

Original Article

EFFECT OF ETHANOL STEM BARK EXTRACT OF *BERLINIA GRANDIFLORA* HUTCH AND DALZ  
ON MARKER LIVER ENZYMES IN RATS TREATED WITH CCl<sub>4</sub>

MARTIN NTIAMOAH DONKOR<sup>1\*</sup>, SAMUEL YAWSON AYIKANLE<sup>1</sup>, SAMUEL ADOESOM DONKOR<sup>1</sup>

<sup>1</sup>Department of Applied Chemistry and Biochemistry, Faculty of Applied Sciences, University for Development Studies, Navrongo Campus,  
P. O. Box 24, Navrongo, Ghana  
\*Email: mndonkor@gmail.com

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ABSTRACT

**Objective:** The objective of this work was to validate the traditional claim of the use of the stem bark of *Berlinia grandiflora* in treating liver problems by investigating the effect of its ethanol stem bark extract on marker liver enzymes *in vivo*.

**Methods:** Groups of Wistar albino rats (6 in each group) were daily treated with CCl<sub>4</sub> (2 ml/kg b.w., diluted with olive oil 1:1 v/v, intravenously), followed by oral administration of ethanol stem bark extract of *Berlinia grandiflora* (ESBG) at doses 100, 300 and 900 mg/kg b.w. respectively, 3 h after the administration of the CCl<sub>4</sub>. Serum biochemical parameters were measured 24 h at the end of the 7-day treatment period and compared to a group intoxicated with CCl<sub>4</sub> (2 ml/kg b.w., diluted with olive oil 1:1 v/v, intravenously) alone.

**Results:** Treatment of rats with ESBG (at doses 100, 300 and 900 mg/kg b.w. orally) reduced the impact of CCl<sub>4</sub>-induced hepatotoxicity on liver marker enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and total bilirubin (TB), with the 900 mg/kg b.w. showing a significant reduction (p<0.05) compared to the group intoxicated with CCl<sub>4</sub>. The extract reversed the impact of the CCl<sub>4</sub>-induced hepatotoxicity by increasing the concentration of the serum Total protein (TP) and albumin (ALB), with the 900 mg/kg b.w. dose showing a significant increment (p<0.05) compared to the group intoxicated with the CCl<sub>4</sub>.

**Conclusion:** The investigation of the effect of *Berlinia grandiflora* on CCl<sub>4</sub> induced liver damage revealed that the ethanol extract of the stem bark of the plant was able to reverse the hepatotoxicity, with the 900 mg/kg b.w. dose showing a significant activity.

**Keywords:** Aminotransferase, *Berlinia grandiflora*, Biochemical, CCl<sub>4</sub>, Hepatoprotective, Phytochemical, Serum

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INTRODUCTION

The liver is the largest organ in the body and the vital end organ for biological processes such as metabolism, excretion, and detoxification. In the process, the hepatocytes get injured resulting in disorders or damaged liver [1]. Hepatic diseases have become one of the major causes of morbidity and mortality all over the world with approximately 20,000 deaths recorded globally every year [2, 3]. Continuous exposure to environmental toxicants, prescribed and over-the-counter drugs, and alcohol abuse make the liver vulnerable to a variety of disorders such as jaundice, hepatitis, and cirrhosis [2].

Even though there have been advances in modern medicine for the treatment of hepatic diseases, these drugs apart from being expensive in resource-poor countries, possess harmful side effects such as insomnia, vomiting, constipation, and depression [4]. On the other hand, natural products of plant origin have been the main source of treatment for many ailments including liver diseases for centuries, with about 80 % of the population in poor resource areas currently depending on traditional medicine for their primary health care [5, 6]. They are considered as rich sources of lead compounds, safe for human use with little side effects and environmentally friendly [7]. Therefore, there is the need to scientifically validate the many plants which are used by traditional medicine practitioners for the treatment of liver-related diseases.

*Berlinia grandiflora* Hutch and Dalz belonging to the family Leguminosae-Caesalpinioideae is a tropical shrub. There are about 20 species which are limited to tropical Africa with almost all the species found in West and Central Africa. It is widespread in countries such as Mali, Guinea, Nigeria, Central Africa Republic, Democratic Republic of Congo and Ghana [8, 9]. In Ghana, it is called "papa" by the Akan ethnic group.

The different parts of the plant have many ethnomedicinal uses. The stem bark is used to reduce labour pain during childbirth and treat

gastrointestinal disorders, while the bark infusion is used as purgative. It is also claimed that the bark sap is used for the treatment of sores and wounds, and bark decoctions are administered to treat hemorrhoids and liver problems. A decoction of the leafy twigs is used as febrifuge, purgative, antiemetic, cholagogue, while leafy decoctions are taken as tonic [10-12].

The crude aqueous, methanol and butanol extracts of the stem bark and leaves of *Berlinia grandiflora* are reported to appreciably inhibit the growth of *Staph. aureus*, *E. coli*, *P. aeruginosa* and *P. vulgaris* which are considered to have multidrug resistance [9]. Other pharmacological properties exhibited by *Berlinia grandiflora* are anti-oxidant, anti-diabetic, anthelmintic and analgesic activities [10, 11]. *Berlinia grandiflora* possess phytoconstituents such as tannins, flavonoids, triterpenes, glycosides and alkaloids [11, 12]. This study reports the effect of the ethanol stem bark extract of *Berlinia grandiflora* (ESBG) on chemical-induced hepatotoxicity to validate its folkloric use as treatment for liver problems. Here hepatotoxicity is induced in rats with carbon tetrachloride (CCl<sub>4</sub>).

MATERIALS AND METHODS

Collection and preparation of plant material

The stem bark of *Berlinia grandiflora* was obtained from Damango in the Northern Region of Ghana in January 2017. The plant sample was identified and authenticated in the Department of Applied Biology, University for Development Studies, Navrongo Campus (voucher specimen number: DAB/EB/M0015/17). The freshly obtained stem bark of *Berlinia grandiflora* was washed and cut into pieces, and air dried under shade for two weeks. The dried sample was pulverized using mortar and pestle. A colander was further used to obtain a fine sample.

Chemicals and reagents

Chemicals, reagents and test kits used were obtained from different companies as follows: carbon tetrachloride, ethanol, sodium

hydroxide, hydrochloric acid, and chloroform–Central Drug House (CDH), India; sulphuric acid, ammonia, acetic anhydride, ferric chloride, Fehling's solution and Wagner's reagent–Sigma, Germany; olive oil–Borges Agricultural and Industrial Edible Oil, S. A. U, Spain); Silymarin (Simepar™ capsules)–Acino Pharmaceuticals, Switzerland; test kits and reagents for biochemical assays [Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB) and Total bilirubin (TB)]–ELITech Group, France. All the chemicals used were of analytical grades.

#### Ethanol extraction of plant material

The powdered sample (500 g) was dispersed in 2.0 l of ethanol (70 % v/v) using the ratio of 1:4 (w/v). The mixture was left standing with periodic shaking to ensure complete extraction within 72 h. The extract was filtered with gauze and three times with pure cotton. The filtrate was concentrated at 60-80 °C using rotary evaporator. The concentrate was further freeze-dried to obtain a solid product which was stored at 4 °C for further use.

#### Phytochemical screening of extracts

The crude ethanol stem bark extract of *Berlinia grandiflora* (ESBG) was qualitatively screened for the presence of various phytochemical constituents by standard protocols [13-15].

#### Experimental animals

Healthy albino rats (Wistar Strain) of both sexes, 12 w old and weighing between 150-223 g body weights (b.w.) were obtained from the animal breeding unit of the animal house, Centre for Plant Medicine Research (CPMR), Mampong-Akuapem, Ghana. They were then brought to the experimental room and allowed to acclimatise for 7 d in their new environment before the start of the experiment. They were maintained under normal ambient conditions of temperature, relative humidity and a 12h/12h day/night cycle [16]. The rats were housed in sanitised metal cages (45×35×18) cm with a base dressing of sawdust as bedding and had free access to standard pellet diet (obtained from GAFCO, Ghana) and sterilised water *ad libitum*. All the experimental procedures were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (Permit number: IEC/STU/0262017).

#### Acute toxicity study

An acute oral toxicity study was carried out according to the procedure described by [2] with some modifications. A total of 6 Wistar albino rats were randomly selected after the acclimatisation period and divided into three groups comprising two animals in each group. The crude ethanol stem bark extract of *Berlinia grandiflora* (ESBG) was administered to the different groups in doses of 500, 1000 and 3000 mg/kg b.w. respectively. The animals were observed for physiological changes due to toxicity such as weakness, feed withdrawal, dizziness, vomiting, and mortality for 72 h. However, no significant physiological changes were observed among the three different treatment groups indicating the rats could be administered with the extracts up to the highest dose of 3000 mg/kg b.w.

#### Hepatoprotective assay

The *in vivo* effect of ESBG was determined using the CCl<sub>4</sub>-induced hepatotoxicity test in rats. A total of 36 Wistar albino rats were randomly selected and divided into 6 groups (n = 6 in each group). The treatment period was for 7 d.

**Group I:** Served as the normal control and received only normal saline (1 ml/kg b.w. daily, orally) for 7 consecutive days.

**Group II:** Received only CCl<sub>4</sub> (2 ml/kg b.w.) diluted with olive oil (1:1, intravenously) for 7 consecutive days.

**Group III:** Received CCl<sub>4</sub> (2 ml/kg b.w.) diluted with olive oil (1:1, intravenously), then the standard drug, Silymarin (100 mg/kg b.w., orally) 3 h after the administration of the CCl<sub>4</sub> daily for 7 d.

**Group IV:** Received CCl<sub>4</sub> (2 ml/kg b.w.) diluted with olive oil (1:1, intravenously), then ESBG (100 mg/kg b.w., orally) 3 h after the administration of the CCl<sub>4</sub> daily for 7 d.

**Group V:** Received CCl<sub>4</sub> (2 ml/kg b.w.) diluted with olive oil (1:1, intravenously), then ESBG (300 mg/kg b.w., orally) 3 h after the administration of the CCl<sub>4</sub> daily for 7 d.

**Group VI:** Received CCl<sub>4</sub> (2 ml/kg b.w.) diluted with olive oil (1:1, intravenously), then ESBG (900 mg/kg b.w., orally) 3 h after the administration of the CCl<sub>4</sub> daily for 7 d.

24 h after the treatment period, the test animals were placed in a wooden box containing a lighted fluorescent bulb to warm them for about 10 m to ensure active blood circulation in the body before blood samples were obtained. The blood samples of the animals were obtained by the tail-cutting method and collected into chemistry gel tubes for serum biochemical analysis.

#### Determination of serum biochemical parameters

This was determined using the procedure described by [2] with some modifications. Blood collected in the chemistry gel tubes were allowed to stand for 30 m at room temperature and then centrifuged at 3000 rpm for 10 m to obtain the sera. The effect of ESBG on CCl<sub>4</sub>-induced liver damage was assessed by measuring the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB) and Total bilirubin (TB) using the semi-automated Biochemistry analyser (MicroLab-300, *Vital Scientific*) according to the manufacturer's instructions.

#### Statistical analysis

The results for serum biochemical parameters were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by the Tukey Multiple Comparison test using GraphPad Prism 5 (GraphPad Software Inc., USA). p<0.05 was considered statistically significant.

## RESULTS

#### Phytochemical screening

Qualitative phytochemical analysis of ESBG showed the presence of flavonoids, steroids, alkaloids, anthocyanins, tannins, coumarins, glycosides, saponins and terpenoids; carbohydrates were not observed (table 1).

**Table 1: Phytochemical screening of ethanol stem bark extract of *B. grandiflora* (ESBG)**

Phytochemical	Ethanol stem bark extract of <i>B. grandiflora</i>
Flavonoids	+++
Steroids	+
Alkaloids	+++
Anthocyanins	++
Tannins	++
Coumarins	++
Glycosides	+
Saponins	++
Carbohydrates	-
Terpenoids	+++

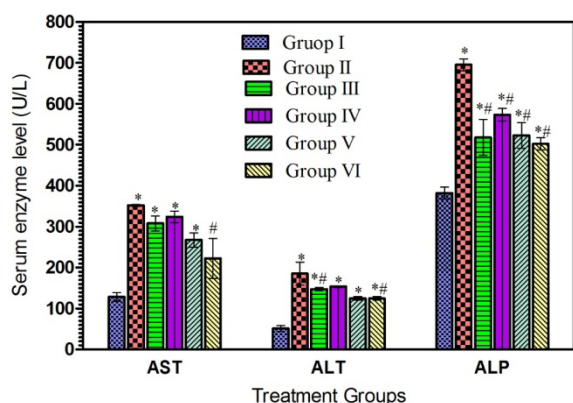
Key: (+++) Abundantly present, (++) Moderately present, (+) Less present, (-) Absent

### Effect of ESGB on serum biochemical parameters

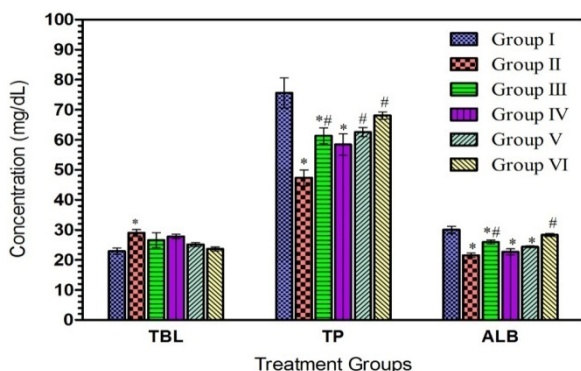
In this study, there were significant elevations ( $p < 0.05$ ) of AST, ALT and ALP in rats induced with 2 ml/kg b.w.  $\text{CCl}_4$  alone (Group II) compared to the normal control group (Group I) (fig. 1). However, it was observed that the levels of these biochemical markers decreased in groups treated with the standard drug 100 mg/kg b.w. Silymarin (Group III) and ESGB (Groups IV to VI) compared to Group II, with Group VI (900 mg/kg b.w. ESGB) showing a significant reduction ( $p < 0.05$ ). The same observation was made for TBL (fig. 2).

On the other hand, there was a significant decrease ( $p < 0.05$ ) in TP and ALB in rats intoxicated with 2 ml/kg b.w.  $\text{CCl}_4$  (Group II) compared to the normal control group (Group I) (fig. 2). Rats administered with the 100 mg/kg b.w. Silymarin (Group III) and ESGB (Groups IV to VI) were observed to have increased levels of TP and ALB compared to Group II animals, with Group III and Group VI (treated with 900 mg/kg b.w. ESGB) showing a significant increment ( $p < 0.05$ ).

It was observed that, generally, there was no significant difference ( $p > 0.05$ ) in all the serum biochemical makers of the group treated with the standard drug Silymarin compared with those treated with ESGB.



**Fig. 1:** Effect of ESGB on serum AST, ALT and ALP levels, Group I = Normal control (1 ml/kg b.w. n-saline); Group II =  $\text{CCl}_4$  (2 ml/kg b.w.); Group III =  $\text{CCl}_4$ +100 mg/kg b.w. Silymarin; Group IV =  $\text{CCl}_4$ +100 mg/kg b. w ESGB; Group V =  $\text{CCl}_4$ +300 mg/kg b.w. ESGB; Group VI =  $\text{CCl}_4$ +900 mg/kg b.w. ESGB; \* $p < 0.05$  = significantly different compared to Group I (normal control); # $p < 0.05$  = significantly different compared to Group II; data presented are the means $\pm$ SEM; n = 6



**Fig. 2:** Effect of ESGB on serum TBL, TP and ALB levels, Group I = Normal control (1 ml/kg b.w. n-saline); Group II =  $\text{CCl}_4$  (2 ml/kg b.w.); Group III =  $\text{CCl}_4$ +100 mg/kg b.w. Silymarin; Group IV =  $\text{CCl}_4$ +100 mg/kg b. w ESGB; Group V =  $\text{CCl}_4$ +300 mg/kg b.w. ESGB; Group VI =  $\text{CCl}_4$ +900 mg/kg b.w. ESGB; \* $p < 0.05$  = significantly different compared to Group I (normal control); # $p < 0.05$  = significantly different compared to Group II; data presented are the means $\pm$ SEM; n = 6

### DISCUSSION

Phytochemical analysis of the ESGB indicates the presence of flavonoids, alkaloids, tannins, saponins, and glycosides. These phytoprinciples have previously been reported to be present in different extracts and parts of the plant [8, 10-12]. Terpenoids, anthocyanins, steroids, and coumarins were the other phytochemicals tested positive in the present work, while carbohydrates tested negative (table 1). The presence of these phytochemicals may account for the traditional use and pharmacological properties exhibited by the stem bark of the plant.

$\text{CCl}_4$  is one of the common chemical agents used to induce acute hepatotoxicity in animal models. It is metabolized by cytochrome  $\text{P}_{450}$  enzymes in the liver to form the highly reactive trichloromethyl free radical ( $\text{CCl}_3\bullet$ ) [17]. The  $\text{CCl}_3\bullet$  can covalently bind to macromolecules to initiate inhibition of lipoprotein secretion, resulting in adduct formation and ultimately cancer initiation. The  $\text{CCl}_3\bullet$  may also be bioactivated by the cytochrome  $\text{P}_{450}$  in the presence of oxygen to produce trichloromethyl peroxy radicals ( $\text{CCl}_3\text{-O}\bullet$ ), which initiate a chain reaction of lipid peroxidation leading to loss of calcium homeostasis and, ultimately, apoptosis and cell death. Both pathways lead to membrane damage and consequently resulting to the leakage of membrane enzymes in the liver [18-20].

In this study, administration of 2 ml/kg b.w.  $\text{CCl}_4$  alone to the rats in Group II for 7 d resulted in a significant elevation ( $p < 0.05$ ) in the hepatic enzymes AST, ALT and ALP in the serum compared to the normal control group (Group I, treated with 1 ml/kg b.w. n-saline) (fig. 1). The same observation was made for TBL (fig. 2). Increased levels of AST, ALT, ALP, and TBL are usually used as indicators of hepatic damage. Administration of Silymarin and ESGB at doses 100, 300, and 900 mg/kg b.w. reduced the levels of these biochemical parameters compared to the group intoxicated with the 2 ml/kg b.w.  $\text{CCl}_4$  alone. The effect of 900 mg/kg b.w. ESGB on these parameters was significant ( $p < 0.05$ ) compared to the 2 ml/kg b.w.  $\text{CCl}_4$  intoxicated group. This suggests that the extract reversed the hepatic damage induced by the treatment of  $\text{CCl}_4$ .

There was a significant decrease ( $p < 0.05$ ) in TP and ALB in the serum in the 2 ml/kg b.w.  $\text{CCl}_4$  intoxicated group compared with the normal control group. Administering Silymarin and ESGB at doses 100, 300 and 900 mg/kg b.w. increased these parameters compared with the  $\text{CCl}_4$  intoxicated group, with Silymarin and 900 mg/kg b.w. ESGB being significant ( $p < 0.05$ ) (fig. 2) suggesting a reversal of hepatic injury.

Elevation of serum marker enzymes such as AST, ALT, ALP and TBL and reduction in TP and ALB are the commonest indicators of hepatocellular damage [18, 21]. Both ALT and AST are considered as hepatic-specific marker enzymes due to their high concentrations in the hepatocytes. However, ALT is specifically located in the cytosol of the hepatocytes. Therefore, it is a sensitive marker for liver injury. AST, on the other hand, is found in the cytoplasm and mitochondria of different cells such as kidney cells, hepatocytes, skeletal muscle cells, cardiac cells, and erythrocytes [22]. The liver injury induced by the  $\text{CCl}_4$  which resulted in the increased activity of AST, ALP, and ALT were reversed by the ESGB. This could be attributed to the ability of the ESGB to scavenge free radicals produced by the  $\text{CCl}_4$  in the liver, thus preventing leakage of intracellular enzymes into the serum.

Serum bilirubin is a remarkable test to ascertain the functional integrity of the liver [23]. Bilirubin is the protein with the highest concentration in plasma, accumulating from the breakup of haemoglobin present in erythrocytes. The liver removes it from the blood and excretes it through the bile. Excess concentration of serum bilirubin may be ascribed to increased haemoglobin breakdown due to haemolysis or failure of the liver to clear it. Jaundice is detected in individuals with liver malfunctions as a result of the accumulation of bilirubin in the blood [24, 25]. The present study showed a reversal of the TBL levels in rats treated with ESGB compared to those intoxicated with  $\text{CCl}_4$ . This could be explained that the liver was able to restore its bilirubin clearance functionality after the ESGB treatment, which might be due to the presence of

active compounds in the extract. The reduction was, however not significant ( $p > 0.05$ ) for both the standard drug Silymarin and all the doses of ESBG.

It was observed that the levels of serum TB and ALB significantly reduced ( $p < 0.05$ ) in the group intoxicated with  $\text{CCl}_4$  alone compared with the normal control group. A healthy functioning liver is required for the synthesis of the serum proteins [26]. Therefore, the significant decrease in TP and ALB observed in the group treated with  $\text{CCl}_4$  alone suggests hepatotoxicity. This could be attributed to the disruption and disassociation of polyribosomes from the endoplasmic reticulum following  $\text{CCl}_4$  administration [2]. Serum TP and ALB levels increased following the administration of ESBG. The increase was significant ( $p < 0.05$ ) for the 900 mg/kg b.w. ESBG. The increment suggests the hepatoprotective effect of ESBG and may be due to stimulation of protein synthesis by stabilization of endoplasmic reticulum causing the acceleration of the regeneration process of liver cells [2].

The Silymarin, which was used as the standard drug, is the collective name of three isomer flavonolignans containing 50–70% silybin as the most bioactive compound. Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation has anti-fibrotic activity and may act as a toxin blockade agent by inhibiting binding of toxins to the hepatocyte cell membrane receptors [27]. The comparable activities ( $p > 0.05$ ) of the Silymarin and the ESBG, especially the 900 mg/kg b.w. ESBG may be explained by the abundance of flavonoids in the extract observed in the phytochemical analysis (table 1).

Phytoprinciples such as flavonoids, terpenoids, steroids, alkaloids, and tannins have been reported to possess hepatoprotective activities. For example, flavonoids are reported to exhibit antioxidant activity, hence they prevent cell damage due to oxidative stress; and also show hepatoprotective activity [28] by reducing lipid peroxidation; reducing and scavenging free radical production; and chelating metal ions. In addition, flavonoids are mentioned to possess anti-inflammatory properties [29]. It is suggested that certain tannins may exert significant liver-protective effects by inhibition of collagen accumulation, oxidative stress, inflammation and apoptosis [30]. Saponins are suggested to protect the structural integrity of hepatocytic cell membrane and regeneration of damaged hepatocytes [31] through modulation of their antioxidant and anti-inflammatory activities. The combination of these phytochemicals in the ESBG may be responsible for the ameliorating effect of  $\text{CCl}_4$  induced liver damage in rats.

## CONCLUSION

The investigation of the effect of *Berlinia grandiflora* on  $\text{CCl}_4$  induced liver damage revealed that the ethanol extract of the stem bark of the plant was able to ameliorate the hepatotoxicity, with the 900 mg/kg b.w. dose very significant and generally showing comparably the same activity as Silymarin. The presence of phytochemicals such as flavonoids, steroids, alkaloids, anthocyanins, tannins, coumarins, glycosides, saponins and terpenoids could be responsible for the hepatoprotective activity and could explain the traditional use of the plant in treating liver problems.

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## AUTHORS CONTRIBUTIONS

MND contributed to the concept and design, study and analyses of data and drafting of the manuscript. SYA and SAD carried out plant extraction and *in vivo* biochemical analyses. All authors read and approved the final manuscript.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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