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Research Article

Effect of Total Saponins and Tannins Isolated from Dialium guineense Stem Bark on Oxidative Status in Rats Exposed to CCl_4

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Abstract

The aim of the present study was to investigate the effect of total saponins and tannins of Dialium guineense stem bark on oxidative status in rats exposed to carbon tetrachloride (CCl_4). Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, total saponins and total tannins groups. Total saponins and tannins were isolated from the plant stem bark using standard methods. 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. Rats in the silymarin group were administered silymarin (standard hepatoprotective drug) at a dose of 100 mg/kg 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 liver homogenate. The results showed that there were no significant differences in the concentrations of liver total protein (TP) among the groups ($p > 0.05$). The activities of the antioxidant enzymes measured as well as levels of reduced glutathione (GSH) and nitric oxide (NO) were significantly lower in CCl_4 control group than in normal control group, but these parameters were increased by extract treatment ($p < 0.05$). However, the level of liver malondialdehyde (MDA) increased by CCl4 was significantly reduced after treatment ($p < 0.05$). These results suggest that total saponins and tannins of D. guineense stem bark may enhance antioxidant defense in rats exposed to CCl_4.

Keywords: Antioxidant enzymes; *dialium guineense*; oxidative stress; saponins; tannins

Introduction

One of the most important causes of liver dysfunction is chemical-induced liver injury, which can lead to a wide spectrum of symptoms ranging from mild non-specific symptoms like asymptomatic transaminitis, acute hepatitis, chronic hepatitis, cholestasis to liver failure [1]. It is caused by a multitude of chemicals, drugs, herbal and dietary supplements. Liver damage can be hepatocellular, cholestatic, or mixed (includes features of both). Cholestatic damage commonly occurs due to the drug or the drug metabolite [2,3]. They inhibit hepatobiliary transporter systems which are essential for bile formation and secretion of cholephilic substances and xenobiotics. Hepatocellular damage occurs through multiple pathways including direct hepatotoxicity, and innate and adaptive immune responses. Chemical-induced liver injury can be dose-dependent/intrinsic, and on most occasions, it is dose-independent/idiosyncratic [4]. Carbon tetrachloride (CCl_4) is an established toxicant used experimentally to induce liver damage. 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 [5]. A secondary mechanism is thought

to link CCl_4 metabolism to the widespread disturbances in organ function.

The secondary mechanism could involve the generation of toxic products directly from CCl_4 metabolism or the peroxidative degeneration of membrane lipids [6,7]. Medicinal plants are plants that generally contain constituents that have been found useful for the treatment and management of diseases. Their use in disease management is as old as man [8,9]. These plants serve as cheap alternative to orthodox medicine since they are readily available [10-12]. 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 [13,14]. Extracts of the plant are reported to be rich in important phytochemicals [15-17]. At present there is dearth of data on the potential of extracts of D. 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. UBH33D).

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 clean mortar and pestle. Total saponins and tannins were isolated from the plant stem bark using standard methods [18].

Experimental Rats

Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 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 – 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 bwt. Rats in the silymarin group were administered silymarin at a dose of 100 mg/kg bwt, while those in the two treatment groups received 150 mg/kg bwt of total saponins or tannins orally for 28 days.

Tissue Sample Collection and Preparation

At the end of the treatment period, the rats were euthanized, and their liver excised, and used to prepare 20 % tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain supernatant which was used for biochemical analysis.

Biochemical Analyses

The activities of catalase, SOD and GPx were determined [19-21]. Levels of total protein, MDA and GSH were also measured [22-24]. The level of NO was determined using a previously described method [25], while the activity of GR was measured as the rate of formation of GSH from GSSG as shown below:

$$Enzyme\ activity = \Delta {}^{[GSH]}/_{time}$$

Statistical Analysis

Data are expressed as mean ± SEM (n = 5). Statistical analysis was performed using GraphPad 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

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 Oxidative Status in Rats

There were no significant differences in the concentrations of liver TP among the groups ($p > 0.05$). The activities of the antioxidant enzymes measured as well as levels of GSH and NO were significantly lower in CCl_4 control group than in normal control group, but these parameters were increased by extract treatment ($p < 0.05$). However, the level of liver MDA increased by CCl_4 was significantly reduced after treatment ($p < 0.05$). These results are shown in Tables 2-4.

Table 1: Relative Organ Weights of Rats Among the Groups.

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: Comparison of the Effect of Total Saponins and Tannins of *D. guineense* Stem Bark on Markers of Oxidative Stress in Rat Liver.

Group	TP (mg/dL)	SOD (Unit/min)x 10^{-5}	MDA (moles/mg tissue) x 10^{-3}	Catalase (Unit/min) x 10^{-3}
Normal Control	18.05 ± 6.97	14.40 ± 0.46	1.95 ± 0.60	33.92 ± 3.41
CCl_4 Control	18.41 ± 0.49	6.82 ± 0.01	10.20 ± 1.81	17.20 ± 5.98
Silymarin	21.73 ± 0.37	18.16 ± 1.17[a]	2.71 ± 0.44[a]	28.88 ± 1.77[a]
T. Saponins	17.80 ± 1.50	19.08 ± 3.81[a]	2.45 ± 0.87[a]	24.24 ± 1.06[a]
T. Tannins	17.02 ± 1.14	18.13 ± 1.21[a]	2.01 ± 0.46[a]	25.59 ± 3.04[a]

Data are oxidative stress markers and are expressed as mean ± SEM. ap < 0.05, when compared with CCl_4 control.

Table 3: Comparison of the 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	71.34 ± 3.13	69.79 ± 0.22	10.29 ± 2.24	14.27 ± 0.63
CCl_4 Control	23.20 ± 0.00	24.27 ± 2.05	2.11 ± 0.10	4.64 ± 0.23
Silymarin	60.32 ± 3.48[a]	46.15 ± 0.00[a]	6.11 ± 0.11[a]	12.06 ± 0.70[a]
T. Saponins	47.40 ± 1.32[a]	40.00 ± 0.00[a]	9.66 ± 1.23[a]	9.48 ± 0.26[a]
T. Tannins	49.88 ± 5.80[a]	41.67 ± 0.20[a]	6.55 ± 1.82[a]	9.98 ± 1.16[a]

Data are oxidative stress markers and are expressed as mean ± SEM. ap < 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	78.39 ± 5.23	497.00 ± 8.39
CCl_4 Control	52.52 ± 6.28	183.12 ± 9.77
Silymarin	71.60 ± 1.60[a]	397.40 ± 12.71[a]
T. Saponins	51.11 ± 0.00	168.25 ± 6.96
T. Tannins	60.65 ± 4.24[a]	255.67 ± 8.33[a]

Data are levels of NO and are expressed as mean ± SEM. ap < 0.05, when compared with CCl_4 control.

Discussion

Carbon tetrachloride (CCl_4) is renowned for its toxicity. As an established toxicant it is used experimentally to induce liver damage, which has far-reaching effects on other organs including the kidney. The liver and kidney are vital organs of the human body involved in metabolism, detoxification, secretion and excretion of various endogenous and exogenous substances [26]. Acute exposure to high levels and chronic inhalation or oral exposure to this chemical causes liver and kidney damage 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 [27]. 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 [28].

It is postulated that secondary mechanisms link CCl_4 metabolism to the widespread disturbances in organ function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degen-

eration of membrane lipids. 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 [29-31]. For the survival of organisms and maintenance of their health, there is usually a balance between the activities and intracellular levels of these antioxidants [32-34]. The bark, leaves and fruits of D. guineense have medicinal properties and are used in Traditional Medicine to treat different diseases [35]. Phytochemical screening of the plant stem bark reveals the presence of bioactive compounds such as alkaloids, flavornoids, phenols, saponins, steroids and tannins. These phytochemicals are believed to be responsible for their various biological and pharmacological effects.

Extracts of the plant have been shown to possess anti-inflammatory, antioxidant, antiplasmodial and hepatoprotective effects. The aim of this study was to investigate the effect of total saponins, and tannins isolated from the stem bark of .D. Guineense on oxidative status in rats exposed to CCl_4. The results showed that there were no significant differences in the concentrations of liver TP among the groups. The activities of the antioxidant enzymes measured as well as levels of GSH and NO were significantly lower in CCl_4 control group than in normal control group, but these parameters were increased by total saponins or tannins treatment. However, the level of liver MDA increased by CCl_4 was significantly reduced after treatment. These results indicate that total saponins and tannins of D. guineense stem bark may enhance antioxidant defense in rats exposed to CCl_4. The capacity of extracts of the medicinal plant to potentiate natural antioxidant defense system has been reported [36-38]. Plants rich in polyphenols are reported to possess good antioxidant capacity [39-41].

Conclusion

The results of this study suggest that total saponins and tannins present in the stem bark of D. guineense enhance antioxidant defense in rats exposed to CCl_4. As important phytochemicals they could form the base for new drug formulation.

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