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Protective Effect of *Erythrina Senegalensis* Stem Bark Extract against Cadmium Toxicity in Rats

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ABSTRACT

The protective effect of *Erythrina senegalensis* extract against cadmium-induced oxidative stress in the liver and kidneys of wister rats was investigated. For the investigation, rats were divided into four groups (control, cadmium alone, 100 mg/kg *Erythrina senegalensis* extract + Cd and 100 mg/kg *Erythrina senegalensis* extract alone). The control rats were administered normal diet and tap water *ad libitum*. *Erythrina senegalensis* (as an extract) in a dose of 3 mg/kg body weight was administered for 5 days after which cadmium (as cadmium chloride) in a dose of 3 mg/kg body weight was administered orally for period of 3 days to cadmium alone and extract + Cd groups. Treatment with cadmium chloride resulted in marked elevation in the level of membrane lipid peroxidation (estimated by amount of MDA), increased activities of enzyme biomarkers in the tissues (liver and kidney) and a decline in the levels of glutathione, urea, creatinine and cholesterol (in the kidney). However, pretreatment with stem bark extract of *Erythrina senegalensis* reduced or reversed the changes caused by cadmium treatment in most parameters. The results indicated that alteration caused by $CdC1_2$ are connected with free radical generation and stem bark extract of *Erythrina senegalensis* protected against cadmium intoxication.

Keywords: Cadmium, enzyme biomarkers, *Erythrina senegalensis*, lipid peroxidation, stem bark extract.

INTRODUCTION

Cadmium, Cd, a non-essential toxic heavy metal has found widespread use in electroplating, production of plastics pigments, fertilizer and other industrial products. This has resulted in the

contamination of the environment; affecting humans through occupational and environmental exposure [1]. The outbreak of itaiitai disease in Japan, a disease characterized by renal tubular lesions and severe bone disorders, awakened public health interest in the toxic effects of environmental Cd by epidemiological evidence linking industrial Cd waste pollution of marine food sources to the disease [2]. Low excretion rate as well as its long biological half-life (up to 30 years) increases Cd toxicity as it bio-accumulates in tissues with the kidney and liver as the main target organs [3]. Cadmium causes toxic lesions in target organs by inducing oxidative stress [4]. Oxidative stress caused by Cd occurs by increase in reactive oxygen species such as singlet oxygen, hydroxyl radical and hydrogen peroxide, which alter tissueantioxidant defense systems and contribute to oxidative stress by increased peroxidation of membrane lipids, gene expression alterations, mutations in DNA and apoptosis [3]. Thus, a number of antioxidant defence systems and antioxidants have been shown to protect cells of target organs from Cd toxicity [5] or to reverse Cd toxicity [6].

Varying results have been reported when chelating compounds (Dimercaprol, disodium versenate and mesomercapto succinic acid) where use in treating cadmium intoxications. However, some safety and effectiveness concerns have been reported about these chelators with no approval for the clinical use of any chelating therapy in reducing Cd toxicity [7, 8]. This has led to the search to develop safe and efficient strategies against Cd toxicity [9] with attention of researches drawn to the potential of medicinal plants reportedly used as therapeutical options for treatment of many human illnesses.

Erythrina senegalensis DC, also known coral tree, is a member offabaceae family.The leaves, stem and root bark are used by traditional healers to cure wide range of illness including malaria, gastrointestinal disorder, fever, dizziness, jaundice, diarrhea, nose bleeding and pain [10,11].

The stem bark has been shown to possessantimicrobial activity, hepatoprotective properties and to a large extentantioxidant properties [12,13].

The present study is aimed at investigating the effect of pretreatment with the stem bark aqueous extract of *Erythrina senegalensis* on cadmium – induced oxidative stress biomarkers in the kidney and liver of rats.

MATERIALS AND METHODS

Fresh stem bark samples of Erythrina senegalensis were collected from Rusau village, Jos North Local Government Area of Plateau State, Nigeria. Identification of the plant was done at the Department of Forestry, Federal College of Forestry Jos, Nigeria. The fresh stem bark samples were dried at room temperature and pulverized to dry power using pestle and mortar.

Preparation

About 100 g of the powered material was weighed and mixed with 500 ml of distilled water and allowed to stand for over 12 hours with continuous shaking at time interval. The mixture was filtered with Whatman filter paper No 1. The filtrate was evaporated to dryness at 50 ºC on a water bath.

Experimental Animals

Sixteen healthy male albino wistar rats weighing 100-200 g were obtained from the Animal House Unit, University of Jos, Plateau State, Nigeria. The rats were acclimatized for one week, kept in plastic cages at room temperature and fed pelleted diet (vital feed, Grand cereal and oil mills Ltd; Kuru, Nigeria) and tap water *adlibitum* throughout the period of this study. The working doses of *Erythrina senegalensis* stem bark aqueous extract and Cd ($CdCl_2$) administered orally to the experiment animals in this study was first determined in a pilot study.

Experimental Design

The rats were divided in 4 groups (A-D) of 4 rats each. Rats in the four groups were fed the standard “vital feed” diet and drinking water *ad libitum*.

Group A: Control

Group B: Cd Alone (3mg/kg b.wt)

Group C: ES pretreatment (100mg/kg b.wt for 5 (five) consecutive days and Cd (3mg/kg b.wt) for the remaining 3 (three) days

Group D: ES Alone (100mg/kg b.wt)

Biochemical Analysis

Membrane lipid peroxidation, glutathione concentration and lipid profile (cholesterol, triglycerides, HDL and LDL) were determined in the kidney and liver supernatant fractions. The liver function enzyme activity (AST, ALT and ALP) were assayed in the liver supernatant fractions while the kidney biomarkers concentration (urea and creatinine) were assayed in the kidney supernatant fractions.

Estimation of lipid peroxidation was by the thiobarbituric acid reaction method as described by Ohkawa *et al* [14]. Glutathione concentration was determined by the Ellman reaction [15] method as described by Beutler *et al* [16]. AST, ALT, ALP, urea, creatinine, Triglycerides, cholesterol, LDL and HDL were determined by the Reflotron Plus method using Reflotron tests reagent strips purchased from Randox laboratories Co. Antrium, UK.

Statistical Analysis

The obtained results from statistical analysis in the study are presented as Mean± SD. Statistical analysis was performed using the statistical package for social science (SPSS) software. One way analysis of variance (ANOVA) with post hoc analysis was used to ascertain the differences between the experimental groups and statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Cadmium Induced Lipid Peroxidation in Kidney and Liver as Determined by Malondialdehyde Levels

Results are summarized on Table 1. The results indicate that lipid peroxidation as determined by MDA level was significantly increased in the kidney and liver of rats exposed to cadmium alone when compared to the control. However, the levels of MDA in the kidney and liver of rats pretreated with stem bark extract of *Erythrina senegalensis* prior to cadmium administration were significantly lower when compared to the rats exposed to cadmium alone. This suggests that pretreatment with stem bark extract of *Erythrina senegalensis* has a modulating effect on cadmium – induced peroxidation at the dose used in the experiment.

Table 1: Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Cadmium Induced Lipid Peroxidation in kidney and liver as Determined by Malondialdehyde Levels

	Malondialdehyde concentration (nmol/g tissue)		
Group	**Treatment**	**Liver**	**Kidneys**
A	Control	23.23±4.26	31.05±2.14
B	Cd alone	58.77±3.61[a]	60.87±1.16[a]
C	Extract +Cd	39.91±3.08[ab]	41.29±0.44[ab]
D	Extract alone	13.46±0.44[ab]	11.96±0.84[ab]

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Effect of Pretreatment with *Erythrina Senegalensis* Stem Bark Extract on Cadmium – Induced Glutathione Concentration in kidney and liverin Cadmium Intoxication

Results are summarized on Table 2 Rats exposed to cadmium alone showed a significant reduction in the concentration of glutathione when compared to the control. This suggests that cadmium depleted glutathione in the kidney and liver examined. However, glutathione concentration was significantly increased in rats pretreated with stem bark extract of *Erythrina senegalensis* than those given cadmium alone, suggesting that stem bark extract of *Erythrina senegalensis* protected against cadmium – induced depletion of tissue glutathione. Pretreatment with stem bark extract of *Erythrina senegalensis* alone was statistically insignificant ($P>0.05$) in all tissues compared to the control.

Table 2: Effect of Pretreatment with *Erythrina Senegalensis* Stem Bark Extract on Cadmium-Induced Glutathione Concentration in Liver and Kidney in Cadmium Intoxication

	Glutathione concentration (µmol/g tissue)		
Group	**Treatment**	**Liver**	**Kidneys**
A	Control	0.49±0.01	0.39±0.01
B	Cd alone	0.19±0.01[a]	0.14±0.02[a]
C	Extract +Cd	0.32±0.01[ab]	0.21±0.13[a]
D	Extract alone	0.43±0.03[b]	0.36±0.01[b]

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* Stem Bark Extract on Liver Function Enzymes Activity in Cadmium Intoxication

Table 3 shows the activities of AST, ALP, and ALT in the tissue examined in this study. The results indicate that the AST, ALP and ALT levels was significantly increased in the liver compared to the control suggesting cadmium induction of enzyme activity. However, pretreatment with stem bark extract of *Erythrina Senegalensis* significantly reduced the activity of ALP and ALT in the tissue examined compared to rats exposed to cadmium alone with AST statistically insignificant ($p>0.05$). This will suggest that pretreatment with *Erythrina Senegalensis* impaired cadmium induction of these enzymes.

Table 3: Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Liver Function Enzymes Activity in Cadmium Intoxication

		Liver function enzyme concentration (U/g tissue)		
Group	**Treatment**	**AST**	**ALT**	**ALP**
A	Control	1273±98	1050±197	136±2.83
B	Cd alone	1638±180[a]	2987±301[a]	154±7.78[a]
C	Extract +Cd	1641±114[a]	778±203[ab]	125±4.95[ab]
D	Extract alone	1640±154[a]	588±105[ab]	168±19.80[ab]

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Effect of Pretreatment with Stem Bark Extract of *ErythrinaSenegalensis*on Kidney Biomarker Concentration in Cadmium Intoxication

Results are summarized on Table 4. The results showed that the concentration of urea and creatinine was significantly lower in the kidney when compared to the control. However, the level of urea and creatinine in kidney on rats pretreated with stem bark of *Erythrina senegalensis*was significantly increased.

Table 4: Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Kidney Biomarker Concentration in Cadmium Intoxication

		Kidney Biomarker Concentration	
Group	**Treatment**	**Urea (mmol/g tissue)**	**Creatinine (µ/mol/g tissue**
A	Control	4.72±0.74	42.75±2.05
B	Cd alone	4.20±1.98[a]	35.80±3.56[a]
C	Extract +Cd	5.70±0.76[ab]	50.60±2.67[ab]
D	Extract alone	5.75±0.12[ab]	41.70±3.39[ab]

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Liver Lipid Profile Concentration in Cadmium Intoxication

Results are summarized on Table 5. The results shows that cholesterol, TG and HDL level was significantly higher in the liver of rats exposed to cadmium alone compared to the control. However, pretreatment with stem bark extract of *Erythrina senegalensis* also significantly increased the cholesterol level in the liver when compared with the control. There was no effect ($P>0.05$) on the liver TG, HDL and LDL level in all the groups on pretreatment with stem bark extract of *Erythrina senegalensis.*

Table 5: Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Liver Lipid Profile Concentration in Cadmium Intoxication

		Lipid profile concentration (mmol/g tissue)			
Group	**Treatment**	**Chol**	**TG**	**HDL**	**LDL**
A	Control	1.49±0.14	0.73±0.04	0.16±0.01	1.73±0.01
B	Cd alone	2.18±0.20[a]	0.79±0.01[a]	0.22±0.02[a]	1.72±0.01
C	Extract +Cd	1.96±0.19[ab]	0.69±0.16	0.21±0.06	1.73±0.04
D	Extract alone	1.55±0.11[b]	0.67±0.04	0.15±0.01	1.73±0.01

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Effect of Pretreatment with Stem Bark Extract of *ErythrinaSenegalensis*on Kidney Lipid Profile Concentration in Cadmium Intoxication

Results are summarized on Table 6. The results shows that cholesterol levels were significantly lower in the kidney of rats exposed to cadmium alone compared with the control. However, pretreatment with stem bark extract of *Erythrina senegalensis* significantly increased the cholesterol, HDL and LDL level in the kidney. There was significance increase in HDL level of rats exposed to cadmium alone when compared with control.

Table 6: Effect of Pretreatment with Stem Bark Extract of *Erythrina Senegalensis* on Kidney Lipid Profile Concentration in Cadmium Intoxication

		Lipid profile concentration (mmol/g tissue)			
Group	**Treatment**	**Chol**	**TG**	**HDL**	**LDL**
A	Control	2.52±0.11	0.79±0.03	0.20±0.003	1.75±0.08
B	Cd alone	2.35±0.02[a]	0.75±0.01	0.13±0.016[a]	1.72±0.01
C	Extract +Cd	2.66±0.23[ab]	0.84±0.01	0.14±0.004[a]	1.86±0.21[a]
D	Extract alone	2.66±0.08[ab]	0.75±0.01	0.18±0.023	1.88±0.05[a]

Values are expressed as mean ± SD, n = 4 for each group
[a]values are significantly different from control ($p<0.05$)
[b]values are significantly different from the group treated with Cd alone ($p<0.05$)

Cadmium has been recognized as one of the most toxic environmental and industrial pollutant that is usually stored in the tissues following exposure to it [3, 17]. One of the main manifestations of oxidative damage is lipid peroxidation which plays an important role in the toxicity of many xenobiotics [3, 18]. The toxicological manifestations of lipid peroxidation include decreased membrane fluidity and function, impaired mitochondrial functions and inhibition of enzymes [19]. MDA is an end product of lipid peroxidation and it is frequently measured as an index of this process [19]. The effects of cadmium intoxication on lipid peroxidation observed in this study demonstrate the ability of cadmium to induce oxidative stress in liver and kidney. The observed induction of lipid peroxidation by cadmium is in agreement with previous reports demonstrating that cadmium induces oxidative stress in tissues by increasing lipid peroxidation [20, 21]. It has been shown that various antioxidant defense systems protect cells from cadmium toxicity by quenching free radicals [19]. *Erythrina senegalensis* has been shown to have antioxidative properties [12]. Antioxidants stop free radical propagation by supplying the missing electron needed by the free radical and in the process

stabilize the unstable free radical [22]. Stem bark extract of *Erythrina senegalensis* act as an antioxidant by reducing and annihilating lipid peroxidation by quenching chain reaction of free radicals.The findings of the study has demonstrated that pretreatment with stem bark extract of *Erythrina senegalensis* protected against cadmium – induced depletion or reduction of chemical antioxidants such as glutathione. The depletion of this glutathione could be due to the formation of Cd – GSH complex with GSH. It has been shown that human beings when suffering from free oxygen radicals; a complex glutathione is activated [23]. This defense system include SOD, CAT, GPx, vitamins and glutathione reductase[23]. The increased activity of AST, ALP and ALT is in agreement with previous findings that exposure to cadmium causes induction of these enzymes [17].

Measurement of the activities of "Marker" biomarkers or enzymes in tissues and body fluids can be used in assessing the toxicity of a chemical compound and degree of assault and the on tissues/ organs [24, 25]. Such measurement can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques [26].

ALP, a "Marker" enzyme for the plasma membrane and endoplasmic recticulum [27], as well as AST and ALT are frequently used to ascertain the integrity of the plasma membrane [26] and the functionality of the liver; such that any alteration in the activity of these enzymes would indicate likely damage to the external boundaries of the cell (plasma membrane) [28].

The increased concentration of AST, ALT and ALP in the liver could be attributed to accumulation of cadmium in this tissue. Therefore, the decrease in the liver ALP and ALT activities in the study following the administration of stem bark extract of *Erythrina senegalensis* could be attributed to either inhibition of the enzyme activity at the cellular/molecular level or inactivation of the enzyme molecules following cadmium accumulation as shown by prior studies [26].

Urea and creatinine are among the indices that can be used to evaluate the normal functioning of animals [29]. In this study, urea and creatininelevels decreased when cadmium was administered alone but increased on administration of the extract. This result agrees with studies that have shown that the non-specific pattern of effect on the urea and creatinine level could possibly suggest physiological and is not likely not be toxicologicallyrelevant, more so when no definite pattern was produced [25].

Cholesterol level significantly increased in the lever of rat exposed to cadmium alone but decreased after the extract was administered while in the kidney, cholesterol level significantly decreased in rats exposed to cadmium alone compared to the control but increased after the extract was administered and this could be physiological.

The absence of an effect by the extract on the TG, HDL and LDL in the liver and the significant difference in the HDL and LDL in the kidney in this study may be an indication that the extract is enzyme and organ selective in exerting its effects.

CONCLUSION

It can be concluded from the results presented in this study that cadmium induced oxidative damage in the tissues examined by enhancing lipid peroxidation as well as alteration of the activities of enzymes biomarkers system. Also, the overall result from the effect of pretreatment with stem bark extract of *Erythrina senegalensis* on parameters determined showed clearly that stem bark extract of *Erythrina senegalensis* has protective effect against cadmium-induced oxidative damage in the kidney and liver of rats.

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