Tropical Journal of Pharmaceutical Research March 2021; 20 (3): 621-626
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i3.26

Original Research Article

Aqueous *Allium sativum* (garlic) extract ameliorates cadmium chloride-induced alterations in blood formation and spermatogenesis in albino rats

Edmund Chidiebere Mbegbu[1]*, Rita Ifeoma Odo[1], Paul Tobechukwu Ozioko[2], Mark Ebubechukwu Awachie[1], Lotanna Gilbert Nwobi[1], Ikechukwu Reginald Obidike[1]

[1]Department of Veterinary Physiology and Pharmacology, [2]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

For correspondence:* ***Email:* *edmund.mbegbu@unn.edu.ng;* ***Tel:*** *+234-8060144975*

Sent for review: 19 August 2020 *Revised accepted: 17 February 2021*

Abstract

Purpose: *To investigate the ameliorative effect of aqueous garlic extract (AGEx) on cadmium chloride ($CdCl_2$-induced) alterations in the blood and testicles of rats.*
Methods: *A total of 24 male rats (160 - 200 g), randomly assigned into 4 groups (A - D; n = 6), were used to investigate the claimed protective effect of AGEx on blood and spermatogenic tissues following $CdCl_2$-intoxication in albino rats. The rats in Group A served as controls and were given 5mg/mL of deionized water. Group B rats were given 300 mg/kg of AGEx. Group C rats were given 2 mg/kg of $CdCl_2$. Rats in Group D first received 2 mg/kg of $CdCl_2$, and 300 mg/kg of AGEx 2 h later. All treatments were administered every 48 h for a period of six weeks.*
Results: *$CdCl_2$ administration to group C rats reduced haematocrit value (PCV), concentration of haemoglobin (Hb), red cell count (RBC), total leucocytes count (tWBC), eosinophil, neutrophil, testicular weight and sperm reserve ($p < 0.05$), but elevated lymphocytes count compared with control ($p < 0.05$). AGEx 300 mg/kg in group D rats significantly reversed ($p < 0.05$) the altered parameters compared with control.*
Conclusion: *The results demonstrate that administration of aqueous Allium sativum (garlic) extract to male rats enhances spermatogenesis, and ameliorates testicular and haematological alterations induced by cadmium poisoning. Therefore, the spermatogenic principle in AGEx is a potential candidate for the clinical management of male infertility.*

Keywords: *Allium sativum, $CdCl_2$ toxicity, Haematology, Spermatogenesis, Rats*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Infertility has a prevalence of approximately 15 % worldwide [1]; and adverse effects of different environmental toxicants on spermatogenesis in rodents and humans have been widely reported [2]. The negative impacts of environmental toxicants on male reproductive physiology could result from production of oxygen free radicals [3],

disruption of endocrine secretions [4], alteration in the expression pattern of genes responsible for production of spermatozoa [2], as well as exerting epigenetic effects in the reproductive potency of the offspring [5].

Cadmium is one of the heavy metals that affect human health in the form of occupational hazards and environmental pollution [6]. The primary means of exposure to cadmium intoxication are through ingestion of contaminated food or water and inhalation of cigarette smoke [7]. In mammals, cadmium negatively affects cell proliferation (leading to increased apoptosis), and International Agency for Cancer Research has classified it as a human carcinogen [6]. Fifty percent of absorbed cadmium is distributed among the blood cellular components, especially the erythrocytes [6].

Garlic *(Allium sativum*) is a medicinal plant that is traditionally associated with therapeutic management of cough, food poisoning, disturbances of the digestive and circulatory systems, snake venom, pile, abdominal discomfort and inflammation of the lungs [8]. In search of cheap and safe remedies for male infertility, *Allium sativum* (garlic) has been accorded a folkloric reputation for reversing reproductive toxicity in male animals and man [9]. The current research was therefore designed to investigate the ameliorative effects of AGE on testicular and hematologic toxicities induced by cadmium chloride in albino rats.

EXPERIMENTAL

Extraction of AGEx

Fresh garlic bulbs were procured from Ogige Market in Nsukka Local Government Area of Enugu State, Nigeria. The clustered garlic bulbs were separated into cloves and the dry outer coverings were removed. Subsequently, 500 g of fresh cloves were ground in a blender containing 200 mL of distilled water and centrifuged at 10,000 g and then filtered through Wattman filter papers (Qualitative papers, 20 - 25 µm porosity, Dawson Ville, USA). The extract was then kept at 4 ^{o}C until and throughout use. The yield of the extract was determined by evaporation to dryness of 100 mL of the aqueous extract.

Animals

Twenty-four albino rats weighing between 160 and 200 g were used in this study. The albino rats used for this study were sourced from breeders in Zoological Garden, University of Nigeria, Nsukka (UNN), Enugu, State Nigeria. Clean metal cages, with wire mesh on top, were used to keep the rats, at prevailing temperatures of between 27 and 32 °C. The work was done at the Animal House Unit of Vet Physiology and Pharmacology Department, UNN. Acclimatization was done for two weeks before treatment ensued. The rats were allowed free and constant access to commercial feed and potable water.

Throughout the period of this research, the rats were naturally exposed to 12 h light/12 h darkness cycle. The Department of Vet Physiology and Pharmacology, UNN approved the experimental protocol (registration no. 09-166012, and international guidelines for animal studies were followed [10]

Experimental design

The twenty-four albino rats were assigned to four groups (n = 6) using simple random sampling method. Rats in group A were given 5 mg/mL of deionized water as placebo and served as the experimental control group. Rats in group B were given 300 mg/kg of AGEx. Group C rats were given 2 mg/kg of $CdCl_2$. Group D rats first received 2 mg/kg of $CdCl_2$, and 300 mg/kg of AGEx 2 hours later. With the aid of 18-guage oral rat feeder, these treatment regimens were administered every 48 h for a period of six consecutive weeks when blood and testes were obtained from all the groups for assessment of different parameters.

Haematological studies

Blood samples for haematology were obtained with the aid of haematocrit tubes from beneath the medial canthus of the eyes (retro bulbar plexus) into EDTA-containing tubes. Analysis for Packed Cell Volume (PCV), blood cells counts (RBC, and WBC), Haemoglobin concentration (Hb) were done using standard procedures [11].

Determination of body and testicular weight

Following determination of the body weights of the rats, humane sacrifice by decapitation was done and testes carefully harvested in order to determine the testicular weights.

Testicular and epididymal sperm count

This was done using the method described by [12], with slight modification [13]. The testicles were excised and trimmed of fats and extraneous tissues. The cauda epididymis or the testis was macerated and mixed with 10 ml of phosphate buffer saline solution of PH 6.8.and filtered.0.1 mL of the homogenate was then diluted with 0.9

mL of WBC fluid and 20 µL was viewed using Leica microscope (x10). Estimation of sperm cell numbers was done as previously described [13].

Histopathological examination

Gonadal tissues (3 – 5 mm thick) were immersed overnight in 75 mL of Bouin's fluid. Then, the tissues were processed for histology as previously prescribed [14].

Statistical analysis

Data obtained from the study were subjected to one-way ANOVA in SPSS. Mean values were separated by Duncan's New Multiple Range test and variations in mean values considered significant at $p < 0.05$. The results are presented as mean ± SEM.

RESULTS

Erythrocytic indices and WBC counts

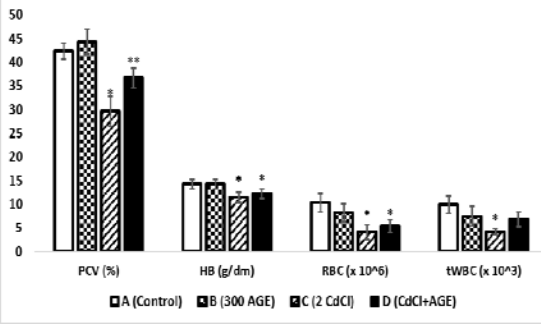

Figure 1: Mean PCV, Hb, RBC and WBC of the rats

Figure 1 showed results of mean PCV, Hb, RBC and WBC Counts. Groups A and B both had significantly *(P<0.05)* higher values of PCV, Hb, RBC count and WBC counts when compared with Group C and D. Administration of $CdCl_2$ (Group C) caused more reduction *(P<0.05)* in PCV and WBC count compared with the group treated first with cadmium chloride, and 300 mg/kg of AGEx after 2 hours (Group D).

Differential WBC count

The results of the differential white blood cells count shown in Table 1 revealed that while there was no variation *($p > 0.05$)* in the numbers of basophils and monocytes, 2 mg/kg of $CdCl_2$ (Group C) reduced *($p < 0.05$)* the numbers of eosinophils and neutrophils counts, but increased lymphocytes count when compared with the controls and Group C rats. It was observed that 300 mg/kg of AGEx ameliorated *($p < 0.05$)* $CdCl_2$-induced eosinopenia, neutropenia and lymphocytosis.

Body and testicular weights

There was no significant variation *($p > 0.05$)* in the mean body weights across the groups. However, 2 mg/kg of $CdCl_2$ significantly *($p < 0.05$)* decreased the testicular weights when compared with the control, rats dosed with 300 mg/kg AGEx, and rats first administered with 2 mg/kg of $CdCl_2$ and then 300 mg/kg of AGEx 2 h later. Treatment with $CdCl_2$ significantly *($p < 0.05$)* lowered the mean testicular allometric weights when compared with the control group and the group treated with 300 mg/kg of AGEx. The mean testicular allometric weight of the $CdCl_2$-treated rats did not vary *($p < 0.05$)* from that of the group first treated with $CdCl_2$ and then 300 mg/kg of AGEx 2 h later (*$p < 0.05$).*

Table 1: Effect of treatment on WBC differential count

Group	Basophils	Eosinophils	Neutrophils	Lymphocytes	Monocytes
A (Control)	0.33±0.33^a	4.67±1.33^a	19.00±1.00^a	68.00±2.00^a	4.00±0.00^a
B (300 AGEx)	0.67±0.33^a	4.00±0.00^a	20.00±0.00^a	70.00±0.00^a	4.33±0.33^a
C (2 CdCl)	0.33±0.33^a	2.67±0.33^b	15.00±0.58^b	74.00±1.00^b	4.00±0.58^a
D (CdCl+AGEx)	0.67±0.33^a	4.67±0.33^a	18.67±0.88^a	70.33±1.33^a	4.67±0.33^a

^{abc} *Varied superscripts alphabets in a column designate significant differences (P<0.05)*

Table 2: Mean values of body weight, testicular weight, and testicular allometric weight (TAW)

Group	Body weight (g)	Testicular weight (g)	TAW (g)
A (Control)	192.40±3.63^a	1.46±0.03^a	0.96±0.01^a
B (300 AGEx)	187.00±18.19^a	1.51±0.03^a	0.86±0.09^a
C (2 CdCl)	223.33±47.56^a	1.27±0.05^b	0.56±0.11^b
D (CdCl+AGEx)	249.00±49.12^a	1.52±0.06^a	0.63±0.08^b

^{abc}*Varied superscripts alphabets in a column designate significant differences in the mean values amongst the groups ($p < 0.05$)*

Testicular and epididymal sperm reserve

300 mg/kg of AGEx in group B rats produced no significant difference *(P>0.05)* in the testicular and epididymal sperm reserves compared with the control (group A) rats*P>0.05*. However, 2 mg/kg of $CdCl_2$ alone (Group C) significantly *(P<0.05)* decreased the mean testicular and epididymal sperm reserves compared with the control group (Group A), and the group treated with 300 mg/kg of AGEx (Group B). Combined administration of cadmium chloride and AGEx (Group D) resulted in significantly *(P<0.05)* higher sperm reserves in the testes and epididymis compared with Group C; but not comparable *(P>0.05)* with the testicular and epididymal sperm reserves of rats in Group A and Group B.

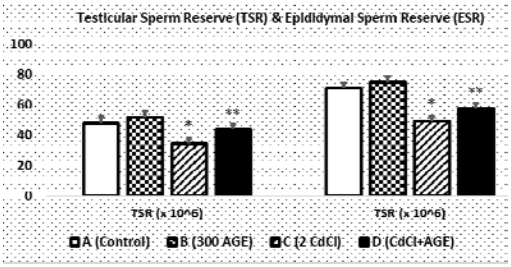

Figure 2: Testicular sperm reserve (TSR) and epididymal sperm reserve (ESR)

Histopathological features

The histological images of the testes (Figure 3 A - D) revealed that Group A rats (Control) had intact testicular interstitium (I) and seminiferous tubules (S) with healthy germinal epithelial layers (G) and lumen (L) richly filled with sperm cells. Rats in Group B (300 mg/kg AGEx) showed normal testicular interstitium (I) and seminiferous tubules (S) with healthy germinal epithelium (G) and sperm-rich lumen (L). There was depleted testicular interstitium (I), seminiferous tubules (S) with scanty germinal epithelium (G) and poorly filled lumen (L) in Group C rats (2 mg/kg $CdCl_2$). Administration of 2 mg/kg $CdCl_2$ and then 300 mg/kg of AGEx 2 hours later reversed the deleterious effect $CdCl_2$ on testicular tissues (Group D rats).

DISCUSSION

Haematological assays provide vital information for diagnostic purposes [15]. The significant reductions in the PCV, haemoglobin concentration and red blood cells count of group C rats when compared with the control group A rats suggest that $CdCl_2$ administration resulted in anaemia [16].

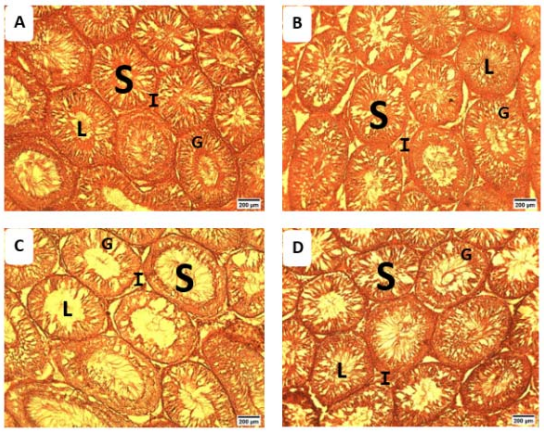

Figure 3: Photomicrograph of sections of the testis. Group A rats (Control) showing normal testicular interstitium (I) and seminiferous tubules (S) with healthy germinal epithelium (G) and richly filled lumen (L). Group B rats (300 mg/kg AGEx) showing normal testicular interstitium (I) and seminiferous tubules (S) with healthy germinal epithelium (G) and richly filled lumen (L). Group C rats (2 mg/kg CdCl2) showing depleted testicular interstitium (I) and seminiferous tubules (S) with scanty germinal epithelium (G) and poorly filled lumen (L). Group D rats (2 mg/kg CdCl2 plus 300 mg/kg AGEx) showing improved testicular interstitium (I) and seminiferous tubules (S) with better germinal epithelium (G) and lumen (L) compared with group C rats. (× 100; H & E)

This could be due to decrease in erythropoietic activities of the bone marrow and lowered haemoglobin-oxygen affinity in the RBCs [17,18]. The significant increase in the PCV in Group D rats when compared with group C rats proved that administration of AGEx ameliorated the adverse effects of $CdCl_2$ on erythropoiesis. The amelioration could not match the control group due to the turn over time of approximately 4 months required for replenishing RBCs [19].

On the other hand, the decreased WBC, eosinophil and neutrophil counts in $CdCl_2$-treated rats could be linked to cytotoxicity and immunosuppression. However, these parameters in group D rats were comparable with control group and AGEx-treated rats, suggesting that garlic *(Allium sativum)* has a remedial effect on the cadmium chloride toxicity. The turn over time of approximately 5 days for neutrophils and eosinophils invariably made this ameliorative impact of AGEx to be felt [19]. It has also been documented that garlic stimulates the immune function, and activates natural killer cells, T-lymphocytes and interleukin-2 [20,21]. The increase in absolute lymphocyte count in $CdCl_2$-treated rats could possibly be due to lymphocytic proliferation for antibodies synthesis in response to $CdCl_2$ intoxication [22].

The obvious reduction in the testicular weight in group C could arise from decrease in the number of epithelial spermatogenic cells nesting the seminiferous tubules, through testicular ischemia and necrosis [23], leading to loss of testicular weight. Aqueous garlic extract ameliorated this deleterious effect possibly by improving spermatogenic cells proliferation, leading to the observed increase in the mean testicular weight in this group. Furthermore, the observed reductions in mean testicular and epididymal sperm reserves could possibly be due to oxidative stress and impairment of spermatogenesis in the testes [24]. Cadmium decreases enzymatic antioxidants (superoxide dismutase (SOD) and catalase). Superoxide dismutase converts superoxide radicals (SOR) into hydrogen peroxide (H_2O_2), while catalase subsequently helps in detoxification of H_2O_2 to water. This mechanism is responsible for protecting the testes against lipid peroxidation [25]. However, garlic (*Allium sativum*) salvaged the negative effect of $CdCl_2$ toxicity, because the mean testicular sperm reserve of rats in group D was higher than the mean testicular sperm reserve of rats in group C. Necrosis and testicular interstitial damage associated with cadmium chloride treatment and increase in spermatogenic cells, normal interstitial spaces and seminiferous tubules lends further credence to the fact that garlic *(Allium sativum)* ameliorated anti-spermatogenic effect of cadmium intoxication.

CONCLUSION

This work has demonstrated that administration of aqueous garlic *(Allium sativum)* extract to male rats enhances spermatogenesis and ameliorates testicular and haematological alterations induced by cadmium poisoning. Therefore, the spermatogenic principle in AGEx can potentially be developed for the clinical management of male infertility.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors, and we accept all liabilities pertaining to claims relating to the content of this article. Edmund Chidiebere Mbegbu, Rita Ifeoma Odo and Ikechukwu Reginald Obidike designed and supervised the research. Paul Tobechukwu Ozioko, Mark Ebubechukwu Awachie and Lotanna Gilbert Nwobi collected and analysed the data. The manuscript was written by Edmund Chidiebere Mbegbu, and all authors edited and approved the manuscript for publication.

REFERENCES

1. *Katole A, Saoji AV. Prevalence of Primary Infertility and its Associated Risk Factors in Urban Population of Central India: A Community-Based Cross-Sectional Study. Indian J Community Med 2019; 44(4): 337-341.*
2. *Ren Y, Shao W, Zuo L, Zhao W, Qin H, Hua Y, Lu D, Mi C, Zeng S, Zu L. Mechanism of cadmium poisoning on testicular injury in mice. Oncol Lett 2019; 18(2): 1035-1042.*
3. *Alahmar AT. Role of Oxidative Stress in Male Infertility: An Updated Review. JHum Reprod Sci 2019; 12(1): 4–18.*
4. *Lauretta R, Sansone A, Sansone M, Romanelli F, Appetecchia M. Endocrine Disrupting Chemicals: Effects on Endocrine Glands. FrontEndocrinol 2019; 10: 178.*
5. *Bitto A, Pizzino G, Irrera N, Galfo F, Squadrito F. Epigenetic modifications due to heavy metals exposure in children living in polluted areas. Curr Genomics 2014; 15(6): 464-468.*
6. *Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metals Toxicity and the Environment. EXS 2012; 101: 133-164.*
7. *Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: An update. Caspian JInternMed 2017; 8(3): 135-145.*
8. *Saied HD, Abdolkarim ZM, Negahdar M, Ehsan A, Razich G, Ehsan D. The effects of fresh garlic on the serum concentration of total cholesterol, total triglyceride and adipose tissue of broilers. Comp Clin Path 2010; 19: 363-365.*
9. *Ayoka AO, Ademoye AK, Imafidon CE, Ojo EO, Oladele AA. Aqueous Extract of Allium sativum (Linn.) Bulbs Ameliorated Pituitary-Testicular Injury and Dysfunction in Wistar Rats with Pb-Induced Reproductive Disturbances. Open Access Maced J Med Sci 2016; 4(2): 200-212.*

10. NRC. Guide for the use of laboratory animals. Institute of Laboratory Animal Research Commission on Life Sciences, National Research Council (NRC). Washington DC: National Academy Press; 1996.
11. Thrall MA, Weiser MG. Hematology. In: Hendrix CM (Ed.) Laboratory Procedures for Veterinary Technicians, 4th ed. Missouri: Mosby; 2002; 29-74.
12. Amann RP, Almquist JO. Reproductive capacity of dairy bulls. Techniques for direct measurement of gonadal and extra gonadal sperm reserves. J Dairy Sci 1986; 44:1537–1545.
13. Mbegbu EC, Odo RI, Edeh DC, Obidike IR. Haematological Profile and Some Reproductive Indices of Male Albino Rats Treated with Ethanolic Stem Bark Extract of Picralima nitida. J Adv Med Pharm Sci 2017; 14(1): 1-14.
14. Slaoui M, Fiette L. Histopathology procedures: from tissue sampling to histopathological evaluation. In: Gautier, J. C. (Ed.), Drug Safety Evaluation: Methods in Molecular Biology (Methods & Protocols), Humana Press, Totowa, New Jersey: 2011; 69-82.
15. Koutsi A, Vervesou EC. Diagnostic molecular techniques in haematology: recent advances. Ann Transl Med 2018; 6(12): 242.
16. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann NY Acad Sci 2019; 1450(1): 15-31.
17. Witeska M, Kosciuk B. The changes in common carp blood after short-term zinc exposure. Environ Sci Pollut Res 2003; 10: 284-286.
18. Andjelkovic M, Buha Djordjevic A, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z. Toxic Effect of Acute Cadmium and Lead Exposure in Rat Blood, Liver, and Kidney. Int J Environ Res Public Health 2019; 16(2): 274.
19. Spalding K, Bhardwaj RD, Buchholz B, Druid H, Frisén J. Retrospective birth dating of cells in humans. Cell 2005; 122: 133-143.
20. Tang Z, Sheng Z, Liu S, Jian X, Sun K, Yan M. The preventing function of garlic on experimental oral precancer and its effect on natural killer cells, T-lymphocytes and interleukin-2. Hunan I Ko Ta Hsueh Pao 1997; 22: 346-348.
21. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L, Ortuño-Sahagún D. Immunomodulation and anti-inflammatory effects of garlic compounds. J Immunol Res 2015; 401630.
22. Hoffman W, Lakkis FG, Chalasani G. B Cells, Antibodies, and More. Clin J Am Soc Nephro 2016; 11(1): 137-154.
23. Zhu Q, Li X, Ge RS. Toxicological Effects of Cadmium on Mammalian Testis. Front Genet 2020; 11: 527.
24. Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review. J Clin Diagnostic Res 2017; 11(5): IE01-IE05.
25. Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci 2018; 12(3): 88-93.