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The Role of Antioxidant Plant Extracts in Cadmium-Induced Testicular Injuries

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ABSTRACT

The effects of cadmium (Cd) on testes were examined in rats injected subcutaneously with cadmium chloride ($CdCl_2$) at a dosage of 7 mg/kg body weight. Following $CdCl_2$ treatment, the rats were euthanized with phenobarbital sodium 24 hours post-injection, and their testes were subsequently removed and homogenized. The extent of hemorrhage was assessed by employing a spectrophotometer to measure the absorbance of hemoglobin at 414 nm in the soluble fraction of the organ homogenates. Exposure to Cd resulted in severe hemorrhage and increased hemoglobin absorbance in the testes of the treated animals. However, oral pre-treatment of different animal groups with various plant antioxidant extracts, such as ginger extract, pure garlic juice, pure broccoli juice, pure mint juice, and curcumin, administered in seven successive doses over seven days, significantly mitigated Cd-induced testicular hemorrhage. Consequently, it was concluded that the antioxidant properties of these plant extracts could effectively prevent cardiovascular damage to rat testes induced by Cd.

KEYWORDS
Broccoli, curcumin, garlic, ginger, testes, toxicity

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1. Introduction

Plant foods and plant-based products, such as cereals, may be contaminated with various heavy metals, leading to serious health problems. The cadmium (Cd) content in plant foods should not exceed permissible limits; however, Cd can accumulate in tissues and has a long half-life (Winiarska-Mieczan, 2014). Consequently, even low levels of Cd could pose health hazards over time. Approximately 2.5 g/kg b.wt./week is a tolerable intake level of Cd. Currently, no methods are employed to minimize the amount of Cd in foods. Thus, chelating agents are utilized to reduce Cd levels by inhibiting absorption and enhancing the body's oxidative capacity. Certain antioxidants, such as curcumin, vitamin C, vitamin E, and rutin, have been employed for this purpose (Tarasub *et al.*, 2012). From a nutritional perspective, examining the antioxidant content of edible foods in daily diets is particularly challenging in terms of mitigating the detrimental effects of toxic metals. The half-life of Cd ranges from 7 to 15 years; therefore, it is excreted extremely slowly, and the residual concentration of Cd can induce severe health effects in the body (Nordberg *et al.*, 2007).

Ginger *(Zingiber officinale)*, a widely consumed food spice in many countries, contains several active dietary components, including trace elements, vitamin C, resins, gingerol, volatile oil, and vitamin B, which can counteract the effects of metals on body tissues. This spice can be used therapeutically rather than prophylactically (Egwurugwu *et al.*, 2007).

Curcuma longa (Zingiberaceae) contains the active chemical curcumin, which is primarily used in India as a coloring agent. Curcumin is effective as an antioxidant (Subramanian *et al.*, 1994) and plays a vital role as a therapeutic agent without dangerous side effects. Sharma and Chetna (2019) reported that curcumin is beneficial to preventing Cd toxicity.

Broccoli *(Brassica oleracea var. capitata)* is rich in antioxidants and can be utilized to prevent the toxicity of metals and their side effects (Eryilmazz *et al.*, 2002). It plays an essential role in the treatment of various human and animal diseases, in addition to having anticarcinogenic, antitumor, antithrombotic, and hepatoprotective properties (Sadek and Al-Qattan, 1995).

In an experimental study, Shagufta *et al.* (2018) reported that garlic *(Allium sativum)* protects against and mitigates Cd's hepatotoxic and teratogenic effects on egg embryos. Garlic aids in the treatment of cancer and other diseases (Setiawan *et al.*, 2005) due to the effects of S-allyl-cysteine, alliin, allicin, and alliinase (Majewski, 2014). It is used as a protective and therapeutic agent against metal toxicity (Ugwuja *et al.*, 2016).

This study investigates the effects of plant antioxidant extracts on testicular blood vessel hemorrhage in male rats.

2. Materials and Methods

2.1. Chemicals:

Cadmium chloride ($CdCl_2$), tris-hydrochloride (HCl), magnesium chloride ($MgCl_2$), and calcium chloride ($CaCl_2$) were obtained from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Animals:

Seventy healthy male albino rats weighing 150–175 g were housed in fiberglass cages and provided with clean food and water ad libitum. They were acclimatized for two weeks. The Institutional Animal Care and Use Committee and the Research Ethics Committee of King Faisal University approved all the experimental procedures used in this study.

2.3. Experimental Design:

The rats were randomly divided into seven groups, each containing 10 animals:

- Group 1 (the control group) received an oral administration of 1 ml of saline and was injected subcutaneously (S/C) 1 hour later with 1 ml of saline.
- Group 2 received an oral administration of 1 ml of saline and was

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injected S/C 1 hour later with 1 ml of $CdCl_2$ at a dose of 7 mg/kg.b.wt. (Niewenhuis and Prozialeck, 1987).

- Group 3 received oral administration of 1 ml of ethanolic ginger extract (500 mg/kg.b.wt.) (Sanghal *et al.*, 2012) for 7 days. On the eighth day, the rats were injected S/C with $CdCl_2$ at the same dosage.
- Group 4 received an oral administration of 0.5 ml of freshly squeezed garlic juice for seven days. On the eighth day, the rats were injected S/C with $CdCl_2$ at the same dose.
- Group 5 received an oral administration of 1 ml of freshly squeezed broccoli juice (Brassica oleracea) for 7 days. On the eighth day, the rats were injected S/C with $CdCl_2$ at the same dose.
- Group 6 received an oral administration of 1 ml of freshly squeezed mint juice (Mentha longifolia) for 7 days. On the eighth day, the rats were injected S/C with $CdCl_2$ at the same dose.
- Group 7 received an oral administration of 15 mg/kg of curcumin for seven days. On the eighth day, the rats were injected S/C with $CdCl_2$ at the same dose.

Following a 24-hour experimental period, the rats from all groups were administered an intraperitoneal injection of phenobarbital sodium (50 mg/kg). Subsequently, the rats were euthanized, and their testes were extracted.

To assess the severity of Cd^{2+}-induced hemorrhaging, both testes from each rat were homogenized in a mortar containing 10 ml of 5-mM Tris-HCl buffer (pH 7.0), 1 ml of $MgCl_2$, and 100 µM of $CaCl_2$. The homogenate was then centrifuged at 5,000 rpm for 30 minutes. A 1-ml aliquot of the resulting supernatant fluid was diluted in 5 ml of buffer. The absorbance of hemoglobin at 414 nm was measured using Spectronic 21 spectrophotometers (Bausch and Lomb), as outlined by Niewenhuis and Prozialeck (1987). Statistical analyses were conducted utilizing SPSS software.

3. Results

The $CdCl_2$ administration led to the development of characteristic hemorrhages and testicular edema. The testes from the Cd-treated rats appeared swollen and dark violet (Fig. 1B). This treatment significantly increased ($p \leq 0.05$) these conditions, as demonstrated by the testicular hemoglobin absorbance (Table 1). In contrast, the testes from the rats pre-treated with various plant extracts for 7 days exhibited reduced levels of visible damage (Fig. 1C and D). Moreover, hemoglobin absorbance significantly decreased ($p \leq 0.05$) compared to the $CdCl_2$-treated group. Notable differences were observed among all the groups treated with Cd. In the curcumin-treated rats, the optical density (OD) of the testes' supernatant fluid (0.427 ± 0.064) was significantly distinct from the OD of the other extracts.

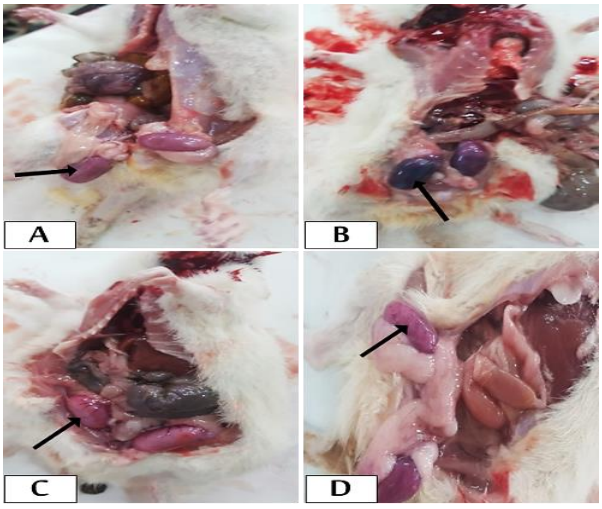

Figure 1: View of the internal organs of the control and treated animals.

1A) Normal testes; B) the testes treated with $CdCl_2$ exhibited severe congestion and subcapsular hemorrhage; C) the testes treated with curcumin demonstrated diminished congestion and hemorrhage, comparable to normal testes; D) the testes treated with broccoli had no abnormalities.

Table 1: Hemoglobin absorbance of testicular OD in the different experimental groups

Groups	Testicular OD Mean ± SE
Control	0.533 ± 0.015c
$CdCl^{2+}$	1.51 ± 0.24a
$CdCl^{2+}$ ginger for seven days	0.743 ± 0.162c
$CdCl^{2+}$ pure garlic juice for seven days	0.632 ± 0.079c
$CdCl^{2+}$ pure broccoli juice for seven days	0.465 ± 0.062c
$CdCl^{2+}$ pure mint juice for seven days	0.956 ± 0.117ab
$CdCl^{2+}$ curcumin for seven days	0.427 ± 0.064d

Mean ± standard error: $p \leq 0.05$.
The means with identical letters in the same column are not significantly different.

4. Discussion

Cadmium toxicity induces cardiovascular diseases, such as atherosclerosis and hypertension. Hypertension resulting from chronic Cd toxicity has been observed in animals (Nwokocha *et al.*, 2013). In addition, Cd toxicity causes an increase in oxidative stress, leading to damage and dysfunction in vascular cells (Liu and Kadiiska, 2009). The mechanism by which Cd induces testicular toxicity remains poorly understood; however, this toxicity may be attributed to blood vessel damage (Jian-Ming *et al.*, 2003). These results concur with those of other researchers who have studied the effects of multiple antioxidant extracts on cd-induced testicular injuries (Kukongviriyapan *et al.*, 2016; Velid et al., 2020).

Curcumin, a phenolic compound, is located within the turmeric rhizome. As an antioxidant, curcumin exhibits strong free radical scavenging activity (Sugiyama *et al.*, 1996). Curcumin's unique structure protects against Cd-induced testicular tissue damage and is crucial to preventing testicular membrane damage (Sharma and Ahuja, 2019).

Cadmium administration induces mild congestion in the seminiferous blood vessels; however, garlic treatment prevents Cd damage to the seminiferous blood vessels of the testes (Abd El-Hamid *et al.*, 2014). The current findings concur with those obtained by Obianime and Robert (2009). In an experimental study, administering aged garlic extract to mice prevented the first stage of atherosclerosis by inhibiting lipid deposition and vascular inflammation (Shang *et al.*, 2019). Elsewhere, Ponnusamy and Pari (2011) suggested that the active component of garlic, di-allyl tetrasulfide, demonstrated antioxidant and cytoprotective activity against Cd-induced toxicity both in vivo and in vitro.

In a further study, Cd caused an increase in malondialdehyde (MDA) and lipid peroxidation (LPO) while reducing the protein content in testicular and prostatic tissues compared with a control group, while conversely, broccoli was useful in reducing the MDA and LPO (Jahan *et al.*, 2014). This reduction was manifested through its natural enzymatic antioxidant, the enzyme, sulforaphane (Dinkova-Kostova and Talalay, 2008). Sulforaphane functions as a free radical scavenger (Kirana *et al.*, 2009).

In another study, cabbage targeted pathological Cd-induced damage through the improvement of hematological indicators, with an increase in the activity of antioxidant enzymes protecting the tissues against Cd damage (Onwuka *et al.*, 2010). Furthermore, a study investigating the effect of *Withania somnifera* (WS), an Indian medicinal plant, on Cd toxicity in testes demonstrated reduced oxidative stress parameters in the sera of the treated rats. In this study, rats were administered Cd mixed with WS or Cd with vitamin E. Interestingly, the WS exhibited similar protective effects to vitamin E against Cd toxicity. This protective effect was attributed to the antioxidant potential of WS (Prithiviraj *et al.*, 2013).

The vegetables used in our experiment are commonly consumed in the Middle East. People have limited protection against the detrimental effects of Cd toxicity, which are reducible, as demonstrated by the descending gradient of the potent effects of curcumin, broccoli, garlic, ginger, and mint, respectively. The current evaluation of the extracts' antioxidant activities presented in this

research aligns with those studied by Al-Sayyed *et al.* (2019). In addition, our results concur with those reported in this field. Certain extracts exhibited high antioxidant content and high total phenolic content. However, some extracts, such as broccoli and turmeric, presented low phenolic content while displaying high antioxidant activity. This occurrence can be attributed to specific phenolic units possessing high antioxidant activity. In contrast, other researchers have reported no correlation between the antioxidant and phenolic content of plant extracts (Kahkonen *et al*., 1999).

5. Conclusion

The findings of this research indicate that all of the examined plant extracts exhibit potential antioxidant properties. It is thus advisable to incorporate vegetables rich in antioxidants as a significant component of daily meals. The results obtained from the investigated vegetables may prove valuable for enriching human diets and informing epidemiological research. The primary objective of this study was to elucidate the role of dietary antioxidants in safeguarding against the effects of $CdCl_2$ on cardiovascular diseases and other conditions associated with oxidative stress. The vegetables employed demonstrated incremental protective effects against Cd-induced testicular damage in rats, which can be attributed to the presence of antioxidants and phenolic compounds.

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References

Abd El-Hamid, A.A., Badr, M., Helal, A.F. and Zaid, A.A. (2014). Protective effect of some medicinal plants and zinc on some serum parameters and histopathological features of liver, kidney, and testis in rats treated with cadmium. *Middle East East j. Appl. Sci.*, **4**(3), 539–54. DOI: 10.1016/j.sjbs.2013.02.004.

Al-Sayyed, H., Al-Kurd, R., Mwalla, M. and Abdel Qader, S. (2019). Determination of antioxidant content and activity in eight Jordanian fresh green leafy vegetables. *Agri Res and Tech, Open Access J.*, **19**(4), 1–8. DOI: 10.19080/ARTOAJ.2019.19.556102

Dinkova-Kostova, A.T. and Talalay, P. (2008). Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol Nutr Food Res*, **52**(1), 128–38. DOI: 10.1002/mnfr.200700195.

Egwurugwu, J.N., Ufearo, C.S., Abanobi, O.C., Nwokocha, C.R., Duruibe, J.O., Adeleye, G.S., Ebunlomo, A.O., Odetola, A.O. and Onwufuji, O. (2007). Effects of ginger (Zingiber officinale) on cadmium toxicity. *Afr. J. Biotechnol.*, **6**(18), 2078–82. DOI: 10.5897/AJB2007.000-2321

Eryılmaz, B., Deliorman, O.D., Aktay, G. and Bingöl, F. (2002). Antioxidant effect of cabbage (brassica oleracea var. capitata) on oxidative stress in cadmium treated mice. *FABAD J Pharm Sci.*, **27**(1), 1–5.

Onwuka, F.C., Erhabor, O., Eteng, M.U. and Umoh, I.B. (2010). Ameliorative effect of cabbage extract on cadmium-induced changes on hematology and biochemical parameters of albino rats. *J. Toxicol. Environ. Health Sci.,* **2**(2), 11–16. DOI: 10.3889/oamjms.2015.120

Jahan, S., Zahra, A., Irum, U., Iftikhar, N. and Ullah, H. (2014). Protective effects of different antioxidants against cadmium- induced oxidative damage in rat testis and prostate tissues. *Syst Biol Reprod Med,* **60**(4), 199–205. DOI:10.3109/19396368.2014.912363

Jian-Ming, Y., Marc, A., Qiong-Yu, C., Xiang-Dong, W., Bing, P. and Xue-Zhi, J. (2003). Cadmium-induced damage to primary cultures of rat Leydig cells. *Reprod. Toxicol,* **17**(5), 553–60. DOI: 10.1016/s0890-6238(03)00100-x

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, **47**(10), 3954–62. DOI: 10.1021/jf990146l

Kirana, H., Agrawal, S.S. and Srinivasan, B.P. (2009). Aqueous extract of Ficus religiosa Linn reduces oxidative stress in experimentally induced type 2 diabetic rats. *Indian J Exp Biol.*, **47**(10), 822–6.

Kukongviriyapan, U., Apaijit, K. and Kukongviriyapan, V. (2016). Oxidative stress and cardiovascular dysfunction associated with cadmium exposure: Beneficial effects of curcumin and tetrahydrocurcumin. *Tohoku J. Exp. Med.*, **239**(1), 25–38. DOI: 10.1620/tjem.239.25

Liu, J., Qu, W. and Kadiiska, M.B. (2009). Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol.*, **238**(3), 209–14. DOI: 10.1016/j.taap.2009.01.029

Majewski, M. (2014). Allium sativum: Facts and myths regarding human health. *Rocz. Panstw. Zakl. Hig,* **65**(1), 1–8.

Niewenhuis, R. and Prozialeck, W. (1987). Calmodulin inhibitors protect against cadmium-induced testicular damage in mice. *Biol. of Reprod,* **37**(1), 127–33.

Nordberg, G.F., Nogawa, K., Nordberg, M. and Friedmann, J.M. (2007). Cadmium. In: G.F. Nordberg, B.A. Fowler, M. Nordberg and L.T. Friberg (eds.) *Handbook on the Toxicology of Metals*. Amsterdam, Netherlands: Elsevier.

Nwokocha, C.R., Baker, A., Douglas, D., McCalla, G., Nwokocha, M. and Brown, P.D. (2013). Apocynin ameliorates cadmium-induced

hypertension through elevation of endothelium nitric oxide synthase. *Cardiovasc Toxicol.,* **13**(4), 357–63. DOI: 10.1007/s12012-013-9216-0

Obianime, A.W. and Roberts, I.I. (2009). Antioxidants cadmium-induced toxicity, serum biochemical and the histological abnormalities of the kidney and testes of the male Wistar rats. *Niger J Physiol Sci.*, **24**(2), 177–85. DOI: 10.4314/njps.v24i2.52910

Ponnusamy, M. and Pari, L. (2011). Protective role of diallyl tetrasulfide on cadmium-induced testicular damage in adult rats: A biochemical and histological study. *Toxicol Ind Health*, **27**(5), 407–16. DOI: 10.1177/0748233710387633.

Prithiviraj, E., Suresh, S., Lakshmi, N.V., Ganesh, M.K., Ganesh, L. and Parkash, S. (2013). Protective effect of Withania somnifera (Linn.) on cadmium-induced oxidative injury in rat testis. *Phytopharmacol*, **4**(2), 269–90. DOI: 10.18203/2319-2003.ijbcp20151340

Sadek, A.F. and Al-Qattan K. (1995), Chemopreventive effects of cabbage on 7,12-dirnethylbenz(a)anthracene-induced hepatocarcinogenesis in toads (Bufo viridis). *Nutr. Sci. Vitaminol.,* **41**(1), 163–8. DOI: 10.3177/jnsv.41.163.

Sanghal, A., Pant, K., Natu, S., Nischal, A. and Nath, R. (2012). An experimental study to evaluate the preventive effect of Zingiber officinale (ginger) on hypertension and hyperlipidaemia and its comparison with Allium sativum (garlic) in rats. *J. Med. Plants Res.*, **6**(25). 4231–8.

Setiawan, V.W., Yu, G.P., Lu, Q.Y., Lu, M.L., Yu, S.Z., Mu, L., Zhang, J.G., Kurtz, R.C., Cai, L., Hsieh, C.C. and Zhang, Z.F. (2005). Allium vegetables and stomach cancer risk in China. *Asian Pac. J. Cancer Prev.*, **6**(3), 387–95.

Shagufta, A., Shabana, S. and Chaman, A. (2018). Protection against cadmium-induced abnormalities and hepatotoxicity in ovo by Allium sativum. *Punjab Univ. J. Zool.*, **33**(1), 34–41. DOI: 10.17582/pujz/2018.33.1.34.41.

Shang, A.O., Shi-Yu, C., Xiao-Yu, X., Ren-You, G., Guo-Yi, T., Harold, C., Vuyo, M. and Hua-Bin, L. (2019). Bioactive compounds and biological functions of garlic (Allium sativum L.). *Foods*, **8**(7), 1–31. DOI: 10.3390/foods8070246

Sharma, S. and Chetna, A. (2019). Curcumin produces protective effects against testicular toxicity induced by cadmium in albino rats. *Int. J. Pharm. Sci. Drug Res*, **11**(5), 255–62. DOI: 10.25004/IJPSDR.2019.110517.

Sharma, S. and Ahuja, C. (2019). Ameliorating efficacy of curcumin on cadmium induced thyroid dysfunction in albino rats. *International Journal of Scientific Research in Biological Sciences*, **6**(1) 187–95. DOI: 10.26438/ijsrbs/v6i1.187195.

Subramanian, M., Sreejayan, R.M.N., Devasagayam, T.P. and Singh, B.B. (1994). Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. *Mutat Res.*, **311**(2), 249–55. DOI: 10.1016/0027-5107(94)90183-x.

Sugiyama, Y., Kawakishi, S. and Osawa, T. (1996). Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem Pharmacol.*, **52**(4), 519–25. DOI: 10.1016/0006-2952(96)00302-4.

Tarasub, N., Junseecha, T., Tarasub, C.H. and Ayutthaya, W.D.N. (2012). Protective effects of curcumin, vitamin C, or their combination on cadmium-induced hepatotoxicity. *J Basic Clin Pharm.*, **3**(2), 273–81. DOI: 10.4103/0976-0105.103821.

Ugwuja, E.I., Erejuwa, O.O. and Ugwu, N.C. (2016). Spice mixture containing garlic, ginger and nutmeg has protective effects on the kidneys and liver of cadmium exposed rats. *Adv. Pharm. Bull.*, **6**(2), 271– 4. DOI: 10.15171/apb.2016.038.

Velid, U., Dalkıran, T., Çiçek, M. and Kölükçü, E. (2020). The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: A review. *Adv Pharm Bull*, **10**(2), 184–202. DOI: 10.34172/apb.2020.023.

Winiarska-Mieczan, A. (2014). Cumulative rate and distribution of Cd and Pb in the organs of adult male Wistar rats during oral exposure. *Environ Toxicol Pharmacol.*, **38**(3), 751–60. DOI: 10.1016/j.etap.2014.08.016.