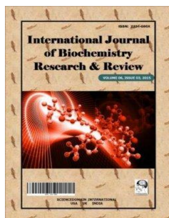

International Journal of Biochemistry Research & Review

21(4): 1-8, 2018; Article no.IJBCRR.41796
ISSN: 2231-086X, NLM ID: 101654445

Cadmium-Induced Testicular Damage in Wistar Rats: Protective Effects of *Hibiscus sabdariffa* L. Anthocyanins

Osuvwe Clement Orororo[1*], Samuel Ogheneovo Asagba[1], Nyerhovwo J. Tonukari[1], Ogheneovo Joy Okandeji[1] and Joseph Johnbusco Mbanugo[1]

[1]Department of Biochemistry, Delta State University, Abraka, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors OCO, SOA and NJT designed the experiment. Authors OCO, JJM and OJO carried out the laboratory work. Authors OCO and SOA wrote and edited the article. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2018/41796
Editor(s):
(1) Enrico Sanjust, Associate Professor, Department of Internal Medical Sciences, University of Cagliari, Italy.
Reviewers:
(1) Veeravan Lekskulchai, Srinakharinwirot University, Thailand.
(2) Noriah Bidin, University of Technology, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/24771

Original Research Article

Received 10th March 2018
Accepted 17th May 2018
Published 24th May 2018

ABSTRACT

Background: The search for potential ameliorators of Cd-toxicity is still on due to the many toxic effects of Cd including testicular damage.
Purpose: Thus the present study was designed to investigate the protective effects of *Hibiscus sabdariffa* L anthocyanins (HSA) on cadmium-induced testicular damage in wistar rats.
Materials and Methods: Twenty four adult male wistar rats (185±5.2g) were randomly divided into four groups and where treated for 15 days: A: control, B: Cd alone, 3 mg/kg b wt, C: HSA alone, 3 mg/kg b wt, Group 4: HSA Pre-CD: HSA (3g/ kg b wt for ten consecutive days) and Cd (3 mg/ kg b wt) for five days.
Results: Exposure to Cd caused significant ($p<0.05$) reduction in weight of testes compared to rats in the control group and those maintained on HSA alone. Exposure to Cd also caused significant reduction in activities of CAT and SOD and in the level of GSH in the testes accompanying increase in lipid peroxidation. However, pre-treatment of Cd-exposed rats with HSA resulted in amelioration

**Corresponding author: E-mail: osuvwec@yahoo.com;*

of Cd-induced reduction in testicular weight, the activities of SOD and CAT and the level of GSH in rat testes. Administration of HSA also reduced Cd-induced testicular lipid peroxidation and could be attributed to the antioxidant properties of HSA.
Conclusion: This study confirms the role of oxidative stress in the toxicity of Cd and the potentials of HSA in protecting tissues against Cd-induced damage and implicates HSA in the reported antioxidant properties of various *H. sabdariffa* extracts.

Keywords: Antioxidants; lipid peroxidation; glutathione; oxidative stress; anthocyanins.

1. INTRODUCTION

Cadmium is an extremely toxic metal commonly naturally found in ores combined with other metals, but has found its way into industrial workplaces and the environment in general due to its use in manufacturing industries and agriculture, smoking of cigarette and the general environmental pervasiveness of heavy metals [1, 2, 3]. Cd has low permissible exposure limit and a long biological half-life and as such overexposure and bioaccumulation occurs in humans and animals [4]. The testis has been reported to be a major target of Cd toxicity being very sensitive to low doses of Cd that may show no toxicological effects in other organs [5-8]. Although the exact mechanism Cd induced reproductive toxicity is still unknown, oxidative stress has been implicated by several studies [9, 8] and Cd has been reported to induce testicular injury by disruption of the blood‐testis‐barrier (BTB) [8]. Alirezaei et al. [5] associated oxidative stress with decline in fertility of spermatozoa and Ige et al. [10] established that Cd exposure causes adverse effects in humans and is a risk factor for infertility. Santos et al. [11] reported that Cd exposure reduces testis weight and causes histopathological lesions leading to reduced sperm counts and impaired sperm motility.

Given the importance of the testes to reproduction and the increasing rate of infertility, many researches have been conducted in search of substances with ameliorative potentials against Cd-induced damage to the testes and reproductive toxicity. Studies in this regard includes the use of vitamin E [12], coenzyme Q10 and vitamin E [9], curcumin [1], *Allium cepa* [10], Egyptian date palm pollen [13], grape juice concentrate [14], Betaine [15], beta carotene [4], *Physalis peruviana* L. [16], and lycopene [17] as exogenous antioxidant agents. Extracts of *Hibiscus sabdariffa* L. have also been reported to possess antioxidant properties capable of scavenging free radicals and reducing Cd-induced oxidative stress [18]. Polyphenolic compounds, flavonoids and other molecules present in these plants and animal materials were implicated in the observed antioxidant effects of these studies, but only few studies have been conducted using the isolated and purified compounds and molecules. The present study was thus designed to investigate the protective effects of *Hibiscus sabdariffa* L. anthocyanins (HSA) on cadmium-induced testicular damage in wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Reagents of analytical grade were used in this study. Cadmium chloride, methanol, trichloroacetic acid, acetonitrile and sodium chloride were purchased from Lobal Chemic Laboratory Regents and Fine Chemicals, Mumbai – India. 2,-thiobarbituric acid, acetic acid, adrenaline, and Ellman's reagent were gotten from BDH Chemical Company (Poole, England).

2.2 Plant Material

Fresh calyces of *H. sabdariffa* L. were obtained from Warri, Delta State and were identified by a specialist in the Department of Botany, Delta State University, Abraka. Thereafter, they were dried under continuous air-flow maintained at room temperature until constant weight was achieved.

2.3 Extraction and Purification *H. sabdariffa* Anthocyanins

Anthocyanins were extracted from *H. sabdariffa* calyces according to the method of Hong and Wrolstad [19] with sight modification as reported by Ologundudu et al. [20]. The extraction began by pulverization of one (1) kg of *H. sabdariffa* calyces. The pulverized calyces were then extracted using ten litres of 0.1% trifluoroacetic acid (TFA) for a period of twelve hours at 40°C. Thereafter, the extract was filtered with Whatman No. 1 filter paper and the filtrate was applied to

silica-gel resin column (120 mesh) for fractionation of the different compounds in the extract. The resin bed became red as it absorbed anthocyanins while sugars, acids and other water-soluble compounds were washed off with three litres of water. Anthocyanin pigments were thereafter eluted with 50% ethanol solution containing 0.1% TFA. The resulting eluate was dried at 40°C under vacuum to obtain a concentrated eluate, which was then was subjected to high-speed liquid chromatography (HPLC) to identify the purified anthocyanins and other active principles. This was done according to the method described by Drust and Wrolstad [21] using Agilent HPLC system (model-LC 1100 series).

2.4 Experimental Animals and Experimental Design

Twenty four (24) adult male wistar rats (185±5.2g) obtained from the animal house of the University of Nigeria, Nsukka were randomly divided into four treatment groups: A: control, B: Cd alone, 3mg/kg b wt, C: HSA alone, 3mg/kg b wt, Group 4: HSA Pre-CD: HSA (3g/ kg b wt for ten consecutive days) and Cd (3mg/ kg b wt) for five days.

H. sabdariffa anthocyanin and cadmium (in form of aqueous solution) were administered to the animals orally by orogastric tube. The animals were handled according to standard laboratory protocols and were fed with clean water and feed *ad libitum*. At the end of the treatment period, the animals were sacrificed by cervical dislocation and from each rat, testes were obtained, weighed and 1 g portion homogenized in ice-cold saline (1:4, w/v) and centrifuged at 5000g for 10 min. Sera collected was stored frozen until used for biochemical analysis.

2.5 Biochemical Assays: Determination of Catalase Activity

Catalase activity of samples was determined by the method of Singha [22]. Optical density was measured at 570nm with a spectrophotometer and a standard Catalase curve was obtained by plotting the concentrations of the standard against absorbance.

2.6 Determination of Superoxide Dismutase Activity

The method of Misra and Fridovich [23] was employed in determining SOD activity in samples based on the inhibition of the autoxidation of adrenaline at pH 10.2 by superoxide dismutase. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds and one unit of SOD activity of being the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adenochrome during 1 minute.

2.7 Estimation of Reduced Glutathione Level (GSH) Level

The method of Beutler et al. [24] was followed in estimating the level of reduced glutathione (GSH). The optical density was measured at 412nm with GSH being proportional to the absorbance at this wavelength as estimated from the GSH standard curve.

2.8 Estimation of Tissue Lipid Peroxidation

The level of Thiobarbituric acid reactive substances (TBARS) which is an index of lipid peroxidation was determined by the method of Varshney and Kale [25]. Values of TBAS were reported in terms of malondialdehyde (MDA) and expressed as µmole MDA/g tissue, the amount of MDA in the samples being quantified using a molar extinction coefficient of $1.56x10^5$m/cm.

2.9 Analysis of Data

Results obtained in the study were presented as Mean ± SD. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software. The one-way analysis of variance (ANOVA) was utilized in comparing the degree of significance of different parameters estimated and the difference between mean were considered to be significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

The effect of *H. sabdariffa* anthocyanin on testicular weight of Cd-exposed rats is presented in Fig. 1. Exposure to Cd caused significant ($p<0.05$) reduction in weight of testes compared to rats in the control group and those maintained on HSA alone. However, when Cd-treated rats were pre-treated with HSA there was increase in testicular weight compared to rats maintained on Cd alone. Changes in organ weight has been used as an indices of toxicity of metals since its reveals swelling in organs, organ deterioration or hypertrophy [26]. Thus, the significant reduction in testicular weight observed in this study (Fig. 1)

can be attributed to the toxic effects of Cd. However, there is conflicting results concerning the effect of Cd on changes in testis weight. While some studies have reported increased testicular weight [27,28], others reported atrophy of testis [13,14,29-34] similar to the findings of the present study. Makanjuola et al. [35] showed that Cd exposure caused a significant decrease in the testicular weight of rats and Rekha et al. [12] indicated that Cd reduces the testicular & epididymal weights. Their findings are in line with the result of this study and it can be concluded, as they did, that a part of the complex Cd-toxicity processes is the induction of necrotic and degenerative changes which damages testes and impair function.

On the other hand, increase in testicular weight caused by administration of HSA is probably due to the nutritional and antioxidant properties of HSA. This observation is in consonance with previous reports [36-38]. Anthocyanins have been reported to have beneficial effects on human health in addition to its nutritional value [39,40].

The level of lipid peroxidation in the testes of Cd-exposed rats pre-treated with HSA is shown in Fig. 2. Rats exposed to Cd alone (Group B) significantly higher MDA levels compared to the control. Rats maintained on HSA alone had MDA levels lower than the control and Pre-treatment of Cd-exposed rats with HSA significantly reduced MDA levels compared to rats maintained on Cd alone. Also, Table 1 shows the effect of *H. sabdariffa* anthocyanin on activities of CAT and SOD and on the level of GSH in the testes of Cd-exposed rats. Exposure to Cd alone (Group B) caused significant reduction in the activities of CAT and SOD and in the level of GSH compared to the control and rats treated with HSA alone.

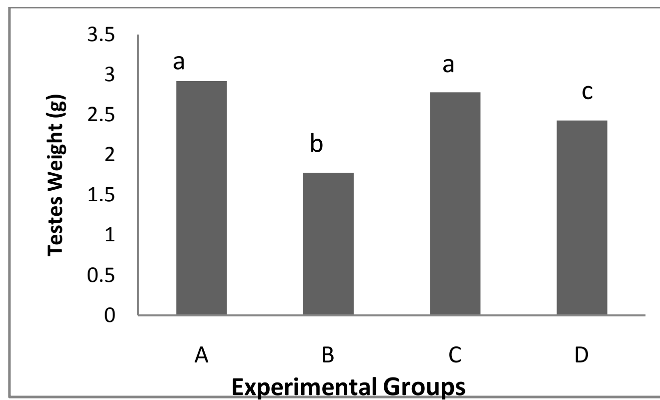

Fig. 1. Effect of *H. sabdariffa* anthocyanin on weight of testes of Cd-exposed rats. Groups: A (Control), B (Cd), C (Anthocyanin), D (Anthocyanin Pre-Cd)

Values with different alphabetic superscripts differ significantly (P<0.05)

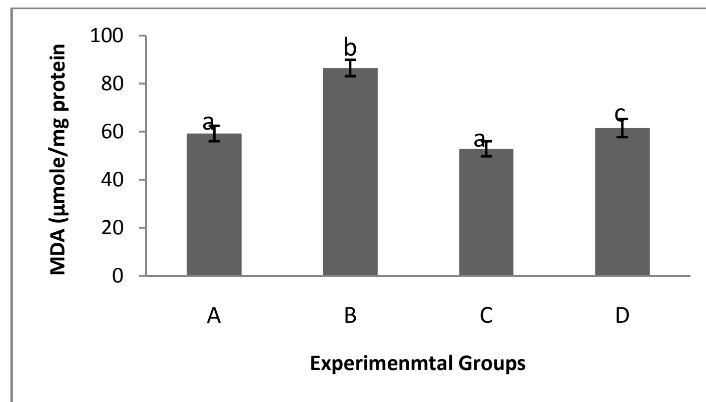

Fig. 2. Effect of *H. sabdariffa* anthocyanin on MDA levels in the testes of Cd-exposed rats. Groups: A (Control), B (Cd), C (Anthocyanin), D (Anthocyanin Pre-Cd)

Values with different alphabetic superscripts differ significantly (P<0.05)

Table 1. Effect of *H. sabdariffa* anthocyanin on activities of CAT, SOD and level of GSH in the testes of Cd-exposed rats

Groups/ parameters	Catalase (mmoles H_2O_2 consumed/min/mg	SOD (µmole/mg protein)	GSH µmole/mg protein
A	28.23±0.43[a]	37.89±1.52[a]	33.60±0.94[a]
B	14.60±0.64[b]	20.90±1.46[b]	19.74±0.45[b]
C	29.04±0.34[a]	37.99±1.57[a]	32.14±0.42[a]
D	20.99±0.29[c]	26.26±1.64[c]	24.02±0.36[c]

Values are presented as Mean ± SD. Values on the same column with different superscripts differ significantly (P<0.05). Groups: A (Control), B (Cd), C (Anthocyanin), D (Anthocyanin Pre-Cd)

However, pre-treatment of Cd-exposed rats with HSA (Group D) significantly increased CAT and SOD activities and the level of GSH relative to rats exposed to Cd alone (Group B). In all the parameters assayed, treatment with HSA alone (Group C) did non-significantly increase the activity of CAT and SOD compared to the control.

Cd-induced increase in lipid peroxidation observed in this agrees with earlier reports [41, 18]. This occurs when Cd-induced generation of free radicals is beyond the buffering ability of the testes antioxidants such as CAT, SOD and GSH. Usoh et al. [42]; Patra et al. [43] and Alirezaei et al. [44] noted that reduced glutathione (GSH), CAT and SOD are involved in the detoxification of reactive intermediates. Thus, the C-induced depletion of GSH and reduction in the activities of CAT and SOD facilitated the production of ROS leading to oxidative stress and increased lipid peroxidation witnessed in this study. This result also agrees with Salem et al. [17] whose worked showed that Cd induces oxidative damage by disturbing the prooxidant-antioxidant balance in tissues. Babaknejad et al. [45] and Salem et al. [17] also reported significant increase in MDA of tissues of Cd-exposed rats compared to the normal control group. In addition Liu et al. [46] reported decrease in testicular antioxidants: SOD, CAT and GSH by treatment with Cd.

However, pre-treatment of Cd-exposed rats with HSA reduced lipid peroxidation with concomitant increase in GSH levels and in the activities of CAT and SOD. This effect of HSA may be due to direct quenching of the reactive metabolites induced by Cd toxicity or its metal chelating ability. Oszmiañski [47] and Mossalam et al. [48] have reported antioxidant and metal ion chelating activities in anthocyanins. Mafulul and Okoye [49] and Mehmet et al. [50] have also reported antioxidant effects of extracts of *H. sabdariffa*. The results of this study confirm that HSA are responsible for such antioxidant effects.

4. CONCLUSION

Pre-treatment of Cd-exposed rats with HSA resulted in amelioration of Cd-induced reduction in testicular weight, the activities of SOD and CAT and the level of GSH in rat testes. Administration of HSA also reduced Cd-induced testicular lipid peroxidation and could be attributed to the antioxidant properties of HSA. This study thus confirms the role of oxidative stress in the toxicity of Cd and the potentials of HSA in protecting tissues against Cd-induced damage. The study also implicates HSA in the reported antioxidant properties of various *H. sabdariffa* extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh P, Deora K, Sankhla V, Mogra P. Curcumin rendered protection against cadmium chloride induced testicular damage in Swiss albino mice. J Cell Mol Bio. 2012;10(2):31-38.
2. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure a review of the

literature and a risk estimate. Scandinavian J Work Environ. Health. 2014;24:11-51.

3. Song JK, Luo H, Yin XH, Huang GL, Luo SY, Lin DR, Yuan DB, Zhang W, Zhu JG. Association between cadmium exposure and renal cancer risk: A meta-analysis of observational studies. Sci Rep. 2015;5: 17976.
4. Rekha DK, Nayanatara AK, Anupama N, Bhaghyalakhmi K, Pai SR, Shetty SB. Beta carotene as an antioxidant in cadmium induced testicular toxicity. Int J Curr Res Biosci. Plant Biol. 2014;1(3):61-66.
5. Alirezaei M, Jelodar G, Ghayemi Z. Antioxidant defense of betaine against oxidative stress induced by ethanol in the rat testes. Int J Pept Res Ther. 2012;18:239–247.
6. Rania ARE, Mohamed EG. Effect of long term cadmium chloride exposure on testicular functions in male albino rats. Am J. Animal Vet Sci. 2014;9(4):182-188.
7. Alaee S, Talaiekhozani A, Rezaei S, Alaee K, Yousefian E. Cadmium and male infertility. J Infert Reproduc. Bio. 2014;2(2): 62-69.
8. Kumar BA, Reddy AG, Kumar PR, Reddy YR, Rao TM, Haritha C. Protective role of N-Acetyl L-Cysteine against reproductive toxicity due to interaction of lead and cadmium in male Wistar rats. J Natural Sci Bio Med. 2016;4(2):414-419.
9. Ognjanović BI, Pavlović SZ, Maletić SD, Zikić RV, Stajn AS, Radojicić RM, Saicić ZS, Petrović VM. Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. Physiol Res. 2010;52(5):563-570.
10. Ige SF, Olalaye SB, Akhigbe RE, Akanbi TA, Oyekunle DA, Udoh UA. Testicular toxicity and sperm quality following Cadmium exposure in rats: Ameliorative potentials of *Allium cepa*. J Hum Reproduct. Sci. 2012;5(1):37-42.
11. Santos FW, Graca DL, Zeni G, Rocha JB, Weis SN. Sub-chronic administration of diphenyl diselenide potentiates cadmium-induced testicular damage in mice. Reprod. Toxicol. 2006;22:546–550.
12. Rekha DK, Tripathi Y, Raghuveer CV, Pai SR, Ramswamy C, Nayanatara AK, Vinodhini NA. Ranade A. Protective role of vitamin E against cadmium chloride induced testicular damage in rats. JPBS. 2009;22(2):12-16.
13. Wafaa AH, Akram ME, Noha A. Egyptian date palm pollen ameliorates testicular dysfunction induced by cadmium chloride in adult male rats. J. Am. Sci. 2012;8(4): 659-669.
14. Pires VC, Andre´a, PB, Gollucke, DA, Ribeiro, LL. Vaˆnia, DA, Odair, A. Jr. Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: A chronic assay. Bri. J. Nutr. 2013;110: 2020–2029.
15. Kheradmand A, Alirezaei M, Dezfoulian O. Cadmium-induced oxidative stress in the rat testes: Protective effects of betaine. Int J Pept Res Ther. 2012;19:337–344.
16. Mohamed SO, Ahmed N, Hassan SZ, Ahmed E, Moneim A. Effect of *Physalis peruviana L*. on cadmium-induced testicular toxicity in rats. Biol. Trace. Elem. Res. 2014;159:278–287.
17. Salem NA, Salem EA. Hepatorenal and testicular protective effects of lycopene against cadmium induced toxicity in male rats. J. Nephrol. Ther. 2016;6:265-270.
18. Al-Groom RM, Al-Kubaisy K. Anthocyanin–rich red dye of *Hibiscus sabdariffa* L. Calyx Modulates CdCl2- induced hypochromic microcytic anaemia and oxidative stress in rat red blood cells. J. Environ. 2016;5(1): 13-18.
19. Hong V, Wrolstad RE. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. J. Agric. Food Chem. 1990;38:708-715.
20. Ologundudu A, Ologundudu AO, Ololade IA, Obi FO. Effect of *Hibiscus sabdariffa* anthocyanins on 2,4-dinitrophenylhydrazine-induced hematotoxicity in rabbits. Afr. J. Biochem. Res. 2010;3:140-144.
21. Drust RW, Wrolstad RE. Separation and characterization of anthocyanins by HPLC. In current protocols in foods analytical chemistry, Wrolstad, R. E., Eds., John Wiley and Sons: New York. 2001;1-13.
22. Sinha AK, Colorimetric assay of catalase. Analy. Biochem. 1972;47(2):389-394.
23. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Bio. Chem. 1972;247:3170-3176.
24. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963;61: 882-888.
25. Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-

induced lipid peroxidation in microsomes. Int J. Radiat. Biol. 1990;58(5):733–743.

26. Amresh GR, Singh PN, Rao VC. Toxicological screening of traditional medicine Laghupatha (*Cissampelos pareira*) in experimental animals. J Ethnopharmacol. 2008;116:454–460.
27. Hwang DF, Wang LC. Effect of taurine on toxicity of cadmium in rats. Toxicology. 2011;167(3):173–180.
28. Adamkovicova M, Toman R, Cabaj M, Massanyi P, Martiniakova M, Omelka R, Krajcovicova V, Duranova H. Effects of subchronic exposure to cadmium and diazinon on testis and epididymis in rats. The Scientific World Journal. (2014): ID 632581:1-9.
29. Gaurav D, Preet S, Dua KK. Chronic cadmium toxicity in rats: Treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. J. Food Drug Analy. 2010;18(6): 464-470.
30. El-Neweshy, MS, El-Maddawy, ZK, and El-Sayed, YS. Therapeutic effects of date palm (*Phoenix dactylifera* L.) pollen extract on cadmium-induced testicular toxicity. Andrologia. 2012;20:1–10.
31. Ekhoye, EI, Nwangwa, EK, Aloamaka, CP. Changes in some testicular biometric parameters and testicular function in cadmium chloride administered Wistar rats. Br. J. Med. Med. Res. 2013; 3:2031-2041.
32. Saeed BT. Effects of Cadmium on sperm parameters, histological and hormonal changes in testes of mature rats. Iraqi J. Embryos Infert. Res. 2013;3(6):45-51.
33. Kaur S, Sharma S. Evaluation of toxic effect of cadmium on sperm count, sperm motility and sperm abnormality in albino mice. Int. J. Adv. Res. 2015;3(3):335-343.
34. Al-Ani NK, Al-Kawaz U, Saeed BT Protective influence of zinc on reproductive parameters in male rat treated with cadmium. Am. J. Med. Med. Sci. 2015; 5(2):73-81.
35. Makanjuola VO, Godam ET, Ipinniwa DA. The effect of methanolic extract of *Dissotis rotundifolia* on cadmium induced testicular damage in whistar rats. IOSR J. Pharm. 2014;4(7):56-65.
36. De Souza Predes, F, Diamante, MA, Dolder, H. Testis response to low doses of cadmium in Wistar rats. Inter J Experim Pathol. 2010;91:125-131.
37. Hashemi JM. *Hibiscus sabdariffa* calyx extract alleviate hepatotoxicity induced by carbon tetrachloride on male albino rats. Nature and Science. 2014;12(6):111-120.
38. Dahiru D, Umaru HA, Vasira B, Agama AM. Antioxidant and antiperoxidative activities of aqueous extract of *Hibiscus sabdariffa* L. calyx on alcohol-induced hepatic lipid peroxidation in rats. Am. J. Biochem. 2013;3(2):62-65.
39. Jaganath IB, Crozier A. Dietary flavonoids and phenolic compounds. In Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology (edited by Cesar G. Fraga). John Wiley & Sons, Inc., Hoboken, New Jersey. 2010;23.
40. Miguel MG. Anthocyanins: Antioxidant and/or anti-inflammatory activities. J. Applied Pharm. Sci. 2011;01(06):07-15.
41. Baiomy AA, Mansour AA. Genetic and histopathological responses to cadmium toxicity in rabbit's kidney and liver: Protection by Ginger (*Zingiber officinale*). Biol. Trace Elem. Res. 2016;170(2):320-9.
42. Usoh IF, Akpan E, Etim EO, Farombi EO. Antioxidant actions of dried flower extracts of *Hibiscus sabdariffa* L. on sodium arsenite – Induced oxidative stress in rats. Pakistan J. Nutri. 2005;4(3):135–141.
43. Patra RC, Rautray AK, Swarup D. Oxidative stress in lead and cadmium toxicity and its amelioration. Vet. Med. Int. 2011; Article ID 457327: 1-9.
44. Alirezaei M, Jelodar G, Ghayemi Z. Antioxidant defense of betaine against oxidative stress induced by ethanol in the rat testes. Int. J. Pept. Res. Ther. 2012;18:239–247.
45. Babaknejad N, Moshtaghie AA, Nayeri H, Shahanipour K, Bahrani S. The protective role zinc and magnesium in cadmium induced renal toxicity in male wistar rats. Biol Trace Elem Res. 2016;174:112-120.
46. Liu XR, Wang YY, Fan HR, Wu CJ, Kumar A, Yang LG. Preventive effects of β-cryptoxanthin against cadmium-induced oxidative stress in the rat testis. Asian J Androl. 2016;18:920-4.
47. Oszmiañski J. Aronia (*Aronia melanocarpa* Elliot) fruits and skullcap Vol. 50 E. Kowalczyk and others 547 (*Scutellaria baicalensis* Georgi) roots are very high in biologically active flavonoids. Farm Pol. 2001;15:726–30.
48. Mossalam HH, Aty OA, Morgan AE, Youssaf EN, Mackawy AMH. Biochemical

and ultra structure studies of the antioxidant effect of aqueous extract of *Hibiscus sabdariffa* on the nephrotoxicity induced by organophosphorous pesticide (Malathion) on the adult albino rats. Life Sci. J. 2011;8(5):561–574.

49. Mafulul SG, Okoye ZSC. Protective effect of pre-supplementation with selenium on cadmium-induced oxidative damage to some rat tissues. Int. J. Biol. Chem. Sci. 2012;6(3):1128-1138.

50. Mehmet KL, Burhan A, Meryem A, Yeter TT, Cevat A, Hamdi U. Vitamin E protects against oxidative damage caused by cadmium in the blood of rats. 2009;154-160.

© 2018 Orororo et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24771