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POTENTIAL OF EXTRACTS OF *Dialium guineense* STEM BARK IN THE MITIGATION OF CARBON TETRACHLORIDE-INDUCED RENAL OXIDATIVE STRESS

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ABSTRACT
The aim of the present study was to evaluate the potential of extracts of Dialium guineense stem bark in the mitigation of carbon tetrachloride (CCl_4)-induced renal oxidative stress. Adult male Wistar rats (n = 20) weighing 160 – 180g (mean weight = 170 ± 10g) were randomly assigned to four groups (5 rats per group): normal control, CCl_4 control, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0mL/kg body weight, bwt. Aqueous and ethanol extracts of the plant stem bark were obtained using cold maceration method. Rats in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated. The results showed that there were no significant differences in the concentrations of total protein among the groups ($p > 0.05$). The activities of all the antioxidant enzymes measured and levels of reduced glutathione (GSH) 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 malondialdehyde (MDA) elevated by CCl_4 intoxication reduced after treatment ($p < 0.05$). These results indicate that extracts of D. guineense stem bark could potentiate the antioxidant defence in the amelioration of CCl_4-induced oxidative stress in the rat kidneys.

KEYWORDS: *Antioxidant enzymes, Dialium guineense, Extract, Organ damage, Oxidative stress*

INTRODUCTION

The pathogenesis of a number of diseases is linked to free radical-induced oxidative damage. Increased lipid peroxidation and decreased antioxidant protection generate epoxides that spontaneously react with nucleophilic centers in cells and thus covalently bind to DNA, RNA, and protein (Yin *et al.,* 1995; Rikans and

Hornbrook, 1997). Such a reaction leads to cytotoxicity, allergy, mutagenicity, and/or carcinogenicity, depending on the properties of the epoxide in question. In addition, oxidative event plays an important role in the mechanism of action of ether lipids, and ability to oxidize them may contribute to cellular drug sensitivity (Wagner *et al.,* 1998). The pathogenesis of CCl_4 - induced renal dysfunction is not completely known. It may be due to the functional state of liver or renal injury may develop independently to hepatic events, or can be attributed to CCl_4 induction of oxidative stress in many settings (Brattin *et al.,* 1985; Parola *et al.,* 1993).

Liver cell injury induced by CCl_4 involves its initial metabolism to trichloromethyl free-radical by the mixed-function oxidase system of the endoplasmic reticulum (Cui *et al.,* 2009). 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 degeneration of membrane lipids (Kim *et al.,* 2010). There is the possible involvement of radical species such as trichloromethyl (CCl_3), trichloromethylperoxy ($OOCCl_3$), and chlorine (Cl) free radicals, as well as phosgene and aldehydic products of lipid peroxidation (toxic intermediates) (Brattin *et al.,* 1985). Studies have shown that CCl_4 enhances lipid peroxidation and reduces the renal reduced/oxidized glutathione ratio in kidney cortex as well as renal microsomes and mitochondria (Abraham *et al.,* 1999). The aim of this study was to evaluate the potential of extracts of *Dialium guineense* stem bark in the mitigation of CCl_4-induced renal oxidative stress.

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.

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. Aqueous and ethanol extracts of the stem bark were obtained using cold maceration method as described previously (Abu *et al.,* 2017).

Experimental Rats

Adult male Wistar rats (n = 20) weighing 160 – 180g (mean weight = 170 ± 10g) 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^{o}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 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 four groups (5 rats per group): normal control, CCl_4 control, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0mL/kg bwt (Abu *et al*., 2015). Aqueous and ethanol extracts of the plant stem bark were obtained using cold maceration method. Rats in the two treatment groups received 1000mg/kg bwt of aqueous or ethanol extract orally for 28 days.

Tissue Sample Collection

At the end of the treatment period, the rats were euthanized. Their kidneys were excised, washed in ice –cold saline, blotted dry and placed in plain containers. Weighted portions of kidney were used to prepare 20% tissue homogenate used for biochemical analyses.

Biochemical Analyses

The activities of catalase, SOD and GPx were determined (Cohen *et al.,* 1970; Misra and Fridovich, 1972; Rotruck *et al.,* 1973). Levels of total protein, MDA and GSH were also measured (Henry *et al.,* 1957; Ellman, 1959; Guttridge and Wilkins, 1982). The level of NO was determined using a previously described method (Marcocci *et al.,* 1994), while the activity of GR was measured as the rate of formation of GSH from GSSG as shown below:

Enzyme activity = Δ [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. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effects of Extracts 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^{-3}
Normal Control	2.82 ± 0.30
CCl_4 Control	2.75 ± 0.22
Aqueous Extract	2.33 ± 0.15
Ethanol Extract	2.56 ± 0.10

Data are relative organ weights and are expressed as mean ± SEM (n = 5).

Effect of Extracts of D. guineense Stem Bark on Oxidative Status in CCl_4-Induced Wistar Rats

There were no significant differences in the concentrations of total protein (TP) among the groups ($p > 0.05$). The activities of all the antioxidant enzymes measured and levels of GSH 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 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 Extracts of *D. guineense* Stem Bark on Markers of Oxidative Stress

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	24.03 ± 5.31	0.40 ± 0.14	13.04 ± 5.31
CCl_4 Control	7.53 ± 0.40	12.79 ± 1.60	3.13 ± 0.60	2.80 ± 0.06
Aqueous Extract	7.12 ± 0.40	20.20 ± 5.89[a]	2.23 ± 1.93[a]	10.02 ± 5.90[a]
Ethanol Extract	8.87 ± 0.60	57.20 ± 0.36[a]	0.80 ± 0.54[a]	46.95 ± 0.36[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 Extracts 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	0.44 ± 0.13	46.42 ± 10.72	10.42 ± 1.08	8.80 ± 1.60
CCl_4 Control	0.17 ± 0.01	35.71 ± 0.00	7.57 ± 0.62	3.40 ± 0.00
Aqueous Extract	0.55 ± 0.02[a]	79.14 ± 7.20[a]	10.44 ± 1.82[a]	11.00 ± 1.40[a]
Ethanol Extract	0.44 ± 0.06[a]	76.18 ± 6.55[a]	26.29 ± 1.24[a]	5.50 ± 0.01[a]

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 Extracts of *D. guineense* Stem Bark on NO Level

Group	% NO Scavenged	NO (µmole/L)
Normal Control	77.62 ± 3.74	157.65 ± 5.91
CCl_4 Control	63.31 ± 3.60	162.25 ± 4.70
Aqueous Extract	61.53 ± 8.47	207.83 ± 23.62
Ethanol Extract	73.74 ± 0.09[a]	155.44 ± 6.00[a]

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

DISCUSSION

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 CCl_4 produces liver and kidney damages in humans. It directly impairs organ function by altering 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 (Yin *et al.,* 1995; Rikans and Hornbrook, 1997). 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 (Wagner *et al.,* 1998).

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration, substrate oxidation or toxicants. Small amounts of ROS, including hydroxyl radicals ($^{\bullet}OH$),

superoxide anions ($O^{2\bullet-}$) and hydrogen peroxide (H_2O_2) are constantly generated in aerobic organisms in response to both external and internal stimuli (Hurst *et al.,* 1997; Jornot *et al.,* 1998; Mills *et al.,* 1998).

The enzymatic and non-enzymatic antioxidant defenses include SOD, GPx, catalase, ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, and vitamin A, which can be evaluated using easy photometric assays (Beaudeux *et al.,* 1996; Hall *et al.,* 1998; Stahl *et al.,* 1998). For the survival of organisms and maintenance of their health, there is usually a balance between the activities and intracellular levels of these antioxidants (Abu and Onoagbe, 2019).

Superoxide dismutase (SOD) detoxifies $O^{2\bullet-}$ which otherwise damage cell membrane and macromolecules (Abu and Onoagbe, 2019). In animals, hydrogen peroxide is detoxified by catalase and GPx. Catalase protects cells from hydrogen peroxide generated within them. Even though catalase is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. The increased sensitivity of transfected catalase-enriched cells to some drugs and oxidants is attributed to the ability of catalase to prevent drug-induced consumption of O_2 (Speranza *et al.,* 1993). Suppressed action of this enzyme results in enhanced sensitivity of cells to free radical- induced cellular damage (Caroline *et al.,* 2008).

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 (Pastore *et al.,* 2003). 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 (Khan *et al.,* 2010).

In this study, the activities of all the antioxidant enzymes measured and levels of GSH were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract 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 mitigate CCl_4-induced renal toxicity. The extract 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 (Abu *et al.,* 2020).

This study has provided first-time experimental evidence for the antioxidant properties of extracts of *Dialium guineense* stem bark in mitigating CCl_4-induced renal damage.

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