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Research Article

Evaluation of the effect of total saponins and tannins isolated from *Dialium guineense* stem bark on CCl_4 - Induced hepatotoxicity in wistar rats

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Abstract

The aim of the present study was to evaluate the hepatoprotective effect of total saponins and tannins of *Dialium guineense* stem bark in Wistar rats exposed to carbon tetrachloride (CCl_4). Adult male Wistar rats (n = 25) weighing 160 – 180 g (mean weight = 170 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, total saponins, and total tannins groups. With the exception of normal control, the rats were exposed to CCl_4 (a single oral dose of 1.0 mL/kg body weight, bwt). Rats in the silymarin group were administered 100 mg/kg bwt silymarin (standard hepatoprotective drug), while those in the two treatment groups received 150 mg/kg bwt of total saponins or tannins orally. Treatment lasted 28 days. Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline Phosphatase (ALP), as well as levels of Total Protein (TP), bilirubin, and albumin, were measured in plasma. The results showed that there were no significant differences in the concentrations of TP among the groups ($p > 0.05$). The activities of the liver enzymes, as well as levels of bilirubin and albumin, were significantly higher in the CCl_4 control group than in the normal control group, but they were reduced by extract treatment ($p < 0.05$). These results indicate that CCl_4 negatively impacted the integrity of the liver cells, and total saponins and tannins of *D. guineense* stem bark conferred some level of protection on the organ.

Introduction

As a major body organ, the liver is involved in the detoxification, distribution, and metabolism of nutrients, and any damage to it causes serious health problems [1]. Chemicals constitute an important cause of liver injury. Carbon tetrachloride is the most commonly used hepatotoxic agent for the induction of liver injuries in experimental animals [2,3]. Acute exposure to high or chronic levels of the substance produces liver and kidney damage in humans. It directly impairs organ function by altering plasma, lysosome, and mitochondrial membrane permeability [4,5].

Dialium guineense (Velvet Tamarind) is a medicinal plant used in folklore medicine for the treatment of infections such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, ulcer, and hemorrhoids [6,7]. It is a tall, tropical, fruit-bearing tree, belonging to the *Leguminosae* family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel [7,8]. The plant is found majorly in Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Equatorial Guinea, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Niger, Nigeria, Sao Tome et Principe, Senegal, Sierra Leone, Sudan and Togo [8]. In Nigeria, it is known by different names: *Icheku* (Igbo), *Awin* (Yoruba), *Tsamiyarkurm* (Hausa) and *Amughen* (Bini) [8].

Extracts of the plant are reported to be rich in important phytochemicals [9-11]. At present, there is a dearth of data on the potential of extracts of *D. guineense* stem bark to protect against CCl_4-induced hepatotoxicity in rats. The aim of this study was to evaluate the hepatoprotective effect of total saponins and tannins of *Dialium guineense* stem bark in Wistar rats exposed to CCl_4.

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Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

Collection of plant material

The stem barks of *D. guineense* were obtained from Auchi, Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330).

Plant preparation and extraction

The stem bark was washed and shade-dried at room temperature for a period of two weeks and crushed into small pieces using a clean mortar and pestle. Total saponins and tannins were isolated from the pulverized stem bark using standard methods [9,12].

Experimental rats

Adult male Wistar rats (n = 25) weighing 160 – 180 g (mean weight = 170 ± 10 g) were obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: temperature of 25 °C, 55% – 65% humidity, and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. Prior to the commencement of the study, the rats were acclimatized to the laboratory environment for one week. The standard experimental protocol was followed for this study.

Experimental design

The rats were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, total saponins, and total tannins groups. With the exception of normal control, the rats were exposed to CCl_4 (a single oral dose of 1.0 mL/kg bwt) [13]. Rats in the silymarin group were administered 100 mg/kg bwt silymarin, while those in the two treatment groups received 150 mg/kg bwt of total saponins or tannins orally. Treatment lasted 28 days.

Blood sample collection and preparation

At the end of the treatment period, the rats were euthanized. Blood samples were collected from the anesthetized rats via cardiac puncture in heparinized sample bottles, and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analysis.

Biochemical analysis

The activities of AST, ALT, and ALP were determined [14,15]. Levels of bilirubin, total protein, and albumin were also measured [16-18].

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM, n = 5). Statistical analysis was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

Results

Effect of total saponins and tannins of *D. guineense* stem bark on relative organ weight

There were no significant differences in relative organ weight among the groups ($p > 0.05$; Table 1).

Effect of total saponins and tannins of *D. guineense* stem bark on liver function

There were no significant differences in the concentrations of plasma TP and globulins among the groups ($p > 0.05$). The activities of the liver enzymes, as well as levels of bilirubin and albumin, were significantly higher in the CCl_4 control group than in the normal control group, but they were reduced by extract treatment ($p < 0.05$). These results are shown in Table 2 and Figures 1, 2.

Discussion

Carbon tetrachloride (CCl_4) is well-known for its toxicity. As an established toxicant, it is used experimentally to induce liver damage, which has a far-reaching effect on other organs including the kidney.

The use of conventional drugs for the management of the liver disease is limited by adverse effects and this has led to increased dependence on complementary and alternative medicine [2,19]. Today, a substantial number of drugs are developed from plants that are active against a number of diseases [20]. In this study, the hepatoprotective effect of total saponins and tannins isolated from the stem bark of *Dialium guineense* in rats exposed to CCl_4 was evaluated. The results

Table 1: Relative Organ Weights of Rats.

Group	Relative organ weight x 10^{-2}
Normal Control	2.98 ± 0.05
CCl_4 Control	2.86 ± 0.06
Silymarin	2.84 ± 0.06
T. Saponins	2.98 ± 0.05
T. Tannins	2.99 ± 0.20

Data are relative organ weights and are expressed as mean ± SEM (n = 5).
Where T. Saponins and T. Tannins = total saponins and total tannins, respectively.

Table 2: Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Liver Function.

Group	AST (IU/L)	ALT (IU/L)	AST/ALT	ALP (IU/L)
Normal Control	37.94 ± 0.00	10.74 ± 4.80	3.53 ± 0.45	12.41 ± 0.00
CCl_4 Control	65.40 ± 1.04	32.87 ± 8.97	1.99 ± 0.12	14.94 ± 0.23
Silymarin	35.87 ± 7.74[a]	12.17 ± 2.08[a]	2.95 ± 0.72[a]	12.13 ± 0.41[a]
T. Saponins	31.78 ± 2.95[a]	13.57 ± 1.07[a]	2.34 ± 0.06[a]	13.74 ± 0.18[a]
T. Tannins	33.42 ± 0.00[a]	18.02 ± 3.29[a]	1.85 ± 0.03	11.84 ± 0.00[a]

Data are liver function parameters and are expressed as mean ± SEM. [a]$p < 0.05$, when compared with CCl_4 control.

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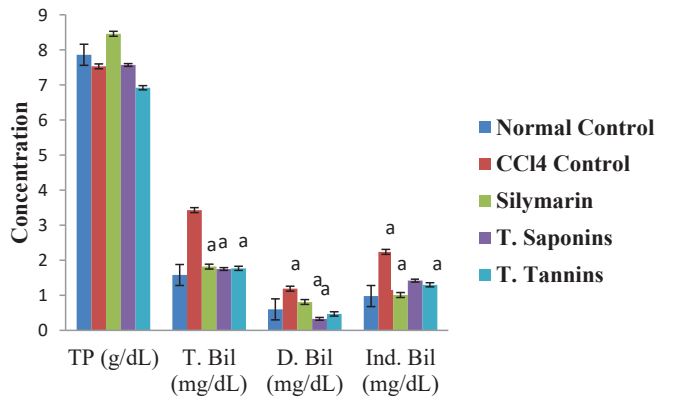

Figure 1: Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Markers of Liver Function. Data are markers of liver function and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control.

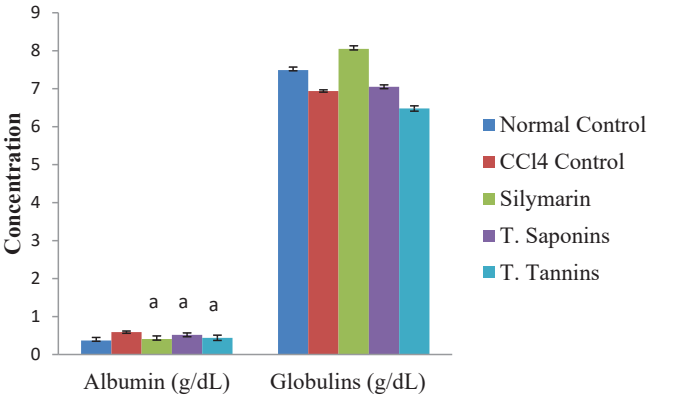

Figure 2: Comparison of the Levels of Liver Proteins Among the Groups. Data are levels of liver proteins and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control.

showed that there were significant increases in the weights of test rats compared to normal control. The liver to body weight ratio of test and control rats was not significantly different.

Liver enzymes are released into the systemic circulation following cellular necrosis and increased cell membrane permeability and are used as a diagnostic measure of liver damage. Most proteins found in the plasma are synthesized by the hepatocytes and secreted into circulation [21]. Alanine aminotransferase (ALT) and AST are important indices for the assessment of liver inflammation and necrosis. The activity of ALT is highest in the liver and lower in kidneys and skeletal muscles. The activity of AST is located in the microsomal and mitochondrial portions of liver cells as well as in the skin, skeletal muscles, pancreas, and kidneys [22].

The ability of the liver to synthesize albumin is reduced when the synthetic function of the organ is affected. The evaluation of plasma total protein alone may not tell the true picture of the metabolic state of an individual, since the concentration of the various proteins is not affected by each other. An elevated level of total protein may be due to dehydration or infection. Plasma concentration may decrease due to impaired synthesis that can result from malnutrition, malabsorption, over-hydration, and some forms of liver diseases [23]. The total protein concentrations of rats in the treatment groups did not show any significant difference when compared to the normal and CCl_4 controls.

Bilirubin is a useful index of the excretory function of the liver and a marker for hemolytic anemia. The results of this study showed that the activities of the liver enzymes, as well as levels of bilirubin and albumin, were significantly higher in the CCl_4 control group than in the normal control group, but they were reduced by extract treatment. It may be stated that the synthetic and excretory functions of the rat's liver were significantly affected by CCl_4. These results are in agreement with previous reports [24-26].

Conclusion

The results of this study have demonstrated that CCl_4 negatively imparted the integrity and ultrastructure of rat liver cells, and total saponins and tannins of *D. guineense* stem bark exhibited some level of hepatoprotection. The health benefits of extracts of the medicinal plant could be due to their phytochemical contents.

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