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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

Peer reviewer

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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.

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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

treated with CCl_4 in olive oil (148 mg/kg body weight) and leaves extract (100 mg/kg) of *V. doniana*. Animals in Group 7 were treated with CCl_4 in olive oil (148 mg/kg body weight) and vitamin E (100 mg/kg). (standard drug). Group 8 was normal animals treated with root bark extract (100 mg/kg) of *V. doniana*. Group 9 was normal animals treated with stem bark extract (100 mg/kg) of *V. doniana*. Group 10 was normal animals treated with leaves extract (100 mg/kg) of *V. doniana*.

2.8. Collection and preparation

At the end of 21 d of treatment, the animals were anesthetized with chloroform and sacrificed by cervical capitation. The liver and the kidney tissues were excised washed and homogenized in suitable buffer and centrifuged at 4000 r/min for 15 min. The supernatant was collected and used for biochemical estimation of different antioxidant enzyme assays.

2.9. Assesement of lipid peroxidation and antioxidant enzyme activities

Catalase was estimated by following the breakdown of hydrogen peroxide according to the method described by Sinha[15]. Superoxide dismutase (SOD) was assayed according the method described by Fridovich[16]. The ability of SOD to inhibit auto oxidation of adrenanline at pH 10.2 forms the basis of this assay. Lipid peroxidation was measured in terms of malondialdehyde (MDA). MDA is a product of lipid peroxidation and is used as an indicator of tissue damage. The MDA form a 1:2 adduct with thiobarbituric acid and produces a pink coloured product which has an absorption maximum of 532 nm[17].

2.10. Statistical analysis

The results of the analysis were expressed as mean±SEM. The SPSS program (version 16.0 SPSS Inc., Chicago, IL, USA) was used for the Analysis of Variance (ANOVA) followed by the new Duncan Multiple.

Range test for multiple comparisons of the means. P values of <0.05 between mean values were considered.

3. Results

3.1. Effects of aqueous Vitex doniana on lipid peroxidation and antioxidant enzymes in liver tissues

Table 1 shows the effect of aqueous root bark, stem bark, and leaves extract of *V. doniana* on lipid peroxidation and some endogenous antioxidant enzymes (catalase and superoxide dismutase) of CCl_4 induced liver damage rats after the administration of extracts for 21 d. There was a significant (P<0.05) increase in the level of Thiobarbituric acid-reactive substances (TBARS) and a significant (P<0.05) decrease in the level of catalase (CAT) and SOD of the CCl_4 induced liver damage rats compared to the normal control. However there was a significant (P<0.05) decrease in the level of TBARS and increase in the level of endogenous antioxidant enzymes of all the induced treated when compared to the untreated CCl_4 induced liver damage control.

Table 1

Effect of aqueous *V. doniana* on lipid peroxidation and endogenous antioxidant enzymes in liver of CCl_4 induced liver damage rats (n=6).

Group	TBARS (mmol/g of tissue)	SOD (mmol/min/g of tissue)	CATALASE (moles of H_2O_2/min/g of tissue)
NC	0.45 ± 0.07^{ab}	0.46 ± 0.10^{b}	0.84 ± 0.07^{b}
VC	0.42 ± 0.64^{ab}	0.55 ± 0.05^{b}	0.92 ± 0.04^{b}
IC	0.74 ± 0.10^{c}	0.26 ± 0.06^{a}	0.40 ± 0.10^{a}
CCl_4+RV	0.49 ± 0.02^{b}	0.48 ± 0.04^{b}	0.83 ± 0.19^{b}
CCl_4+SV	0.45 ± 0.06^{ab}	0.54 ± 0.05^{b}	0.85 ± 0.05^{b}
CCl_4+LV	0.32 ± 0.03^{a}	0.52 ± 0.07^{b}	0.89 ± 0.02^{b}
CCl_4+Std	0.47 ± 0.02^{ab}	0.54 ± 0.07^{b}	0.91 ± 0.06^{b}

Values are means±SEM. determinations with different superscript along the row are significantly different (P<0.05).

NC: Normal control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 induced liver damage control rats, CCl_4+RV: CCl_4 induced liver damage rats+100 mg of aqueous root bark extract, CCl_4+SV: CCl_4 induced liver damage rats+100 mg of aqueous stem bark extract, CCl_4+LV: CCl_4 induced liver damage rats+100 mg of aqueous leaves extract, CCl_4+Std: CCl_4 induced liver damage rats+100 mg of vitamin E.

The effect of daily oral administration of aqueous root bark, stem bark and leaves extracts of *V. doniana* on lipid peroxidation and endogenous antioxidant (catalase and SOD) enzymes in liver of normal rats is also shown in Table 2. The result shows that the extracts had no significant (P<0.05) effect on the lipid peroxidation and the endogenous antioxidant enzymes.

Table 2

The effect of aqueous extracts of *V. doniana* on lipid peroxidation and endogenous antioxidant enzymes in liver of normal rats (n=6).

Groups	TBARS (mmol/g of tissue)	SOD (mmol/min/g of tissue)	CATALASE (moles of H2O2/min/g of tissue)
NC	0.45 ± 0.07^{a}	0.46 ± 0.10^{a}	0.84 ± 0.07^{a}
N+RV	0.55 ± 0.04^{a}	0.62 ± 0.06^{a}	0.99 ± 0.05^{a}
N+SV	0.56 ± 0.02^{a}	0.46 ± 0.08^{a}	0.44 ± 0.07^{a}
N+LV	0.64 ± 0.13^{a}	0.47 ± 0.06^{a}	0.87 ± 0.06^{a}

Values are means± SEM. Determinations with different superscript along the row are significantly different (P<0.05).

NC: Normal rats Control; N+RV: Normal rats+aqueous root bark extract; N+SV: Normal rats+aqueous stem bark extract; N+LV: Normal rats +aqueous leaves extract.

3.2. Effects of aqueous V. doniana on lipid peroxidation and antioxidant enzymes in kidneys tissues.

The effect of daily oral administration of aqueous

V. doniana and vitamin E on lipid peroxidation and endogenous antioxidant enzymes in the kidneys of CCl_4 induced liver damage rats is shown in Table 3. The result showed that there was no significant ($P<0.05$) difference between the TBARS of normal control, induced not treated and the induced treated groups. However the SOD and catalase of CCl_4 induced liver damage control group were significantly ($P<0.05$) lowered than the normal control group. There was no significant ($P<0.05$) difference between the induced treated groups and induced not treated group in the level of SOD except for the standard drug that significantly ($P<0.05$) increase the level of SOD. Root extract and standard drug significantly ($P<0.05$) increase CAT when compared to induced not treated animals. Table 4 also shows the effect of daily oral administration of aqueous root bark, stem bark and leaves extracts of *V. doniana* on lipid peroxidation and endogenous antioxidant enzymes in the kidneys of normal rats. The result showed no significant ($P>0.05$) difference in the level of TBARS and the endogenous antioxidant of the normal control group and all the extracts treated groups except in the group treated with stem where TBARS was significantly ($P<0.05$) lowered.

Table 3

Effect of aqueous *V. doniana* on lipid peroxidation and endogenous antioxidant enzymes in kidney of CCl_4 induced liver damage rats.

Group	TBARS (mmol/ g of tissue)	SOD (mmol/ min/g of tissue)	CATALASE (moles of H_2O_2/ min/g of tissue)
NC	0.065 ± 0.005^{ab}	2.38 ± 0.61^{c}	2.66 ± 0.22^{b}
VC	0.055 ± 0.015^{a}	2.03 ± 0.07^{bc}	2.59 ± 0.37^{b}
IC	0.095 ± 0.005^{b}	0.95 ± 0.05^{a}	1.28 ± 0.11^{a}
CCl_4+RV	0.067 ± 0.009^{ab}	1.87 ± 0.23^{abc}	2.39 ± 0.32^{b}
CCl_4+SV	0.067 ± 0.007^{ab}	1.41 ± 0.05^{ab}	2.03 ± 0.28^{ab}
CCl_4+LV	0.075 ± 0.005^{ab}	1.81 ± 0.08^{abc}	2.25 ± 0.28^{ab}
CCl_4+Std	0.073 ± 0.009^{ab}	2.02 ± 0.33^{bc}	2.36 ± 0.35^{b}

Values are means±SEM, $n=6$, determinations with different superscript along the row are significantly different ($P<0.05$).

NC: Normal Control rat, VC: Vehicle control rats, CCl_4: Carbon tetrachloride, IC: CCl_4 induced liver damage control rats, CCl_4+RV: CCl_4 induced liver damage rats+100 mg of aqueous root bark extract, CCl_4+SV: CCl_4 induced liver damage rats+100 mg of aqueous stem bark extract, CCl4+LV: CCl_4 Induced liver damage rats+100 mg of aqueous leaves extract, CCl_4+Std: CCl_4 Induced liver damage rats+100 mg of vitamin E.

Table 4

Effect of aqueous extracts of *V. doniana* on lipid peroxidation and endogenous antioxidant enzymes in kidneys of normal rats.

Groups	TBARS (mmol/ g of tissue)	SOD (mmol/ min/g of tissue)	CATALASE (moles of H_2O_2/ min/g of tissue)
NC	0.065 ± 0.005^{b}	2.38 ± 0.61^{a}	2.67 ± 0.22^{ab}
N+RV	0.045 ± 0.005^{ab}	1.99 ± 0.70^{a}	2.90 ± 0.44^{ab}
N+SV	0.035 ± 0.005^{a}	1.27 ± 0.37^{a}	2.26 ± 0.17^{a}
N+LV	0.050 ± 0.010^{ab}	1.73 ± 0.20^{a}	3.94 ± 0.46^{b}

Values are means±SEM, $n=6$, determinations with different superscript along the row are significantly different ($P<0.05$).

NC: Normal Control rats; N+RV: Normal rats+aqueous root bark extract; N+SV: Normal rats+aqueous stem bark extract; N+LV: Normal rats+aqueous leaves extract.

4. Discussion

CCl_4 is one of the most used hepatic toxins for experimental induction of liver fibrosis on rats[18]. The chronic liver damage induced by carbon tetrachloride in rats produces liver fibrosis and biochemical patterns that resemble human liver cirrhosis. Within the body, CCl_4 is metabolized to produce highly toxic trichloromethyl (CCl_3.) and trichloromethyl peroxyl (CCl_3O_2.) free radicals by cytochrome P450 enzyme and causes damage to hepatocytes[19-22]. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage. Therefore the increased level of TBARS in the liver tissue of the rats administered CCl_4 may be as a result of the enhanced membrane lipid peroxidation by free radicals generated and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals[23,24].

Antioxidant activity or scavenging activity of the generated free radicals is important in the curative effect of CCl_4-induced hepatotoxicity. The body has an effective defence mechanism to prevent and neutralize free radicals-induced damage. This is accomplished by a set of endogeneous antioxidant enzymes such as superoxide dismutase, catalase. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury[25], CAT is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver.

SOD catalyses the dismutation of superoxide anions while CAT catalyses the reduction of H_2O_2 and able to prevent the tissue from reactive free oxygen and hydroxyl radicals[26].

In CCl_4-induced hepatotoxicity, the balance between ROS production and antioxidant defenses may be lost hence oxidative stress results. The decreased activity of SOD and CAT in the liver tissues of CCl_4 treated rats may be due to these free radicals generated by the CCl_4 or inactivation of the antioxidative enzymes[27]. Treatment with aqueous root bark, stem bark and leaves extracts of *V. doniana* significantly ($P<0.05$) increase the level of SOD, CAT level and a reduction in TBARS. The effects of the extracts were comparable to the standard drug (vitamin E most important lipophilic antioxidant). Thus, this result suggests that aqueous root bark, stem bark and leaves extracts of *V. doniana* contains free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide radicals indicating the regeneration of damaged liver cells.

The extracts had no significant ($P>0.05$) effect on the lipid peroxidation and endogenous antioxidants enzymes of the liver. This may suggest that the extracts had no adverse effect on the endogenous antioxidant enzymes of the liver in a normal condition; this may explain the fact that the plant

mechanism of action in the liver is efficient in a disease condition.

There was significant ($P<0.05$) decrease in the kidney level of SOD, CAT and a non significant ($P>0.05$) increase in the level of TBARS when compared to normal control group.

It was demonstrated that liver is not the only target organ of CCl_4, it also causes free radical generation in other organs, such as kidneys, heart, lung, testis, brain and blood in various studies by researchers[28-30]. The decrease in the kidneys activities of SOD and CAT after exposing rats to CCl_4 was also reported by Ragip *et al*[31]. The non significant increase in TBARS of kidney tissue as compared to liver may be as a result of the fact that CCl_4 systemically applied on rats is found to be distributed in a higher concentration in the liver when compared to the kidneys[32]. There was no significant ($P>0.05$) difference between the kidney level of TBARS in the normal control and all the induced groups. Also no significant ($P>0.05$) difference between the kidney level of SOD in the CCl_4 group and all the induced treated groups except the vitamin E group, which shows a significant ($P<0.05$) increase in the level of SOD when compared to the CCl_4 group. Vitamin E and root extract significantly ($P<0.05$) increased the level of CAT compared to the induced not treated group. It could be considered that the non significant ($P>0.05$) increase in SOD and CAT values observed in the groups which received extracts could be related to the application period of the extracts, a more significant increase could be observed in the long-term periods of application. Moreover there was no significant ($P>0.05$) difference between all the extracts treated and normal control groups in the kidney level of TBARS, SOD and CAT when the extracts were administered to normal rats for 21 d except in the stem group where there was a significant ($P<0.05$) decrease in the level of TBARS. This suggests that the stem extract could improve normal renal function.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The potential toxicity of synthetic drugs has drawn worldwide attention to natural, environment-friendly herbs for medicinal purposes. Hence the need for intensive investigation of the efficacy and toxicity of African traditional herbs. *V. doniana* is a commonly used traditional herb in Nigeria. The current study is therefore quite important as it sought to prove the antioxidant efficacy of different parts of a highly consumed plant and their toxicities to experimental animals and consequently human being. The investigation is therefore of medicinal and public health significance.

Research frontiers

Investigation was performed into the antioxidant properties of aqueous root bark, stem bark and leaves of *V. doniana*. The results revealed that the three parts of the plant has antioxidant properties comparable to those of commonly used antioxidants drugs. In fact the stem bark has potential to improve renal functions in experimental rats.

Related reports

The data obtained by the authors on generation of free radicals in the liver and kidneys of rats induced with oxidative stress, following CCl_4 exposure in Wistar, are in agreement with the results obtained by Liu *et al* (2009) and Kim *et al* (2011). The decrease in SOD and catalase activities in rats exposed to CCl_4 was also obtained by Ragip et al. (2008). The insignificant increase in thiobarbituric acid reactive substance in the kidneys as compared to the liver of oxidative-stressed animals agreed with the result obtained by Mukai *et al* (2002).

Innovations and breakthroughs

The data generated in the current study proves for the first time the protective effect of parts of *V. doniana* against oxidative damage of the liver and kidney as evident in the decrease in reactive oxygen species, and maintenance of normal levels of antioxidant enzymes (SOD and CAT) in CCl_4 treated rats.

Applications

The results suggest that the aqueous root bark, stem bark and leaves are promising candidates for development of antioxidant drugs which supports its common usage for medicinal purposes in Nigeria.

Peer review

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.

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