Total Saponins and Tannins of Dialium guineense Stem Bark Protect against CCl4-induced Oxidative Stress in Rats Liver

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Abstract

The present study investigated the capacity of total saponins and tannins of *Dialium guineense* stem bark to protect against Carbon Tetrachloride (CCl_4) induced oxidative stress in rats' liver. Adult male Wistar rats (n = 25) weighing 160g to 180g (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 at a single oral dose of 1.0 mL/kg body weight, BWT. Total saponins and tannins were isolated from the plant stem bark using standard methods. Rats in the silymarin group were administered silymarin (standard hepatoprotective drug) at a dose of 100 mg/kg body weight, BWT, while those in the two treatment groups received 150 mg/kg BWT of total saponins or tannins orally for 28 days. Activities of antioxidant enzymes such as catalase, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) were evaluated in plasma. The results showed that there were no significant differences in the concentrations of plasma Total Protein (TP) among the groups ($p > 0.05$). The activities of all the antioxidant enzymes measured as well as levels of GSH and Nitric Oxide (NO) were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract treatment ($p < 0.05$). However, the level of plasma MDA increased by CCl_4 intoxication reduced after treatment ($p < 0.05$). These results suggest that total saponins and tannins of *D. guineense* stem bark could enhance the antioxidant defense in the protection of rat's liver against CCl_4-induced oxidative stress.

Keywords: Antioxidant enzymes, *Dialium guineense*, Lipid peroxidation, Oxidative stress, Saponins.

Introduction

The liver is the metabolic hub of higher animals. It functions in metabolism, detoxication and excretion. Some medicinal agents, chemicals and even herbal remedies may cause liver injury [1,2].

Carbon Tetrachloride (CCl_4) is an established toxicant used experimentally to induce liver damage [3]. Liver cell injury induced by this chemical involves its initial metabolism to trichloromethyl free-radical by the mixed-function oxidase system of the endoplasmic reticulum [4]. Secondary mechanisms are thought to link CCl_4 metabolism to the widespread disturbances in organ function. The secondary mechanisms could involve the generation of toxic products directly from CCl_4 metabolism or the peroxidative degeneration of membrane lipids [5,6].

Medicinal plants are plants that generally contain constituents that have been found useful for the treatment

and management of diseases. Their use in the management of diseases is as old as man [7,8]. These plants serve as cheap alternative to orthodox medicine since they are readily available [9-11]. *Dialium guineense* 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 [12]. Extracts of the plant are reported to be rich in important phytochemicals [13-15]. At present there is dearth of data on the potential of extracts of *Dialium guineense* stem bark to protect against CCl_4 induced oxidative stress in rats. The aim of this study was to investigate the capacity of total saponins and tannins isolated from the stem bark of *D. guineense* to protect against CCl_4-induced oxidative stress in rats liver.

Materials and Methods

Chemicals

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 Area of Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_{33D}).

Plant Preparation and Extraction

The stem bark was brushed and shade- dried at 30 ˚C for a period of two weeks and crushed into small pieces using clean mortar and pestle. Total saponins and tannins were isolated from the plant stem bark using standard methods [13,16].

Experimental Rats

Adult male Wistar rats (n = 25) weighing 160g to 180g (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 ºC, 55% to 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 commencement of the study, the rats were acclimatized to the laboratory environment for one week. The study protocol was approved by the University of Benin Faculty Of Life Sciences Ethical Committee on Animal Use.

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 at a single oral dose of 1.0 mL/kg body weight, BWT. Total saponins and tannins were isolated from the plant stem bark using standard methods. Rats in the silymarin group were administered silymarin at a dose of 100 mg/kg body weight, bwt, while those in the two treatment groups received 150 mg/kg BWT of total saponins or tannins orally for 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 through cardiac puncture in heparinized sample bottles, and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analysis.

Biochemical Analyses

The activities of catalase, SOD and GPx were determined [17-19]. Levels of total protein, MDA and GSH were also measured [20-22]. The level of NO was determined using a previously described method [23], while the activity of GR was measured as the rate of formation of GSH from GSSG as shown below:

Enzyme activity = [GSH]/time

Statistical Analysis

Data are expressed as mean ± SEM (n = 5). Statistical analysis was performed using Graph Pad Prism Demo (6.07). 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

As shown in Table 1, there were no significant differences in relative organ weight among the groups ($p > 0.05$).

Table 1: Relative Organ Weights of Rats Induced with CCl_4

Group	Relative organ weight x 10^{-2}
Normal Control	2.98 ± 0.05
CCl4 Control	2.86 ± 0.06
Silymarin	2.84 ± 0.06
T. Saponins	2.85 ± 0.15
T. Tannins	2.93 ± 0.06

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.

Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Oxidative Status in CCl_4-Induced Wistar Rats

There were no significant differences in the concentrations of plasma TP among the groups ($p > 0.05$). The activities of all the antioxidant enzymes measured in rat plasma as well as levels of GSH and NO were significantly lower in CCl_4 control group than in normal control group, but they were increased by total saponins and tannins treatment ($p < 0.05$). However, the level of plasma MDA increased by CCl_4 intoxication reduced after treatment ($p < 0.05$). These results are shown in Tables 2 and 3.

Table 2: Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Markers of Oxidative Stress in Plasma

Group	TP (mg/dL)	SOD (Unit/min) x 10^{-4}	MDA (moles/mg tissue) x 10^{-6}	Catalase (Unit/min) x 10^{-3}
Normal Control	7.86 ± 0.30	2.87 ± 0.46	1.14 ± 0.14	3.77 ± 0.66
CCl_4 Control	7.53 ± 0.40	0.84 ± 0.03	3.26 ± 0.59	0.99 ± 0.07
Silymarin	8.46 ± 0.36	1.82 ± 0.13[a]	0.14 ± 0.04[a]	2.71 ± 0.90[a]
T. Saponins	7.57 ± 0.72	2.80 ± 0.15[a]	1.42 ± 0.27[a]	2.42 ± 0.19[a]
T. Tannins	6.92 ± 0.59	3.19 ± 0.17[a]	1.31 ± 0.69[a]	3.90 ± 0.89[a]

Data are oxidative stress markers, and are expressed as mean ± SEM. [a]$p < 0.05$, when compared with CCl_4 control.

Table 3: Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Rat Oxidative Status

Group	GSH (mg/dL)	% GSH	GPx (Unit/min)x 10^{-4}	GR (Unit/min)x 10^{-2}
Normal Control	39.44 ± 11.60	45.96 ± 9.60	35.35 ± 0.45	7.89 ± 1.32
CCl4 Control	15.08 ± 1.16	27.27 ± 0.00	11.33 ± 1.70	3.02 ± 0.23
Silymarin	32.48 ± 2.05[a]	40.00 ± 2.08[a]	19.20 ± 0.00[a]	6.50 ± 0.41[a]
T. Saponins	39.44 ± 6.70[a]	38.18 ± 1.82[a]	30.15 ± 2.95[a]	7.89 ± 1.34[a]
T. Tannins	37.12 ± 1.34[a]	38.18 ± 1.80a	31.00 ± 2.40[a]	7.42 ± 0.2[7a]

Data are oxidative stress markers, and are expressed as mean ± SEM. [a]$p < 0.05$, when compared with CCl_4 control.

Table 4: Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on NO Level

Group	%NO Scavenged	NO (µmole/L)
Normal Control	77.62 ± 3.74	170.50 ± 1.00
CCl4 Control	58.86 ± 0.86	151.12 ± 3.38
Silymarin	74.00 ± 0.00a	166.75 ± 6.63a
T. Saponins	72.79 ± 0.20a	164.50 ± 3.62a
T. Tannins	72.06 ± 4.96a	164.25 ± 3.17a

Data are levels of NO and are expressed as mean ± SEM. [a]$p < 0.05$, when compared with CCl_4 control.

Discussion

Certain drugs and chemical agents are notorious for causing liver injury. Such injuries may have far-reaching effect on other organs.

Carbon tetrachloride is the most commonly used hepatotoxic agent for the induction of liver injuries in experimental animals. Acute exposure to high levels and chronic inhalation or oral exposure to this chemical cause's liver and kidney damages in humans. It directly impairs organ function via alteration in the permeability of the plasma, lysosome and mitochondrial membranes. Carbon

tetrachloride is metabolized to the noxious trichloromethyl radical (CCl_3) by cytochrome p4502E1 (cyp2E1) in hepatocytes [24,25]. The CCl_3 causes lipid peroxidation and membrane damage. The radical undergoes anaerobic reactions to form chloroform or carbon monoxide, as well as bind directly to lipid, proteins and DNA [26].

Aerobic organisms possess antioxidant defense systems that deal with Reactive Oxygen Species (ROS) produced by aerobic respiration, substrate oxidation or toxicants. Small amounts of ROS, including hydroxyl radical ($^{\bullet}OH$), superoxide anion ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2) are constantly generated in aerobic organisms in response to both external and internal stimuli [27-29].

The enzyme and non enzyme antioxidant defenses include SOD, GPx, catalase, ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, and vitamin A [30- 32]. For the survival of organisms and maintenance of their health, there is usually a balance between the activities and intracellular levels of these antioxidants [8].

Superoxide Dismutase (SOD) detoxifies $O^{2\bullet-}$ which otherwise damage cell membrane and macromolecules [8]. In animals, hydrogen peroxide is detoxified by catalase and GPx. Catalase protects cells from hydrogen peroxide generated within them. It plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Catalase prevents drug-induced consumption of O_2 [33]. Suppressed action of this enzyme results in enhanced sensitivity of cells to free radical-induced cellular damage [34].

Reduced glutathione (GSH) is a major non-protein thiol in living organism, which act against xenobiotics and neutralize ROS, and disturbances of its intracellular level in biological system has been reported to lead to serious consequences [35].

Malondialdehyde (MDA), a commonly used biomarker of lipid peroxidation, is synthesized from the breakdown of lipid peroxyl radicals during oxidative stress. Measured level of MDA is considered a direct index of oxidative injuries associated with lipid peroxidation [36,37].

In this study, the activities of all the antioxidant enzymes measured in rat plasma as well as levels of GSH and NO were significantly lower in CCl_4 control group than in normal control group, but they were increased by total saponins and tannins treatment. However, the level of MDA increased by CCl_4 intoxication reduced after treatment. It is likely that the extracts potentiated the antioxidant system of the rats so as to protect against CCl_4 induced hepatotoxicity. The extracts may be used as a potential crude drug for conditions that result from oxidative stress. The observed enhanced antioxidant effect may be due to the presence of many important phytochemicals in the extract of this medicinal plant [15].

Conclusion

The results obtained in this study suggest that total saponins and tannins of *D. guineense* stem bark could enhance the antioxidant defense in the protection of rat's liver against CCl_4 induced oxidative stress.

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