



Advanced Research in Life Sciences

Journal Details



Format: Journal

eISSN: 2543-8050

First Published: 15 Jun 2017

Publication timeframe: 1 time per year

Languages: English

Open Access

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Published Online: 30 Oct 2020

Volume & Issue: Volume 4 (2020) - Issue 1 (January 2020)

Page range: 36 - 40

Received: 29 Feb 2020

Accepted: 30 Sep 2020

DOI: <https://doi.org/10.2478/arls-2020-0016>

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DOI: 10.2478/arls-2020-0016
Research Article

In vivo* Antioxidant and Hepato-Protective Properties of Stem Bark Methanol Extract of *Vitex doniana

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Received March 2020; Revised August, 2020; Accepted October, 2020

Abstract

The harmful effects that accompany the use of orthodox antioxidant medicine have necessitated the hunt for inherent antioxidants from plants extracts. In the present study, the *in vivo* antioxidant and hepato-protective activities of *Vitex doniana* against carbon tetrachloride (CCl₄) induced liver damage in albino rats were investigated. The hepato-protective activities of the methanol extract of *Vitex doniana* stem bark were compared with Silymarin, a known hepatoprotective drug. Twenty-five (25) male albino adult rats were grouped into five (5) each. Group 1 and 2 was used as the normal and negative control respectively. Group 3-5 were treated with 200 mg/kg, 400 mg/kg methanol extract of *Vitex doniana* stem bark and 100 mg/kg Silymarin respectively. Results indicated that elevated levels of serum ALT, AST and ALB, and reduced serum SOD, GST and CAT in CCl₄-hepatotoxic rats was an evidence of impairment in liver function. Administration of methanol extract of *Vitex doniana* stem bark (200 and 400 mg/kg body weight) and standard control drug Silymarin (100 mg/kg) have no significant (P>0.05) effect on CCl₄- induced elevations of the ALT and AST levels while the reduction in albumin concentration, total proteins, SOD, GST and CAT due to CCl₄ was reversed. In conclusion, *Vitex doniana* exhibited significant antioxidant and hepatoprotective properties in CCl₄ induced liver damage in rat, and thus could be used and incorporated in the development of new and effective antioxidant drugs.

Keywords: Antioxidant, Hepato-protective, *Vitex doniana*, carbon tetrachloride.

Introduction

According to World Health Organization (WHO) more than eighty percent of the world population depends on folklore medicine as an important alternative in health care delivery system [1]. These ethno-medicines plays an important role in the native systems of health management. They have an advantage of easy administration, cost effective and no cause of noticeable resistance [2]. They are also considerably recognized for their bioactive compounds (phytochemicals) that could serve as a margin in the

development of novel and effective drugs for the treatment of several ailments [3,4].

Free radicals are unbalanced intermediates produced during breakdown of macromolecules or by environmental factor such as tobacco, smoke and radiation with unpaired electron [3]. These free radicals cause tissue damage when the rate of free radical production is greater than that of its clearance via oxidative stress. Oxidative stress plays an essential role in the pathology of various chronic diseases such as diabetes, cardiovascular diseases, decline in immune system, neurodegenerative diseases and osteoarthritis [4, 5]. Improved antioxidants system helps to reduce oxidative damage and delay or prevent pathological changes.

Antioxidants protect the body from the effects of free radicals by inhibiting the oxidation reactions and can be

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classified as non-enzymatic and enzymatic antioxidants [6]. In the body, the mechanism of action of antioxidant network includes: reactive oxygen-free radical species (ROS) scavenging, lipid peroxidation, and the inhibition or chelation of metals. Studies have shown the potential of medicinal plants in free radical scavenging, their effectiveness as natural antioxidants and safety [7]. *Vitex doniana* (black plum, family, Verbenaceae) also known as black plum "Or -ila" by the yoruba's, dinya (Hausa) and uchakoro (igbo) [8]. The plant is widespread in the south western Nigeria as a perennial tree [9]. Young leaves of *Vitex doniana* are fit for human consumption as vegetable or in sauces [10]. The blackish flesh/pulp of the fruits is sweet, edible and can be eaten uncooked. The fruit juice can be used to make beverage and the boiled fruit can be used for alcoholic liquor and wine [11]. Studies have shown that in Nigeria, dried leaves are used to treat dizziness and hot water extract of dried stem bark is used as stimulant, antihypertensive, against dysentery, diarrhea [11] and *in vitro* study of the extracts of the stem bark shown efficacy in trypanocidal activity against *Trypanosoma brucei* [12]. This study evaluates the *in vivo* antioxidant and hepato-protective activities of *Vitex doniana* against carbon tetrachloride (CCl₄) induced liver damage in albino rats

Material and Methods

Chemicals and Reagents

The chemicals/reagents used for the analysis were of analytical grade obtained from Sigma Aldrich.

Plant material

The stem bark and leaves of *Vitex doniana* were collected from Kaduna state, they were cleaned, dried and air-dried in the laboratory and pulverized into a coarse powder. A 150g of *Vitex doniana* was refluxed at 64°C for four hours in a condenser using methanol as solvent and filtered. The filtrate was concentrated and extracts were stored in the refrigerator until required for use.

Experimental Animals

Twenty-five (25) male wistar rats within the range of (110–120 g) were obtained from the animal husbandry unit of the School of Life Sciences, Federal University of Technology Minna, Niger State, and divided into five equal groups. They were allowed unrestricted access to rat pellets and water. This study was carried in compliance with the Guide for the Care and the Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA.

Experiment Design

Experimental design for hepato-protective study was described by previous study [13,14]. A total of twenty-five (25) rats were grouped into 5 of five animals each. Group A serve as normal control, groups B and C serve as positive control (100mg/kg body weight of Silymarin) and negative control (0.2 mL of distilled water). Groups D and E were treated with 200mg/kg and 400mg/kg body weight of methanol extract of *Vitex doniana*. All treatments were administered for 7 days. On the seventh day, groups B-E were treated with 0.2% carbon tetrachloride (CCl₄) 10ml/kg body weight in olive oil.

Collection of Blood Samples

Samples were collected after 24 hours of last treatment for biochemical analyses according to the method previously described [15, 16]. The animals were anesthetized with chloroform and blood was collected through cardiac puncture into clean, dry centrifuge tubes. The blood sample was allowed to stand for 10 minutes at room temperature and then centrifuged at 1000 rpm (503 x g) for 15 minutes to get the serum.

Analysis of Serum Biochemical Parameters

Serum biochemical activities of alanine transferase (ALT) and aspartate transferase (AST) were determined using method described by Reitman & Frankel [17]. The concentrations of serum total proteins and albumins were also calculated using standard methods [18]. Oxidative stress markers; catalase (CAT), glutathione transferase (GST) and superoxide dismutase (SOD) activities were determined according to the methods described previously [19-21].

Statistical Analysis

Data generated were analyzed using ANOVA and presented as mean ± SEM and data was analyzed using ANOVA. Difference within the groups were compared statistically at p<0.05, using the Duncan Multiple Range Test.

Results and Discussion

Results of the present study indicated that the liver damage was indicated by the elevated levels of serum ALT, AST and ALB (figure 1), and reduced serum albumin, total proteins (figure 1), SOD, GST and CAT (figure 2) in CCl₄-hepatotoxic rats. Administration of methanol extract of *Vitex doniana* stem bark (200 and 400 mg/kg body weight) and standard control drug Silymarin (100 mg/kg) have no significant (P>0.05) effect on CCl₄-induced elevations of the levels of ALT and AST while the reduced concentration of albumin, total proteins, superoxide dismutase (SOD), catalase glutathione transferase (GST) due to CCl₄ was reversed.

Liver tissue damage arises as a result of oxidative stress imposed by free radicals. Carbon tetrachloride (CCl₄) a hepatotoxin is one of the compounds capable of eliciting liver damage [13]. Carbon tetrachloride (CCl₄) is metabolized within the body to produce highly toxic compounds, trichloromethyl and trichloromethyl peroxy free radicals by cytochrome P450 enzyme which leads to destruction of hepatocytes [22]. The first-line defense against such impairment is antioxidant enzymes and thus offers protection against the deteriorating effect. Therefore, assessment of antioxidant enzymes and other biochemical parameters of liver integrity will be useful in determining the treatment outcome of *V. doniana* against toxicant induced liver damage.

Serum total protein is highly important and efficient in defending the body against free radical attack and also the main target of attack by free radicals. Albumin is very important in transporting different substances in the body and also blocks the production of free radicals from the system [23]. CCl₄ untreated rats shows a decrease level of total proteins and albumin, however, treatment with *V. doniana* stem bark produce a

significant ($P < 0.05$) restoration of serum total protein and albumin when compared to untreated control. This is in agreement with the observation of James et al. [24]

who worked with the root and stem bark extracts of *V. doniana*.

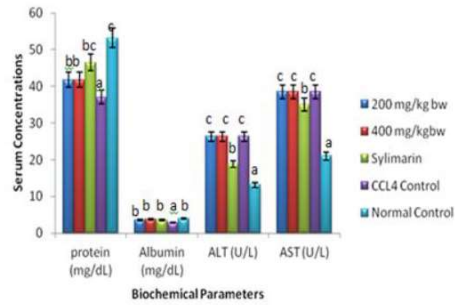


Figure 1. Effect of methanol stem bark extract of *Vitex doniana* on liver biomarkers parameters in CCL₄ induced liver damage

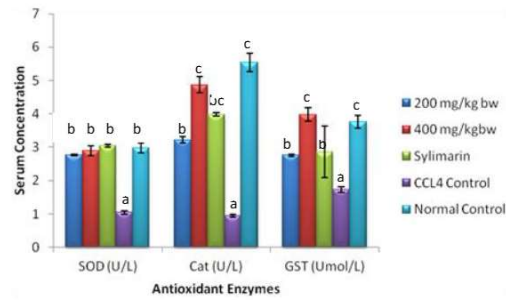


Figure 2. Effect of methanol stem bark extracts of *Vitex doniana* on antioxidant enzymes in CCL₄ induced liver damage

Serum alanine transferase (ALT) and aspartate transferase (AST) are enzymes actively found in the cytoplasm at higher concentration and are released into the serum in accordance with the level of liver damage; therefore their concentrations are elevated in response to liver assault [25, 26]. In the study, high level of AST in the serum as seen in the untreated group confirms the hepatocellular damage caused by the induced carbon tetrachloride (CCL₄). However, treatment with

the extract produced no ameliorative effect on CCL₄ induced elevation of AST and ALT which is an indication of non-protective impact of the extract on the liver which result in the leakage of these enzymes (ALT and AST) from the liver into the serum. James et al. [24] however, reported that root bark and stem bark aqueous extracts of *Vitex doniana* significantly ($P < 0.05$) lowered ALT and AST in CCL₄ induced liver damage in rats, these differences may be attributed to the different

part of the used plant, solvent for extraction and treatments dose.

Superoxide dismutase (SOD) catalyzes superoxide anion, the first toxic species produced from oxygen into H_2O_2 which is then converted into H_2O and O_2 by the catalase [27]. Glutathione transferase are agent that catalyzes the detoxification of alkylating agents with the SH group of glutathione, thereby deactivating their electrophilic sites which result into formation of water-soluble product [28]. In the present study, CCl_4 was found to induce high levels of oxidative stress as evident by decreased levels of superoxide dismutase (SOD), glutathione transferase (GST) and catalase (CAT) in CCl_4 toxicant rats. However, treatment with stem bark methanol extracts of *Vitex doniana* produce significant and dose dependent antioxidant and hepatoprotective effect as evident by increased levels of antioxidant enzymes (SOD, GST and CAT). The extract was able to preserve and prevent the loss of the antioxidant cellular defense systems at their normal cell level [29]. The antioxidant activities of the extracts may be reflected to the bioactive compounds identified in the extract which includes: flavonoids, tannins and phenols which are the major groups of compounds that act as free radical scavenger [30]. These phytochemicals have been implicated singly or synergistically in the antioxidant's activities of medicinal plants.

Conclusions

The stem bark methanol extract of *Vitex doniana* exhibited significant antioxidant and hepato-protective properties in CCl_4 induced liver damage in rat, and thus could serve as natural source novel antioxidants drug.

Competing interests

We do not have any competing interest.

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