African Scientist Vol. 26, No. 2 June 30, 2025
Printed in Nigeria

1595-6881/2025 $80.00 + 0.00

https://africansciientistjournal.org

afs2025026/26204

Nutritional, Elemental Mineral Composition and Modulatory Activity of *Entandrophragma utili* on Some Liver Enzymes and Biomolecules in Carbon Tetrachloride-Intoxicated Wistar Rats

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(Received June 19, 2025; Accepted in revised form June 26, 2025)

ABSTRACT: This study investigated the nutritional, elemental mineral and modulatory activity of the crude stem back extract of *Entandrophragma utile* (EU) on the plasma levels of some liver enzymes and biomolecules in carbon tetrachloride-intoxicated albino Wistar rats. The EU extract was screened for its nutritional and elemental mineral properties. Modulatory activity of EU was tested using twenty-five male rats (120 – 150 g), which were randomized into five groups and fed rat chow and water *ad libitum* throughout the experiment. Group I (control) was administered distilled water only. Groups II – V were intoxicated with a single dose of carbon tetrachloride- CCl_4 (1.25 mL/kg bw in olive oil (1:1); intraperitoneally) to induce liver injury. While rats in Group II were not treated, Groups III – V were treated with silymarin (50 mg/kg bw), EU crude extract 200 mg/kg bw and 400 mg/kg bw, respectively, once daily, for eight days. Subsequently, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin and bilirubin were evaluated. All experiments were done using standard procedures. The liver architecture was also examined for histopathological changes. Nutritional analysis showed reasonable amounts of nitrogen free extract (NFE), crude fat, crude protein, moisture, crude ash and crude fibre. Also, the presence of P, Mg, Ca, Na, K, Se and Zn were detected. Meanwhile, there were significant increases ($p < 0.05$) in plasma hepatic enzyme (ALT, AST and ALP) activities and bilirubin concentration, with a reduction in total protein and albumin levels of intoxicated rats when compared to control group. Administration of EU and silymarin ameliorated the toxic assault from CCl_4. Similarly, EU significantly mitigated the aberrations caused by CCl_4 to the liver architecture. Findings from this study, suggest a rich nutritional status and a hepatoprotective activity of EU crude extract against CCl_4 -induced liver injury in rats.

Keywords: *Entandrophragma utile*, Nutritional status, Elemental minerals, Hepatotoxicity, Carbon tetrachloride

Introduction

The application of herbs, otherwise known as medicinal plants, in folklore medicine in places like Africa, Asia, Europe, etc., with rich plant flora predates modern history. These plants have served the purpose of cure and management of several ailments due to the biological activities of their phytoconstituents (Yuan *et al*., 2016). A notable example of these medicinal plants is a member of the Meliaceae family, *Entandrophragma utili*, which is endemic in tropical Africa. The tree can reach heights of 60 m and a trunk diameter of 2 m. The leaves, which possess acuminate tip, are pinnate, with 5–9 pairs of leaflets, each being 8–10 cm long (Orwa *et al*., 2009). The fruit, which has numerous winged seeds, is described as a five-valve capsule (Happi *et al*., 2018). Other species of the genus include *E. angolense, E. bussei, E. caudatum, E. candollei, E. congolense, E. cylindricum, E. delevoyi, E. palustre, E. spicatum,* etc (Happi *et al*., 2018). Some of its local names are Ìjebò (Yoruba), Okeong (Igbo), while its common names are Dawe and Sprague Sprague, African cedar, etc. (Aigbokhan, 2014). *Entandrophragma* spp. are extensively used for the treatment of several diseases including malaria, rheumatism, joint and abdominal pains, wounds, dysentery, gonorrhoea, syphilis, trypanosomiasis, cancer, gastrointestinal ulcer, sickle cell disease, e.t.c. (Bickii *et al*., 2006; Kouam *et al*., 2012; Happi *et al*., 2015).

Despite the potentials of these medicinal plants, diseases continue to ravage significant percentage of the population of third world countries, where there exists broken infrastructure (including healthcare), low purchasing power, poor sanitation, etc. (WHO, 2023; NBS, 2025). For instance, liver disease has remained a huge concern in the global health circle. This disease caused by the impact of deleterious substances such as infections and toxins from lifestyle activities and environmental factors, has taken its toll on individuals cutting across continents of the world (Wu *et al*., 2019). In the US alone, over 4.5 million adults suffer from liver disease, while about 56,000 people die from the disease annually (CDC, 2024). In Nigeria, liver diseases accounts for 38,326 mortality per annum, thus making it the 6th leading cause of death in the country (WHO, 2020). This increase in disease burden has reinforced the need for the exploitation of therapeutic alternatives such as herbal remedies, believed to hold great potentials for new drug targets. Therefore, this study examined the nutritional, elemental mineral composition, and modulatory activity of the crude stem bark extract of *Entandrophragma utili* on some liver enzymes and biomolecules in carbon tetrachloride-intoxicated albino Wistar rats.

Materials and methods

Chemicals and reagents: All kits were products of Agape Diagnostics (Cham, Switzerland), ethanol, carbon tetrachloride (Sigma Aldrich, England), silymarin (Silver Health Diagnostics, Nigeria). The chemicals and reagents were of analytical grade, and procured from accredited suppliers.

Plant collection and preparation: Stem bark of *E. utili* was collected from the forest area of Ijebu-ode, Ogun State, Southwest Nigeria. Identification of the plant material was done at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. The sample was cleaned of debris, air-dried under shade, and pulverized using mortar and pestle. Thereafter, the pulverized sample was stored in moisture-free, air tight container until ready for use.

Extraction: Weighed portion (500 g) of the pulverized plant material was macerated in ethanol (1.2 L) for 3 days under repeated stirring. It was filtered with Whatman filter paper No. 1 (Whatman, England) and dried *in vacuo* at 45 °C using a rotary evaporator (Buchi R-200, Germany) to obtain the crude extract.

Determination of nutritional content: Proximate analysis to determine the nutritional composition of the plant sample was done according to the methods of AOAC (2000). The following components were estimated; moisture content, crude protein, crude ash, ether extract (crude fat), crude fibre and nitrogen free extract (NFE).

*Elemental mineral analysis:*Elemental minerals of interest in the sample were analyzed by first dry ashing the sample, followed by dissolution of the ash by a mixture of acids and absorbance read against blank using Atomic Absorption Spectrometer according to the methods of Hseu (2004). Sodium, calcium, magnesium, potassium, selenium, zinc and phosphorus, were determined.

Experimental animals: Twenty-five male albino Wistar rats weighing 120 – 150 g were used for this study. They were acquired from the Animal House of Igbinedion University, Okada, Edo State and kept under a 12 h light-dark cycle in wooden cages. The rats were acclimatized for 7 days and fed rat chow and water *ad libitum* for the period of the experiment.

Experimental design: The rats were randomized into five groups of five (5) rats each. Intoxication was done by administering a single dose of CCl_4 (1.25 mL/kg in olive oil 1:1) through the intraperitoneal route, while treatments were administered orally, once daily for 8 days (24 h post-intoxication), as described below;

- Group I (baseline control) was given distilled water and olive oil only.
- Group II (negative control) was administered CCl_4, but not treated.
- Group III (reference drug control) was administered CCl_4 and treated with sylimarin (50 mg/kg bw).
- Group IV was administered CCl_4 and treated with EU extract (200 mg/kg bw).
- Group V was administered CCl_4 and treated with EU extract (400 mg/kg bw).

Sample collection: At the end of the treatment period, blood samples were collected from overnight fasted rats under anesthesia, through cervical decapitation, for biochemical assays such as; alanine transaminase (ALT), aspartate transaminase (AST) (Reitman and Frankel, 1957); alkaline phosphatase (ALP) (Kochmar and Moss, 1976); total protein (TP) (Tietz, 1995), albumin (ALB) and bilirubin (Jendrassik and Grof, 1938). A portion of the liver was preserved in 10% formalin for histology. The sections were processed and stained with hematoxylin and eosin (Drug and Wallington, 1980) and examined under a microscope for histopathological changes.

Data analysis: GraphPad Prism 10 was used for statistical analysis and data presented as mean ± SEM. One-way analysis of variance (ANOVA) was used to compare differences in means, while differences in significance was assessed by Turkey's multiple range tests. Significance was set at $p < 0.05$.

Results

Nutrient composition

The percentage nutrient composition of *E. utili* stem bark is presented in Figure 1. Proximate analysis revealed a high presence of nitrogen free extract (NFE) and relatively low composition of crude fibre, crude ether extract and crude ash. However, crude fibre, crude protein, and moisture were sufficiently present.

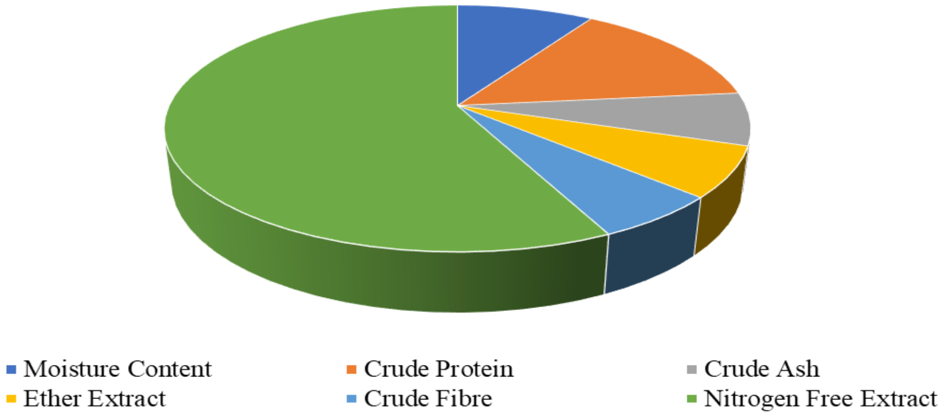

Figure 1: Nutritional Content of *E. utili* Stem bark. Data represent percentage mean ± SEM (n = 3).

Elemental mineral composition: Figure 2 shows the elemental mineral content of *Entandrophragma utili* stem bark. From the result, all the elements tested were relatively present in the plant sample. Phosphorus, potassium, magnesium, calcium and sodium were more in quantity compared to selenium and zinc which were also present though in reduced quantity.

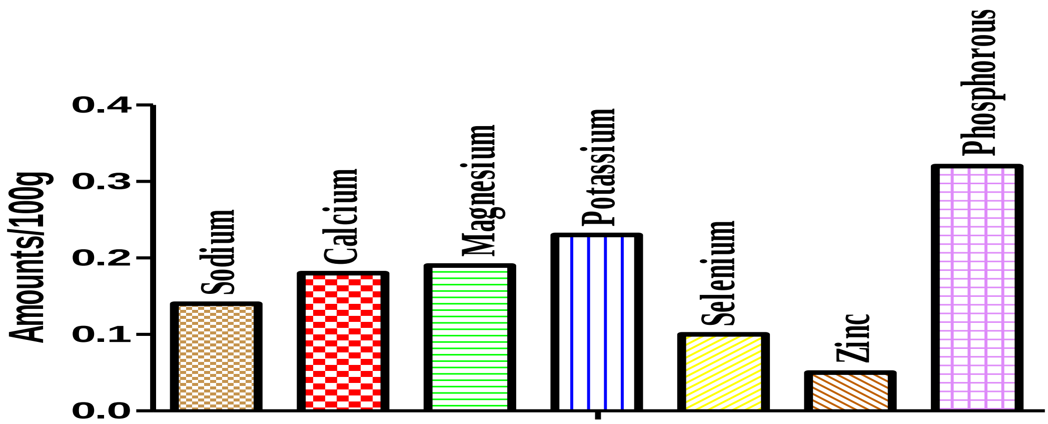

Figure 2: Elemental Mineral Content of *E. utili* Stem bark. Data represent percentage amounts/100 g, (n = 3).

Effect of crude stem bark extract of E. utili on some plasma hepatic enzymes in CCl_4- induced liver damage in Wistar rats: The effect of the crude stem bark extract of EU on plasma ALT, AST and ALP activities in CCl_4-intoxicated rats is shown in Figure 3. Administration of CCl_4 significantly ($p < 0.05$) increased the activities of ALT and AST by 1.31 and 1.28-folds, while ALP was non-significantly ($p > 0.05$) increased by 1.06-fold when compared with normal control. Treatment post-intoxication with EU at doses of 200 and 400 mg/kg body weight for 7 days significantly ($p < 0.05$) reduced the activities of ALT and AST when compared with the CCl_4-only administered group. Silymarin at a dose of 50 mg/kg also significantly prevented the elevation of plasma ALT and AST.

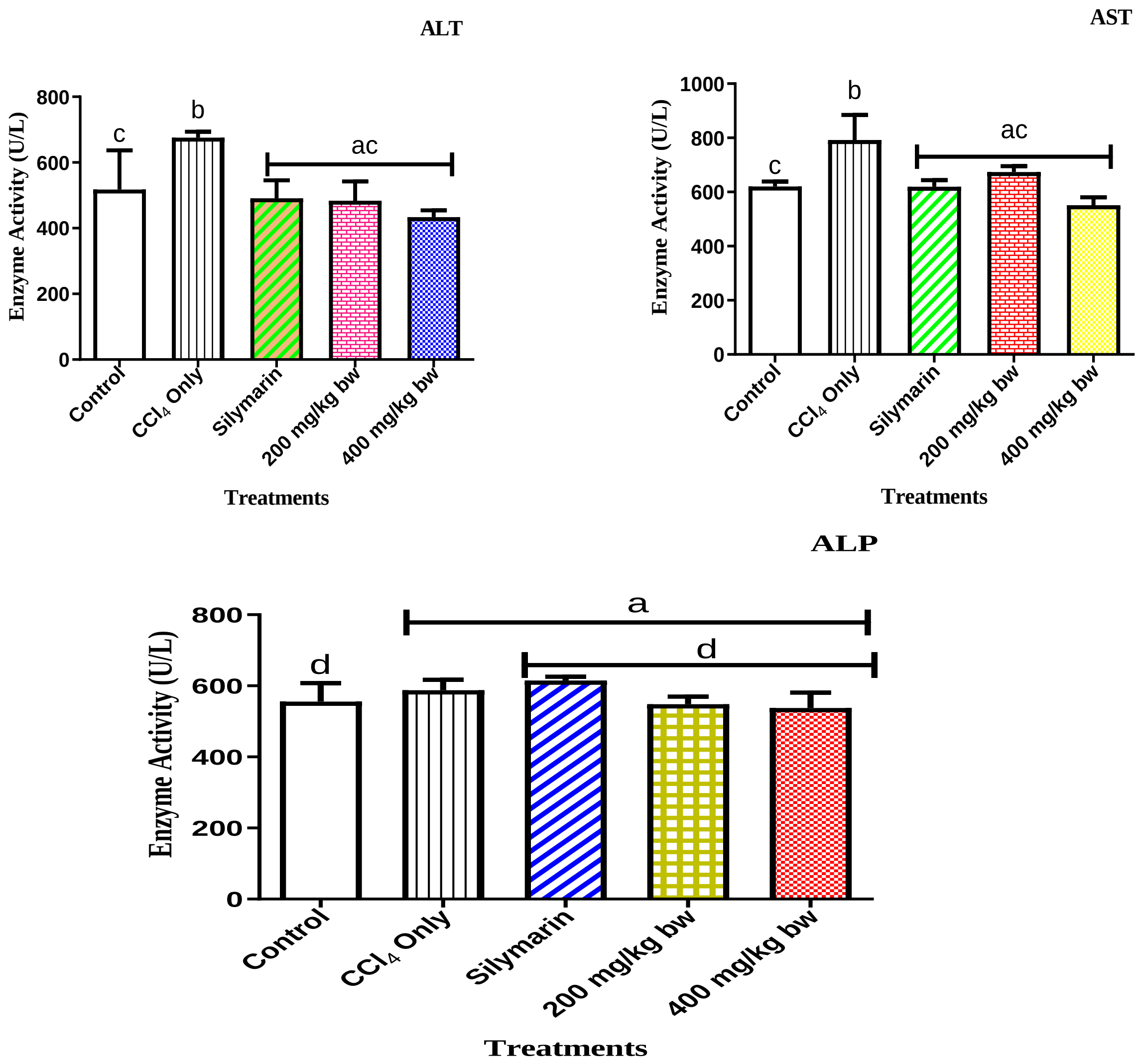

Figure 3: Effect of crude EU extract on ALT, AST and ALP Activities in CCl_4-induced liver damage in Wistar rats. Values represent the mean ± SEM, (n= 5). CCl_4: Carbon tetrachloride. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the CCl_4 group. d = non-significant compared to the CCl_4 group.

*Effect of crude stem bark extract of E. utili on plasma total protein, albumin and bilirubin concentrations in CCl_4-induced liver damage in Wistar rats:*The effect of crude EU extract on total protein, albumin, and total, direct and indirect bilirubin concentrations in CCl_4-induced liver damage in Wistar rats is presented in Figure 4. The result indicated a significant ($p < 0.05$) decrease in total protein, and a significant ($p < 0.05$) increase in total, direct and indirect bilirubin concentrations in rats induced with CCl_4-only when compared to the control rats. However, treatment with EU extract showed a significant ($p < 0.05$) increase in total protein and a non-significant increase in albumin levels, while bilirubin concentrations (total, direct and indirect) decreased significantly ($p < 0.05$) in the group administered 400 mg/kg bw compared to the CCl_4 only administered rats. Silymarin significantly ameliorated the distortions caused by CCl_4 and restored the values of total protein, albumin, and bilirubin to levels comparable to control.

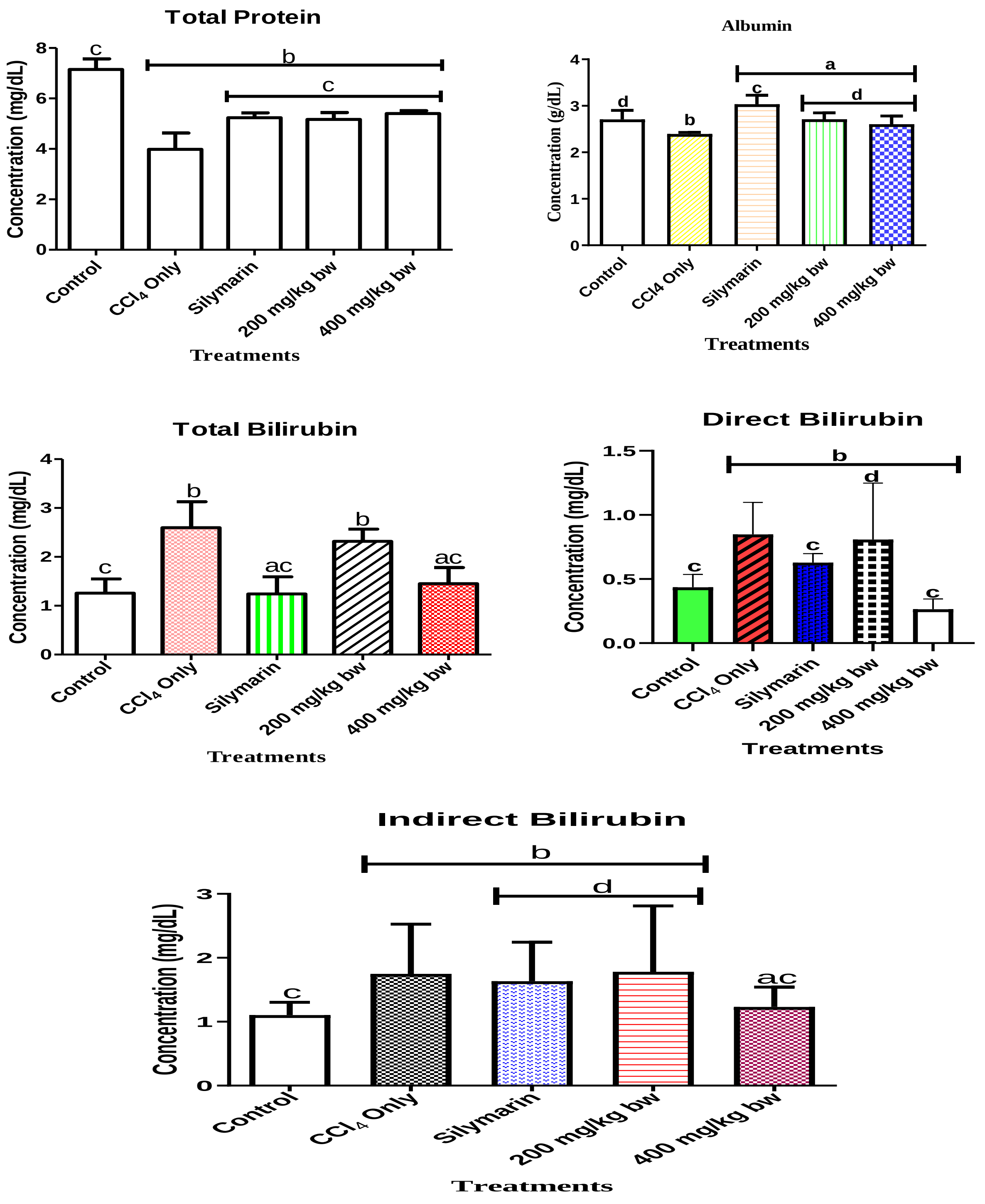

Figure 4: Effect of Crude EU Extract on Total Protein, Total Bilirubin, Direct Bilirubin and Indirect Bilirubin Concentrations in Carbon Tetrachloride-Intoxicated Wistar Rats. Values represent the mean ± SEM, (n= 5). CCl_4: Carbon tetrachloride. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the CCl_4 group., d = non-significant compared to the CCl_4 group.

Effect of crude stem bark extract of E. utili on liver architecture of carbon tetrachloride-intoxicated Wistar rats: Histopathological examinations of liver sections from the control group showed normal cellular architecture with normal hepatocytes, sinusoidal spaces, portal vein, bile duct and hepatic artery (Plate 1). However, rats administered CCl_4 only showed zonal necrosis, activation of Kupffer cells, heavy periportal infiltrates of inflammatory cells and vascular congestion. But treatment with silymarin and EU extract at a dose of 50, 200 and 400 mg/kg, respectively, reduced the architectural distortion, decreased fatty acid degeneration and zonal necrosis. Also, it was observed that EU at a dose of 400 mg/kg showed normal hepatocytes, portal vein, vasodilatation and congestion and Kupffer cells mobilization.

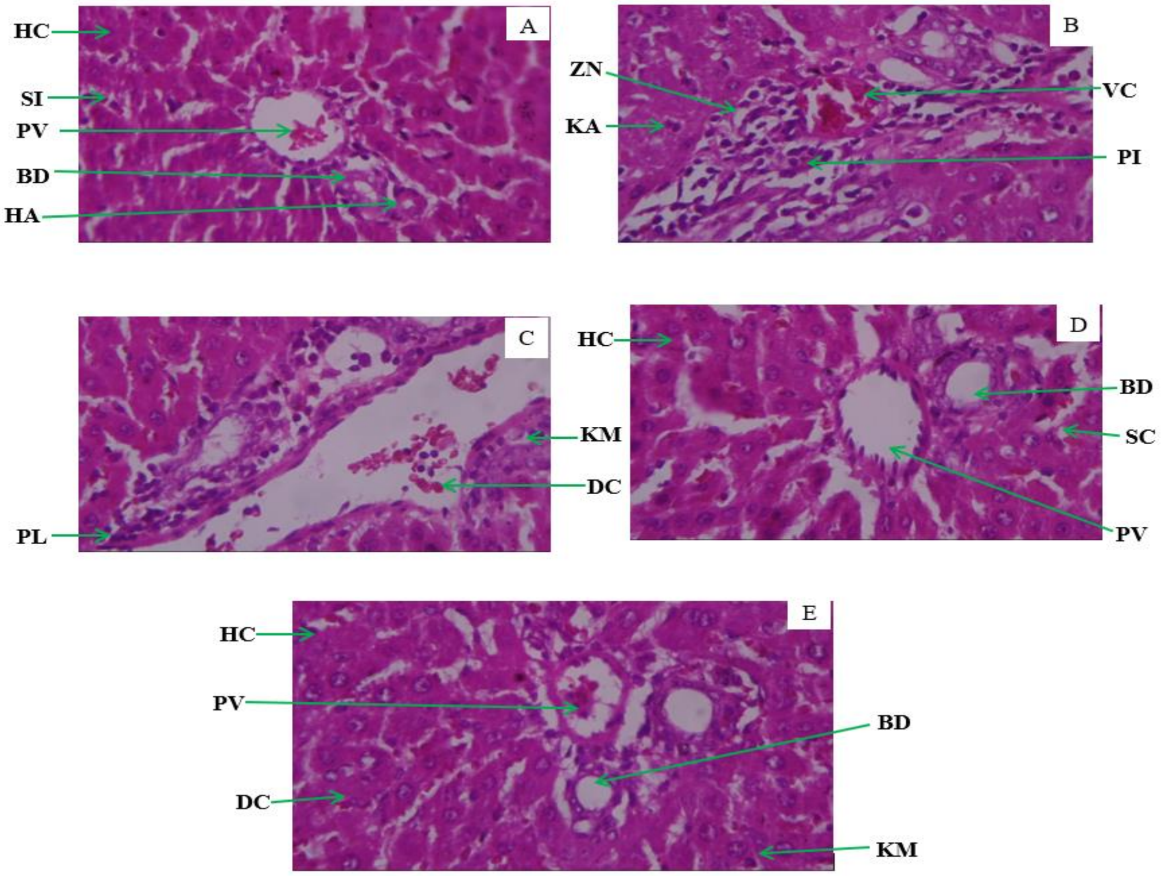

Plate 1: Photomicrographs of Liver Sections. **(A)** Control rats administered water only showing HC: hepatocyte, SI: sinusoid, PV: portal vein, BD: bile duct, HA: hepatic artery **(B)** Rats administered CCl_4 only showing ZN: zonal necrosis, KA: Kupffer cell activation, PI: heavy periportal infiltrates of inflammatory cells VC: vascular congestion **(C)** Rats administered CCl_4 + Silymarin (50 mg/kg bw) showing PL: periportal mobilization of lymphocytes cells, DC: vasodilatation and congestion, KM: Kupffer cell mobilization **(D)** Rats administered CCl_4 + EU (200 mg/kg bw) showing HC: hepatocyte, BD: bile duct, SC: active sinusoidal congestion, PV: portal vein **(E)** Rats administered CCl_4 + EU (400 mg/kg bw) showing HC: hepatocyte, PV: portal vein, DC: vasodilatation and congestion, BD: bile duct, KM: Kupffer cell mobilization (H&E x 400).

Discussion

As the hub of detoxification, metabolism and synthesis of vital compounds, the liver stands as a prime target for injury inflicted possibly by an array of xenobiotics, spanning pharmaceuticals, environmental pollutants and industrial compounds (Grimes *et al*., 2023; Zhang *et al*., 2023). Studying the complex processes involved in liver toxicity is crucial for developing effective strategies to prevent and treat these ensuing abnormalities. The complementary role of medicinal plants in ensuring wellness in a largely impoverished sub-Saharan population is reassuring, considering Africa's vast flora. Plants like *Entandrophragma utili* have been exploited for their medicinal gains amongst others uses and, in this study, we deepen the scope by evaluating its nutritional, elemental mineral and modulatory potentials on some liver enzymes and biomolecules in CCl_4-intoxicated albino Wistar rats.

One of the crucial qualities of a good herb is its ability to provide essential food nutrients to support the process of healing and recovery. *Entandrophragma utili* was found to contain rich food components capable of boosting the nutrient supply of the body. The results shows that the plant is rich in carbohydrate, protein and fat. It also possesses considerable amounts of moisture, ash and fibre necessary to sustain good health. Also, a cursory look at its elemental mineral profile suggests that *E. utili* contains vital mineral elements that supports various metabolic functions including enzyme activities. For instance, phosphorus and magnesium (needed for the formation and maintenance of hard tissues such as bones and teeth), calcium and sodium (implicated in several metabolic reaction including playing roles as second messengers in signal transduction), selenium and zinc (required by some important enzymes as co-factors), were all detected in reasonable amounts in the stem bark of *E. utili*. These properties could support the used of *E. utili* in trado-medical practices.

Meanwhile, besides profiling the nutritional and elemental mineral status of *E. utili*, its modulatory properties in carbon tetrachloride-induced liver injury was also determined. Carbon tetrachloride- CCl_4 is a toxicant renowned for its ability to induce liver injury and perturb liver markers (Lee *et al*., 2014; Oriakhi and Orumwensodia, 2021). This it does through its radical species (trichloromethyl radicals-$^{-}CCl_3$ and trichloromethyl peroxyl radicals-$^{-}OOCCl_3$), both being products of enzymatic catalysis by cytochrome p450 oxidase in the liver. These radicals abstract electrons from fatty acids and intrinsic proteins of cell membranes, thereby leading to lipid peroxidation, fibrosis and necrosis (Chiu and Jin, 2019). An assault to the liver cells results in a loss of hepatic membrane integrity and the emptying of its content, hence, the elevation of enzymes such as ALT and AST, which are markers of liver injury (Uadia *et al*., 2015; Johra *et al*., 2023). In this study, an intoxication of rats with CCl_4 significantly ($p < 0.05$) increased ALT, AST and ALP activities. This rise in the activity of hepatic biomarkers could be attributed to CCl_4-induced oxidative stress owing to its established metabolic consequence (Chiu and Jin, 2019; Oriakhi and Orumwensodia, 2021). However, treatment of the rats with EU significantly decreased ALT, AST and ALP activities in a dose-dependent fashion and the results compared favorably with the control rats given distilled water and olive oil only.

To further confirm the hepatoprotective effect of EU stem bark extract against the negative impact of CCl_4, plasma levels of other liver parameters such as total protein, albumin, bilirubin (total, direct and indirect bilirubin) were determined. There were decreased total protein and albumin concentrations as well as increased bilirubin levels in the CCl_4-intoxicated group. This impaired level of vital liver markers signifies chronic liver challenge akin to cirrhosis, a late-stage hepatic fibrosis, which is associated with widespread distortions of normal hepatic function (Abdel-Moneim *et al*., 2015). Nonetheless, EU was able to restore the plasma levels of these biomolecules in a dose-dependent manner and comparable to the effect seen with silymarin. Similarly, CCl_4-intoxicated rats treated with silymarin at a dose of 50 mg/kg significantly ($p < 0.05$) reduced ALT, AST and ALP activities, as well as bilirubin concentrations. Just as it increased the levels of plasma protein and albumin comparably to the naïve rats. This activity can be ascribed to the plant's rich nutrients and phytochemical composition which helped in scavenging free radicals responsible for the liver damage. Histopathological results further support these findings as treatments with EU extract and silymarin significantly resolved aberrations like Kupffer cell activation, vascular congestion, heavy periportal infiltrates of inflammatory cells and zonal necrosis noticed in rats administered CCl_4.

Conclusion

Our findings show a rich nutrient and elemental mineral composition of *E. utile* (EU). Also, EU positively modulated plasma levels of some liver enzymes and biomolecules thus, suggesting a possible hepatoprotective activity. This activity was comparable to that of the reference drug, silymarin, thus affirming its potentials as a natural alternative for the management of liver injury. However, the evaluation of its long-term safety index and the investigation of the bioactive compound(s) responsible for its hepatoprotective activity may facilitate the development of standardized herbal preparations for therapeutic applications.

Authors' contributions

KOO conceptualized the study, optimized the protocols, carried out bench work, performed statistical analysis, wrote, reviewed, and edited the manuscript. AEF carried out bench work, performed statistical analysis, wrote, reviewed, and edited the manuscript.

Funding statement

This study was self-sponsored by the authors

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors appreciate the Natural Products Research and Diseases Control Laboratory (NPRDC), University of Benin, Benin City, for its facilities and Mr Daniel Ovilomarie for his technical assistance.

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