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In vivo antioxidant effect of aqueous root bark, stem bark and leaves extracts of *Vitex doniana* in CCl_4 induced liver damage rats

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PEER REVIEW

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Comments

This is a very good study in which the investigators evaluated and established the antioxidant characteristics of *V. doniana* with regards to oxidative damages elicited in the liver and kidney of rats. The generated data also suggest that the aqueous extract of the stem bark of the plant could enhance normal renal function.

(Details on Page 399)

ABSTRACT

Objective: The antioxidant effects of aqueous root bark, stem bark and leaves of *Vitex doniana* (*V. doniana*) were evaluated in carbon tetrachloride (CCl_4) induced liver damage and non induced liver damage albino rats. **Methods:** A total of 60 albino rats (36 induced liver damage and 24 non induced liver damage) were assigned into liver damage and non liver damage groups of 6 rats in a group. The animals in the CCl_4 induced liver damage groups, were induced by intraperitoneal injection with a single dose of CCl_4 (148 $mg \cdot ml^{-1} \cdot kg^{-1}$ body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 h before the subsequent treatment with aqueous root bark, stem bark and leaves extracts of *V. doniana* and vitamin E as standard drug (100 mg/kg body weighy per day) for 21 d, while the animals in the non induced groups were only treated with the daily oral administration of these extracts at the same dose. The administration of CCl_4 was done once a week for a period of three weeks. **Results:** The liver of CCl_4 induced not treated group showed that the induction with CCl_4, significantly ($P<0.05$) increased thiobarbituric acid reactive substance (TBARS) and significantly ($P<0.05$) decreased superoxide dismutase (SOD) and catalase (CAT). However there was no significant ($P>0.05$) difference between TBARS, SOD and CAT in the liver of the induced treated groups and normal control group. In the kidney, TBARS showed no significant ($P>0.05$) difference between the normal and the induced groups, SOD was significantly ($P<0.05$) reduced in the CCl_4 group compared to standard drug and normal control groups, CAT was significantly ($P<0.05$) increased in root and vitamin E groups when compared to induced not treated group. The studies also showed that when the extracts were administered to normal animals, there was no significant ($P>0.05$) change in the liver and kidney level of TBARS, SOD and CAT compared with the normal control except in the kidney of animals treated with stem extract where TBARS was significantly ($P<0.05$) lowered compared to control group. **Conclusion:** The result of the present study suggests that application of *V. doniana* plant would play an important role in increasing the antioxidant effect and reducing the oxidative damage that formed both in liver and in kidney tissues. However stem bark has potential to improve renal function in normal rats.

KEYWORDS
Antioxidant, *Vitex doniana*, Carbon tetrachloride

1. Introduction

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) formation and scavenging by antioxidants. The overproduction of harmful ROS can cause severe damage to membrane lipids, protein synthesis, and DNA[1]. Liver is the organ in charge of many important life functions, including food digestion, glycogen storage, control of metabolism, drug detoxification and hormone production[2]. It has great capacity to detoxicate toxic substances and synthesize useful principles. *In vivo* liver systems represent a better experimental approach to generate free radicals and to investigate the effects of antioxidant agents. Liver cell lines are characterized

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by unlimited subcultivation and cell availability in large number[3].

Carbon tetrachloride (CCl_4) is an archetype of hepatotoxin used commonly in experimental models to induce oxidative stress in liver[4]. The liver is the major target organ of CCl_4 toxicity owing to its high content of cytochrome P-450. Antioxidants are used to antagonize the deleterious action of free radicals (or ROS) and to protect hepatocytes from damage. Vitamin E (α-tocopherol), a fat soluble antioxidant is a powerful chain-breaking antioxidant and resides primarily in biologic membranes, protecting membrane phospholipids from peroxidation[5,6].

Medicinal plants ultilization and conservation has attracted global attension[7]. The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years[8]. In contrast to the use of synthetic drugs in modern medicine, the potential toxicity of the use of herbal remedies has not been fully investigated scientifically[9]. The damaging effect of herbal remedies to the human body is generally considered to be lower than synthetic drugs and as such may be Generally Regarded As Safe[10]. Because of the increasing use of herbal formulation in nutraceuticals, there is a compelling need for evaluation and standardization of medicinal plants[11,12].

Vitex doniana (*V. doniana*)is a common medicinal plant in southwestern Nigeria. It is commonly known as black plum or African olive, Dinya in Hausa, Oori-nla inYoruba, Ucha coro in igbo. However the present study was design to specifically investigate the antioxidant efficacy of root bark, stem bark and leaves extracts by investigating antioxidant enzymes and lipid peroxidation in the liver and kidney of normal and CCl_4 induced liver damage wister albino rats when it is administered *in vivo*.

2. Materials and method

2.1. Plant samples collection and identification

The fresh root barks, stem barks and leaves of *V. doniana* were collected from the Institute of Agricultural Research, Ahmadu Bello University, Zaria Kaduna State, Nigeria in April 2012. The plant was identified at the herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher specimen (1162) was deposited.

2.2. Experimental animals

Adult albino rats of both sexes weighing between 150-200 g were purchased from University of Jos, Plateau State, Nigeria. The animals were acclimatized for period of two weeks under ambient environmental conditions. They were allowed free access to grower's mash (Vital feeds Grand Cereal Plc, Bukuru, Jos, Plateau State) and water *ad libitum*.

2.3. Preparation of plant

The collected plant samples were rinsed in clean water and shade dried under ambient temperature for two weeks. The dried plant sample was ground into powder using a mortar and pestle, the powder obtained was then used to prepare the extracts.

2.4. Extractions

One hundred gram of each of the powdered root bark, stem bark and leaves were weighed into three sterilized conical flasks and 500 ml of distilled water was poured into each of the flasks. The contents of the three flasks were shaken and the tops were covered with aluminium foil and kept at ambient temperature for 48 h after which the extracts were obtained by filtering using clean cloth with fine pore. The extracts were then concentrated in crucibles using water bath set at a temperature of 45 °C. The weight of the concentrated extracts were taken and then stored in an air-tight sample bottles in a refrigerator until required for analysis[13].

2.5. Acute toxicity (LD_{50}) test

The mean lethal dose of aqueous and root bark, stem barks and leaves extracts of *V. doniana* were determined in albino rats (weighing 150g-200g) using the method described by lorke[14]

2.6. Induction of liver damage

The liver damage was induced by the administration of CCl_4 (Sigma chemicals Co., St. Louis USA). Rats were injected intraperitoneally with a single dose of CCl_4 (148 mg/kg body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 h before the administration of the extracts. This was done once a week for a period of three weeks.

2.7. Animal grouping and treatment

A total of 60 rats were used. The rats were randomly divided into 10 groups of 6 rats each. Group 1 was normal control animals given feed and water only. Animals in Group 2 were treated with olive oil and served as vehicle control. Animals in Group 3 were treated with CCl_4 in olive oil (148 mg/kg body weight). Animals in Group 4 were treated with CCl_4 in olive oil (148 mg/kg body weight) and root bark extract (100 mg/kg) of *V. doniana*. Animals in Group 5 were treated with CCl_4 in olive oil (148 mg/kg body weight) and stem bark extract (100 mg/kg) of *V. doniana*. Animals in Group 6 were

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