Aqueous *Allium sativum* (garlic) extract ameliorated CdCl₂-induced alterations in blood formation and spermatogenesis in albino rats

Edmund Chidiebere Mbegbu¹*, Rita Ifeoma Odo¹, Paul Tobechukwu Ozioko², Mark Ebubechukwu Awachie¹, Lotanna Gilbert Nwobi¹, Ikechukwu Reginald Obidike¹

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

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

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**Abstract**

**Purpose:** To investigate the ameliorative effect of aqueous garlic extract (AGEx) on cadmium chloride (CdCl₂-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 CdCl₂-intoxication in albino rats. The rats in Group A served as controls and were given 5 mg/mL of deionized water. Group B rats were given 300 mg/kg of AGEx. Group C rats were given 2 mg/kg of CdCl₂. Rats in Group D first received 2 mg/kg of CdCl₂, and 300 mg/kg of AGEx 2 h later. All treatments were done every 48 h for a period of six weeks.

**Results:** CdCl₂ administration to group C rats reduced (p < 0.05) haematocrit value (PCV), concentration of haemoglobin (Hb), red cells count (RBC), total leucocytes count (tWBC), eosinophil, neutrophil, testicular weights and sperm reserves; but elevated (p < 0.05) lymphocytes count compared with the controls. AGEx 300 mg/kg in group D rats significantly reversed (p < 0.05) the altered parameters compared with the controls.

**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*, CdCl₂ toxicity, Haematology, Spermatogenesis, Rats

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.

METHODS

Extraction of AGE

Fresh garlic bulbs were procured from Ogige Market in Nsukka Local Government Area of Enugu State. 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 °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 with registration number 09-166012, and the animals were humanely handled [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 AGE. Group C rats were given 2 mg/kg of CdCl2. Group D rats first received 2 mg/kg of CdCl2 and 300 mg/kg of AGE 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 cells 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 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 hours later. $P<0.05$; $P<0.05$.

**Table 1:** Effect of treatment on WBC differential count

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

abc Varied superscripts alphabets in a column designate significant differences ($P<0.05$)

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

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. However, 2 mg/kg of CdCl₂ 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.

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 CdCl₂ administration resulted in anaemia [16].

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 CdCl₂). Administration of 2 mg/kg CdCl₂ and then 300 mg/kg of AGEx 2 hours later reversed the deleterious effect CdCl₂ on testicular tissues (Group D rats).

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 CdCl₂ 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 CdCl₂-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 CdCl₂-treated rats could possibly be due to lymphocytic...
proliferation for antibodies synthesis in response to CdCl₂ 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 (H₂O₂), while catalase subsequently helps in detoxification of H₂O₂ to water. This mechanism is responsible for protecting the testes against lipid peroxidation [25]. However, garlic (Allium sativum) salvaged the negative effect of CdCl₂ 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.

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