

Hepatoprotective Effects of The Methanol Extracts of The Leaves of *Napoleonae imperialis* Against Carbon TetraChloride (CCl₄)- Induced Hepatic Damage in Albino Rats

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ABSTRACT

This study was aimed at investigating the effect of methanol extract of Napoleonae imperialis leaves against carbon tetrachloride (CCl₄) induced hepatic damage in albino rats. Thirty (30) male albino rats of mean weight 130 g were used for this study. The animals for the study were grouped into five (5) of six (6) rats each. Group A received feed and water only and Group B was induced with CCl₄ without treatment. Test groups (C and D) were orally given 250 mg and 500 mg/kg b.wt of leaves extract, and group E was given orally the extract only (500 mg/kg b.wt) respectively for 14 days. All the rats were initially subjected to hepatocyte damage using 2.0 ml/kg of CCl₄ except the normal control group. The rats were sacrificed after 14 days and the blood samples were collected for biochemical analysis. The result obtained showed a significant decrease ($p < 0.05$) in (Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alanine phosphatase (ALP), albumin, total protein and total cholesterol) in the test groups treated with 250 mg and 500 mg/kg body weight of the plant extract, unlike the positive control where the liver parameters were still high which could be as a result of hepatocellular damage by CCl₄ without treatment. Also there was a significant ($p < 0.05$) decrease in comparison of the control groups and the group that received the extract only (500 mg/kg b.wt). The results of this study indicate that the methanol extract of Napoleonae imperialis may have exerted hepatoprotective effects in albino rats. Moving forward, this plant should be further studied for drug formulation, geared toward more effective management of organ toxicity and hepatoprotective drugs.

Keywords

Hepatic damage, Carbon tetrachloride, Aspartate amino transferase, Alanine amino transferase, Alanine phosphatase.

Introduction

In recent years, many researchers have examined the effects of bioactive components of plants origin, used traditionally by indigenous healers to support treatment of liver diseases. There are no effective drugs (that we are aware of) that are available in modern medicine that confer protection to the liver against damage or regenerate hepatic cells [1]. Due to the dearth of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders [2]. Scientific validations are being made globally to get evidences for traditionally reported herbal plants. Unfortunately, synthetic drugs used in the treatment of liver diseases are inadequate and

sometimes have side effects hence, many researchers turn to complementary and alternative medicine [1].

There is an increasing interest in the biochemical and mechanistic basis of action of protective effect of naturally occurring antioxidants in biological systems. Several plant constituents have shown anti-oxidative activity and have been empirically proven to be useful in the clinical treatment of liver disorders [3-6]. Higher plants produce a wide array of secondary metabolites that have therapeutic and pharmaceutical applications. Flavonoids and other phenolic compounds of plant origin have roles as scavengers and inhibitors of lipid peroxidation [7].

Carbon tetra chloride (CCl₄), whose catabolism produces radicals, is commonly used for induction of liver damage in rats. The radicals cause lipid peroxidation and necrosis of hepatocytes [8].

Consequently, this study was designed to monitor the effect of methanol extract of *Napoleonae imperialis* against CCl₄ hepatic damage in albino rats with a view to understanding, and establish scientifically its usage in traditional medicine as an ethanolic extract for hepatic disease management.

Materials and Methods

Analytical tools

Using the UV-Visible Spectrophotometer (Cole-Parmer UV-7504), the quantitative and qualitative of all samples were carried out. Biochemical parameters measurement were carried out using a Colorimeter (CO75 digital colorimeter, Binton Cambridge, UK), and all reagents used were of analytical grades. Samples were centrifuged using, Hettich universal centrifuge (Hettich Tuttlinger, Germany).

Assay Kits

Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine phosphatase (ALP), total protein, albumin, total cholesterol, were purchased from Randox, UK. All other chemicals were of analytical grade.

Plant material

Fresh leaves, of the plant *Napoleonae imperialis* were locally sourced in Abia State, Nigeria, and were identified at the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The fresh leaves of the plant collected were washed and dried under shade at room temperature and then blended to powdery using a blender.

Extraction

The powdered leaves of *Napoleonae imperialis* (100 g) were soaked in methanol for 48 hours, after, which the extract was filtered using a Whatman no. 1 filter paper, the filtrate, allowed to evaporate to dryness, under a water bath with a temperature set at 40°C.

Animals

Healthy male albino rats of mean weight of 130 g were used for the study. All animals were kept in the animal house under normal room conditions, acclimatized for two (2) weeks, and were used, following the approval of institutional animal ethical committee. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

Induction Of Hepatotoxicity And Nephrotoxicity

All the rats (except the normal control group) used for this study were initially subjected to hepatocyte damage using 2.0 ml of carbon tetrachloride (CCl₄) intraperitoneal (i.p) except the normal control group.

Experimental Design

Thirty (30) male albino rats of mean weight 130 g were used for this study. The animals for the study were grouped into five (5) groups of six (6) rats each. Group (A) and (B) were the control groups, group (C) and (D) were the test groups, and group E was the

group that receive the extract only. Group (A), the normal control group received feed and water only and Group (B), the positive control group, was induced with (CCl₄) without treatment. Groups C and D were orally given 250 mg and 500 mg/kg body weight of *Napoleonae imperialis* leaves extract respectively, whilst group E orally received the extract only (500 mg/kg b.wt).

The hepatocyte damage was established due to increase in the level of the liver parameters, which could be due to the leakage of these enzymes in the liver. Treatment lasted for 14 days and after, which the animals were sacrificed on day 15 under mild anesthesia (10% formosaline). Blood samples were collected in plain bottle for the analyses of the effects of the methanol extract on the liver biochemical parameters.

Evaluation of The Various Biochemical Parameters Studied

Assay of alanine amino transferase (ALT) activity

Serum ALT activity were estimated by the method [9].

Principle: This method is based on the production of pyruvate by the transamination activity of alanine amino transferase. Pyruvate reacts with 2, 4 dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazone that is measured colorimetrically at 546 nm

$$\text{a-oxoglutarate} + \text{L-alanine} \xrightleftharpoons{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

Reagent composition

- R1 is a reagent containing Phosphate buffer (100 mmol/L, pH7.4), L- Alanine (200 mmol/L) a-oxoglutarate (2 mmol/L).
- R2 is a reagent containing 2, 4dinitrophenyl hydrazine (2 mmol/L).

Procedure: In two separate test tubes, a volume, 0.1 ml of serum and water were mixed with 0.5 ml of R1 as test and blank, respectively. The solutions were mixed and incubated, respectively for 30 minutes at 37°C. Subsequently, 0.5ml of R2 was added to both test-tubes, incubated for another 20 minutes at 25°C, and followed by addition of 5ml of sodium hydroxide (NaOH) solution. The resultant solutions were mixed and the absorbance of test sample against reagent blank were read after 5 minutes at 546 nm.

Assay of serum aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined according to the method [9].

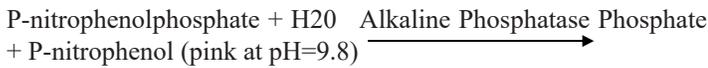
Principle: Oxaloacetate reacts with AST and is decarboxylated spontaneously to pyruvate. The pyruvate is measured by hydrazone formation after pyruvate reacts with 2,4 dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazone which can be measured at 546nm using spectrophotometer.

$$\text{a-oxoglutarate} + \text{L-Aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate}.$$

Determination of Alkaline Phosphatase (ALP)

Determination of alkaline phosphatase (ALP) was carried out according [9].

Principle: The principle of this method is based on the reaction of alkaline phosphate and a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns pink that can be determined spectrophotometrically.



Methods

The blank and sample test tubes were set up in duplicates and 0.05 ml of sample was pipetted into the sample test tubes. 0.05 ml of distilled water was pipetted into the blank tube. 3.0 ml of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405 nm. The stopwatch was started and the absorbance of the sample and the blank read again three more times at one-minute intervals.

Calculation: alkaline phosphatase activity was calculated as follows:

$$\text{Activity of ALP (in U/L)} = \frac{\text{Absorbance of Sample} \times 3300}{\text{Absorbance of Standard}}$$

Determination of total cholesterol

Total cholesterol was determined according [10]. A portion of the sample (10 µl) was mixed with 1000 µl of reagent (4-aminoantipyrine, phenol, peroxidase, Cholesterol esterase, Cholesterol oxidase, Pipes buffer) and incubated for 10 mins at 25°C.

Absorbance of the sample was read against reagent blank within 60 mins at 546 nm using the spectrophotometer.

Calculation

$$\text{Conc. of cholesterol in sample} = \frac{\Delta A \text{ sample} \times \text{conc. of standard}}{\Delta A \text{ standard}}$$

Determination of albumin

Albumin was determined according [11]. A portion of the sample (0.01 ml) and 3.00 ml of sodium hydroxide was put into a test tube, mixed and incubated at 25°C for 30 mins. The absorbance of the sample and that of the standard against the reagent blank was determined using a spectrophotometer at 630 nm.

$$\text{Albumin concentration (g/dl)} = \frac{A_{\text{sample}} \times \text{concentration of standard}}{A_{\text{standard}}}$$

Statistical Analysis

The data were expressed as mean ± standard deviation and analysed using statistical package for the social sciences (SPSS 22.0). Comparison was made between the test groups and the control groups using One way Anova and $p \leq 0.05$ was considered statistically significant.

Results and Interpretation

This study was designed to monitor the effects of methanol extract

of *Napoleonae imperialis* against carbon tetrachloride induced hepatic liver damage using albino rats as the animal model. The Figure 1 shows the results of the effects of methanol extract of *Napoleonae imperialis* by comparing the mean of AST between the control groups and the test groups.

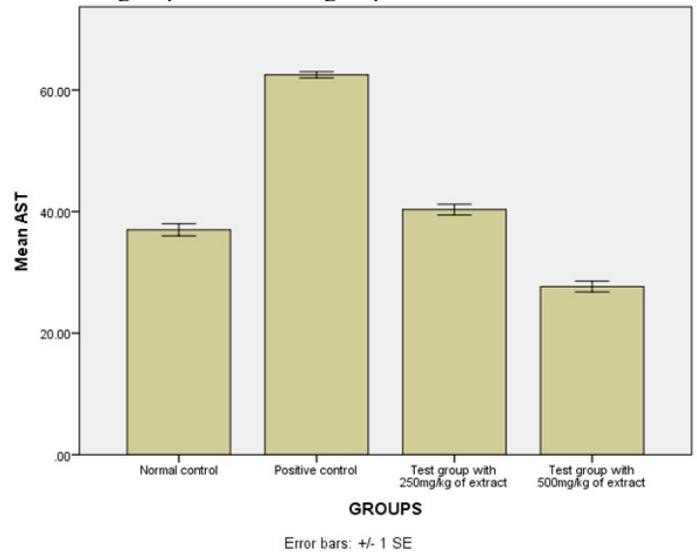


Figure 1: Mean values Comparison of AST between the Control groups and the Test groups.

In Figure 1, there is a significant decrease ($p < 0.05$) between the control groups (normal and positive control) and the test groups that received 250 mg/kg b.wt and 500 mg/kg b.wt of the plant extract, showing that the leaves extract had an ameliorating effects on the rats.

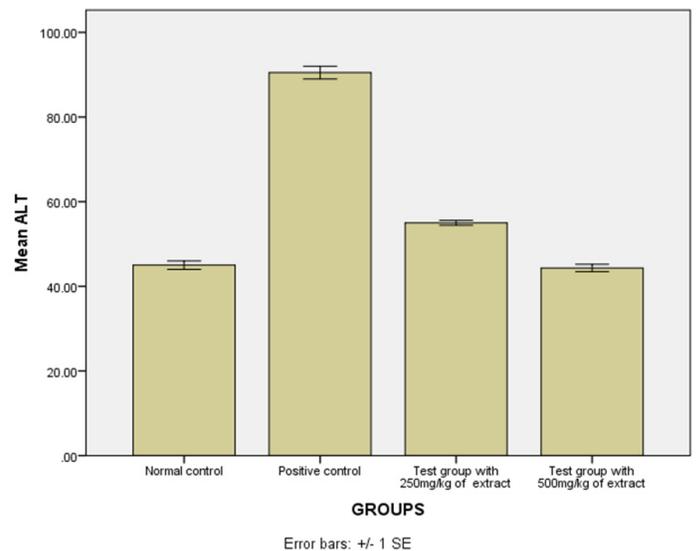


Figure 2: Mean values Comparison of ALT between the Control groups and the Test groups.

Also, the Figure 2 is the results showing the effects of the extract on ALT. The Fig indicated a significant decrease ($p < 0.05$) between the control groups (normal and positive control) and the test group that orally received 250 mg/kg body weight of the leaves extract.

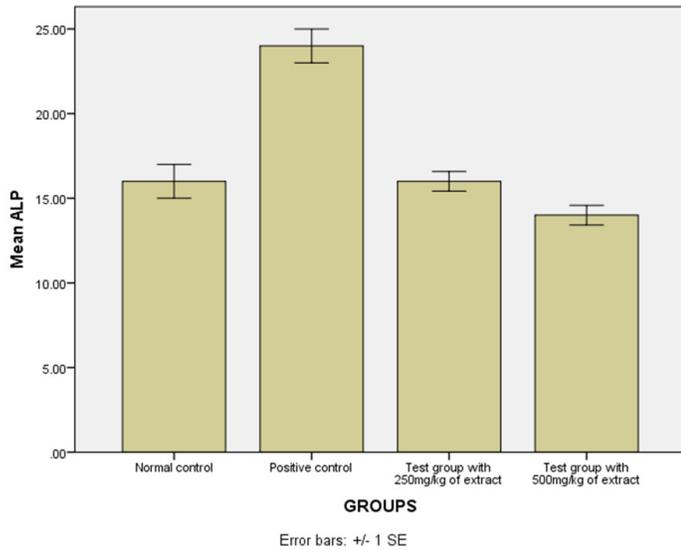


Figure 3: Mean values Comparison of ALP between the Control groups and the Test groups.

The Figure 3 shows the effects of Methanol Extract of *Napoleonae imperialis* on ALP. In the results there are a significant decrease ($p < 0.05$) between the positive control and the test groups that received 250 mg/kg and 500 mg/kg body weight of the leaves extract.

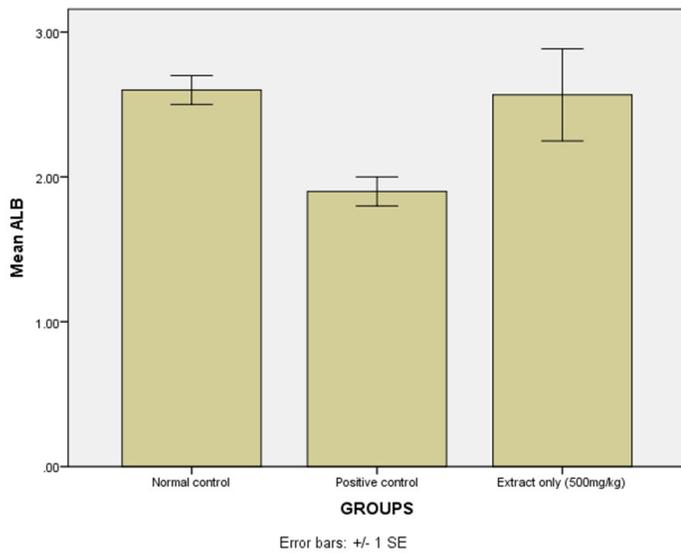


Figure 4: Mean values comparison of Albumin between the control groups and the group that received the extract only (500 mg/kg body weight).

The Figure 4 is the effects of Methanol Extract of *Napoleonae imperialis* on Albumin with a significant increase ($p < 0.05$) between the positive control and the group that received the extract only (500 mg/kg b.wt).

The Figure 5 is the outcome of the extract effects on Total cholesterol (T. Chol). It shows a significant decrease ($p < 0.05$) between the normal control group and the group that received the extract only (500 mg /kg body weight).

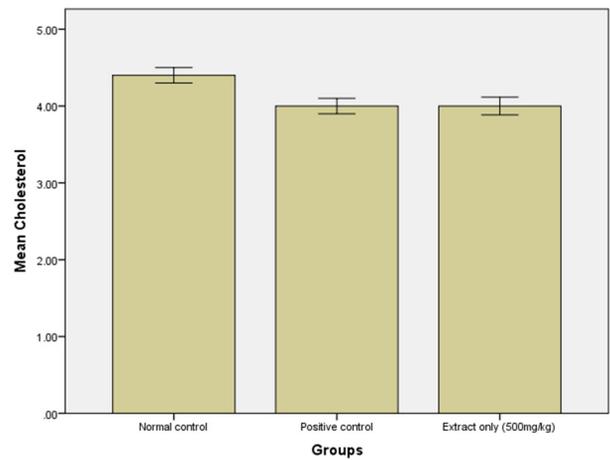


Figure 5: Mean values comparison of total cholesterol between the control groups and the group that received the extract only (500 mg/kg body weight).

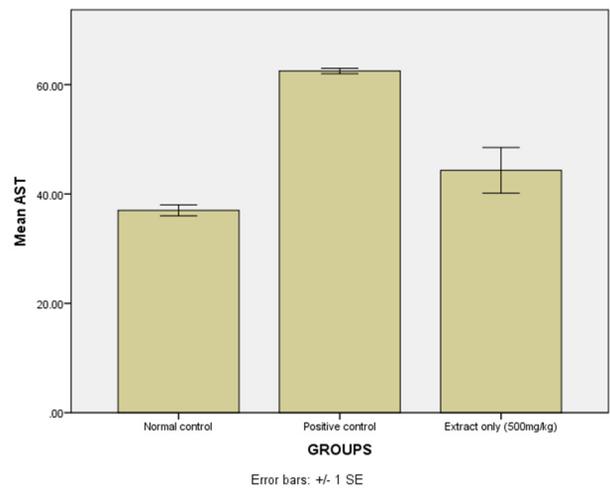


Figure 6: Mean values comparison of AST between the control groups and the group that received the extract only (500 mg/kg body weight).

The effects of Methanol Extract of *Napoleonae imperialis* on AST shows a significant decrease ($p < 0.05$) between the control groups especially with positive control against the group that received the extract only (500 mg.kg b.wt).

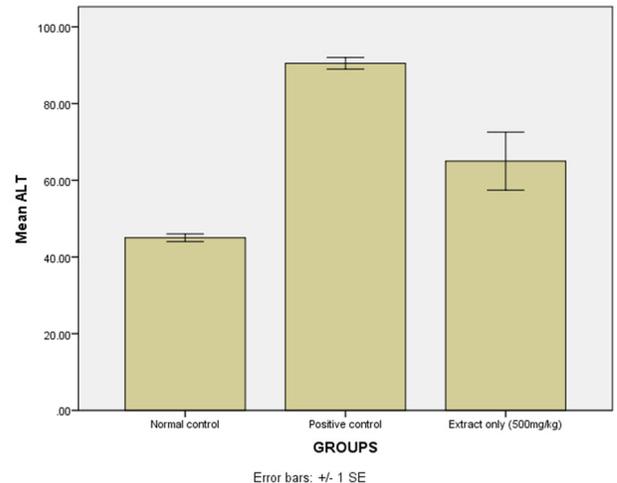


Figure 7: Mean values comparison of ALT between the control groups and the group that received the leaves extract only (500 mg/kg body weight).

Figure 7 shows the effects of Methanol Extract of *Napoleonae imperialis* on ALT with a significant decrease ($p < 0.05$) between the positive control and the group that received the leaves extract only (500 mg/kg b.wt).

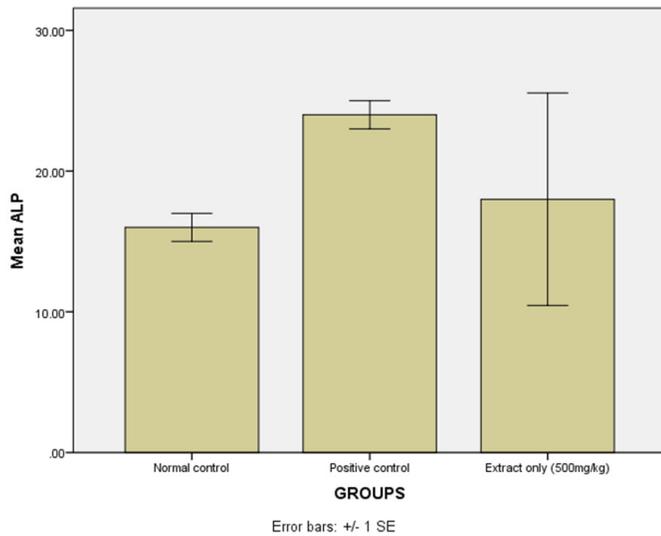


Figure 8: Mean values comparison of ALP between the control groups and the group that received the extract only (500 mg/kg b.wt).

The Figure 8 is showing the effects of Methanol Extract of *Napoleonae imperialis* on ALP, there are no significant decrease ($p > 0.05$) between the control group (normal), and the group that received the leaves extract only (500 mg/kg b.wt).

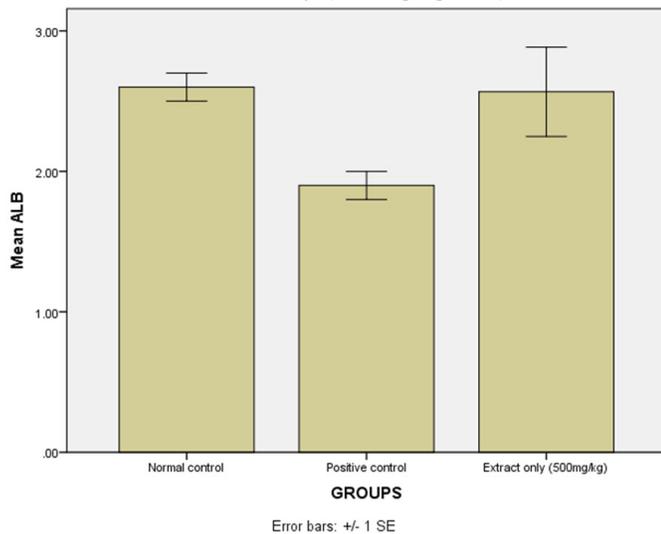


Figure 9: Mean values comparison of Albumin between the control groups and the group that received the extract only (500 mg/kg body weight).

In figure 9 above, the effects of Methanol Extract of *Napoleonae imperialis* on Albumin shows a significant increase ($p < 0.05$) between the positive control and the group that received the extract only (500 mg/kg b.wt).

Discussions

The liver performs an essential function in various metabolic processes, which makes it prone to the toxic effects of many exogenous/endogenous compounds [12]. Hepatotoxicity, a disorder to the hepatocyte leads to damaged hepatic function caused by free radicals derived from oxygen on exposure to drugs, chemical and non-infectious agents [13]. Various reports confirmed that CCl_4 induces hepatic injury by producing free radicals [14,15]. The beginning and development of CCl_4 induced toxicity is tightly linked to the generation of reactive oxygen species (ROS), which have the ability to cause spontaneous DNA damages [16]. Earlier, it has been established that CCl_4 metabolized to a highly reactive intermediate, trichloromethyl radical (CCl_3) [17]. Trichloromethyl radical and trichloromethylperoxyl radical are known to be involved in a number of deleterious interactions with biological molecules, which includes proteins, lipids and DNA [18].

This is believed to be part of the critical processes by which, some of these toxicants (chemicals), perturb some key signaling pathways via differential gene expression leading to alteration in protein expression, and thus, play an increasing role in the development of disease phenotype [16]. Thus the increase in the concentration of the liver marker enzymes (AST, ALT and ALP) after CCl_4 administration as observed from the experiments may be adduced to the escape of these enzymes emanating from protein distortion leading to damages of structural integrity of the underlying liver genes located in the cytoplasm [19].

Plasma levels of ALT and AST have been revealed to be essential factor in the treatment of hepatic disorder [20]. Liver cells are particularly rich in the transaminases (ALT and AST), as well as alkaline phosphatase and glutamyltransferase. Increased activities of plasma ALP can be attributed to its increased synthesis in the existence of aggregate biliary pressure. Plasma alkaline phosphatase is recognized to rise as a result of biliary obstruction as found in cholestasis disorder of the hepatocytes. Gamma Glutamyl Transferase (GGT) is a membrane bound enzyme and elevated plasma concentration is a gauge of tissue impairment [21].

The vulnerability of the hepatocytes to oxidative stress occasioned by CCl_4 can be attributed to the inability of the antioxidant to effectively scavenge free radical, thereby leading to lipid peroxidation (Lipid peroxidation is a marker of oxidative stress), and ultimately tissue damage [22].

The concentration of liver marker enzymes such as AST, ALT and ALP are frequently used to measure the status of liver function, however, only ALT activity is remarkably specific for liver function. In this study, there were significant ($p < 0.05$) increase observed in these liver marker enzymes (AST, ALT and ALP) activity in the hepatic control which was CCl_4 induced but not treated suggesting that the CCl_4 might have compromised the liver integrity and function possibly through the mechanism of free radical generation by the carbon tetrachloride. These might have damaged the liver membrane and resulted to the increased permeability of the membrane leading to the escape of the liver

enzymes to the extra hepatic tissues. The significant decrease ($p < 0.05$) observed in these liver marker enzymes of the extract treated groups relative to the CCl_4 untreated group could be attributed to antioxidant activities exhibited by phytochemicals such as flavonoids, phenols and alkaloids against free radicals generated by CCl_4 . It is most probable that these bioactive molecules present in the extract may be involved or have ability to induce the restoration of cellular pathways, which promote cellular senescence via some mechanisms involving some epigenetic alterations [16].

Previously, we established the median lethal dose (LD50) of the oral administration of up to 5000 mg/kg body weight of the *Napoleonae imperialis* methanol leaf extract, without observing any effects on the rat as there were, no acute toxicity or instant death in any of the rats used [23]. Also, analyzing the phytochemical of the methanol leaf extract of *Napoleonae imperialis* we showed, it contains; alkaloids, tannins, terpenoids, reducing sugar, glycosides and flavonoids, and all are bioactive components that are thought to be hepatoprotective [24]. This work on the methanol leaf extract of *Napoleonae imperialis* further lead credence to other previous studies that suggests medicinal herbs can exhibit hepatoprotective effects through additive and synergistic actions of antioxidant activities of their phytochemicals constituents such as phenol and flavonoids [25]. The extracts could have restored the membrane permeability thereby preventing leakages of the liver enzymes to the extra hepatic tissue.

Our earlier study [23], suggest no acute toxicity using this extract, these results showed that the extract exhibited more hepatoprotective activity at low doses as the hepatoprotective activity decreases with increasing doses, indicating that the extract may contain other non-hepatoprotective components that could have interfered with its hepatoprotective activity. Further identification, separation and refining of the active hepatoprotective components in this extract is essential, as it would enhance functions for the improvement of hepatoprotective drug.

Carbon tetrachloride adversely inhibits protein digestion probably by hindering the production of proteins such as albumin and total protein [26], which may be ascribed to the decreased levels of total protein and albumin observed in this study.

Conclusions

The findings of this study indicate that methanol leaves extract of *Napoleonae imperialis* possess hepatoprotective properties capable of maintaining liver functions through stabilization of membrane as noticed in the decreased amount of liver marker enzymes. The extract was most useful in the treatment of liver damage at a lower dose 250 mg/kg body weight, as the hepatoprotective activity decreases with increasing doses which could be an indication that the extract may contain other components that could have interfered with its hepatoprotective properties. The study discovers the possible synergistic effect of bioactive flavonoids, total phenolics, saponins, and alkaloids present in the methanol extract of *Napoleonae imperialis* leaves that can be beneficial in

maintaining liver integrity and functions in carbon tetrachloride induced liver damage.

Generally, therapy against liver damage using phytochemicals could gain more acceptability, and hence, more research works are required to establish the mechanistic processes for their effects. Moving forward, this plant should be further studied in the formulation of more effective hepatoprotective drugs. This could, herald in, a new approach in the use of plant extracts as a possible dietary supplement in the treatment and management of hepatic malfunction and other disease phenotype leading to improvement in the lives of patients.

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