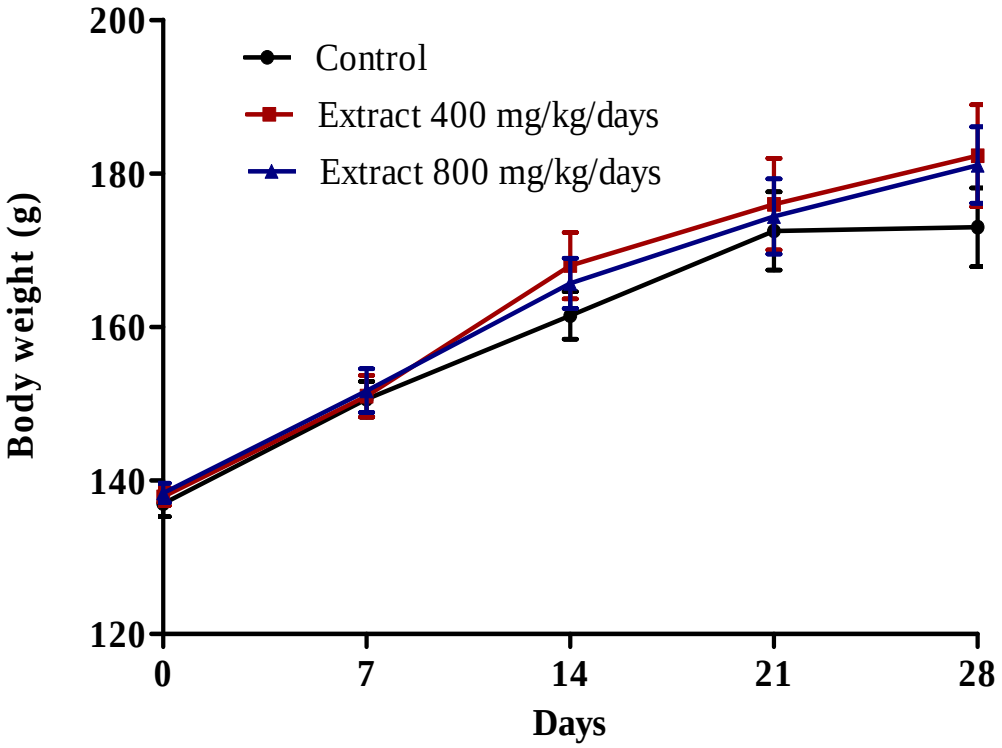


Figure 3. Mean body weight of rats during 28 days treatment with ethanolic root extract of *Byrsocarpus coccineus.* No statistical difference between the control and treated groups (P > 0.05).

findings of Akindele and Adeyemi (2006b). Adeyemi et al. (2010) who demonstrated that aqueous leaf extract of *B. coccineus* did not cause oral toxicity, respectively in rats and mice at the dose of 10000 mg/kg. The same trend was observed by Akpan et al. (2012) and Dada et al. (2013) using respectively ethanolic leaf extract of the plant at the dose of 5000 mg/kg in mice and hydro-ethanolic leaf extract at the dose of 10000 mg/kg in rat.

Generally, reductions in body weight gain and internal organs weights are sensitive indices of toxicity after exposure to toxic substances (Teo et al., 2002; Ouedraogo et al., 2013). The results obtained in the sub-chronic toxicity study indicated that EEBc did not affect neither the weight of the whole animal nor the weight of the specific vital organs, as previously described by Adeyemi et al. (2010).

Hematological indices in animals are important to determine the toxicity risk since the changes in the blood system have a higher predictive value for human toxicity (Ouedraogo et al., 2013). The hematological indices obtained in this study suggest that the EEBc is not toxic on hematological parameters. However, the increase in the level of RBC and HGB in treated groups confirms the traditional use of this plant against anemia.

The serum biochemical parameters were studied to evaluate the possible alterations in hepatic, renal and pancreatic functions influenced by EEBc. ALP, AST and ALT are usual markers of liver toxicity (Costa-Silva et al., 2008). Results obtained in this study indicated that EEBc did not induce liver injury.

Serum level of bilirubin was also not altered significantly. Oboh (2005) has reported that increase in bilirubin levels suggests increase in hemolysis intensity. The water solubility of bilirubin allows the bilirubin to be excreted in the bile; the bile is then used to digest food. As the liver becomes irritated, then TB may rise (Muhammad et al., 2011). Results indicated that the extract did not interfere with the metabolism of TB and TP in the liver. The liver is also the site of cholesterol degradation and an increase in cholesterol levels is considered as a sign of hepatic damage (Subhangkar and Rana, 2012). In this study a significant decrease in cholesterol and triglycerides levels was observed. These results may be attributed to the presence of hypolipidemic agents in the extract.

It is well known that almost all drugs, chemicals, xenobiotics are eliminated through renal excretion (Biswas et al., 2010); hence, it was found necessary to

estimate the effects of EEBc on kidney functions. EEBc had no adverse effect on the concentration of creatinine and urea. This is suggestive of no kidney damage specifically by renal filteration mechanism (Crook, 2006).

Conclusion

It can be concluded from the aforementioned results that the ethanol extract from roots bark of *B. coccineus* has a good antioxidant activity and do not produce any toxic signs or evident symptoms in acute and sub-chronic oral toxicity. Hence, *B. coccineus* root may be exploited as a natural antioxidant and health promoting agent for the treatment and prevention of free radicals associated diseases. These results confirm the utilisation of *B. coccineus* in traditional medicine many centuries ago. However, more investigations are needed before its use for clinical purpose.

Abbreviations

Nitric oxide (NO) and Malondialdehyde (MDA)

Conflict of interest

The authors have not declare conflict of interest.

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