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Aqueous Extract of *Hibiscus Sabdariffa* Ameliorates Cadmium-Induced Liver and Kidney Injuries in Male Wistar Rats

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Abstract
This study investigated the ameliorative effect of aqueous extract of Hibiscus sabdariffa on cadmium-induced liver and kidney injuries in Wistar rats. Twenty-five (25) adult male Wstar rats were randomly assigned to five groups of five rats each. With the exception of group A (normal control group), rats in groups B to E received 50 mg/kg body weight, bwt, of cadmium orally once every three days. Rats in group B were not treated with extract or silymarin, group C received 100 mg/kg bwt silymarin, group D received 250 mg/kg bwt of extract, while group E received 500 mg/kg bwt of extract. After 21 days of treatment, the rats were sacrificed, and blood and tissue samples collected for biochemical analysis. The result obtained showed that cadmium significantly increased the concentration of malondialdehyde (MDA), and significantly reduced the activities of superoxide dismutase (SOD) and catalase in the kidney of rats ($p < 0.05$). However, treatment with aqueous extract of Hibiscus sabdariffa or silymarin significantly increased the activities of SOD and catalase, and significantly reduced the concentration of MDA in these tissues ($p < 0.05$). The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were significantly increased in serum of rats induced with cadmium. However, treatment with aqueous extract of H. sabdariffa significantly reversed the effect of cadmium on the activities of these enzymes ($p < 0.05$). The concentration of total protein (TP) was significantly higher in extract-treated groups than in group B (negative control) ($p < 0.05$). The concentrations of creatinine and urea were also significantly higher in group B (negative control) than in extract-treated groups ($p < 0.05$). These results suggest that H. sabdariffa protects liver and kidney against cadmium- induced hepatorenal toxicity comparable to the effect of silymarin.

Keywords: Cadmium, *Hibiscus sabdariffa*, Kidney, Liver, Silymarin, Wistar rats

Introduction
Cadmium (Cd) is a naturally-occurring metal released into the environment through natural activities such as mining, smelting, tobacco smoking, incineration of municipal waste and production of fertilizers [1]. Environmental exposure to cadmium is via contamination of groundwater released from smelting of lead and zinc ores, industrial wastes and use of sewage sludge as fertilizer [2]. Very little cadmium is excreted in urine. The rate of excretion increases slowly with increasing body burden, but as renal dysfunction develops, it increases even more sharply leading to a decrease in hepatic and renal cadmium concentrations [3].

Plants are good sources of food and medicines [4]. *Hibiscus sabdariffa* is a herb cultivated for its leaf, fleshy catyx, seed or fiber [4]. In Nigeria, a decoction of *H. sabdariffa* leaf is consumed as a common local drink popularly known as "zobo". In folk medicine, *H. sabdariffa* is used in the treatment of hypertension [5, 6]. The plant is also popular for its laxative effect. *Hibiscus* anthocyanin, a group of phenolic natural pigments present in dried flower of *H. sabdariffa* have been shown to confer protection on the cardiovascular system (CVS) [7, 8]. It also possesses hypocholesterolemic [9, 8], antioxidant and hepatoprotective effects in animals [5, 10].

Silymarin is an antioxidant compound ten times more potent than vitamin E [11]. It exerts membrane-stabilizing and antioxidant effects, promotes hepatocyte regeneration, reduces inflammatory reaction and inhibits fibrogenesis in the liver [12].

Materials and Methods
Plant material
Fresh calyces of *H. sabdariffa* were obtained from a market at Ndoro, Ikwuano Local Government Area of Abia State. They were identified by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike as *H. sabdariffa.* Herbarium specimen was prepared, and specimen number issued (MOUAU/COLNAS/PSB/17/118).

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Plant extraction

Dried calyces of *H. sabdariffa* were pulverized using mechanical blender and a portion (100 g) of the fine powder was weighed into a clean beaker and dissolved in 500 ml of distilled water. The content was mixed thoroughly and allowed to stand for 72 h with intermittent shaking to increase the rate of extraction. The suspension was filtered using Whatman filter paper No 1 and the filtrate concentrated using a rotary evaporator.

Experimental rats

The adult male Wistar rats used for this study were obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. They were housed in aluminum cages in a well-ventilated room and had free access to feed and clean drinking water. The rats were maintained in accordance with the recommendation of the Guide for the Care and Use of Laboratory Animals [13]. They were acclimatized for one week.

Experimental design

Twenty-five (25) adult male Wistar rats were randomly assigned to five groups of five rats each. With the exception of group A (normal control group), rats in groups B to E received 50 mg/kg body weight, bwt, of cadmium orally once every three days. Rats in group B were not treated with extract or silymarin, group C received 100 mg/kg bwt silymarin, group D received 250 mg/kg bwt of extract, while group E received 500 mg/kg bwt of extract. After 21 days of treatment, the rats were sacrificed, and blood and tissue samples collected for biochemical analysis. Portions of the excised kidneys were used to prepare 10 % tissue homogenate using phosphate buffer.

Biochemical analysis

The activities of AST, ALT and ALP, and concentrations of total bilirubin, TP, creatinine and urea were determined using Randox kits. The activities of SOD and catalase, and concentration of MDA were determined in kidney homogenate.

Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was analyzed by one-way analysis of variance (ANOVA) using SPSS (20.0). Groups were compared using Duncan multiple test range and values of $p < 0.05$ were considered statistically significant.

Results

Indices of liver function

The activities of AST, ALT and ALP, and concentration of total bilirubin were significantly higher in group B (negative control) than in groups C, D and E ($p < 0.05$). However, there were no significant differences in the concentrations of TP among the groups ($p > 0.05$). The activities of AST and ALP in group A (normal control) were not significantly different from those of groups C, D and E ($p > 0.05$). However, the activity of ALT and concentration of total bilirubin were significantly higher in groups C, D and E than in group A (normal control) ($p < 0.05$). There were no significant differences in the activities and concentrations of the measured parameters between group D and E ($p > 0.05$).

Table 1: Comparison of indices of liver function among the different groups

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	TP (mg/dL)
A (normal control)	19.18 ± 0.29^a	18.96 ± 1.13^a	51.50 ± 0.93^a	0.21 ± 0.02^a	5.07 ± 0.06
B (negative control)	30.87 ± 0.71^b	32.78 ± 1.41^b	62.02 ± 2.33^b	0.84 ± 0.07^b	4.72 ± 0.02
C (silymarin control)	19.81 ± 0.57^a	23.39 ± 3.00^a	53.20 ± 2.89^a	0.38 ± 0.10^a	5.44 ± 0.25
D(250 mg/kg bwt extract)	22.78 ± 1.69^a	25.41 ± 3.40^a	55.93 ± 1.54^a	0.50 ± 0.04^a	5.73 ± 0.02
E (500 mg/kg bwt extract)	19.66 ± 1.00^a	25.79 ± 3.29^a	52.86 ± 3.38^a	0.42 ± 0.03^a	5.55 ± 0.05

Data are expressed as mean ± SEM; n = 5

Within each column, superscripts with different letters are significantly different at $p < 0.05$.

Indices of kidney function

Treatment with aqueous extract of *H. sabdariffa* significantly reduced the concentrations of creatinine and urea in serum of rats ($p < 0.05$). However, there were no significant differences in the concentrations of creatinine and urea between group D and E ($p > 0.05$).

Table 2: Concentrations of creatinine and urea in the different groups

Groups	Creatinine (mg/dL)	Urea (mg/dL)
A (normal control)	0.63 ± 0.38^{a}	31.95 ± 1.39^{a}
B (negative control)	0.98 ± 0.03^{b}	27.09 ± 2.60^{b}
C (silymarin control)	0.88 ± 0.02^{a}	32.27 ± 1.64^{a}
D (250 mg/kg bwt extract)	0.91 ± 0.03^{a}	31.65 ± 2.49^{a}
E (500 mg/kg bwt extract)	0.85 ± 0.04^{a}	30.80 ± 0.50^{a}

Data are expressed as Mean ± SEM; n = 5
Within each column, superscripts with different letters are significantly different at $p < 0.05$.

Rats oxidative status

The activities of SOD and catalase were significantly higher in groups C, D and E than in group B (negative control), but the concentration of MDA was significantly reduced in groups C, D and E, when compared with group B ($p < 0.05$). However, there were no significant differences in the activities of SOD and catalase, and concentration of MDA between group A (normal control) and groups D and E ($p < 0.05$). There were no significant differences in the activities of SOD and catalase between group D and E ($p > 0.05$). However, treatment with aqueous extract of *H. sabdariffa* significantly and dose-dependently reduced the concentration of MDA ($p < 0.05$).

Table 3: Activities of SOD, catalase and concentration of MDA in the different groups

Groups	SOD (unit/mg protein)	Catalase (unit/mg protein)	MDA (μmole/mg protein)
A (normal control)	12.76 ± 0.61^{a}	14.35 ± 0.35^{a}	1.96 ± 0.05^{a}
B (negative control)	10.69 ± 1.36^{b}	10.92 ± 0.30^{b}	3.53 ± 0.42^{b}
C (silymarin control)	12.75 ± 0.53^{a}	14.07 ± 0.58^{a}	2.06 ± 0.04^{a}
D (250 mg/kg bwt extract)	12.95 ± 1.38^{a}	13.89 ± 0.33^{a}	1.85 ± 0.12^{a}
E (500 mg/kg bwt extract)	12.61 ± 0.45^{a}	13.67 ± 0.51^{a}	1.02 ± 0.06^{a}

Data are expressed as mean ± SEM; n = 5
Within each column, means superscripts with different letters are significantly different at $p < 0.05$, when compared with negative control group.

Discussion

Cadmium is a toxic heavy metal and exposure occurs mainly via inhalation or contamination of the food chain [14]. It is mostly toxic to the liver and kidney and the underlying mechanism involves induction of oxidative stress [15]. It has been reported that cadmium induces oxidative stress by promoting lipid peroxidation, depletion of glutathione level and distortion of membrane structures [16].

In the present study, there were significant reductions in the activities of SOD and catalase in rats treated with cadmium only, an indication that cadmium may suppress the activities of antioxidant enzymes in the kidney of rats. These results agree with those of previous studies [17].

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion into hydrogen peroxide and oxygen, while catalase catalyzes the breakdown of hydrogen peroxide to water and oxygen [18], thereby protecting biological systems from oxidative damage.

In this study, the concentration of MDA was significantly higher in group B (negative control) than in groups C, D and E. Malondialdehyde (MDA) is an index of lipid peroxidation. Cadmium promotes generation of reactive oxygen species (ROS) which in turn react strongly with membrane lipids, thus leading to lipid peroxidation and cell necrosis [19]. Lipid peroxidation affects membrane fluidity, damages membrane structures and proteins, and deactivates membrane receptors [20].

It is likely that aqueous extract of *H. sabdariffa* ameliorated the oxidative stress induced by cadmium by preventing lipid peroxidation. The antioxidant capacity of aqueous extract of *H. sabdariffa* may be attributed to

its rich phenolic content. Dietary sources of polyphenols possess high superoxide anion scavenging capacity in biological systems [21].

In this study, cadmium induced hepatic damage. Liver damage increases the concentrations of liver enzymes in the blood due to increased membrane permeability and necrosis. These enzymes leak from the damaged cells and find their way to the blood. The activities of AST, ALT and ALP, and concentration of total bilirubin were significantly higher in group B (negative control) than in groups C, D and E. There were no significant differences in the concentrations of TP among the groups. However, administration of aqueous extract of *H. sabdariffa* ameliorated the harmful effects of cadmium on the indices of liver function.

Treatment with aqueous extract of *H. sabdariffa* also significantly reduced the concentrations of creatinine and urea in serum of the *rats.* Creatinine is synthesized in the liver and it is a breakdown product of creatine phosphate in muscle, and it serves as a biomarker for kidney injury. Creatinine is removed mainly via the kidney, and its concentration in biological systems can be altered by various muscle sizes or decreased muscular activity [22]. The increased concentration of creatinine in group B (negative control) suggest that cadmium is nephrotoxic, and the low concentration of urea may be because of decreased production of urea by the liver. However, this only occurs when there is significant liver damage or disease.

Conclusion

The results obtained in this study suggest that aqueous extract of *H. sabdariffa* is effective in ameliorating liver and kidney injuries induced by cadmium.

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References

1. Chakraborty S, Dutta AR, Sural S, Gupt D and Sen S: Ailing bones and failing kidneys: a case of chronic cadmium toxicity. *Ann of Clin Biochem,* 50 (5): 492 - 495. 2003.
2. Gregory JM, Ingram WJ, Palmer MA, Jones GS, Stott PA, Thorpe RB *et al*: A new method for diagnosing radiative forcing and climate sensitivity. *Geophys Res Lett,* 31 (3): L03205. 2004.
3. Nordberg GF, Nordberg M, Piscator M and Vesterberg O: Seperation of two forms of metallothionein by isoelectric focusing. *Biochem J,* 126 (3): 491 - 498. 1972.
4. Dalziel JM: The useful plants of west tropical Africa. The Crown Agents for the Colonies, London. Pp 314 - 315. 1937.
5. Wang CJ, Wang JM, Lin WL, Cha CY, Chou FP and Tseng TH: Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem Toxicol,* 38 (5): 411 - 416. 2000.
6. Odigie IP, Ettarh RR and Adigun SA: Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. *J Ethnopharmacol,* 86 (2-3): Pp 181 - 185. 2003.
7. Jonadet M, Bastide J, Bastide P, Boyer P, Carnat AP and Lamaison JL: *In vitro* enzyme inhibitory and in vivo cardioprotective activities of hibiscus *(Hibiscus sabdariffa)* L. *J. Pharm. Belg,* 45 (2): 120 - 124. 1990.
8. Olaleye MT: Cytotoxicity and Antibacterial Activity of Methanolic Extract of *Hibiscus sabdariffa*. *J.Med.Plants,* 1: Pp 9 - 13. 2007.
9. Chen CC, Hsu JD, Wang SF, Chiang HC, Yang MY, Kao ES *et al*: *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *J Agric Food chem,* 51(18): 5472 - 5477. 2003.
10. Amin A and Hamza AA: Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation. *Toxicology,* 208 (3): 367 - 375. 2005.
11. Morales-González JA, Gayosso-Islas E, Sánchez-Moreno C, Valadez-Vega C, Morales-González A, Esquivel-Soto J. *et al*: Protective effect of silymarin on liver damage by xenobiotics. In: Oxidative stress and chronic degenerative diseases-a role for antioxidants, editor. *Rijeka: Croatia InTech, Pp* 500. 2013.
12. Feher J and Lengyel G: Silymarin in the prevention and treatment of liver diseases and primary liver cancer. *Curr Pharm Biotechnol,* (13): 210 - 217. 2012.
13. National Institutes of Health Office of Laboratory Animal Welfare Public Health Service policy on the humane care and use of laboratory animals. Bethesda, MD: NIH, 2002.
14. Abernethy D, DeStefano A, Cecil T, Zaidi K and Williams R: Metal impurities in food and drugs. *J Pharm Res,* 27: 750 – 755. 2010.

15. Matovi´c V, Buha A, Bulat Z and Duki´c-Cosi´c D: Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. *Arhiv za Higijenu Rada i Toksikologiju,* 62: 65–76. 2011.
16. Valko M, Morris H and Cronin D: Metals, toxicity and oxidative stress. *Curr Med Chem,* 12: 1161 – 1208. 2005.
17. Shagirtha K, Muthumani M and Prabu M: Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. *Eur Rev Med Pharmacol Sci,* 15: 1039 – 1050. 2011.
18. Kaushik D and Aryadeep R: Reactive oxygen species (ROS) and response of antioxidnats as ROS-scavengers during environmental stress in plants. *Front Env Sci,* 2: 53. 2014.
19. Wijeratne S: Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco- 2 human colon cells. *J Agric Food Chem,* 53: 8768 - 8774. 2005.
20. Arulselvan P and Subramanian S: Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic β-cells in experimental diabetes in rats. *Chem Biol Interact,*165: 155 – 164. 2007.
21. Emmanuel UE, Ebhohon OS and Omeh YN: Effect of fermented and unfermented cocoa bean on some liver enzymes, creatinine and antioxidant in wistar albino rats. *Carpathian J Food Sci Tech,* 7: 132 - 138. 2015.
22. Ndukaku OY, Ejiofor EU, Loveth UE and Oluchi OI: Valuation of some serum kidney functions and lipid profile of malaria patients in South Eastern Nigeria. *Rom J Biochem,* 52: 39 – 49. 2015.