Int J Physiol Pathophysiol Pharmacol 2020;12(4):107-114
www.ijppp.org /ISSN:1944-8171/IJPPP0112735

Original Article

Hibiscus sabdariffa extract protects against cadmium-induced ovarian toxicity in adult Wistar rats

Adeoye O Oyewopo[1], Kehinde S Olaniyi[2,4], Samuel O Olojede[3], Sodiq K Lawal[3], Oluwatobi A Amusa[4], Isaac O Ajadi[2]

[1]Department of Anatomy, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria; [2]School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag X54001, Congella 4013, Westville, Durban, South Africa; [3]Discipline of Clinical Anatomy, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag X54001, Congella 4013, 719 Umbillo Road, Durban, South Africa; [4]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

Received April 17, 2020; Accepted June 30, 2020; Epub August 25, 2020; Published August 30, 2020

Abstract: *Hibiscus sabdariffa* (HS) is native to tropical and subtropical regions, and its enrichment as a source of antioxidants and phytoestrogen has been documented. The present study investigated effects of HS on ovarian toxicity induced by cadmium. Adult female Wistar rats were grouped into 4 (n=5/group): Group A received HS (100 mg/kg), group B received cadmium sulphate (5 mg/kg), group C received cadmium sulphate and HS, and group D (control) received 1 ml of distilled water. Cadmium sulphate was administered for five days (*i.p*) followed by oral administration of HS for 28 days. Results showed distortion in the cytoarchitecture of the follicular cells in the ovary of cadmium-treated rats while there was mild or no distortion recorded for the ovary of the rats treated with cadmium and HS. There was also a significant reduction in the serum level of Luteinizing and follicle stimulating hormone of the rats treated with cadmium (group B) when compared with control rats. However, these alterations were attenuated when treated with HS. We concluded that HS has an ovarian protective effect in cadmium-treated adult female rats. Hence the present results suggest that HS extract would be a potential therapeutic agent in ovarian dysfunction.

Keywords: Cadmium sulphate, *Hibiscus sabdariffa*, luteinizing and follicle stimulating hormone, ovary

Introduction

In the recent years, activities such as cigarette smoking, waste burning, use of fossils fuels and metal ore combustion have put humans in great risk of exposure to heavy metals with potential toxicities [1]. The reports on potential toxicity of cadmium (CD), one of the heavy metals are rising yearly, possibly due to its wide range of applications in plastic industries, in production of battery and electroplating that results in environmental contamination and subsequently to environmental exposure [2, 3].

Cadmium toxicity and poisoning have been reported to be one of the global health issues due to their effects on major organs of the body, making it a major contributor to morbidity and mortality worldwide. Different route of cadmium entry into the body have been earlier described, such as through food, water, soil, air and chronic/acute exposure to cadmium have been implicated in several organ and systemic toxicity [4-6]. The cadmium toxicity increases as it accumulates in the liver and the kidney owing to its low elimination rate [7]. In previous studies that examined the effect of cadmium on female reproductive performance, histopathological alterations in the uterus and ovaries were reported, resulting from cadmium toxicity and this has also been suggested to be an independent contributor to female infertility [8-10]. Though Zhang et al. stated that mechanism of organ toxicity and ovarian dysfunction in cadmium exposure remains unknown [9]. However, recent studies have linked cadmium-induced ovarian dysfunction/organ toxicity to oxidative stress through reactive oxygen spe-

cies, alteration of gene expression, DNA damage, apoptosis and increase in membrane lipids peroxidation [11-13]. However, in quest for safe and effective therapeutic agent in combating the deleterious effects of cadmium, particularly on reproductive organs, researchers are diverting attentions to medicinal plants with potential therapeutic values.

Hibiscus sabdariffa Linn (*H. sabdariffa)* is an annual shrub commonly used to make jellies, jams and beverages. It is generally referred to as Roselle and locally known as "Zobo plant" in Nigeria. Various species of *H. sabdariffa* are widely distributed in tropical and subtropical regions of the world [14, 15]. The plant reaches about two meters in height with beautiful bright white to pale yellow flowers and a stout fleshy and bright red as the fruit matures. It is famous for producing edible calyx that remains the most frequently used portion of the plant, the leaves and seeds are often made into salads, curries and potherbs [16, 17]. Traditionally, *H. sabdariffa* is used as a source of drink and medicinal plant [18]. The leaves and calyces are prepared as a local dish for consumption and the plant is used as antiseptic, diuretic, purgative, sedative, anti-infertility and emollient [18, 19]. The leaves are also used for the treatment of hypertension and improvement of health immune system thus in the prevention of disease or infection [20]. Reports have it that *H. sabdariffa* is attracting the attention of food and beverage manufacturers and pharmaceutical concerns who feel it may have exploitable possibilities as a natural food product for herbal medicine and as a colorant to replace some synthetic dyes [21, 22].

Investigations have documented that the plant is enriched with vital minerals and nutrients such as iron, copper, calcium, magnesium, manganese required for healthy growth in humans, while the phytochemical analysis revealed its main constituents which include alkaloids, flavonoids, phenols, saponin, steroid, tannin, terpenoids, glycosides, phlobatannins and cardiac glycosides [21, 23]. In addition, a number of studies has investigated *H. sabdariffa* and found to contain many classes of secondary metabolites, including, anthocyanins, terpenoids, polysaccharides, amino acids, lipids, sesquiterpene, quinones, and naphthalene groups. Some of these compounds have been shown to have antibacterial, anti-inflammatory, antihypertensive, antifertility, hypoglycemic, antifungal, and antioxidative activities [19, 20]. In vitro and in vivo studies of *H. sabdariffa extract* revealed a potent antioxidant property [24]. Likewise, *H. sabdariffa extract* in experimental animals has been reported to protect against reactive oxygen and free radicals [24, 25], xanthine oxidase activity, lipid peroxidation-induced cell damage [26]. It was also demonstrated to improve glutathione level [27] and mitigated against hepatic injuries [20]. However, its ovario-protective effect has not been documented. In view of this, the present study was designed to investigate the ameliorative role of *H. sabdariffa* extract on cadmium-induced ovarian toxicity. The study also examined the effect of the treatments on gonadotropic hormones and hematological parameters.

Materials and methods

Preparation of the extract

Mature dry dark-red calyces of *Hibiscus sabdariffa* were locally obtained. These were botanically authenticated by Mr Bolu in the Department of Plant Biology, University of Ilorin, Ilorin. Authentication number was issued (UILH/001/646) and the plant was deposited at the herbarium. After drying, 100 g of the petals of *Hibiscus sabdariffa* was brewed in boiled tap water for 45 minutes. Using a filtration sieve of pore size 0.5 mm diameter, resulting decoction was filtered in steam bath until substantial water has been removed. It was later dried in the oven at 37°C and the extract concentration was prepared.

Animals, grouping and protocol

Twenty adult female Wistar rats weighing 150-180 g were obtained from the animal house, College of Medicine and Health Sciences, University of Ilorin, Ilorin, Nigeria. The rats were housed in wire mesh cages and maintained in a well-ventilated room at 25 ± 2°C, on a 12-h light/12-h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatized for one weeks, the rats were randomly allotted into groups (n=5 each); Group A was given HS (100 mg/kg *bw*), group B was given cadmium sulphate (5 mg/kg *bw*; Tianjin Kermel Chemical Reagent Co., Ltd.,

Table 1. Effects of *H. sabdariffa* on body weight in cadmium-treated Wistar rats

	A	B	C	D
Body weight (g)				
Initial	150.5 ± 8.4	152.0 ± 7.7	163.5 ± 8.42	154.3 ± 6.2
Change	47.5 ± 5.6*	3.7 ± 3.6*	16.2 ± 5.3#	25.5 ± 4.2

Data are expressed as mean ± S.E.M. n=5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*P<0.05 *vs.* D; #P<0.05 *vs.* B). A; *H. sabdariffa*-treated, B; Cadmium-treated, C; Cadmium + *H. sabdariffa*-treated and D; Control group.

China, c22892472) and group C was given cadmium sulphate and HS, and group D (control) was given 1 ml of distilled water. Cadmium sulphate was administered for five days (*i.p*) and the dose was chosen as previously described [5, 6] followed by oral administration of HS for 28 days [18]. The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of University of Ilorin, Ilorin, and every effort was made to minimize both the number of animals used and their suffering. Initial and final body weights were monitored using animal weighing balance (Olympia SCL66110 model, Kent Scientific Corporation, Torrington, CT06790, USA) and the body weight change was estimated.

Sample preparation and biochemical analysis

At the end of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p*). Blood was collected from the apex of the heart into EDTA and plain sample tube and centrifuged at 3000 rpm for 15 minutes using a bench centrifuge and the serum was stored frozen until it was required for biochemical assay. Biochemical analysis of serum gonadotropic hormones (Follicle stimulating hormone, FSH; Luteinizing hormone, LH) were performed using ELISA kits obtained from Roche (Switzerland). The blood collected with EDTA bottles were used for the analysis of hematological parameters (RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; PLT, platelet; WBC, white blood cell; PMN, polymorphonuclear; LYM, lymphocyte).

Histology

The ovaries were excised, blotted and weighed. After weighing, ovarian tissues were fixed in 10% buffered formol saline for histological examination using hematoxylin and eosin (H&E) staining techniques and examined microscopically at magnification of ×400.

Statistical analysis

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS, version 22 of statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values between the groups. Statistically significant differences were accepted at P< 0.05.

Results

Effects of H. sabdariffa on body weight in cadmium-treated adult female rats

Table 1 depicts the effect of administration of *H. sabdariffa* and cadmium on body weight. The results showed significant loss in body weight during treatment with cadmium sulphate alone when compared with control group. However, concomitant treatment with extract of *H. sabdariffa* during treatment with cadmium sulphate significantly improved the body weight. Treatment with *H. sabdariffa* alone significantly increased body weight when compared with control group.

Effects of H. sabdariffa on gonadotropic hormones (FSH and LH) in cadmium-treated adult female rats

Serum levels of gonadotropic hormones (FSH and LH) significantly decreased in cadmium-treated group when compared with control group. However, treatment with extract of *H. sabdariffa* significantly restored FSH and LH serum levels (**Table 2**).

Effects of H. sabdariffa on hematological parameters in cadmium-treated adult female rats

Treatment with cadmium sulphate significantly reduced red blood cells, hematocrit and platelet compared with control group. Whereas cad-

Table 2. Effects of *H. sabdariffa* on serum gonadotropic hormones in cadmium-treated Wistar rats

	A	B	C	D
FSH (mIU/mL)	0.582 ± 0.030	0.451 ± 0.020*	0.590 ± 0.030#	0.692 ± 0.010
LH (mIU/mL)	0.085 ± 0.002	0.043 ± 0.003*	0.056 ± 0.004*,#	0.076 ± 0.002

Data are expressed as mean ± S.E.M. n=5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*P<0.05 *vs.* D; #P<0.05 *vs.* B). A; *H. sabdariffa*-treated, B; Cadmium-treated, C; Cadmium + *H. sabdariffa*-treated and D; Control group. FSH, Follicle stimulating hormone; LH, Luteinising hormone.

Table 3. Effects of *H. sabdariffa* on hematological parameters in cadmium-treated Wistar rats

	A	B	C	D
RBC (10^{12} cells/l)	6.60 ± 0.18	4.22 ± 0.61*	5.87 ± 0.29#	5.96 ± 0.49
Hb (g/l)	12.15 ± 0.21	12.27 ± 0.53	10.90 ± 0.51	10.52 ± 1.89
HCT (%)	42.50 ± 0.55	30.75 ± 0.45*	37.25 ± 0.29#	40.75 ± 0.15
PLT (10^9 cells/l)	64.90 ± 2.3	42.1 ± 3.8*	55.9 ± 4.5*,#	66.7 ± 3.6
WBC (10^9 cells/l)	9.77 ± 1.83	10.22 ± 1.79	8.00 ± 1.32	8.85 ± 0.75
PMN (10^9 cells/l)	6.4 ± 0.4	5.4 ± 3.7	5.9 ± 3.2	5.7 ± 2.4
LYM (10^9 cells/l)	74.00 ± 4.74	76.25 ± 3.22	72.25 ± 4.35	68.00 ± 6.01

Data are expressed as mean ± S.E.M. n=5. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*P<0.05 *vs.* D; #P<0.05 *vs.* B). A; *H. sabdariffa*-treated, B; Cadmium-treated, C; Cadmium + *H. sabdariffa*-treated and D; Control group. RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; PLT, platelet; WBC, white blood cell; PMN, polymorphonuclear; LYM, lymphocyte.

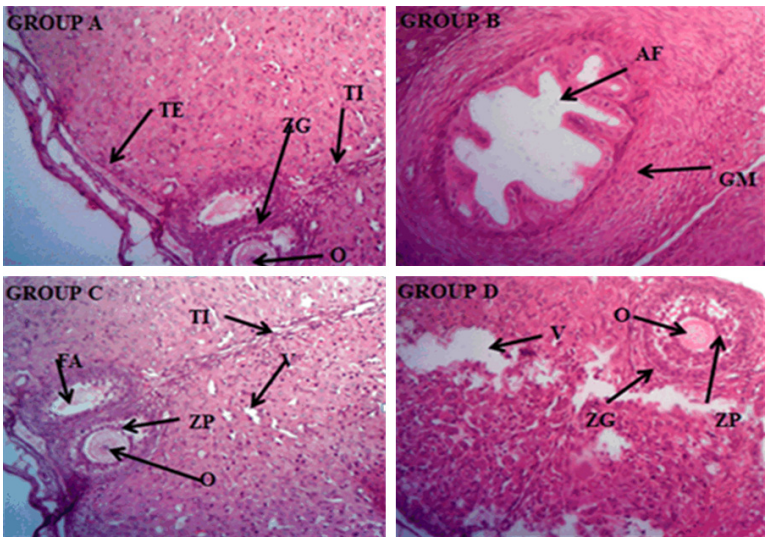

Figure 1. Effects of *H. sabdariffa* on the histology of ovary in cadmium-treated Wistar rats. O = Oocyte, ZP = Zonapellucida, ZG = Zonagranulosa, V = Blood vessels, TI = Theca interna cells, TE = Theca externa cell, FA = Follicular antrum, AF = Atretic follicles, GM = Glassy membrane. Group A: (H. sabdariffa) normal cytoarchitecture with oocyte, primordial and primary follicles, Group B: (Cadmium) showing severe deterioration of ovarian follicles and poor vascularization, Group C: (H. sabdariffa and Cadmium) showing normal cytoarchitecture with oocyte and proliferative follicles, Group D: (Distilled water) showing normal cytoarchitecture, matured oocyte, proliferative follicles (H and E stain; ×200, transverse section).

mium + *H. sabdariffa*-treated group showed a significant increase in red blood cells, hematocrit and platelet compared with cadmium-treated group. Hemoglobin, white blood cells, lymphocyte and polymorphonuclear remained unchanged in all the treated groups compared with control group (**Table 3**).

Effect of H. sabdariffa on the histology of ovary in cadmium-treated adult female rats

Histopathological changes in the ovaries have been shown to influence the function of this organ. H & E stained section of ovaries of *H. sabdariffa*-treated rat shows normal cytoarchitecture with oocyte, primordial and primary follicles (**Figure 1A**), cadmium-treated rat shows severe deterioration of ovarian follicles and poor vascularization (**Figure 1B**), cadmium + *H. sabdariffa*-treated rat shows normal cytoarchitecture with oocyte and proliferative follicles (**Figure 1C**) and control rat shows normal cytoarchitecture,

matured oocyte, proliferative follicles (**Figure 1D**).

Discussion

The search for safe and effective medicinal plants with an antioxidant property that can combat and caters for reproductive toxicity upon exposure to heavy metals are ongoing. Cadmium is a toxic heavy metal resulting in environmental contamination and subsequently affects human health. Moreover, studies on female rats revealed accumulation of cadmium in the female reproductive organs due to its long half-life [28, 29]. However, a medicinal plant such as *Hibiscus sabdariffa* that has been reported to exert antioxidant properties and chemo-protection are capable of combating toxicity upon exposure [30].

The results of this present study showed significant weight gain for group A animals administered with *Hibiscus sabdariffa*. The group B animals lost weight after the administration of cadmium prior to sacrifice. The body weight loss reported in this study is in consonance with previous study that documented body weight loss on experimental rats upon cadmium exposure [21, 31]. In another study, body weight loss observed in experimental rats owing to cadmium toxicity was linked to effect of cadmium on the intestinal absorption [32]. The group C animals that were administered with *Hibiscus sabdariffa* and cadmium gained weight when compared with cadmium-treated group but not with control group.

Analysis of serum LH levels of animals showed significant decrease in group B (cadmium) compared with control group (D) and this was restored when treated with *H. sabdariffa* (cadmium + *H. sabdariffa*). No significant difference was recorded between group A (*H. sabdariffa*) and group D (control). This finding was in line with the result given by Ali et al. [33]. Previously, cadmium is referred to as endocrine disruptor which indicates the possibility of causing reproductive toxicity, especially disruption of female reproductive hormone but the mechanism of reproductive toxicity is not fully understood [34, 35]. Analysis of FSH levels of rats showed decreased serum FSH level in group B rats that received cadmium only compared to the control group. Hormonal imbalance observed in group B of this study was consistent with recent study by Nasiadek et al. [35] that revealed ovarian damage in oral exposure of rats to cadmium and consequently resulted in hormonal imbalance. The serum FSH level of rats in group C (cadmium + *H. sabdariffa*) was shown to be lower and significant when compared with group D (control). The serum FSH of rats in group A (*H. sabdariffa*) was slightly lower but not significant to group D the control group. Similar results were documented in separate studies by Ali et al. [33] and Orisakwe et al. [36].

The hematological report of rats in group B that received cadmium sulphate showed a significant decrease in some hematological parameters such as red blood cells, hemoglobin concentration hematocrit and platelets when compared with control group D. These observations are consistent with earlier findings by Al-Asgah et al. [37] where cadmium was described as stressor that causes changes in biochemical and hematological parameters owing to its accumulation in different tissues of the body. These alterations were significantly recovered when treated with *H. sabdariffa,* suggesting that extract of *H. sabdariffa* possibly possesses the capacity to improve erythropoiesis.

The photomicrograph of rats in group A (*H. sabdariffa*), showed proliferative follicles with oocyte and was highly vascularized when compared to that of rats in group D (control). Group B (cadmium), showed atretic follicles, severe deterioration of the ovarian follicles and epithelia and also poor vascularization when compared with rats of the control group (D). The results in the present study are in consonance with findings that reported histological changes in the architecture of ovaries following administration of cadmium to experimental rats [38, 39] and similar to Nasiadek et al. [35] where cadmium administration was reported to lead to degeneration of granulosa cells, degeneration of corpus lutea, damage and less numerous oocytes. The ovarian distortion observed in the present study is also similar to previous observation and changes observed have been linked to the release of reactive oxygen species (ROS) and reactive nitrogen species as a result of exposure to cadmium, which causes oxidative stress to the reproductive tissues [40].

Group C (cadmium + *H. sabdariffa*), had little or no distortion of the ovarian follicle, showing oocyte, the primordial follicles and blood ves-

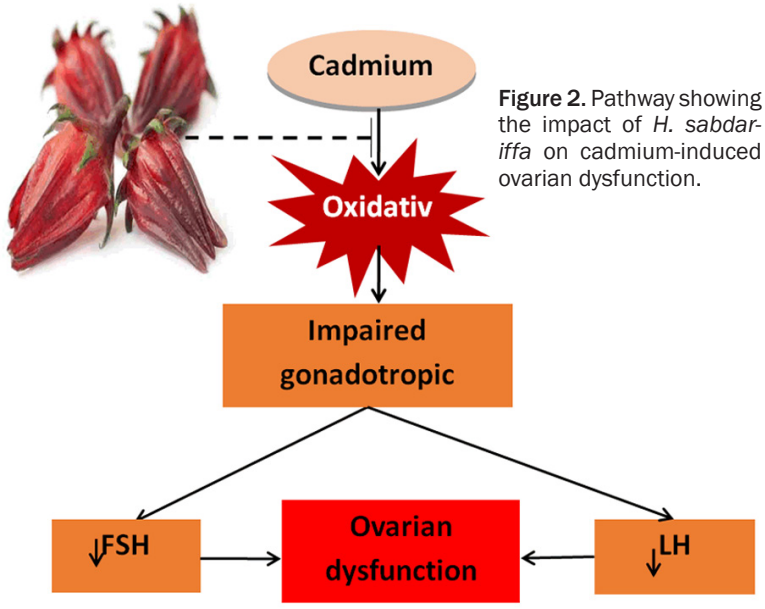

Figure 2. Pathway showing the impact of *H. sabdariffa* on cadmium-induced ovarian dysfunction.

sels when compared to the control group (D). The result obtained on the histology of rats' ovaries treated with *Hibiscus sabdariffa* upon exposure to cadmium clearly indicates the protective effects of *H. sabdariffa* which was in agreement with previous findings that shows mild effects of *H. sabdariffa* extract on rat reproductive hormones after cadmium exposure [41]. The protective effects observed upon administration of cadmium may be due to the antioxidant properties of the *H. sabdariffa* [42].

Conclusion

Results obtained from this experiment following administration of cadmium shows deleterious effect on the cytoarchitecture of the ovary, and *H. sabdariffa* protects against ovarian toxicity induced by cadmium sulphate. Further study is required to isolate the active ingredient of *Hibiscus sabdariffa* that is responsible for these effects and confirm the possible mechanism of action (**Figure 2**).

Disclosure of conflict of interest

None.

Address correspondence to: Kehinde S Olaniyi, School of Laboratory Medicine & Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Private Bag X54001, Congella 4013, Westville, Durban, South Africa. E-mail: olaniyisk@abuad.edu.ng

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