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Hepatocurative potential of *Vitex doniana* root bark, stem bark and leaves extracts against CCl_4-induced liver damage in rats

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PEER REVIEW

Peer reviewer

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Comments

This is a good research work in which the authors demonstrated the hepatocurative property of *V. doniana* root bark, stem bark and leaves against rats with CCl_4-induced liver injury. Rats serum samples were analysed to estimate the liver and kidney function parameters of the experimental and control groups.

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ABSTRACT

Objective: To evaluate the hepatocurative effects of aqueous root bark, stem bark and leaves of *Vitex doniana* 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 (1 mL/kg 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 *Vitex doniana* and vitamin E as standard drug (100 mg/kg body weight 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 3 weeks.

Results: There was significant ($P<0.05$) increase in concentration of all liver marker enzymes, alanine aminotransferase, aspartate aminotransferase and alkaline aminotransferase (ALT, AST and ALP) and significant ($P<0.05$) decrease in albumin in the CCl_4 induced liver damage control when compared to the normal control. The extracts caused a significant ($P<0.05$) reduction in the serum activities of liver marker enzymes (ALT, AST and ALP) and a significant ($P<0.05$) increase in albumin of all the induced treated groups. Only stem bark extract and vitamin E significantly ($P<0.05$) increased total protein. All the extracts significantly ($P<0.05$) lowered serum creatinine whereas only root bark extract significantly ($P<0.05$) lowered serum level of urea in the rats with CCl_4 induced liver damage.

Conclusion: Hepatocurative study shows that all the plant parts (root bark, stem bark and leaves) possess significant hepatocurative properties among other therapeutic values justifying their use in folklore medicine.

KEYWORDS

Vitex doniana, Hepatocurative, Liver damage , Creatinine, Carbon tetrachloride

1. Introduction

Liver diseases remain one of the major threats to public health and a worldwide problem[1]. The World health organization estimates that 46% of global disease and 59% of mortality are due to chronic disease[2] and the management of liver disease is still a challenge to the modern medicine since modern medicine has little to offer alleviation of hepatic ailments[3].

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is the organ in charge of many important life functions, including food digestion, glycogen storage, control of metabolism, drug detoxification and hormone

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production[4]. Since liver is so vital to life, malfunction or failure of the organ often results in high rates of morbidity and mortality.

Various substances are known to cause liver damage, and one of them is carbon tetrachloride (CCl_4), which is a well-known hepatotoxin[5]. Within the body, CCl_4 breaks down to highly toxic trichloromethyl and trichloromethyl peroxyl free radicals by cytochrome P450 enzyme[6] which cause damage to hepatocytes[7].

The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy[8]. Herbs have been used for centuries to treat illness and improve health and account for approximately 80% of medical treatments in the developing world[9]. It is extremely widespread in tropical Africa. It is commonly known as Black Plum or African olive, Dinya (Hausa), Galbihi (Fulani), Oori-nla (Yoruba), Ucha coro (Igbo), and is wide spread in the southwestern Nigeria as a perennial tree. In Nigeria, from information available from the indigenous traditional healers, a decoction of the chopped stem bark part of *Vitex doniana* (*V. doniana*) is prepared and taken orally for treatment of gastroenteritis. It is administered for ailments including diarrhoea and dysentery. It is also taken to improved fertility and the juice may be squeezed into the eyes to treat eye troubles. It is also used in the treatment of liver disease.

The anti-hypertensive effect of extract of the stem bark of *V. doniana* has been reported. The extract exhibited a marked dose related hypotensive effect in both normotensive and hypertensive albino rats[10]. Extracts of stem bark of *V. doniana* have also demonstrated some level of *in vitro* trypanocidal activity against Trypanosoma brucei brucei[11].

Our previous study expressed that root bark, stem bark and leaves extracts possessed good *In vivo* antioxidant activity[12]. In this study we report the hepatocurative activity through serum analysis. Hence, the present study focused on evaluating the potential *In vivo* hepatocurative effect of aqueous extracts from root bark, stem bark and leaves in CCl_4 induced liver damage rats and the effect of the extracts were also investigated in normal rats.

2. Materials and methods

2.1. Plant samples collection and identification

The fresh root barks, stem barks and leaves of *V. doniana* were harvested from its natural habitat, Institute of Agricultural Research (IAR), Ahmadu Bello University (ABU), Zaria Kaduna State, Nigeria in the month of 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 2 weeks under ambient environmental conditions in a well aerated cages in Veterinary Public health Department Animal House, Ahmadu Bello University, Zaria Kaduna State. 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

To 100 g of each of the powdered root bark, stem bark and leaves were weighed into 3 sterilized conical flasks and 500 mL of distilled water was poured into each of the flasks. The contents of the 3 flasks were shaken and the tops were covered with aluminium foil and kept at ambient temperature for 48 h (2 d) 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 root bark, stem barks and leaves extracts of *V. doniana* were determined in albino rats (weighing 150-200 g) using the method described by Lorke[14].

2.6. Induction of liver damage

The liver damage was induced by the administration of Carbon tetrachloride (CCl_4) (Sigma chemicals Co., St. Louis USA). Rats were injected intraperitoneally with a single dose of CCl_4 (1 mL/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 3 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 as follows:

Group 1: Normal control animals given feed and water only; Group 2: Animals were treated with olive oil and served as vehicle control; Group 3: Animals were treated with CCl_4 in olive oil (1 mL/kg body weight); Group 4: Animals were treated with CCl_4 in olive oil (1 mL/kg body weight)+root bark extract (100 mg/kg) of *V. doniana*; Group 5: Animals were treated with CCl_4 in olive oil (1 mL/kg body weight)+stem bark extract (100 mg/kg) of *V. doniana*; Group 6: Animals were treated with CCl_4 in olive oil (1 mL/kg body weight)+ leaves extract (100 mg/kg) of *V. doniana*.
Group 7: Animals were treated with CCl_4 in olive oil (1 mL/kg body weight)+vitamin E (100 mg/kg). (standard drug); Group 8: Normal animals treated with root bark extract (100 mg/kg body weight) of *V. doniana*; Group 9: Normal animals treated with stem bark extract (100 mg/kg body weight) of *V. doniana*; Group 10: Normal animals treated with leaves extract (100 mg/kg body weight) of *V. doniana*.

2.8. Treatment of samples and tissues examination

At the end of 21 d of treatment, the animals were anesthetized with chloroform and sacrificed by cervical capitation. Blood was obtained through cardiac puncture by means of hypodermal syringe and needle. The collected blood samples were placed in ice-cold micro centrifuge tubes. The blood was allowed to coagulate and centrifuged at 4 000 r/min for 10 min. The serum samples were collected and utilized for some biochemical parameters analysis.

2.9. Biochemical determinations

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, urea and creatinine were determined by the standard method using commercially available kits (from Randox laboratories Ltd. Ardmore, Co. Antrm UK)

2.10. Statistical analysis

The results of the analysis were expressed as mean± Standard deviation. 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 extracts of V. doniana on liver function parameters

Table 1 shows ALT, AST, and ALP concentrations in the serum of CCl_4 induced liver damage rats after the daily oral administration of aqueous root bark, stem bark and leaves of *V. doniana* for 21 d. There was significant ($P<0.05$) increase in concentration of all these liver marker enzymes (ALT, AST and ALP) in the CCl_4 induced liver damage control when compared to the normal control. The levels of ALT, AST and ALP in the induced treated groups were however significantly ($P<0.05$) reduced when compared to induced not treated group. Table 1 also shows the serum total protein and albumin of induced liver damage rats treated with aqueous root bark, stem bark and leaves extracts of *V. doniana* and vitamin E for 21 d. The result showed that stem extracts and vitamin E were able to significantly ($P<0.05$) increase the serum total protein more than the other induced treated groups and the induced not treated group. Also serum albumin of the induced not treated was significantly ($P<0.05$) lowered than the normal control, but there was no significant ($P>0.05$) difference between the serum albumin of all the induced treated groups and the normal control groups.

On the other hand the effect of the extracts on the liver of normal rats is shown in Table 2. The result showed that root bark and stem bark extracts significantly ($P<0.05$) lowered ALT, the lowering effect was more in root bark extract. Also root bark extract was able to significantly lowered ALP, whereas there was no significant ($P>0.05$) effect on AST. The stem bark and leaves extracts significantly ($P<0.05$) increased total protein whereas all the extracts had no

Table 1
Effects of aqueous extracts of *V. doniana* on liver function parameters in liver of CCl_4 induced liver damage rats.

Group (n=6)	ALT (U/l)	AST(U/l)	ALP(U/l)	Total protein (g/L)	Albumin (g/L)
Normal control	14.50 ± 2.5^{abc}	43.33 ± 29.8^{a}	89.70 ± 25.3^{a}	66.28 ± 3.4^{ab}	37.24 ± 3.0^{b}
Vehicle control	8.67 ± 3.1^{a}	47.00 ± 15.3^{a}	77.28 ± 14.0^{a}	72.12 ± 12.2^{ab}	35.59 ± 4.0^{b}
CCl_4 induced liver damage control	26.00 ± 2.0^{d}	70.00 ± 5.0^{b}	147.20 ± 15.9^{b}	58.48 ± 3.4^{a}	26.58 ± 1.4^{a}
CCl_4+RE	17.00 ± 2.6^{bc}	46.67 ± 11.5^{a}	87.17 ± 9.1^{a}	62.38 ± 3.4^{a}	33.46 ± 3.0^{b}
CCl_4+SE	9.50 ± 4.1^{a}	46.67 ± 10.4^{a}	95.68 ± 33.6^{a}	79.92 ± 6.7^{b}	33.96 ± 0.9^{b}
CCl_4+LE	20.00 ± 7.3^{cd}	47.50 ± 14.4^{a}	96.60 ± 31.4^{a}	70.18 ± 5.9^{ab}	33.96 ± 0.9^{b}
CCl_4+Std	12.50 ± 13.8^{ab}	45.00 ± 9.3^{a}	107.30 ± 4.8^{a}	79.92 ± 9.0^{b}	37.73 ± 2.9^{b}

Values are means±SD. Values with different superscript down the colomn are significantly different ($P<0.05$).
CCl_4+RE: CCl_4 Induced liver damage rats+100 mg/kg body weight of aqueous root bark extract, CCl_4+SE: CCl_4 Induced liver damage rats+100 mg/kg body weight of aqueous stem bark extract, CCl_4 + LE: CCl_4 induced liver damage rats+100 mg/kg body weight of aqueous leaves extract, CCl_4+Std: CCl_4 induced liver damage rats+100 mg/kg body weight of Vitamin E.

Table 2
Effects of aqueous extracts of *V. doniana* on liver function parameters in normal rats.

Groups (n=6)	ALT (U/l)	AST(U/l)	ALP(U/l)	Total protein (g/L)	Albumin (g/L)
Normal control	14.50±2.5^{c}	43.33±29.8^{a}	89.7±25.3^{b}	66.28±2.0^{a}	37.24±2.9^{a}
N_+RE	4.50±1.9^{a}	22.5±10.4^{a}	43.37±7.0^{a}	70.17±3.4^{ab}	32.48±2.8^{a}
N_+SE	9.33±1.1^{b}	25.0±7.9^{a}	79.12±10.5^{b}	83.82±3.9^{b}	37.56±1.6^{a}
N_+LE	16.00±4.0^{c}	37.50±23.3^{a}	90.16±12.0^{b}	81.87±6.8^{b}	35.76±1.9^{a}

Values are means±SD. Values with different superscript down the colomn are significantly different (P<0.05)
N_+RE: Normal rats $_+$100 mg/kg body weight of aqueous root bark extract; N_+SE: Normal rats $_+$100 mg/kg body weight aqueous stem bark extract; N_+LE: Normal rats $_+$100 mg/kg body weight aqueous leaveas extract.

significant (P>0.05) effect on the albumin when compared to the normal control.

3.2. Effects of Aqueous extracts of V. doniana on kidney function parameters

Figure 1 shows Creatinine concentrations in the serum of normal and CCl_4 induced liver damage rats after the oral administration of aqueous root bark, stem bark and leaves extracts of *V. doniana* and vitamin E for 21 d. The results showed that the concentration of creatinine in the serum of CCl_4 induced not treated rats was significantly (P<0.05) higher when compare to normal control rats. However, there was no significant (P>0.05) difference between the concentration of creatinine in all the induced treated groups when compared to the normal control group.
The effect of the aqueous root bark, stem bark, and leaves extracts of *V. doniana* on serum creatinine in normal rats. The result showed that all the extracts significantly reduced (P<0.05) the concentration of serum creatinine when compared to the normal control animals

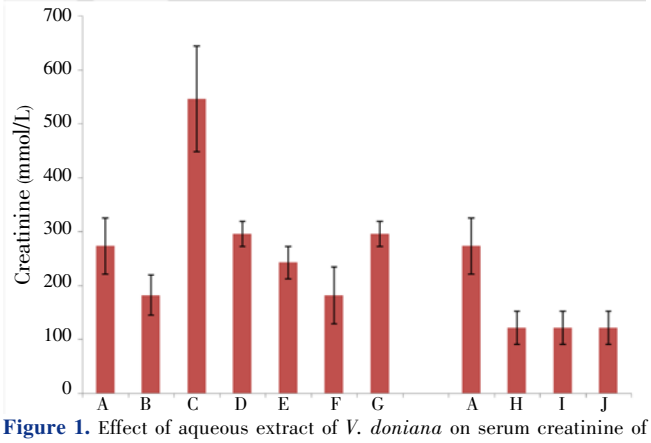

Figure 1. Effect of aqueous extract of *V. doniana* on serum creatinine of normal and CCl_4 induced liver damage rats.
A: Normal Control rat, B: Vehicle control rats, C: CCl_4 Induced liver damage control rats, D: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous root bark extract, E: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous stem bark extract, F: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous leaves extract, G: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of Vitamin E, H: Normal rats$_+$100 mg/kg body weight of aqueous root bark extract; I: Normal rats$_+$100 mg/kg body weight aqueous stem bark extract; J: Normal rats$_+$100mg/kg body weight aqueous leaves extract.

Figure 2 depicts the urea concentrations in the serum of normal and CCl_4 induced liver damage rats after the oral administration of aqueous root bark, stem bark and leaves extracts of *V. doniana* and vitamin E for 21 d. The results showed that the concentration of urea in the serum of CCl_4 induced not treated rats was significantly (P<0.05) higher when compared to normal control rats. However, there was no (P>0.05) significant difference between the concentration of urea in the serum of all the induced treated groups when compared to the induced not treated group, except the group treated with root which showed no significant (P>0.05) difference when compared with the normal control group.

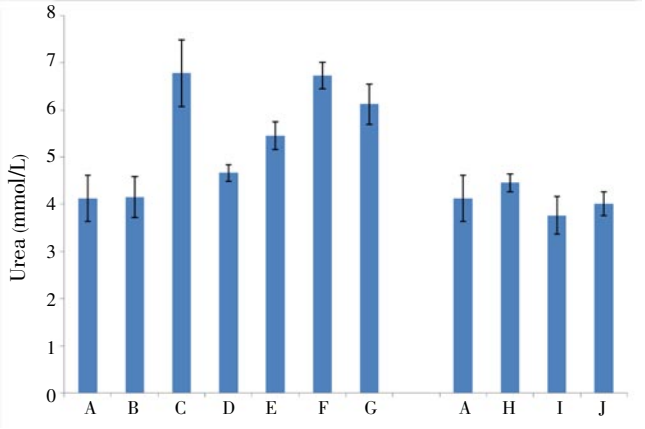

Figure 2. Effect of aqueous extract of *V. doniana* on serum urea of normal and CCl_4 induced liver damage rats.
A: Normal Control rat, B: Vehicle control rats, C: CCl_4 Induced liver damage control rats, D: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous root bark extract, E: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous stem bark extract, F: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of aqueous leaves extract, G: CCl_4 Induced liver damage rats$_+$100 mg/kg body weight of Vitamin E, H: Normal rats$_+$100 mg/kg body weight of aqueous root bark extract; I: Normal rats$_+$100 mg/kg body weight aqueous stem bark extract; J: Normal rats$_+$100 mg/kg body weight aqueous leaves extract.

The effect of daily oral administration of aqueous root bark, stem bark and leaves extracts of *V. doniana* on serum urea concentration in normal albino rats showed that there was no significant (P>0.05) difference between the serum urea concentrations of all the extracts treated animals and the normal control animals.

4. Discussion

Liver fibrosis represents chronic wound repair following liver injury[15]. In this study, we examined the response of the liver and kidney to hepato-/nephrotoxicants in rats with a preceding liver injury. Hepatotoxicity of the CCl_4 in the rats was determined by changes in serum parameters.

Assessment of liver was made by estimating the activities of serum ALT, AST and ALP which are enzymes originally present at higher concentration in the cytoplasm[16]. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage[17,18]. Administration of CCl_4 caused a significant ($P<0.05$) elevation of enzyme levels such as AST, ALT, ALP and decrease in the level of albumin when compared to normal control. The elevated level of these entire marker enzymes and decrease in albumin observed in the CCl_4-induced not treated group (positive control) corresponded to the extensive liver damage induced by CCl_4. These results are in agreement with previous finding that the activity levels of serum ALT and AST were significantly elevated in rats after CCl_4 administration[19-21]

There was significant ($P<0.05$) restoration of these enzyme levels and albumin on administration of the extracts and vitamin E at a dose of 100 mg/kg. The reversal of serum enzymes in CCl_4-induced liver damage towards a near normalcy by the extracts observed in this study may be due to the prevention of the leakage of intracellular enzymes by the presence of polyphenol in the extracts and their membrane stabilizing activity[22,23]. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[24]. It is therefore, a clear manifestation of hepatocurative effects of the extracts especially root and stem bark extracts.

Injury to liver, resulting in loss of its normal physiological/biochemical functions, may adversely affect a secondary organ like kidneys. The kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. Creatinine is a waste product formed in muscle by creatine metabolism. Creatine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment.

Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases which diminish the glomerular filteration lead to urea retention. Administration of CCl_4 causes nephrotoxicity as indicated by significant ($P<0.05$) elevation in serum level of urea and creatinine. These results were in agreement with earlier findings[25,26]. From the present study it is evident that elevation in plasma urea and creatinine levels can be attributed to the damage of nephron structural integrity[27].

All the extracts significantly ($P<0.05$) lowered creatinine in CCl_4 induced liver damage and normal rats indicating that the extracts improve renal function in diseased and normal animals. Whereas only root bark extracts significantly ($P<0.05$) lowered serum urea in CCl_4 treated animals. This may indicate that root extracts is more efficient.

In the present study, *V. doniana* root bark, stem bark and leaves extracts possessed *In vivo* hepatocurative activity in a rats model of CCl_4 induced liver damage. The hepatocurative activity of *V. doniana* root bark, stem bark and leaves extracts may be due to its various bioactive compounds in the extracts. Further studies are in progress to better understand the mechanism of action of *V. doniana* that is responsible for the hepatocurative and antioxidant.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Liver is a vital organ that metabolises and regulates most of the chemicals in the blood. Interestingly, liver disorder is still a major global threat in this modern era. Hence it is pertinent to scientifically explore the hepatocurative potential of traditional plant extracts in reducing the problem.

Research frontiers

The present research work reveals the hepatocurative activities of aqueous extracts of *V. doniana* root bark, stem bark and leaves against CCl_4-induced hepatic injury in rats by estimating the liver and kidney function parameters.

Related reports

CCl_4 has been reported to induce hepatic necrosis in laboratory animals. There were numerous reports on the efficacies of plant extracts in treating various liver disorders.

Innovations and breakthroughs

V. doniana is a common plant in tropical Africa and has been used by indigenous traditional healers to treat gastroenteritis. In the present study, the authors have demonstrated the hepatocurative activities of *V. doniana* root bark, stem bark and leaves in CCl_4-induced hepatic

injury in a rat model.

Applications

Different parts of *V. doniana* have been used by the indigenous traditional healers in Nigeria for various ailments. This scientific study shows that its root bark, stem bark and leaves possess significant hepatocurative properties, besides its other therapeutic values as practiced by folklore medicine.

Peer review

This is a good research work in which the authors demonstrated the hepatocurative property of *V. doniana* root bark, stem bark and leaves against rats with CCl_4-induced liver injury. Rats serum samples were analysed to estimate the liver and kidney function parameters of the experimental and control groups.

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