

Anti-Oxidant and Hepatoprotective Effects of *Senecio biafrae* on CCl₄-induced Liver Damage in Rats

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ABSTRACT

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Background:

The present study was performed to explore whether the aqueous extract of *Senecio biafrae* (*S. biafrae*) roots provide any *in vivo* protective activity against carbon tetrachloride (CCl₄)-induced hepatotoxicity in male albino rats.

Methods:

Rats (150-200 grams) were grouped into five groups (A-E) of six rats each and were treated orally for twelve days with 72 hourly administration of CCl₄ (1 mL/kg) as follows: *Group A* received distilled water only (negative control), *Group B* was administered distilled water plus CCl₄ (positive control), *Group C* was administered 400 mg/kg extract and CCl₄, *Group D* received 200 mg/extract and CCl₄, while *Group E* was administered standard drug (Silymarin 25mg/kg, PO).

Results:

Pre-treatment with the extract of *S. biafrae* (200 or 400mg/kg) or Silymarin (25mg/kg) caused significant restoration in the biomarkers as evaluated by reducing the levels of malondialdehyde, transaminases and elevating the levels of superoxide dismutase, catalase and glutathione peroxidase activities, which were altered by CCl₄ toxicity. The extract at a dose of 400mg/kg demonstrated similar activities comparable to the standard drug (Silymarin).

Conclusion:

The results of this study indicate that the root extract of *S. biafrae* possesses hepatoprotective and anti-oxidant properties which may be due to the presence of phytochemicals in it.

Keywords:

Anti-Oxidant; Carbon Tetrachloride; Hepatoprotective; *Senecio Biafrae*; Silymarin.

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INTRODUCTION

Carbon tetrachloride (CCl₄)-induced liver damage is an oxidative-stress model employed for screening of hepatoprotective compounds (1). Carbon tetrachloride is known to induce hepatotoxicity by exerting oxidative stress on the organ. A relationship between lipid peroxidation and oxidative stress has previously been described (2). Trichloromethyl radical (CCl₃•), a metabolite (toxic intermediate) from the cytochrome P₄₅₀ system of enzymes in liver microsomes, is reported to give rise to liver damage. CCl₄-induced liver damage progresses from steatosis to centrilobular necrosis, thereby developing fibrosis and cirrhosis (3).

The liver has an important role in nutrients metabolism and chemicals. It is also involved in the

excretion of xenobiotics from the body and providing protection against harmful foreign materials through detoxification and elimination (4). Liver injury arising from several etiologies are said to cause steady failure in hepatic functions. Free radicals, food additives, alcohol, pollutants and xenobiotics are the key risk factors leading to cirrhosis, alcoholic liver diseases and hepatitis (5).

Treatment of liver-related diseases is important and should be handled with utmost care. Not all conventional drugs available for regeneration of liver cells are without hepatotoxic side effects (6). Therefore, it is essential to explore safe and hepatoprotective agents, in the context of traditional and medical knowledge (7). *S. biafrae* is a naturally occurring herb found in African forest areas. It is used as a green leafy vegetable in Sierra Leone, Benin,

Nigeria, Ghana, Cameroon and Gabon. It contains several metabolites like sesquiterpenes, dihydroisocoumarins, amino acids and terpenoids (8). It is used in Nigeria for the treatment of pulmonary conditions and women infertility (9). The plant has been reported to have antimicrobial (10) and antihypertensive properties (11). Similarly, its antidiabetic and physicochemical properties have previously been reported (12). Our findings from traditional healers about the herb in the Niger Delta revealed that the root has been used for the treatment of liver-related diseases amongst others. Considering that other species of *Senecio* have shown hepatoprotective activity (13) and the phytochemical profile of the plant (10, 14), the present study was performed to investigate whether the aqueous extract of *S. bialfrae* roots demonstrate any *in vivo* protective activity against CCl₄-induced liver damage in rats.

MATERIALS AND METHODS

Reagents and Chemicals: Silymarin and CCl₄ were purchased from Sigma chemicals (St. Louis, USA). Diagnostic kits for serum total bilirubin (TBIL), Albumin (ALB), Total protein (TP), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were purchased from Randox Laboratories Ltd. (Ardmore, UK). All other chemicals for this experiment were obtained locally, and were of analytical grade.

Plant Material: The roots of *S. bialfrae* were collected from Akoko, Ondo State, Nigeria, and authenticated by Dr. H.A. Akinnobosun of the Department of Plant Science (Reference voucher #: UBHs 0149), University of Benin, Edo State, Nigeria.

Preparation of Plant Materials: The collected, shade dried roots were pulverized to fine powder by pestle and mortar. They were extracted with distilled water by soaking fifty grams in 200mL of the solvent in airtight flask and shaken daily for three days. The mixture was filtered through muslin cloth and Whatman No. 1 filter paper and concentrated under reduced pressure and low temperature using a rotary evaporator. The resultant extract was kept at 4°C before use. Afterwards, the dried extract were suspended in distilled water to prepare the two concentrations (400 & 200mg/ml) for the purpose of this study.

Animals: The male albino rats (weighing 150-200g) were bought from the Anatomy Department of Delta State University, Abraka, Nigeria. They were fed grower's mash (Top Feeds, Ltd, from Sapele in Delta State, Nigeria) and water *ad libitum*. The animals were maintained in agreement with the guidelines of the institutional Committee for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Grouping and Treatment of Animals: The rats were divided into five groups (A-E) of six rats each and were treated orally for twelve days as follows: *Group A* received distilled water only (negative control), *Group B* was administered distilled water plus CCl₄ (positive

control), *Group C* was administered 400mg/kg extract and CCl₄, *Group D* received 200mg/extract and CCl₄, while *Group E* was administered the standard drug (Silymarin 25mg/kg, PO). The CCl₄ was given at a dose of 1mL/kg of body weight (BW; 30% concentration in liquid paraffin) 72 hours after the first extract or Silymarin administration and subsequently every other 72 hours. The animals were sacrificed by decapitation 24 hours after the last administration to obtain their blood and organs, which were used for the biochemical assays. The blood was allowed to clot after collection and centrifuged at 3000×g (15min), while the liver tissues were homogenized in phosphate buffer (pH 7.4) and centrifuged at 3000g for 10 min. Thereafter, the supernatants were collected and used for the assays.

Determination of Serum Hepatospecific

Markers: The activities of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total bilirubin (TBIL), albumin (ALB) and total protein (TP) were tested by assay kits, obtained from Randox Laboratories Ltd. (Ardmore, UK).

Determinations of Antioxidant Activity In Vivo:

The levels of lipid peroxidation was evaluated based on the reaction of two moles of N-methyl-2-phenylindole, a chromogenic reagent, with one mole of either malondialdehyde (MDA) or

4-HNE at 45°C for 60 min to yield a stable chromophore with a maximum absorbance at 586 nm as described elsewhere (15). Catalase activity was evaluated according to the method of Aebi (16). Glutathione peroxidase activity was determined following NADPH oxidation at 340nm in the presence of excess reduced glutathione, glutathione reductase and corresponding peroxide (17). Liver superoxide dismutase (SOD) activity was evaluated according to the method described by Kakkar *et al.* (18), while glutathione-S-transferase (GST) activity was estimated by the method of Habig *et al.* (19).

Statistical Analysis: The results were calculated by the Graph pad Prism 6 software using one-way ANOVA followed by Tukey-Kramer multiple comparisons test. A confidence level of $p < 0.05$ was considered significant. The values shown in Tables 1 and 2 are as means \pm standard deviation (SD).

RESULTS

Biochemical Changes in Serum Markers: The effects of aqueous extracts of *S. bialfrae* on the liver function of CCl₄ intoxicated rats, after twelve days of treatment, are shown in Table 1. The administration of CCl₄ significantly ($p < 0.05$) increased ALT and AST levels in the positive control group B, compared to those in negative control group A. Also, a significant increase ($p < 0.05$) in ALP and TBIL levels were found in group B rats relative to those in normal control group A. On the other hand, treatment with either the extract or the standard drug significantly ($p < 0.05$) reduced the levels of serum hepatic enzymes ALT, AST & ALP and TBIL, compared with those in positive control group B. However, lower levels of albumin and total protein

were noticed in group B rats, compared with those in negative control group A. Significantly ($p < 0.05$) higher levels of these two parameters were detected in the rats that received either a higher dose of extract (group C) or the standard drug (group E).

Effect of Aqueous Root Extracts of *S. Biafrae* on Liver Biochemical Parameters: Liver biochemical parameters such as SOD, CAT, GST and glutathione peroxidase (GPx) were reduced while lipid peroxidation increased in CCl₄-induced rats (Group B) compared to the negative controls (Group A) as shown

in Table 2. Treatment with either the extract or the standard drug restored the level of these oxidative stress markers to near normal. Thus, a striking increase in the MDA level was seen in the liver of Group B (CCl₄-exposed) rats relative to normal controls in Group A rats (Table 2). The increase in MDA was statistically significant ($p < 0.05$). Likewise, the group treated with 400 mg/kg of *S. biafrae* extract had lower MDA levels compared to those in the standard group. Thus, the highest dose (400mg/kg) of *S. biafrae* extract and Silymarin showed the best results.

Table 1. Effect of *S. biafrae* aqueous extract on the biochemical parameters in carbon tetrachloride-induced hepatotoxicity.

Parameters	Group A	Group B	Group C	Group D	Group E
ALT (U/L)	36.32±0.86 ^b	93.32±2.86 ^a	40.25±1.76 ^{ab}	56.05±2.13 ^{ab}	32.4±1.25 ^{ab}
AST (U/L)	48.24±0.89 ^b	162.42±5.74 ^a	45.19±2.88 ^b	71.19±1.93 ^{ab}	43.01±2.56 ^{ab}
ALP (U/L)	72.29±1.88 ^b	195.37±6.02 ^a	78.53±3.47 ^b	94.44±2.72 ^{ab}	63.25±3.29 ^{ab}
TP (mg/dl)	7.53±0.81 ^b	4.09±0.68 ^a	6.74±0.38 ^b	5.2±0.64 ^a	7.31±0.47 ^b
ALB (g/dl)	2.49±0.31 ^b	1.18±0.09 ^a	2.64±0.37 ^b	1.49±0.22 ^a	2.38±0.25 ^b
TBIL (mg/dl)	1.13±0.07 ^b	3.12±0.17 ^a	1.45±0.12 ^{ab}	2.15±0.11 ^{ab}	2.00±0.17 ^{ab}

Key: ^a $p < 0.05$ vs. negative controls; ^b $p < 0.05$ vs. positive controls.

Table 2. Effect of *Senecio biafrae* aqueous extract on SOD, CAT, GPx, GST and MDA in CCl₄-induced hepatotoxicity.

Parameters	Group A	Group B	Group C	Group D	Group E
SOD (U/mg protein)	73.4±1.61 ^b	28.24±0.66 ^a	61.65±1.31 ^{ab}	51.8±2.27 ^{ab}	66.19±1.13 ^{ab}
CAT (U/mg Protein)	132.39±1.13 ^b	40.43±2.03 ^a	109.06±2.14 ^{ab}	76.34±2.84 ^{ab}	120.19±2.98 ^{ab}
GPx (U/mg protein)	14.25±0.51 ^b	6.72±0.5 ^a	9.81±0.6 ^{ab}	7.62±0.37 ^a	10.92±0.43 ^{ab}
GST (U/mg protein)	8.15±0.49 ^b	3.07±0.53 ^a	6.11±0.59 ^{ab}	4.15±0.46 ^a	7.04±0.22 ^{ab}
MDA (nmol/mg protein)	6.08±0.64 ^b	12.79±0.82 ^a	6.20±0.43 ^b	8.48±0.46 ^{ab}	6.73±0.56 ^b

Key: ^a $p < 0.05$ vs. negative controls; ^b $P < 0.05$ vs. positive controls.

DISCUSSION

The maintenance of body homeostasis is one of the major functions of the liver. Carbon tetrachloride-induced liver damage is an oxidative stress model employed for screening of hepatoprotective chemicals and drugs (20). Liver transaminases, such as AST and ALT still remain the best standards for the evaluation of liver injury and have been the preferred biomarkers for decades (21). In this study, significant liver necrosis supported by increased levels of total bilirubin, liver marker enzymes activities (ALT, AST and ALP), and decreased levels of albumin and total protein were observed in the rats treated with CCl₄ (Group B). But pretreatment with *S. biafrae* extract (200 and 400mg/kg, BW) for twelve days resulted in a significant decrease of the CCl₄-induced elevation of enzyme markers in serum, comparable to the effect of the standard drug, Silymarin. This drug is a well-known hepatoprotective compound derived from Silybum marianum and its protective effect on the plasma membrane of hepatocytes has been shown (22). The significant reduction in the total protein and albumin observed in CCl₄-treated rats might be as a result of functional failure in the cytochrome-P₄₅₀ complexes (23). Thus, the result indicate that pre-treatment of *S. biafrae* extract, caused restoration to normal, the activities of serum marker enzymes and the levels of total protein, total bilirubin and albumin, thus indicating that *S. biafrae* extract maintained the structural integrity of hepatocellular constituents and kept the liver from CCl₄-induced injury *in vivo* (24).

An essential mechanism in the protection of liver against CCl₄-induced damage is the inhibition of excess reactive oxygen species (ROS) production. MDA is commonly used as a major parameter and marker of lipid peroxidation when assessing the status of oxidative stress (25). In this study, we observed an elevated level of liver MDA in the CCl₄-treated rats, which implies an increase in lipid peroxidation with the consequent damage to tissue and breakdown of the antioxidant defense mechanisms to stop formation of excess free radicals. This result is in agreement with the previous reports that CCl₄ caused hepatic injury by elevating the MDA level (26). However, we also observed that pretreatment with the extract significantly decreased the CCl₄-induced liver MDA elevation. Therefore, the mechanism of hepatoprotection of *S. biafrae* extract may be due its antioxidant potential (12,27).

Super oxide dismutase functions by removing reactive oxygen radicals and preventing the production of more hydroxyl radicals, thus considered as the initial line of cellular defense against oxidative stress (28). Catalase is a heme-containing enzyme localized largely in the subcellular organelles (peroxisomes) that converts H₂O₂ to water and O₂, and protects the cell from oxidative damage caused by H₂O₂ and OH group. It has been established that CCl₄, in addition to initiating lipid peroxidation, also reduces CAT and SOD activities in the tissue, which may be caused by oxidative alteration of these proteins (29). In this study, a decrease in the activities of SOD and CAT was noted in the CCl₄-treated groups, thus suggesting liver injury in the rats.

However, the rats treated with 200 and 400mg/kg BW of *S. bialifrae* showed significant increase in the level of these enzymes, suggestive of the antioxidant effect of the extract (12).

Glutathione peroxidase (GSH-Px) is an essential enzyme which catalyzes the reduction of lipid hydroperoxides and H₂O₂ into H₂O and resultant alcohols and thereafter, terminating the processes of lipid peroxidation (30). Glutathione-S-transferase (GST) is an enzyme that catalyzes the conjugation of GSH through the sulfhydryl group. It participates in the detoxification process resulting from conjugation reaction between xenobiotics and GSH (31). The activity of GST is valuable in the endogenous detoxification of compounds, such as peroxidized lipids (32). In this study, a decrease in the activity of this enzyme, in CCl₄-treated rats and a reversal close to normalcy in the extract or the standard drug treated rats were shown. Thus, pretreatment with *S. bialifrae* led to a rise in GST activity and a decrease in the liver MDA, thereby suggesting that the protective effect *S. bialifrae* may be linked to its anti-oxidative stress function.

The observed effects of *S. bialifrae* extract in this study may be attributed partly to several phytoconstituents present in it, such as alkaloids, saponins, tannins, glycosides, phenols, β Carotenes, flavonoids, cardenolides, anthraquinones and steroids (10,14). These phytoconstituents could generally have contributed to the detected hepatoprotective and antioxidant activities of the plant (33-35).

CONCLUSION

Administration of CCl₄ at 1 ml/kg body weight to Wistar male rats resulted in an increase in lipid peroxidation and reduction in enzymatic antioxidants. *S. bialifrae* extract, at 200 or 400mg dosage showed hepatoprotective effects, but 400 mg/kg was more effective, compared with the effect of standard drug, Silymarin. We conclude that *S. bialifrae* extract had antioxidant and protective activities against CCl₄ hepatotoxicity in Wistar albino male rats, which is likely due to its ability to scavenge free radicals and reduce inflammatory responses. However, further studies are highly recommended to identify the active components of the extract and the molecular mechanisms responsible for the hepatoprotective effect.

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest in conducting this study.

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